Effect of Three Dimensional Substratum Features on Benthic Algal Biomass Productivity

by

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Abstract

Algae has emerged as a promising and valuable source for biofuels, waste water remediation and bio-products such as fertilizers, bioplastics and aquaculture feed. Algae's emergence can be attributed to its high growth rates and the ability to use the cultivation of algae for the dual purposes of a biofuel source and as an agent for wastewater remediation. For cultivated algae, the yield is strongly dependent on the characteristics of the substratum used for cultivating algae. Substratum features such as topography and roughness affect algal attachment and colonization, thereby influencing the growth rate of algae grown on such substratum. Traditionally, two dimensional substrata have been the substratum of choice for algal cultivation, with significant effort being invested towards optimizing features such as the surface roughness. Recently, the suitability of three dimensional substrata has been tested, with results showing strong algal yield performances. However, how three dimensional substratum supports algal biomass yield is not fully understood. This study seeks to investigate the effect of three dimensional substratum features such as fiber density on algal biomass productivity, with the goal of understanding how such features impact algal cultivation. Additionally, the three dimensional substratum are deployed in both low and high nutrient concentration environments to explore their performance under different nutrient environments. Also, the importance of substratum base roughness is investigated. In two dimensional substrata, the substratum roughness feature is vital. However, for three dimensional substrata, the introduction of vertical structures to support algal attachment and colonization activities may minimize the need for optimizing the roughness of the substratum base. To carry out these experiments, bioreactor systems were designed, constructed and put to use. Also,

experimental methods for growing, harvesting, drying and measuring algal biomass were developed and implemented. Findings obtained from the studies showed that three dimensional substratum features such as fiber density significantly affect algal biomass productivity, while features such fiber height may not have so much effect on algal yield. The introduction of three dimensional substrata also significantly increased algal yield under different nutrient concentration environments. Under low nutrient environments, the switch to three dimensional substratum led to a 174% increase in yield, while under high nutrient environments the yield increased by 89%. Finally, results suggest that when using three dimensional substrata for algal cultivation, the substratum base roughness may not be a significant factor for algal biomass productivity.

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1. Introduction

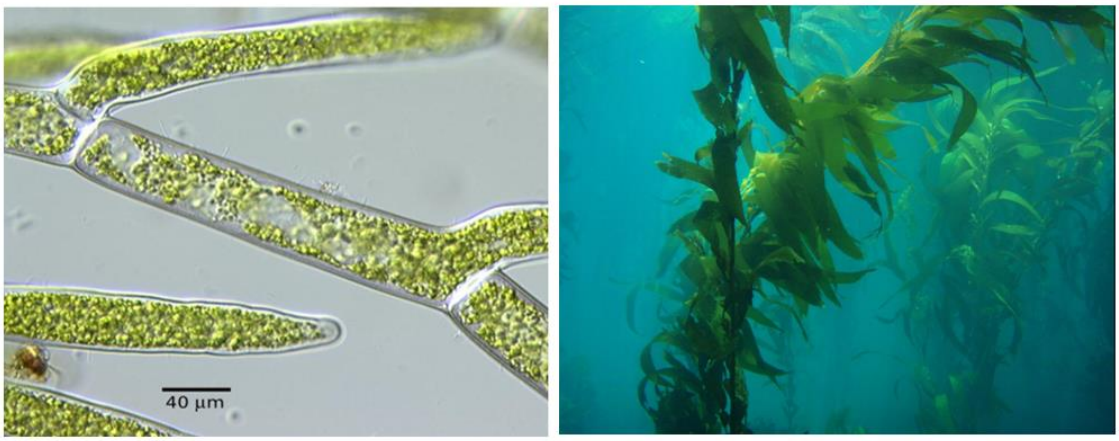
Biomass has been identified as having the potential to substantially contribute to meeting future energy demand in a sustainable manner and currently makes the largest contribution to renewable energy [1]. Among the sources for biomass, algae have received significant attention. Part of the reason for the interest in algal biomass cultivation is the potential of using it for multiple purposes such as producing bio-products (such as animal feed supplement, bioplastic and cosmetics) [2-4] and for nutrient removal in wastewater treatment systems [5].

Algae are a diverse group of organisms typically found in all natural waters, from fresh to marine water systems containing chlorophyll which are able to photosynthetically convert sunlight, water and carbon dioxide in the atmosphere into a wide range of metabolites and chemicals such as proteins, hydrogen, polysaccharides and lipids, found in algal biomass [6-9]. They have sizes ranging from single cells to large sea weeds (Figure 1). In general, reported sizes for algae cells are mostly between 1 and 50 μm [10].

Algae can be classified broadly into two categories: macroalgae and microalgae. Macroalgae can further be divided into three classes: Phaeophyceae (Brown seaweed), Rhodophyceae (Red seaweed) and Chlorophyceae (Green seaweed) based on pigmentation [11-13], and often include forms that are benthic and filamentous. Microalgae often include phytoplankton and suspended algae and can be classified further into four classes: Bacillariophyceae (diatom), Chlorophyceae (green algae), Cyanophyceae (blue-green algae) and Chrysophyceae (golden algae) [14].

Large-scale cultivation of algae is typically done in designed bioreactors such as raceways or flow-ways. For cultivation purposes, the bioreactor design largely depends on the type of algae to be cultivated, benthic (filamentous) algae or planktonic (suspended) algae (Figure 2). Bioreactor

designs intended for benthic algae offer several benefits over those designed for planktonic algae. In benthic algae bioreactors, the algae attach to the substratum and grow out from the attachment point. As filaments grow, they take up nutrients from water flowing around them. Biomass harvesting can then be performed with relative ease through some mechanical process (simple mechanical cutting or scraping) to detach the algae at the point of attachment and gather the algae. Figure 3 illustrates attached filamentous algae's growth form and the process of water remediation with it.



(a) (b)

Figure 1: Different sizes of algal species: (a) *Cladophora glomerata* [15] and (b) *Macrocystis pyrifera* [16].

The Algal Turf Scrubber (ATS) has been developed as a cost effective system for cultivating attached benthic algae for the dual purposes of harvesting biomass and water remediation. The ATS system typically consists of sloping surfaces made up of substratum screens in a shallow flow-way trough with naturally seeded filamentous algae attached to the screens. Impacted or polluted water is diverted from the source to the highest point on the surface of the ATS system for unidirectional flow. The attached algae then takes up nutrients and gives off oxygen as the water flows down the ATS flow-way [17].

The overall yield of algal biomass in the ATS is dependent on several factors, including algal species mix, light, temperature, pH, nutrient concentration, flow velocities, and substratum characteristics [18-20]. For attached growth systems such as the ATS, the physical characteristics of the substratum play a crucial role in the algal biofilm colonization process, algal filament loss during the growth period, and in the ability for regrowth after harvesting [21, 22].

(a) (b)

Figure 2: Bioreactor designs for (a) benthic (filamentous) algae [23] and (b) planktonic (suspended) algae [24]

Substratum topography, as defined by irregularities such as depressions, protrusions and crevices on the substratum surface [25-27] and substratum effective surface area (total available surface area for algal attachment per square area) plays an important role in biomass accumulation. Previous studies have investigated the impact of substratum topography on the productivity of algal biomass [28-30]. However, the impact of substratum effective surface area on biomass productivity remains relatively unexplored.

Adey et al. [31] presents one of the earliest efforts to improve algal biomass productivity in a benthic algae cultivation system through the enhancement of the substratum effective surface area

with the introduction of three dimensional substratum. Three-dimensional substratum has the added advantage of permitting algal cell packing in the vertical direction, such that sustainable growth occurs on different levels of the substratum. In the pilot study by Adey et al., diatom algal growth on traditional 2-D screens and novel 3-D substratum were compared. Results showed that algal productivity observed on the three-dimensional substratum was up to 3 times greater than that observed on the traditional two-dimensional substratum, as measured by repeated harvesting over multiple seasons.

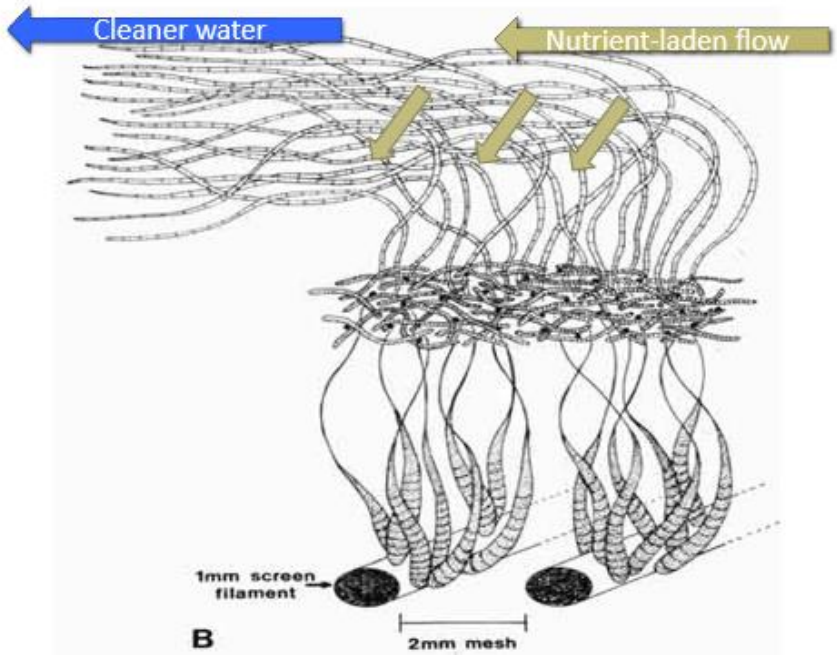


Figure 3: How the concept of the ATS works with benthic filamentous algae [32]

Although the results of this pilot study showed promise for 3-D substratum, the study did not explore the features of the substratum to understand which might be most relevant for increased algal productivity. Also, the pilot study was carried out in a single geographic location, with the 3-D substratum being exposed to a single growth environment.

The 3D substratum design of Adey et al [31] inspires further consideration for optimization, yet, to date, no research effort has investigated the effect of 3D substratum features. This study seeks to investigate the effect of three dimensional substratum features on algal biomass productivity. The goal is to understand how these features impact algal biomass productivity and how the features could be optimized to improve algal biomass cultivation purposes using ATS.

2. Literature Review

As a result of the goals set by the US government to replace 20% of the market share of fossil-based transportation fuels with biofuels--an equivalent of 51 billion gasoline-equivalent gallons--by the year 2030, and the US government's commitment to this goal as demonstrated by a recent $35 million investment in research efforts [33], biofuel sources have naturally received a lot of attention recently. Various promising biofuel sources such as animal fat, canola oil, corn oil, palm oil, soybeans oil, and vegetable oil, have been investigated [34, 35]. Sources such as perennial crops cannot be used in a practical manner as they would require an exorbitant amount of harvesting area, thereby directly competing for land meant for food production [36]. Palm oil, which is one of the best oil sources grown on land, would require about 18% of the existing US crop area to meet the stated goal [37, 38].

Because of these challenges, algae have emerged as a viable biofuel source for achieving the stated goal. Algae offer several advantages that make it attractive as a bioenergy source. Their ability to be cultivated on non-arable land and in brackish water eliminates any competition with food crops for resources. Certain algae also have high oil contents, and generally have high growth rates such that their population can be doubled in about 7-8 hours [39-41]. Apart from its potential as a source for biofuel energy, algal biomass can be used for a number of commercial bio-products such as supplement in animal feed [2, 42], bioenergy production [31, 43], bioplastics [4], fertilizers and aquaculture feed [44-47], as well as for cosmetic products [3].

Major drawbacks of using algae as a biofuel source include the high costs associated with algal farming and the difficulty in scaling production up to desirable levels [37, 48]. Fortunately, the costs associated with algal farming can be ameliorated by benefits from wastewater remediation [49]. The cultivation of algal biofilms for nutrient recovery has the potential to provide effective

treatment of wastewater, while producing valuable algal biomass products [5]. A study conducted by the United States Department of Energy's Aquatic Species Program concluded that, from an economic standpoint, a coupling of wastewater treatment with production of algal biomass is a compelling scenario, as the entire system could be funded simply by the value of wastewater treatment [50].

Algal growth systems such as the algal turf scrubber (ATS) have been shown to effectively treat wastewater while generating large amounts of biomass [17, 51-53]. Available cost analyses performed for algal production systems suggest that the ATS approach may be an effective choice for low cost algae production [5, 46]. The ATS system, developed in the early 1980s [54], is typically made up of downward sloping flow-ways containing a "turf" formed by a mixed algal community attached to a surface (substratum) in a shallow trough flow-way. Influent wastewater is pumped through the flow-way, and the wastewater is remediated through the uptake of nutrient compounds by the sessile algal community, causing the release of dissolved oxygen through the process of photosynthesis [17]. The attached periphytic algal turf encourages further entrapment of suspended solids from the wastewater [55]. At the end of the ATS flow-way, the treated wastewater emerges with a higher quality, with a lower concentration of nutrients and a higher concentration of dissolved oxygen [56].

In the ATS system, the growing algal community is frequently harvested, approximately weekly, to ensure the complete removal of nutrients taken up by algae from the water body. Harvest also serves to revitalize the algal community, thereby increasing the overall production rate. Algal growth in ATS systems is also stimulated by introducing pulsed water movement using surge devices. This prevents the development of mass transfer limitations by encouraging water and nutrient exchange within the algal turf layers [51]. The ATS systems' capability for wastewater

treatment and biomass production have already been demonstrated for several pollution sources such as agricultural [57-59], industrial [60] and municipal [61-63] wastewaters. ATS systems have been shown to have reliably high biomass production rates for natural and managed ecosystems [64].

Several factors affect algal biomass productivity in attached growth systems [65]. Some of these factors include temperature, pH, nutrient, light and substratum characteristics. Temperature is known to play a significant role in the growth and chemical composition of algal species; for example, it has been shown that the ratio of saturated to unsaturated fatty acids decreases with decrease in temperature [66]. It is challenging to select the right temperature for algal species since different species tend to have different temperature ranges for optimal growth rates. The pH value also affects algal growth as it directly impacts cell growth and lipid accumulation in cultivated algae, with a pH value around 8 considered most suitable for algal growth [67, 68]. As in the case of temperature, the effect of pH differs from species to species.

Nutrient concentration is also a crucial factor for algal biomass productivity, as it serves as the source of nutrition for algae. Nitrogen, phosphorous, and carbon are the essential macro-nutrients required for algal production, while other elements such as silica, calcium, magnesium, potassium, iron, manganese, sulfur, zinc, copper, and cobalt are necessary at the microscale [69]. A typical optimal ratio of carbon, nitrogen, and phosphorous required by algae for growth is 106:16:1 based on global averages [70]. However, it is known that this ratio differs from species to species, depending upon local conditions and environments [71].

Light plays a critical role in algal biomass productivity as well, as it is a vital requirement for photosynthetic activities. Both the wavelength and intensity level of incident photons are important, as they drive photosynthesis of algal cells and directly influence algal productivity [31].

As with other factors, different species have different optimal light intensity ranges for optimal algal growth. Outside of such ranges, the light intensity may become detrimental for algal growth [72, 73].

Substratum characteristics are known to be an important factor for initial adhesion of algal cells to surfaces [74], especially for attached growth systems such as in an ATS. The physical and chemical characteristics of the substratum have been shown to be influential in algal biofilm colonization process [28, 75, 76], while filamentous periphyton have been shown to display dissimilar colonization densities based on the substratum type [77]. For instance, the growth rate and biomass accumulation of periphyton on stable substrata such as stones and gravel are typically higher than for surfaces such as sand and organic materials [78].

Substratum topography, defined by irregularities such as depressions, protrusions (also known as asperities), and crevices on the substratum surface may impact cell attachment and retention, as well as biomass harboring and accumulation on the substratum [25-27, 79, 80]. Substratum topography may also affect the water flow dynamics around the substratum, thereby changing the diffusive boundary layers and nutrient delivery kinetics [25]. The effect of surface topography on the substratum's ability to receive and retain algal cells is especially significant when the substratum features are considerably larger than the microbial cell dimensions, due to stronger adhesion to the substratum because of increased contact area with the substratum surface [81-83].

Previous studies have reported a positive correlation between algal cell attachment and increased surface roughness [84, 85], suggesting that the degree of roughness of the substratum could be influential on algal biomass productivity. In addition, the heterogeneity of characteristics of substratum may be critical in supporting diversity in attached algal communities which enhances biomass productivity [30]. The orientation of the substratum relative to the direction of water flow

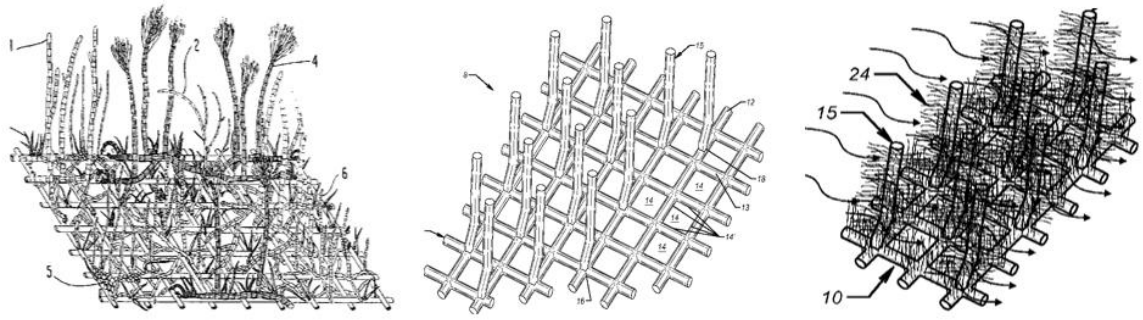
is another element that has been shown to impact algal biomass productivity. Substratum surfaces deployed vertically tend to accumulate significantly less biomass than those deployed horizontally or at 45^0 [86].

Finally, substratum surface area plays an important role in biomass accumulation especially in nutrient deficient water bodies. Ahn et al. [81] concluded that an increase in the substratum surface area resulted in increased periphyton biomass in nutrient deficient water bodies, whereas in nutrient sufficient environments, the effect of substratum surface area increase did not appear to have a significant impact on periphyton biomass. Substratum surface area has also been reported to influence microbial biodiversity, where taxa richness and number of species increased as the surface area increased [87].

Three dimensional substratum surfaces (Figure 4-1) increase the total substratum effective surface area by providing additional area in the vertical direction. This allows for better support of larger and loosely attached algal species, which tend to acquire greater light and nutrients through vertical growth, but also tend to be more vulnerable to loss of biomass due to grazing or scouring [88, 89].

Adey et al. [31] deployed several three dimensional substrata in an environment rich in diatoms (Great Wicomico River near Reedville, Virginia) with the objective of comparing algal growth dynamics between traditional two dimensional screens and three dimensional substratum. Results showed that algal biomass productivity, defined as areal biomass recovered by repeated sacrificial harvest over time, was nearly 3 times greater on the three-dimensional substrata than that observed on the traditional two dimensional substrata. Based on this work, patents have been developed for apparatus designs that utilize the concept of three dimensional substratum for algal production [21, 90]. Cooke et al. [90] introduced an apparatus for algae production that employs an array of two-dimensional algae production substratum configured vertically relative to the surface of the water.

Their findings suggest that the three-dimensional configuration substantially increased algal biomass productivity per area of open water.



(a) (b) (c)

Figure 4-1: Substratum for algae colonization (a) two dimensional substratum with algal growth (b) structure of three dimensional substratum (c) three dimensional substratum with algal growth [21, 32].

The seemingly superior performance of three dimensional substratum in producing algal biomass can be attributed to its enhanced structural strength, involving both tensile and shear strength, the ability to allow greater algal cell packing, and increased surface area available for algal colonization [21, 31]. Three dimensional substratum allows for the retention of filamentous diatoms along with filamentous algae. Opportunities exist to further increase the algal biomass productivity performance of three-dimensional substratum. Adey et al. [31] pointed out that future engineering of three dimensional substratum to optimize its features (such as fiber density) for algal biomass production could result in substratum surfaces that maximize algal photosynthesis and productivity in bioreactors such as the ATS.

Recent studies have shown that periphyton communities have the potential to colonize customized engineered artificial substratum [91-95]. Such substratum designs may be utilized to influence algal species composition and dominance relationships in engineered periphyton cultivation

systems [96]. The above-mentioned studies focused on 2-D substratum, but studies have not considered such investigations for 3-D substratum. A possible explanation for this gap is that the concept of three-dimensional substrata for algal biomass cultivation is relatively new and as such has not been vigorously studied.

In this study, the effect of three-dimensional substratum features on the productivity of algal biomass is investigated. Different features of 3-D substratum are explored with the goal of understanding the effects on the colonization and growth of algae.

3. Research Questions and Objectives

The work of Adey et al. [31] has shown that the use of three dimensional substratum could be critical for significantly increasing the productivity of cultivated algae. However, further investigation is required to understand how 3-D substratum supports algal biomass cultivation, and how the substratum can be improved to increase algal productivity. For example, what property of a three dimensional substratum makes it a better surface for supporting algal cultivation? Is it possible to increase the algal productivity in an ATS by altering some features of the three dimensional substratum?

In ATS systems, the rates at which pollutants (typically nutrients or metals) are recovered from wastewaters depend on the productivity of the algal turf community. This productivity is dependent on the limiting factors that affect the growth of the algal community [64, 97]. As such, the interaction of substratum surfaces features with the supply of limiting factors (for example, light, nutrient availability or mass transport dynamics) may determine the biomass productivity. From a design perspective, it may be desirable to incorporate the knowledge of three dimensional substratum interactions with limiting factors into the substrata design process to maximize algal productivity.

In algal cultivation systems, algae are exposed to various environmental factors that influence the overall growth. However, based on Liebig's law of the minimum, which states that the growth of an organism is most strongly controlled by the availability of the most limited resource requirement (limiting factor), the algal biomass productivity is dependent on limiting factors within the cultivation system. As such, the ATS is typically designed and operated in a way that minimizes factor limitations.

Drawing inspiration from Liebig's law of the minimum, models have been developed to study growth dynamics of organisms. For algal growth systems, the process by which algae takes up and uses nutrients for growth can be represented by mathematical expressions representing the processes of nutrient uptake, internal nutrient storage, and algal growth kinetics [98]. The Michaelis-Menten model [98], describing the kinetics of enzyme function, forms the basis for describing the relationship between nutrients concentration and algal cell growth, as the Monod formulation. For the process for internal storage of nutrients, the Droop model [98] is used to describe the dynamics involved.

The Monod model [99], one of the earliest and most extensively used models for studying algal growth, is used to describe the relationship between nutrient concentration and algal growth. The original version of the Monod model [100] is expressed as

$$\mu = \mu_{max}\left(\frac{s}{k+s}\right) \quad (1)$$

where μ is the specific growth rate of microorganism (d^{-1}); μ_{max} is the maximum specific growth rate of microorgansism (d^{-1}); s is the concentration of the limiting nutrient ($mg \cdot L^{-1}$); and k is the half-saturation coefficient ($mg\ L^{-1}$)

Over time, several modifications of the Monod model have been developed to include factors other than limiting nutrients. For example, Ye et al. [101] modified the Monod model by assuming that μ_{max} is a function of environmental factors such as temperature and light. Based on this assumption, an alternative model was proposed and expressed as

$$\mu = A \cdot b^{T} \cdot \frac{s}{k+s} \quad (2)$$

where *A* is the maximum specific growth rate at a certain temperature (d^{-1}); b is the activation energy dependent constant, and *T* is the temperature (^0C). For a given substratum condition, which might include 3-D element structure, the maximum specific growth rate A is a descriptive parameter directly related to the structure composition and topographic arrangement.

Based on this modified version of the Monod model, it is hypothesized that, at a given level of limiting nutrient concentration, there should be a correlation between the features of the three dimensional substrata and the observed algal biomass productivity on the 3D substratum. Specifically, this study explores the effects of three features of three dimensional substrata - fiber density, fiber height, and substratum base roughness (Figure 4-2)—on the productivity of algal turf biomass in a flow environment. A customized carpet designed specifically for algal cultivation is used as the three dimensional substratum. Details of this carpet is presented in Section 4.1.

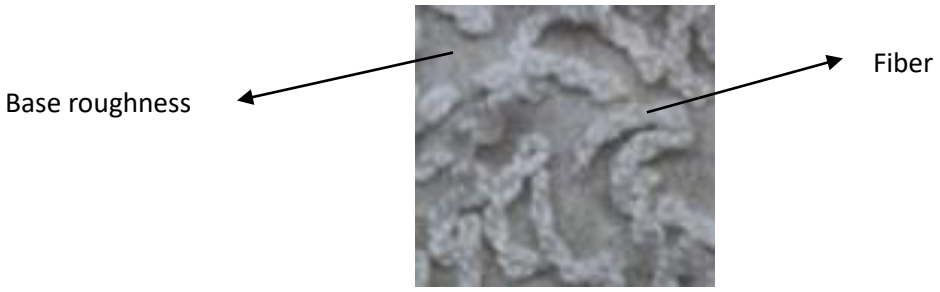


Figure 4-2: Description of three dimensional substratum features

In this study, fiber density is defined as the number of vertically extending fibers per area of the carpet surface used as the 3D substratum. It is expressed in stitches per square cm (the fibers are stitched unto the carpet). The fiber height is measured as the height of each fiber, while the substratum roughness describes the roughness characteristics of the base of the 3D substratum

(either in its original version having “fuzzy” attributes which encourages roughness or having the “fuzzy” attribute scraped off, in which case the roughness is lowered).

In this research, the following objectives were explored:

1. Determine the impact of three dimensional substratum features such as fiber density and fiber height on the growth of algae.

2. Explore the effect of nutrient concentration as limiting factor on the productivity of algal biomass cultivated on three dimensional substratum surfaces.

3. Investigate the impact and contribution of substratum base roughness as an attribute of three dimensional substrata for algal biomass productivity.

To accomplish these objectives, three experiments were conducted. The first experiment investigated the effect of different levels of substratum fiber density of the productivity of algal biomass. In the second experiment, the effectiveness of three dimensional substrata as a surface for cultivating algal biomass is explored under different nutrient concentrations. Finally, the third experiment studies the impact of substratum roughness on algal biomass productivity for two dimensional and three dimensional substrata.

4. Experiment 1: Impact of fiber density of three dimensional substrata on benthic algal productivity

The purpose of the first experiment was to investigate the effect of fiber density of the three-dimensional substratum on algal biomass productivity. For the first experiment, fiber density was selected based on preliminary observations that suggested that substratum fiber density may have a significant effect on algal biomass productivity. It is possible that differences in algal biomass productivity observed between different levels of fiber density may be attributed to limiting factors such as light (due to shading effect) and nutrient availability (due to nutrient transport via hydrodynamic mechanism).

4.1 Preliminary Study

A preliminary study was carried out to better understand the effect of three dimensional substratum features on algal productivity in a cultivation scenario. The objectives of the preliminary investigations were:

(1) To design, construct, and test a flow lane bioreactor system to be used for experimentation
(2) To develop experimental protocols for growing, harvesting, drying, and measuring algal biomass.
(3) To screen the features of three dimensional substratum surfaces that may have a significant effect on algal biomass productivity and that could be feasibly studied in a laboratory setting.
(4) To perform preliminary experimental runs to test the workability of the constructed system, validate the developed experimental methods, and draw preliminary observations on the viability of proposed experiments.

Details of the construction of the bioreactor system used and the experimental methods developed for this experiment are presented in Section 4.2. To carry out this preliminary investigation and subsequent experiments, substrata developed by Hydromentia LLC (Ocala, FL, USA) and Interface, Inc. (La Grange, GA, USA) for algal biomass cultivation were used. In some cases, the original substratum was modified to suit experimental design needs. Figure 5 shows a sample of the original version of both substratum types obtained from the two companies. Figure 5(a) shows the substratum obtained from Hydromentia. The substratum is basically a two dimensional substratum made of open nylon fabric. The substratum can also be considered to have a three dimensional component in that the fabric is woven at the top. However, the substratum does not have any fiber extending in the vertical direction. In Figure 5(b), the substratum obtained from Interface is shown. This is a three dimensional substratum having 2-3 cm long loose fibers stitched to the base of the carpet, having on average 0.67 stitches per square cm. Each fiber was made by weaving and twisting on average 60 single filaments together. The fibers were made of nylon/wool material and on average each fiber had a thickness of 0.5 cm. Also the base has “fuzzy-like” nylon/wool material attached to it, thereby enhancing the base roughness of the substratum. Both substratum surfaces were ideal for the experiments because of their material make up, making them have desirable properties for algal cultivation such as being durable, having large surface area and being a supportive material for dense periphyton formation [58, 102, 103].

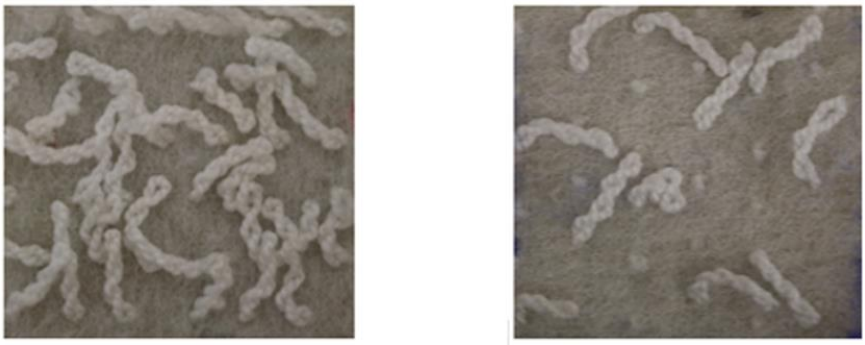
(a) (b)

Figure 5: Samples of original substratum surfaces developed for industrial use for cultivating algae:

(a) Hydromentia company substratum and (b) Interface company substratum

For the preliminary investigation, fiber density and fiber height were the two features of 3-D substratum selected for study. To obtain the two levels of each features, modifications of the original version (Figure 5b) of the three dimensional substratum obtained from Interface (La Grange, GA, USA) were incorporated. For fiber density, the first level (Treatment A) was the original version of the substrate, in which there are 6 fibers per 9 cm^2 (0.67 stitches per square cm), while the second level (Treatment B) was modified to have 2 fibers extending in the vertical direction for each 9 cm^2 (0.22 stitches per square cm) area of the substratum. This was accomplished by trimming some fibers from the original version of the substratum.

For the fiber height feature, the fiber density was maintained at 0.22 stitches per square cm of the substratum, with the height levels being the default height of 2.50 cm (Treatment A) and 1.25 cm (Treatment B). Figures 6 and 7 show samples of the treatment levels used for preliminary investigation of fiber density and fiber height, respectively.



(a) Treatment A (0.67 stitches per cm^2) (b) Treatment B (0.22 stitches per cm^2)

Figure 6: Experimental treatment levels used for preliminary investigation of fiber density

For the preliminary experiment, the different substratum treatments were introduced to the flow lane bioreactor system, which was seeded with algae collected from a local stream, Chewacla Creek at Chewacla State Park in East Alabama. The system was then allowed to run for two full weeks to allow for system inoculation before data was gathered. Following the two-week inoculation period, three weeks of harvest data were collected and used as the data source for observational purposes.

(a) Treatment A (2.50 cm) (b) Treatment B (1.25 cm)

Figure 7: Experimental treatment levels used for preliminary investigation of fiber height

Based on the results obtained over the three-week period, it was observed that the effect of fiber density on algal biomass productivity appeared to be more pronounced than the effect of fiber height. For fiber density, the mean algal biomass productivity observed on Treatment A was (0.38 ± 0.02 mg dW cm^{-2} d^{-1}, n = 3), while the mean productivity observed on Treatment B was (0.32 ± 0.01 mg dW cm^{-2} d^{-1}, n = 3). Similarly, for fiber height, the mean algal biomass productivity observed on Treatment A was (0.29 ± 0.01 mg dW cm^{-2} d^{-1}, n = 3), while the mean productivity observed on Treatment B was (0.28 ± 0.01 mg dW cm^{-2} d^{-1}, n = 3). The results obtained from the three weeks of data collected during this preliminary phase are presented in Figure 8. This shows that fiber density significantly impacts algal biomass productivity and based on this finding, fiber density was chosen for further investigation.

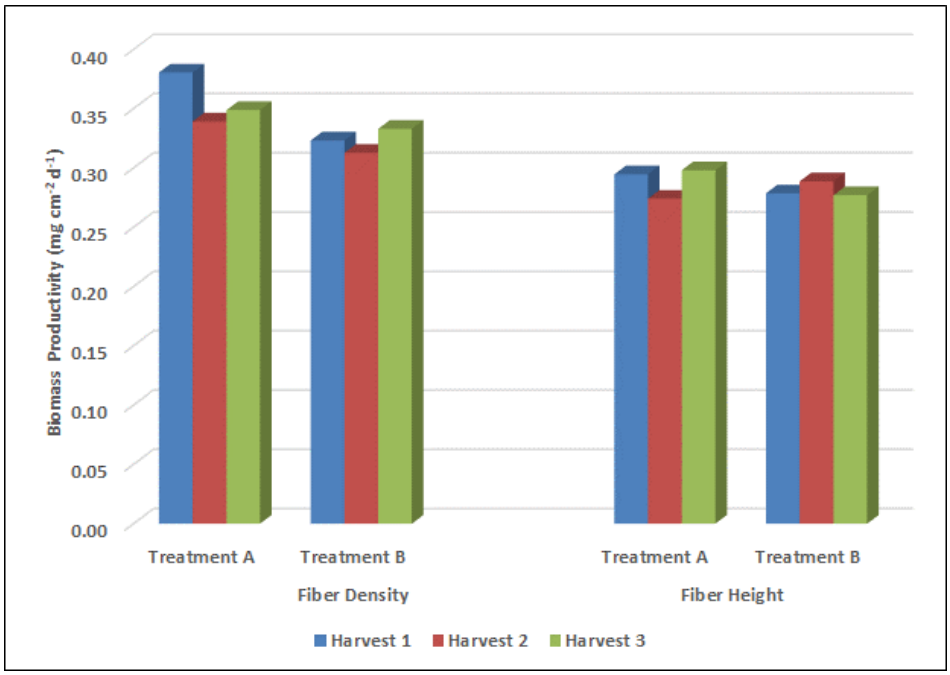


Figure 8: Preliminary results on fiber height and density

4.2 Materials and Methods

Experiment 1 was carried out over a 12-week period. For these experiments, a 4-lane bioreactor system was designed and constructed. The four lanes of the bioreactor system were made using 10-foot long Genova half round gutters (made of PVC plastic) supported by a framework constructed using PVC pipes and wood at a slope of 1.35%. The dimensions of each of the four lanes of the bioreactor system was 304.8 cm L x 7.6 cm W. The whole area was used as the growing area. Figures 9 and 10 presents the bioreactor system and a schematic representation of the 4-lane bioreactor system respectively.

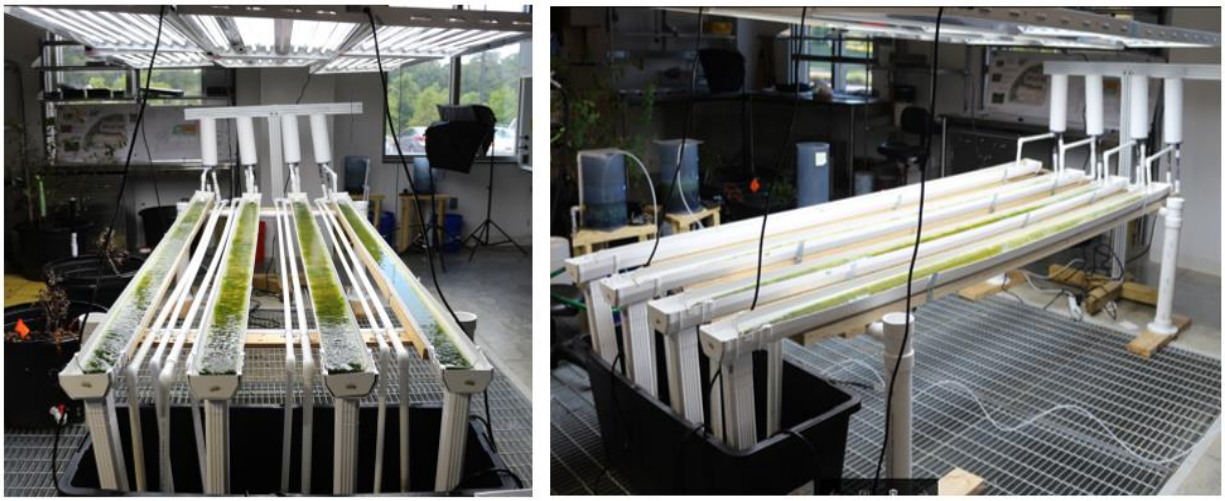


Figure 9: Four-lane bioreactor system

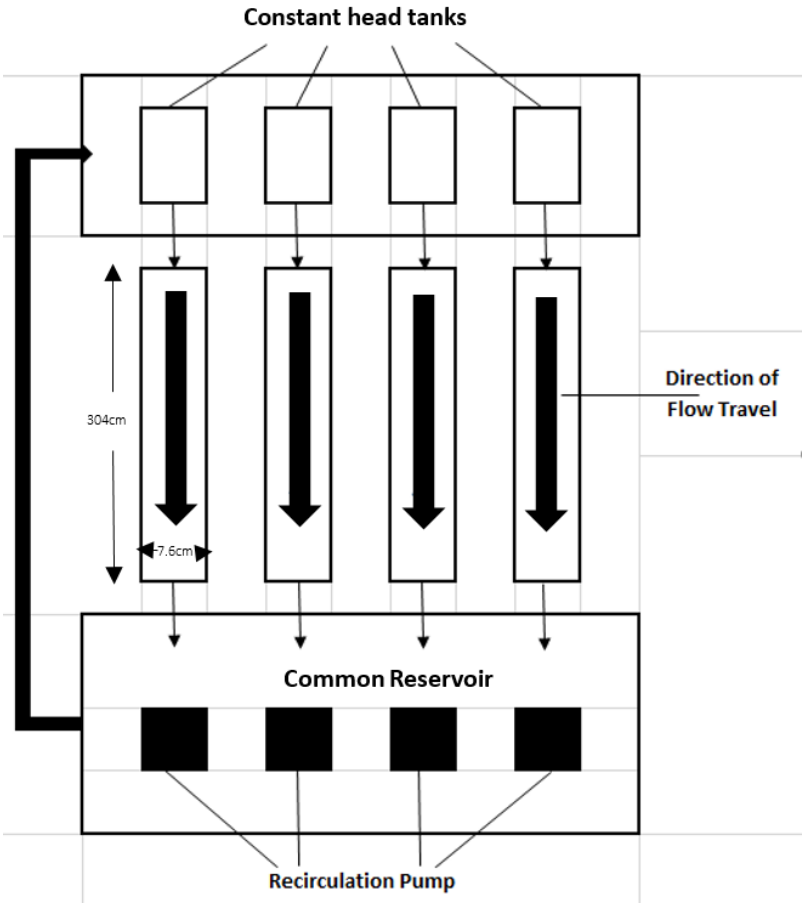


Figure 10: Top view schematic representation of the four-lane bioreactor system used in experiment 1

The surface of each Genova half round gutter was covered with a substratum treatment level on which algae grew. Both two-dimensional and three-dimensional substratum surfaces were used for algal attachment and colonization on the four lanes of the system for this experiment. The three-dimensional (3D) substratum was obtained from the carpet company Interface Inc. (La Grange, GA, USA), while the two-dimensional (2D) substratum was obtained from Hydromentia LLC (Ocala, FL, USA).

With knowledge from the preliminary work, three treatment levels of fiber densities were considered to investigate the effect of fiber density of 3D substratum on algal biomass

productivity. All three levels were obtained using variations of the original 3D substratum by cutting off some of the fibers on the substratum. The 2D screen was included as a control, as it is the standard substratum commonly used in industrial applications.

Table 1 describes the different substratum treatment levels used. In all, four treatment levels for substratum were used for this experiment (3 levels of 3D substrata and 1 level of 2D substratum). Each lane of the four-lane bioreactor system was entirely covered with one of the four treatment level substratum. A 100% silicone all-purpose adhesive sealant (DAP, UNSPSC 31201606, USA) was used to adhere the substratum surfaces to the lanes of the bioreactor system and then allowed to cure for several days. Figure 11 shows the four experimental treatment levels of substratum surfaces used for the experiment.

(a) Treatment Level A (b) Treatment Level B (c) Treatment Level C (d) Treatment Level D

Figure 11: Photographs of samples representing treatment levels for substratum surfaces.

Table 1: Experimental treatment levels for substratum surfaces.

Treatment level	Substratum dimension	Fiber density level	Number of fibers (per 9 cm^2)	Stitches per square cm^2
A	2D	Control Substratum	0	0
B	3D	Low	1	0.11
C	3D	Medium	2	0.22
D	3D	High	6	0.67

Six fluorescent light fixtures (Sun System Sun Blaze T5 High Output), located directly above the 4-lane bioreactor system, were used to provide lighting for the bioreactor system. The dimensions of each of the fixtures were 119.4 cm (length) x 57.2 cm (width) x 6.4 cm (height). Each fixture uses 8 bulbs (Spectralux 901618, China), each with a wattage of 54W. The bioreactor system was operated under continuous light (24 hours of daily light). The height and location of light fixtures were adjusted until moderate light intensity levels were achieved at the cultivation surface. Figure 12 shows the light illumination maps across the bioreactor system before and after light fixtures were calibrated. Photosynthetic photon flux density on the algal growth substratum averaged 288 ± 55 (range 179 – 355) µmol m^{-2} s^{-1} over the whole bioreactor system. Light intensity measurements were taken using a quantum flux meter and probe (LI-250 Light Meter and LI-190 Quantum Sensor, LI-COR Biosciences, Lincoln, Nebraska, USA).

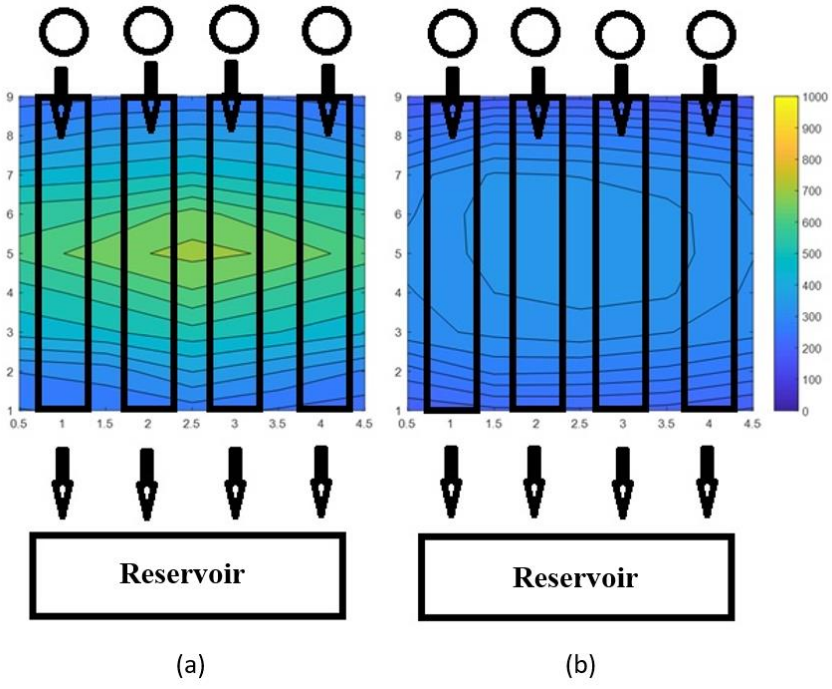


Figure 12: Light illumination map (Photon flux density) for (a) before and (b) after adjustment to desired levels

The system was operated in a continuous mode by recirculating 90 L (24 gal) of freshwater nutrient solution to a common rectangular reservoir (Figure 13). Water flow through each lane was implemented using a separate centrifugal submersible pump (Supreme Mag Drive, Model MD 18, Danner Manufacturing, Islandia, New York, USA) submerged in the reservoir to pump water through PVC pipe lines into a constant head tank that then release water into the lane at a constant flow rate. The constant overhead tanks (Figure 14) were used to minimize flow variability across the 4 lanes. The flow rates across the 4 flow lanes averaged 9.22 (range 8.58-9.60) L/min. The mean depth was 3.8cm.

Figure 13: Common rectangular reservoir for all four lanes

The water level in the reservoir was kept constant by replacing daily water losses through evaporation with distilled water. Interruption of water flow only occurred during daily data collection for temperature, pH, and conductivity values, as well as during non-drying harvests.

Figure 14: Constant head tanks

The flow-way reactor was inoculated with an algal community that was collected from local streams in Eastern Alabama. The dominant species were *Microspora floccose* and *Mougeotia scalaris* (Figure 15). This was determined by species identification of samples taken from the local streams. The species identification was conducted via digital microscopy. A Motic optical microscope (Motic Corp., Richmond, BC) was used at a magnification of 400X to 1000X. The reactor was dosed daily with commercial F/2 media (Pentair Co., Apopka, FL) at the recommended loading concentration rate (1 ml nutrient per 1 gallon of water). Also, daily temperature, pH and conductivity were measured by hand using a handheld combination pH/EC probe (HI 98130, Hanna Instruments, Woonsocket, Rhode Island). Throughout the duration of the experiment, the temperature averaged 77.9 ± 0.8 (range 75.2 – 80.5, n = 85) ^{o}F, the pH averaged 8.45 ± 0.21 (range 7.89 – 8.89, n = 85) while the conductivity had an average value of 0.43 ± 0.06 (range 0.26 – 0.57, n = 85). Details of temperature, pH, and conductivity records for the reservoir are provided in Appendix I. The dissolved N concentrations was measured as NO3-N: 2.12 ± 0.55 ppm, using Insta-Test testing pads (LaMotte Co., Chestertown, MD).

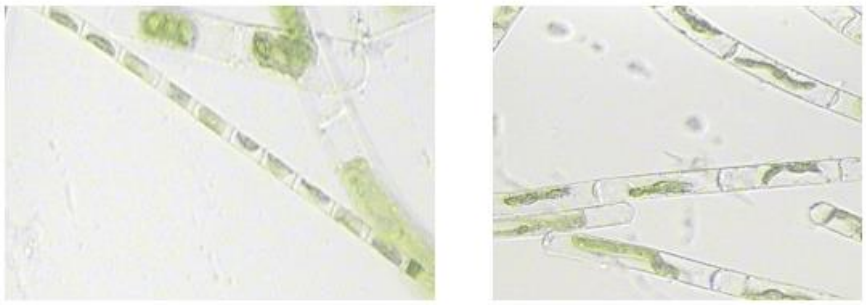


Figure 15: Algal species that occurred and were observed to be dominant in community used to inoculate reservoir (a) *Microspora floccose* (b) *Mougeotia scalaris*

To mitigate possible effects of variability in flow rates and light intensity distribution across the 4 lanes of the bioreactor system, the positions of the treatments on the 4 lanes of the system were alternated during a 4-week period (Table 2). This approached ensured that any experimental noise was evenly spread across the four treatments used, as each treatment was exposed to all four lanes during each 4-week period.

Table 2: Placement of substratum treatment levels (A, B, C and D described in Table 1) on the lanes of the bioreactor system during the 12-week experimental period.

	LANES			
	Lane 1	Lane 2	Lane 3	Lane 4
Week 1	D	A	C	B
Week 2	C	B	A	D
Week 3	A	D	B	C
Week 4	B	C	D	A
Week 5	B	D	A	C
Week 6	A	C	B	D
Week 7	D	A	C	B
Week 8	C	B	D	A
Week 9	A	B	C	D
Week 10	D	C	A	B
Week 11	C	D	B	A
Week 12	B	A	D	C

Algal biomass was sacrificially harvested every 7 days from the entire growing area of each of the flow lanes. On harvesting days, the pumps were turned off to stop the flow of water on the four lanes of the bioreactor system and the water was allowed to drain out for about 10 minutes. The lanes of the bioreactor system were not allowed to become fully dry so as not to compromise the viability of the remaining algal community on the bioreactor system after harvesting [31]. Each of the four lanes was then harvested mechanically [5, 22] using a commercial wet/dry vacuum (Rigid, Model WD1637, Emerson Electric Company, St. Louis, Missouri, USA) shown in Figure 16. Each lane to be harvested was first detached from the flow-way system and then

water was further drained by inclining the lane at an angle. The reason for this was to get as much water out of the lane as possible so that the required drying time for the algae could be minimized. Once water drained out, the wet/dry vacuum was used to harvest and collect the algae off the entire growing area of substratum in the lane. The vacuum removed a significant portion of the algae, while leaving a small remnant (typically less than 2% of harvested biomass) of the algal filaments attached to the substratum. This remnant of algae filaments would serve as a means of re-initiating algal growth on the substrate for the next harvesting period.

Figure 16: Commercial wet/dry vacuum attached to 5-gallon bucket during harvesting operation

The harvested biomass from the 5-gallon bucket was poured into a separate drying pan immediately after harvesting from each lane of the bioreactor system. The bucket was also rinsed to ensure complete recovery of harvested biomass. All four drying pans with harvested biomass collected from each substratum treatment level were then put into a fume hood where they were allowed to dry out (Figure 17). Two fans were included to aid the drying process which lasted for 72 hours. Biomass weights were measured by taking the difference of the weights of the pans before the harvested biomass was poured into the pans and the weights of

the pans after the drying process had been concluded. An Acculab analytical laboratory balance (ALC-80.4, Arvada, Colorado, USA) was used for these weights measurement procedures. The accuracy of the laboratory balance was 0.0001g.

Figure 17: Drying operation for harvested biomass in fume hood

4.3 Results

Figure 18 shows the daily biomass productivity for each of the 4 substratum treatment levels used in this experiment and across the 12 harvests carried out during the experimental period. To analyze the data statistically, an analysis for underlying ANOVA assumptions of homoscedasticity was first carried out. This included tests for normality and analyses of residual plots. Plots and histograms for residual analyses on harvested algal biomass are shown in Figure 19. A one-way ANOVA test was conducted using Minitab 18. As shown in Figure 19, the data were normally distributed. Raw data showing the details of harvested biomass for all substratum treatment levels, measured after the drying process, is presented in Appendix II.

As observed in Figure 18, algal productivities on the 3-D substratum (Treatments B, C and D) were always greater than the productivity of the 2D substrate (Treatment A). The highest daily productivity was observed during harvest 3 on 3D substratum; Treatment C (0.541 mg dW cm^{-2} d^{-1}), while the lowest daily productivity was observed during harvest 6 on the 2D substratum; Treatment A (0.087 mg dW cm^{-2} d^{-1}). During the 12 harvests performed, higher productivities were observed on Treatment C.

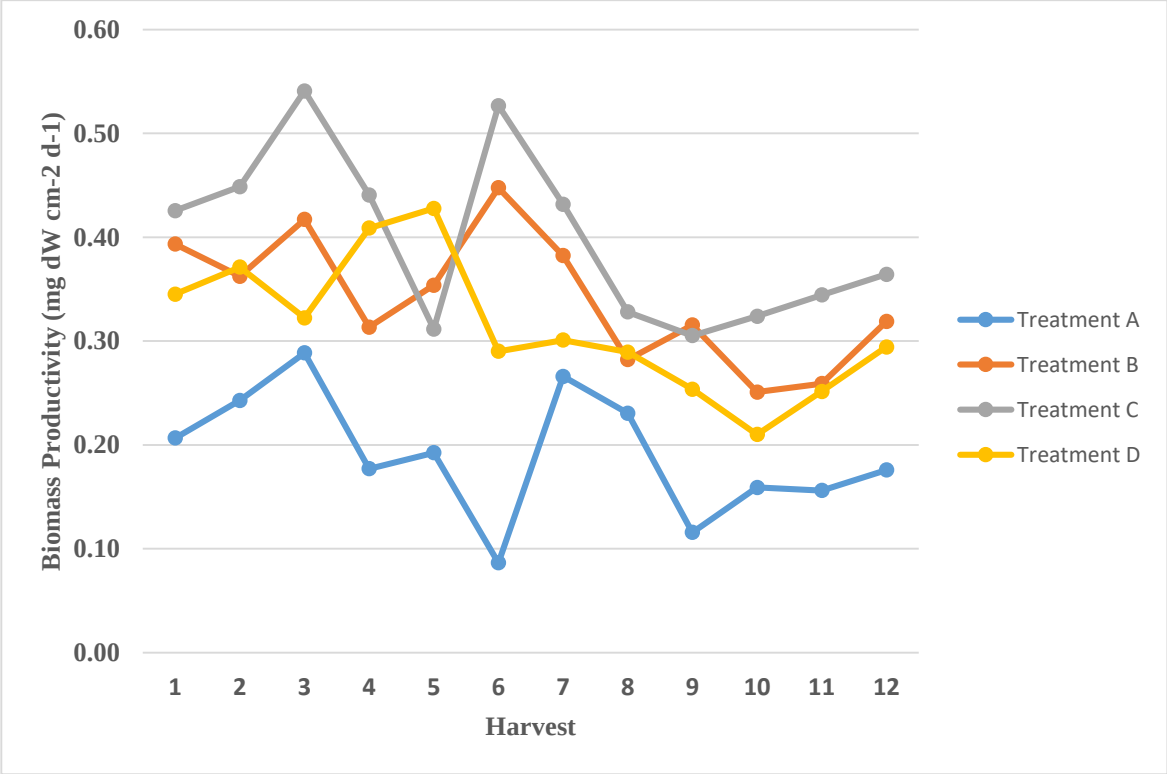


Figure 18: Algal biomass productivity (mg dW cm^{-2} d^{-1}) versus substratum type for entire duration of experiment

Across the whole experiment period, the greatest mean productivity was observed on treatment level C, the medium density 3D substratum (0.40 ± 0.08 mg dW cm^{-2} d^{-1}, n = 12), while the lowest productivity was observed on treatment level A, the 2D substratum (0.19 ± 0.06 mg dW cm^{-2} d^{-1}, n = 12). The main effects of algal biomass productivity of each substratum type is

presented in Figure 20.

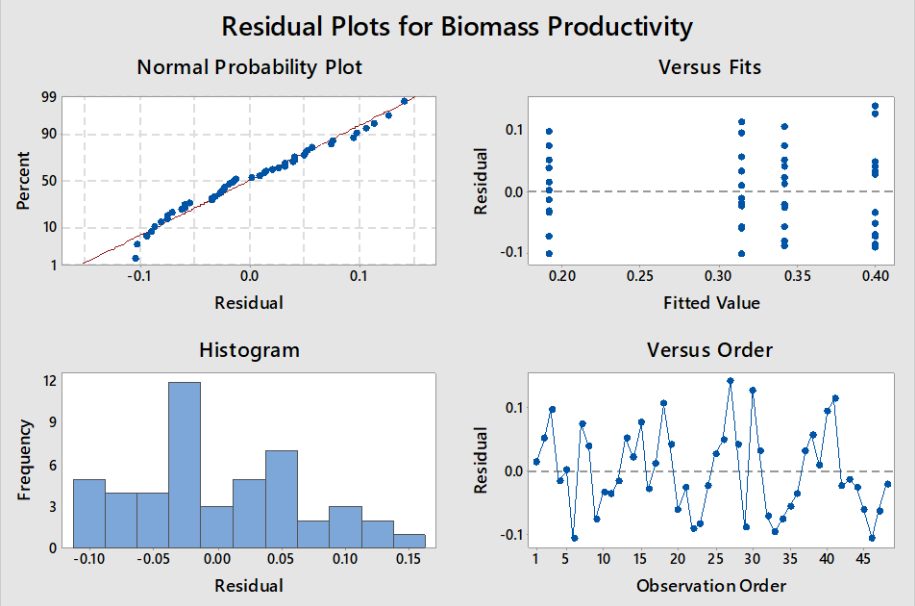


Figure 19: Residual plots for algal biomass productivity (mg dW cm-2 d-1)

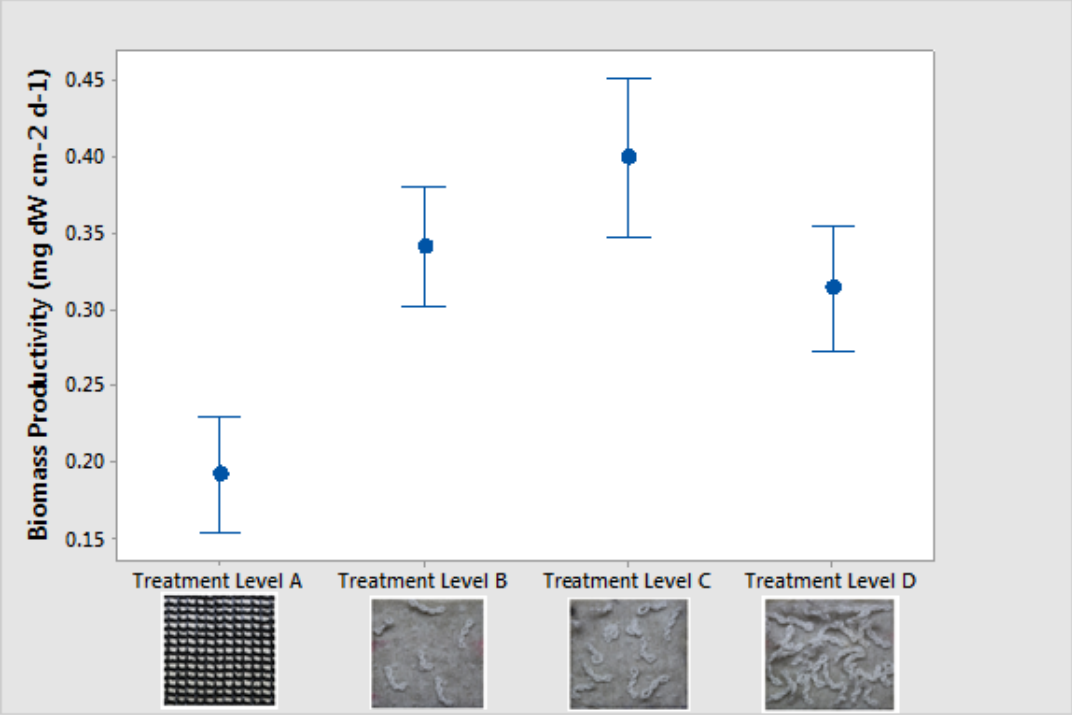


Figure 20: Mean algal biomass productivity versus substratum treatment level

Results of ANOVA test (Table 3) showed that the effect of substratum density on algal productivity was significant at the 5% statistical significance level ($F[3, 44] = 20.11$, $p < 0.01$). A paired t-test was used for pairwise comparison for differences between all pairs of substrates type. Results showed that the low density (Treatment B) and high density (Treatment D) 3D substratum types were not significantly different (p-value = 0.201) from each other. All other pairwise comparisons were significantly different (p-value ≤ 0.008).

Table 3: Summary of one-way ANOVA for algal biomass productivity for experiment 1

Factor Information

Factor	Levels	Values
Substratum	4	Treatment Level A, Treatment Level B, Treatment Level C, Treatment Level D

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Substratum	3	0.2761	0.092038	20.11	0.000
Error	44	0.2013	0.004576		
Total	47	0.4774			

5. Experiment 2: Effect of nutrient concentration on algal biomass productivities of two dimensional and three dimensional substratum surfaces

The second experiment involved exposing both two dimensional and three dimensional substrata to low and high nutrient concentration environments. The purpose of this experiment was to investigate the effect of nutrient concentration on the productivities of algal biomass cultivated on two dimensional and three dimensional substrata. Specifically, the aim was to observe the performance of three dimensional substrata for algal cultivation in different nutrient concentration environments and to compare the performance with two dimensional substrata (control substrata). As with experiment 1, a preliminary study was conducted before the actual experiment was carried out.

5.1 Preliminary Study

Based on observations from the first experiment, it became obvious that there were opportunities to improve upon the bioreactor system and the methodology used for biomass handling operations to reduce biomass drying time. Changes were made to the bioreactor system to optimize laboratory space and to have the light intensity more evenly distributed across the four lanes of the bioreactor system. Although the impact of light intensity variability was reduced in experiment 1 by alternating the substratum treatment levels across the lanes of the bioreactor system, mitigating this variability in the design of the reactor would be a better approach. By shortening the lanes and changing the orientation of the system relative to the lights, the variability in light intensity could be reduced further.

The objectives of the preliminary investigation were as follows:

(1) To modify the bioreactor system and to test the changes introduced to the experimental methods for growing, harvesting, drying and measuring algal biomass

(2) To perform preliminary experimental runs to test the performance of the modified bioreactor system, validate the changes made in the experimental methods and to draw preliminary observations on the viability of the proposed experiment.

(3) To test the levels of the experimental factors

Details of the modifications made to the bioreactor system and the changes introduced to the experimental methods for this experiment are presented in Section 5.2.

For the preliminary investigation, two treatment levels were used for the substratum. The first level was a two dimensional substratum (Treatment A), and the second level was a three dimensional substratum (Treatment B). The two substratum treatment levels (Figure 26) were exposed to two nutrient concentrations environments (low and high). Low nutrient environments were characteristic of water bodies having low nutrient concentrations, typically having total nitrogen (TN) values less than 10 mg L^{-1} [104, 105], while high nutrient environments were characterized by waters with total nitrogen (TN) values greater than 10 mg L^{-1}.

Following similar protocols as in the first preliminary study, the flow-way system was allowed to run for two weeks to inoculate and stabilize system to steady state before observational data were collected. After the two-week period, three weeks of harvest data were collected and served as the data source used for observational purposes.

Figure 21 shows the results obtained during the three weeks of observation. The mean algal biomass productivity observed on the 2D substratum (Treatment A) under low and high nutrient concentration were (0.11 ± 0.01 mg dW cm^{-2} d^{-1}, n = 3) and (0.26 ± 0.02 mg dW cm^{-2} d^{-1}, n = 3) respectively, while the mean algal biomass productivity observed on the 3D substratum

(Treatment B) under low and high nutrient concentration were (0.27 ± 0.02 mg dW cm^{-2} d^{-1}, n = 3) and (0.40 ± 0.02 mg dW cm^{-2} d^{-1}, n = 3) respectively. The results suggest that three dimensional substratum performs better than two dimensional substrata with regards to algal biomass productivity. This initial observation is consistent under both low and high nutrient concentration environments. Based on the observations from this preliminary study, a more detailed experiment was carried out to obtain sufficient data to draw conclusions statistically.

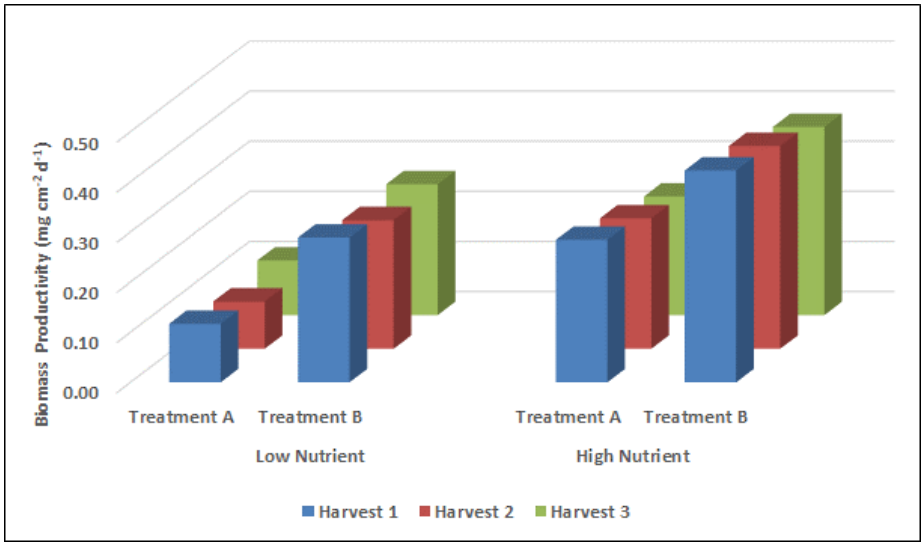


Figure 21: Preliminary results on substratum type and nutrient concentration

5.2 Materials and Methods

The second experiment was carried out over a 12-week period. As mentioned before, the 4-lane bioreactor system used for the first experiment was modified and reconstructed for use in carrying out this experiment. The modified 4-lane bioreactor system was designed and constructed to carry out a 2 x 2 experimental design. The four lanes of the system were fabricated using identical detachable Genova Half Round Gutters supported by a framework constructed using PVC

pipes and wood at a slope of 2.4%. Each of the four lanes contained (152.4 cm x 7.6 cm) of growing area. Figures 22 and 23 show the modified 4-lane bioreactor system and a schematic representation of the modified system respectively.

Figure 22: Modified four-lane flow-way system

The four lanes were split into two identical groups, with each group consisting of two lanes operated in a continuous mode by recirculating 38 liters (10 gal) of water nutrient solution each to a separate reservoir (Figure 24) using a centrifugal pond pump (Supreme Mag Drive, Model MD 18, Danner Manufacturing, Islandia, New York, USA).

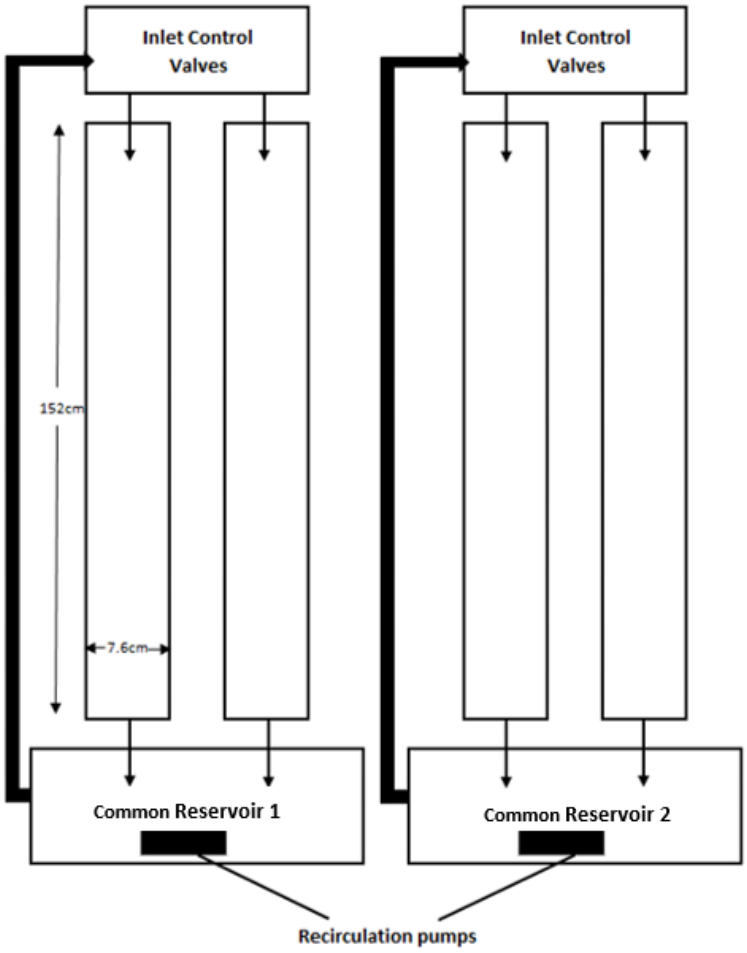


Figure 23: Schematic of the modified 4-lane flow-way system

Adjustable PVC valves were used to regulate the flow rates in each of the lanes. Collimators were also fixed at inlets of each flow lane to ensure laminar flow of water. The collimators were constructed using cut 1/2-inch diameter straws glued together (Figure 25) using 100% silicone all-purpose adhesive sealant (DAP, UNSPSC 31201606, USA).

As mentioned in Section 5.1, two treatment levels were used for the substratum. The first substratum treatment level (Treatment A) is the same two dimensional substratum surface used in experiment 1, while the second substratum treatment level (Treatment B) is the modified medium density version of the three dimensional substratum surface, having two fibers extending

in the vertical direction for each 9 cm^2 (0.22 stitches per square cm) area of the substratum.

Figure 24: Separate reservoirs for different nutrient treatments

This variation of the three dimensional substratum was selected for experiment 2 due to its superior performance in experiment 1. The two treatment levels for the substratum surfaces are shown in Figure 26.

Figure 25: Adjustable valves and collimators included to regulate water flow

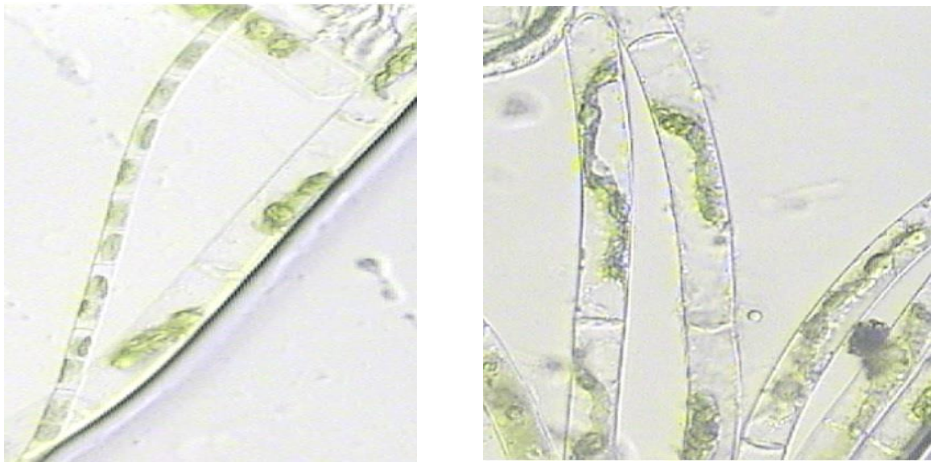
The two substratum treatment levels were exposed to the two nutrient concentration treatment levels (Low and High), such that each nutrient concentration treatment level of the bioreactor system had the two dimensional substratum (Treatment A) on one lane and the three dimensional substratum (Treatment B) on the other lane. The substratum surfaces were adhered to the lanes of the bioreactor system using 100% silicone all-purpose adhesive sealant (DAP, UNSPSC 31201606, USA).

(a) 2D Substratum Treatment (Level A) (b) 3D Medium Density Substratum Treatment (Level B)

Figure 26: Experimental treatment levels for substratum surfaces

Each reservoir of the bioreactor, which each had a 38 L (10 gal) capacity, was inoculated with the algal community collected from local streams in eastern Alabama. Following a similar protocol as in the first experiment, the dominant species were identified to be *Microspora floccose* and *Mougeotia scalaris* (Figure 27). Both reservoirs were dosed daily with commercial F/2 media (Pentair Co., Apopka, FL). Daily temperature, pH, and conductivity were measured by hand for both reservoirs using a handheld combination pH/EC probe (HI 98130, Hanna Instruments, Woonsocket, Rhode Island). To avoid reservoir concentrations increasing over time, half of each reservoir volume (19 L or 5 gal) was replaced with distilled water every day. Recommended doses of commercial F/2 media (Pentair Co., Apopka, FL) was added for each

gallon of water replaced to keep the nutrient level constant. The temperature averaged 77.1 ± 0.5 (range 73.7 – 77.3, n = 85) ^{o}F for the low nutrient reservoir and 75.0 ± 0.4 (range 73.6 – 76.6, n = 85) ^{o}F for the high nutrient reservoir. The conductivity averaged 0.17 ± 0.05 (range 0.11 – 0.26, n = 85) for the low nutrient reservoir and 0.36 ± 0.03 (range 0.28 – 0.42, n = 85) for the high nutrient reservoir, while the pH had an average value of 8.02 ± 0.13 (range 7.77 – 8.26, n = 85) for the low nutrient reservoir and 8.17 ± 0.15 (range 7.86 – 8.44, n = 85) for the high nutrient reservoir. Details of temperature, pH, and conductivity records for the two reservoirs are provided in Appendix III. Dissolved N concentrations was measured as NO3-N: 1.68 ± 0.39 ppm and 12.69 ± 0.82 ppm respectively for the low and high reservoirs, using Insta-Test testing pads (LaMotte Co., Chestertown, MD). These were measured weekly.



(a) (b)

Figure 27: Algal species used for inoculating reservoir (a) *Microspora floccose* (b) *Mougeotia scalaris*

The illumination for the bioreactor system was provided by placing three Sun System Sun Blaze T5 High Output fluorescent fixtures, suspended from the ceiling perpendicularly across the flow lanes. The dimensions of each of the fixtures are 119.4 cm (length) x 57.2 cm (width) x 6.4

cm (height). Each fixture uses 8 bulbs (Spectralux 901618, China), with each having a wattage of 54W. As mentioned in Section 5.1, the orientation of the bioreactor system was changed and placed such that each light fixture covered all four lanes of the bioreactor system. As a result of this change, the bioreactor provided a more homogeneous light intensity distribution across the four lanes of the system as demonstrated by the heat map for the light intensity across the lanes of the modified bioreactor system (Figure 28). Photosynthetic photon flux density on the algal growth substratum averaged 252 ± 38 (range 194 – 321) µmol m^{-2} s^{-1} over the whole bioreactor system. Light intensity measurements were taken using a quantum flux meter and probe (LI-250 Light Meter and LI-190 Quantum Sensor, LI-COR Biosciences, Lincoln, Nebraska, USA).

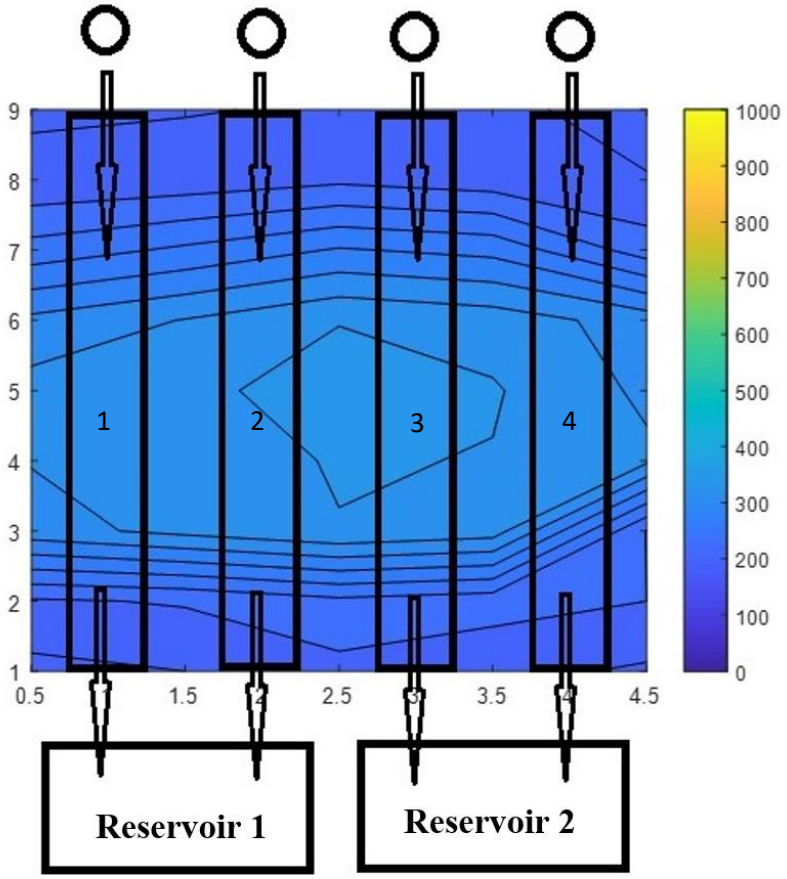


Figure 28: Light illumination map for modified bioreactor system

The bioreactor system was operated continuously (24 hours of daily light). Light intensity measurements were taken using a photon flux sensor (LI-250 Light Meter and LI-190 Quantum Sensor, LI-COR Biosciences, Lincoln, Nebraska, USA). Photosynthetic photon flux density on the algal growth substratum averaged 265 ± 50 (range 193 – 342) µmol m^{-2} s^{-1} over the whole bioreactor system.

Similar to the first experiment, the channels for the two treatment levels for the substratum treatment were swapped between the two lanes within each nutrient concentration treatment level (Table 4). This was implemented to mitigate any effect of variability due to flow rate and light intensity that may still be experienced across the lanes of the bioreactor system.

Table 4: Placement of substratum treatment levels on the lanes of the bioreactor system during the 12-week period for experiment 2 [A, B represents 2D and 3D substratum respectively, with L, H represents low and high nutrient concentrations respectively].

	LANES			
	Lane 1	Lane 2	Lane 3	Lane 4
Week 1	A_L	B_L	A_H	B_H
Week 2	B_L	A_L	B_H	A_H
Week 3	A_L	B_L	A_H	B_H
Week 4	B_L	A_L	B_H	A_H
Week 5	B_L	A_L	A_H	B_H
Week 6	A_L	B_L	B_H	A_H
Week 7	A_L	B_L	A_H	B_H
Week 8	B_L	A_L	B_H	A_H
Week 9	A_L	B_L	A_H	B_H
Week 10	B_L	A_L	A_H	B_H
Week 11	B_L	A_L	B_H	A_H
Week 12	A_L	B_L	B_H	A_H

The harvesting, drying and measurement protocols used in experiment 1 were also modified in experiment 2. First, a vacuum flask was used for harvesting rather than the mechanical vacuum used in experiment 1. Secondly, in an effort to reduce the drying period, which was 72 hours in experiment 1, harvested biomass slurry was allowed to settle for 12 hours by sedimentation and decanted using a 25 ml pipette before drying was carried out. To ensure that no biomass was lost through the decantation process, a filtration and drying protocol was introduced to estimate

biomass loss through decantation. Also, to further accelerate the process, a heat lamp was introduced into the fume hood during the actual drying process. The detailed protocol used in experiment 2 for harvesting, drying and measuring algal biomass will now be presented in the remaining part of this section.

Algal biomass was sacrificially harvested every 7 days from the substratum on each lane. On harvesting days, the pumps were turned off to stop the flow of water on the four lanes of the bioreactor system. The bioreactor system was not allowed to become fully dry to not compromise the viability of the remaining algal community on the flow-way system after harvesting. Each of the four lanes was then harvested mechanically using a tube connected to a vacuum source (Figure 29). Each channel was harvested thoroughly, with 1000mL of water added across the substratum as a wash to aid the recovery process of attached biomass from the surface of the substrate.

Biomass samples were also collected from each lane and stored separately in formalin vials to monitor if there were changes in the algal community within the reservoir. Biomass samples were removed from each lane using tweezers. The species identification was conducted through digital microscopy utilizing a Motic optical microscope (Motic Corp., Richmond, BC). The process involved mixing the content of each vial thoroughly and then taking a sample out of the vial using a tweezer and placing it on a glass slide. From each vial, three sub samples were drawn. The glass slide was then placed under the microscope for observation. The Motic optical microscope was used at 400 X to take 3 random images from each subsample and the number of times each species appeared in the micrograph was counted and analyzed for differences.

Figure 29: Biomass harvesting operation using vacuum flask

After each lane was harvested, the biomass was poured into a graduated cylinder (Figure 30). Additional water was used to rinse residual biomass from the filtering flask, which is then poured into a graduated cylinder. The harvested biomass was then allowed to settle by sedimentation for 12 hours.

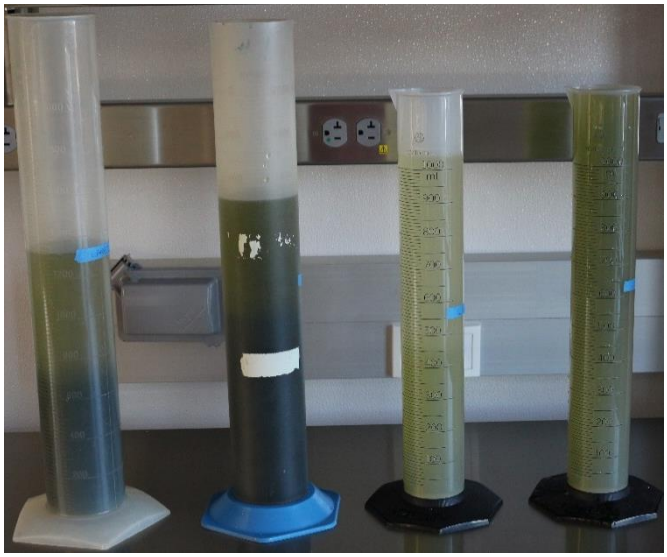


Figure 30: Harvested biomass put in graduated cylinder for sedimentation purposes

After the settling period, a 25 mL pipette was used to remove excess water from the top of the graduated cylinder. The recovered water was placed in a graduated cylinder and measured. The recovered water was stirred thoroughly, and three 10 mL samples were taken and stored in a vial at -20^{o}F (Figure 31). The purpose of taking these samples is to use them for estimating the overall weight of biomass in the recovered water.

Figure 31: Three samples of decanted liquid taken from a lane

The recovered biomass (biomass slurry) left in the graduated cylinder was then transferred into a pre-weighed pan lined with aluminum for drying purposes. Different drying protocols are followed for drying the recovered biomass slurry and for the biomass samples taken from the decanted water. They were stored in vials because of the difference in biomass quantity in both cases.

Figure 32: Recovered biomass put in drying pan in preparation for drying operation

For the recovered biomass slurry, once containing the biomass slurry (Figure 32), the pans were placed into a fume hood and allowed to dry for a period of 36 hours. A heat lamp and two Honeywell fans (HT-908, USA) were introduced into the fume hood to accelerate the drying process (Figure 33). After removing the drying pans from the fume hood, the biomass was then air dried for an additional 24 hours. The pans were then weighed again after the air-drying process. The weight of the biomass was calculated as the difference between the weights of the empty pan and the pan after the drying process (Biomass 1 (g)).

Figure 33: Drying protocol setup for drying recovered biomass in fume hood

To measure the biomass trapped in the excess water removed, the three 10 ml subsamples taken from the decanted water were processed to extract suspended algae by filtration and drying process. For the filtration process, 4.7 cm extra thick glass fiber filter papers (Pall Corporation, Ann Arbor, Michigan) were used. The filter papers used for the filtering process were first pre-heated in the oven at 105^0C for 24 hours and then placed in a glass desiccator and allowed to cool for 24 hours. Each sample was then filtered using the glass fiber filter and laboratory vacuum pressure. The glass fiber filter was then placed on a pan and oven dried at 105^0C for 24 hours. After oven drying, the glass fiber filter is retrieved and placed in the glass desiccator for cooling. Figure 34 shows the drying pans before and after the drying process.

The weight of biomass in each 10 ml subsample was then calculated as the difference between the weights of the dry glass fiber filter paper before filtering and after oven drying. The average weights of the biomass for the three 10 ml subsamples (Biomass2 (g)) then becomes the biomass weight for each 10 ml of decanted water for that particular lane in the flow-way system.

The overall biomass harvested from each lane of the flow-way system was then measured as the sum of the dry biomass obtained from the dried biomass slurry and from the decanted water as expressed in equation 4.

Total Biomass (g) = Biomass 1 (g) + ([Total volume of decanted water (mL)]/10 (mL)) * Biomass2 (g). (3)

Figure 34: Algal sample drying process before and after oven-drying

5.3 Results

The raw data obtained for the daily biomass productivity for the two substratum treatment levels (2D and 3D) used under the two nutrient treatment levels (low and high nutrient concentrations) in experiment 2 over the 12 harvests performed for the experiment are presented in Figure 35. Detailed records of harvested biomass for each substratum treatment level, measured after the drying process, are presented in Appendix IV.

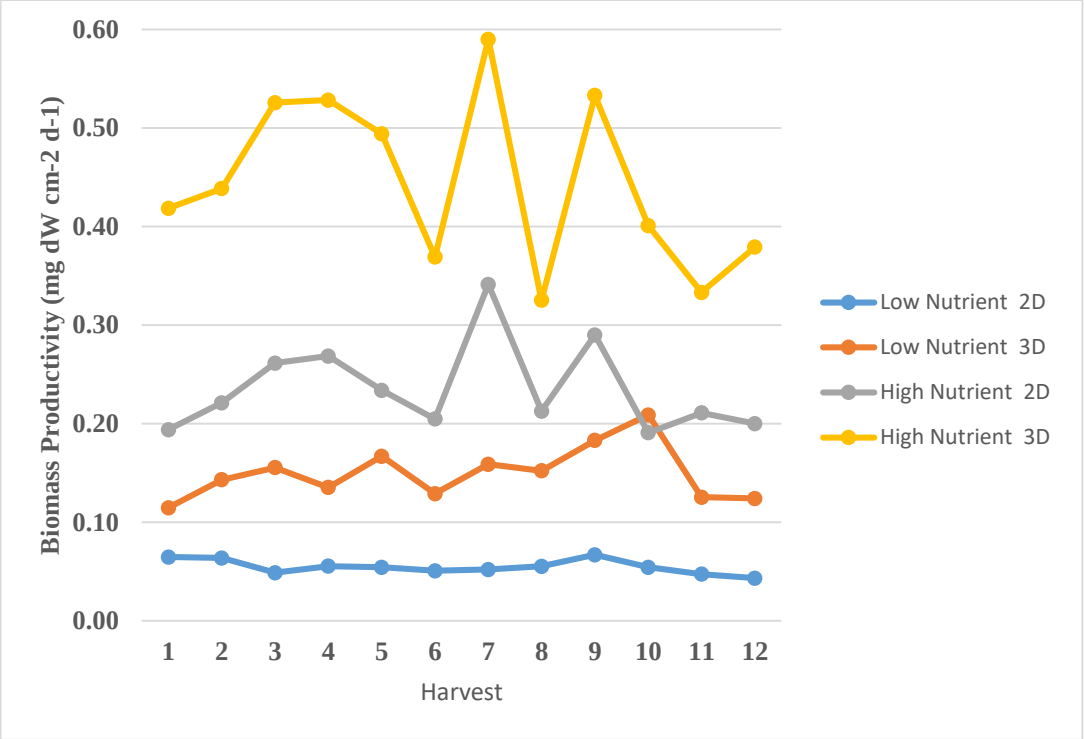


Figure 35: Algal biomass productivity (mg dW cm^{-2} d^{-1}) versus substratum type for two nutrient concentrations.

An analysis of underlying ANOVA assumptions of homoscedasticity was performed to determine a suitable approach to analyze the data statistically. Tests of normality and analyses of residual plots were conducted. As shown in Figure 36, the data was normally distributed. Parametric statistical tests were employed after checking conformity of ANOVA assumptions. A two-way ANOVA test was conducted using Minitab 18. All the plots and histograms for residual analyses on harvested algal biomass are presented in Figure 36.

The algal biomass productivities for both 2D and 3D substratum generally increased as the nutrient condition changed from low nutrient concentration to high nutrient concentration (Figure 37). Similarly, the productivity also generally increased as the dimension of the substratum type increased from two dimensional to three dimensional.

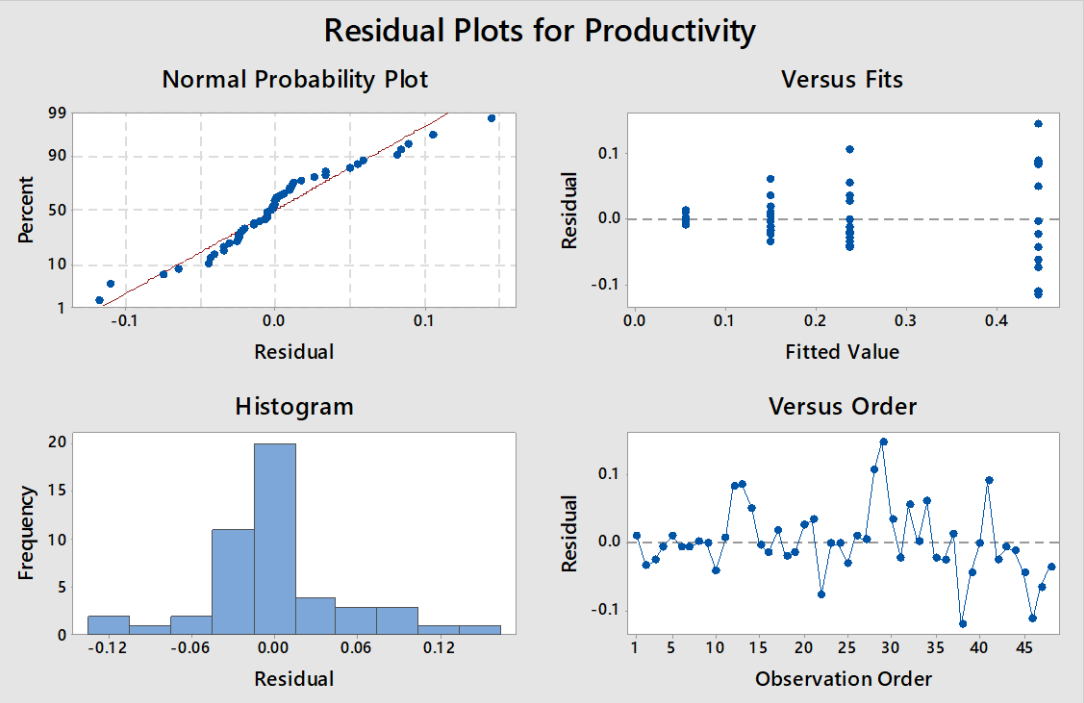


Figure 36: Residual plots for algal biomass productivity (mg dW cm^{-2} d^{-1})

Across all treatments combinations, the highest productivity was observed consistently on the 3D substratum and high nutrient concentration treatment with the greatest productivity value being (0.44 ± 0.09 mg dW cm^{-2} d^{-1}).

The lowest productivity was observed consistently on the 2D substratum and low nutrient concentration treatment with the lowest productivity value being (0.0547 ± 0.01 mg dW cm^{-2} d^{-1}). The result of ANOVA testing showed that the main effect of substratum type was significant ($F[1,44] = 104.8$, $p < 0.001$).

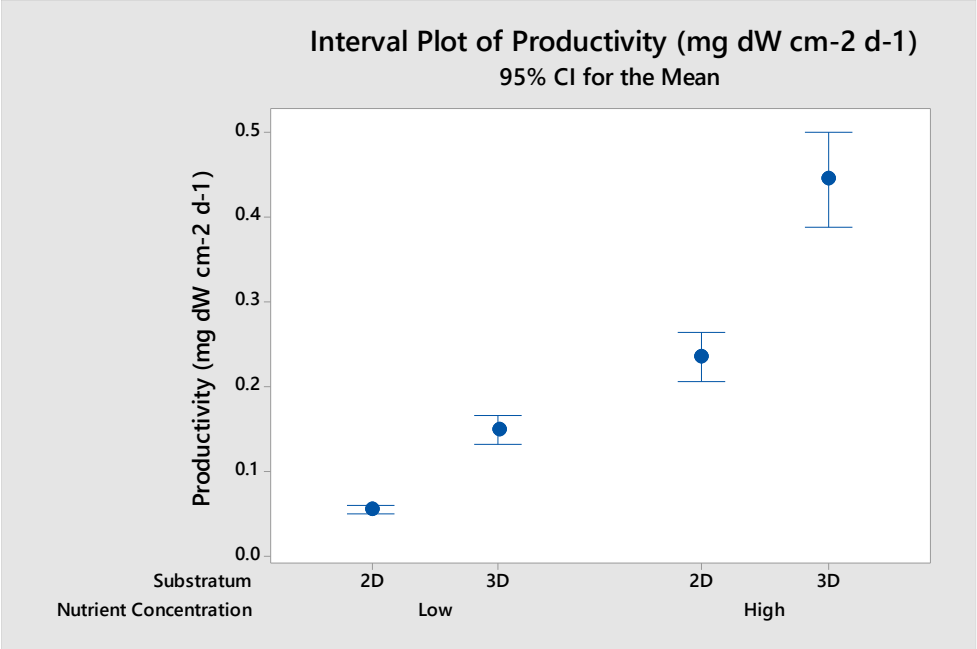


Figure 37: Interaction plot for algal biomass productivity versus nutrient concentration for both 2D and 3D substratum

Also, the main effect of nutrient concentration was significant ($F[1,44] = 257.3$, $p < 0.001$). The interaction effect of substratum type and nutrient concentration was also significant ($F[1,44] = 14.7$, $p < 0.001$). Results of the two-way ANOVA are shown in Table 5. Results also showed that under low nutrient conditions, algal biomass productivity increases by 174% when three dimensional substrata are used rather than 2 dimensional substrata. Under high nutrient concentrations, the productivity increased by 89%. Also, when two dimensional substrata are used under high nutrient concentrations rather than under low nutrient concentrations, algal biomass productivity increases by 330%, while productivity increases by 197% when three dimensional substrata are used under high nutrient concentrations rather than under low nutrient concentrations.

Table 5: Summary of two-way ANOVA for algal biomass productivity for experiment 2

Factor Information

Factor	Type	Levels	Values
Substratum	Fixed	2	2D, 3D
Nutrient Concentration	Fixed	2	Low, High

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Substratum	1	0.27691	0.276914	104.83	0.000
Nutrient Concentration	1	0.67965	0.679646	257.30	0.000
Substratum*Nutrient Concentration	1	0.03894	0.038936	14.74	0.000
Error	44	0.11622	0.002641		
Total	47	1.11172			

6. Experiment 3: Impact of three dimensional substratum base roughness on algal biomass productivity

The third experiment focused on understanding how important the surface roughness of the substratum base is for algal biomass productivity when algae is cultivated using three dimensional substrata in comparison to two dimensional standards. In two dimensional substrata, the roughness of the substratum is an important factor that influences algal biomass productivity and significant efforts have been invested to optimize the surface roughness to improve algal biomass productivity. However, for three dimensional substrata, the incorporation of fibers extending in the vertical direction should provide better support for the algae as they attach to the substratum and grow on it. As such it is hypothesized that the roughness of the substratum base should not be as critical for algal biomass productivity as it is for two dimensional substrata. The aim of this experiment was to investigate the impact of substratum base roughness on three dimensional substrata and compare the results with those obtained from two dimensional substrata.

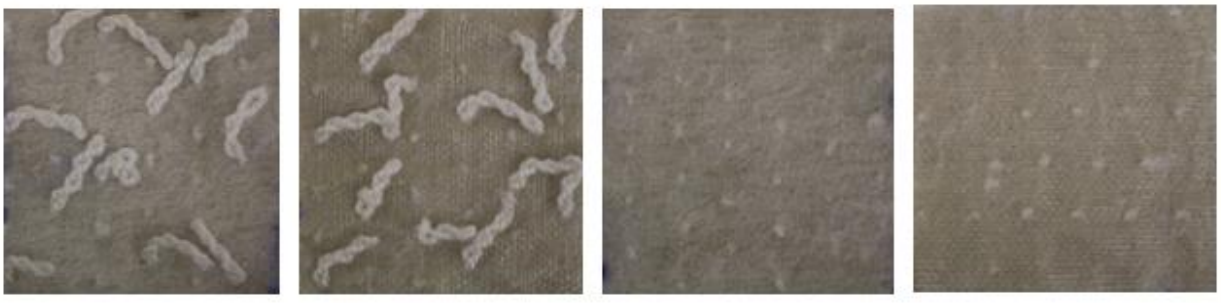
6.1 Preliminary Study

A preliminary study for this experiment was not conducted since the bioreactor system and the protocol for handling biomass was already in place.

6.2 Materials and Methods

The third experiment was carried out over a 12-week period, with harvesting operations performed every 5 days to generate more data. Based on observations from the previous two experiments, it is expected that this change would not significantly affect results, but would

provide the opportunity for more slots. The substratum treatment levels were formed by modifying the three dimensional substratum surface obtained from Interface Inc. that was used in both experiments 1 and 2 (Treatment C in experiment 1 and Treatment B in experiment 2). Further modifications were made to the substratum to suit the experimental needs of experiment 3. In all, four treatments, classified into two groups of the substratum were utilized. In the first group consisting of two substrata, the vertical fibers extending from the base were left intact and these substrata were classified as three dimensional. Of the two substratum in the three dimensional category, the first substratum has the "fuzzy" base left intact (Treatment A), while the second substratum has the "fuzzy" base scraped off (Treatment B) as shown in Figure 38 (a) and (b). The second group has the vertical fibers cut off, and these substrata are classified as two dimensional. As in the first group, one of the substratum has the "fuzzy" base left intact (Treatment C), while the other has the "fuzzy" base scraped off (Treatment D) as shown in Figure 38 (c) and (d). All four experimental treatment levels for the substratum surfaces are shown in Figure 38. The four substratum surfaces treatments covered the entire growing area of the lanes of the system and provided the surface for algal attachment and colonization. They were glued to the lanes using aquarium safe adhesives (DAP, UNSPSC 31201606, USA).



(a) Treatment A (b) Treatment B (c) Treatment C (d) Treatment D

Figure 38: Experimental treatment levels for substratum surfaces in experiment 3

The bioreactor system used for experiment 2 was used for this experiment, however a common reservoir having 57 L (15 gal) replaced the two reservoirs used in experiment 2. Water flow through the lanes of the flow-way system was implemented using two centrifugal pond pumps (Supreme Mag Drive, Model MD 18, Danner Manufacturing, Islandia, New York, USA) submerged in the reservoir. Similar to experiment 2, half of the reservoir volume (38 L or 10 gal) was replaced with distilled water every day to avoid the reservoir concentration from increasing over time. The reactor was dosed daily with commercial F/2 media (Pentair Co., Apopka, FL). The recipe for the F/2 media is provided in Appendix VIII. Daily temperature, pH and conductivity were measured by hand using a handheld combination pH/EC probe (HI 98130, Hanna Instruments, Woonsocket, Rhode Island). Temperature, pH, and conductivity values measured daily all through the period of the experiment showed that these values varied slightly over the life of the experimental time-frame. The temperature averaged 76.1 ± 1.0 (range 74.5 – 78.9, n = 86) ^{o}F, and the pH averaged 7.26 ± 0.43 (range 6.49 – 7.91, n = 86) while the conductivity had an average value of 0.07 ± 0.03 (range 0.04 – 0.14, n = 86). Dissolved N concentration was measured as NO3-N: 1.56 ± 0.38 ppm, using Insta-Test testing pads (LaMotte Co., Chestertown, MD). Details of temperature, pH, and conductivity records for the two reservoirs are provided in Appendix V.

A low nutrient level was used so that nutrient concentration could constitute a limiting factor for the experiment. Based on Ye et al [101] modified version of Monod model, it is hypothesized that under a limiting nutrient concentration (s), the maximum specific growth rate of algae would be a function of the substratum type on which the algae grow. As such, different channels of the bioreactor system would experience different maximum specific growth rates based on the substratum type placed on the channel. This would account for the differences in algal biomass

productivity observed for different substratum types.

Also, similar to experiments 1 and 2, the four factor levels for substratum surfaces were alternated between the four lanes to mitigate any effect of variability due to flow rate and light intensity across the lanes of the bioreactor (Table 6).

The harvesting, drying, and measurement protocols used in processing biomass follow the same protocols put in place while handling biomass in experiment 2. However, harvesting was performed every 5 days. Detailed explanation of these protocols are outlined in Section 5.2.

Table 6: Treatment placement on flow-way system during experiment 3 [A, B, C and D represents substratum treatments A, B, C and D respectively].

	LANES			
	Lane 1	Lane 2	Lane 3	Lane 4
Week 1	A	B	C	D
Week 2	D	A	B	C
Week 3	C	D	A	B
Week 4	B	C	D	A
Week 5	D	C	B	A
Week 6	B	A	C	D
Week 7	A	B	D	C
Week 8	C	D	A	B
Week 9	C	D	A	B
Week 10	A	B	D	C
Week 11	D	C	B	A
Week 12	B	A	C	D
Week 13	B	A	D	C
Week 14	D	C	B	A
Week 15	C	D	A	B
Week 16	A	B	C	D

6.3 Results

Figure 39 presents the raw data for daily productivity for all four treatment combinations for experiment 3 over the 16 harvests performed. Detailed records of harvested biomass for each treatment combination, measured after the drying process, are presented in Appendix VI.

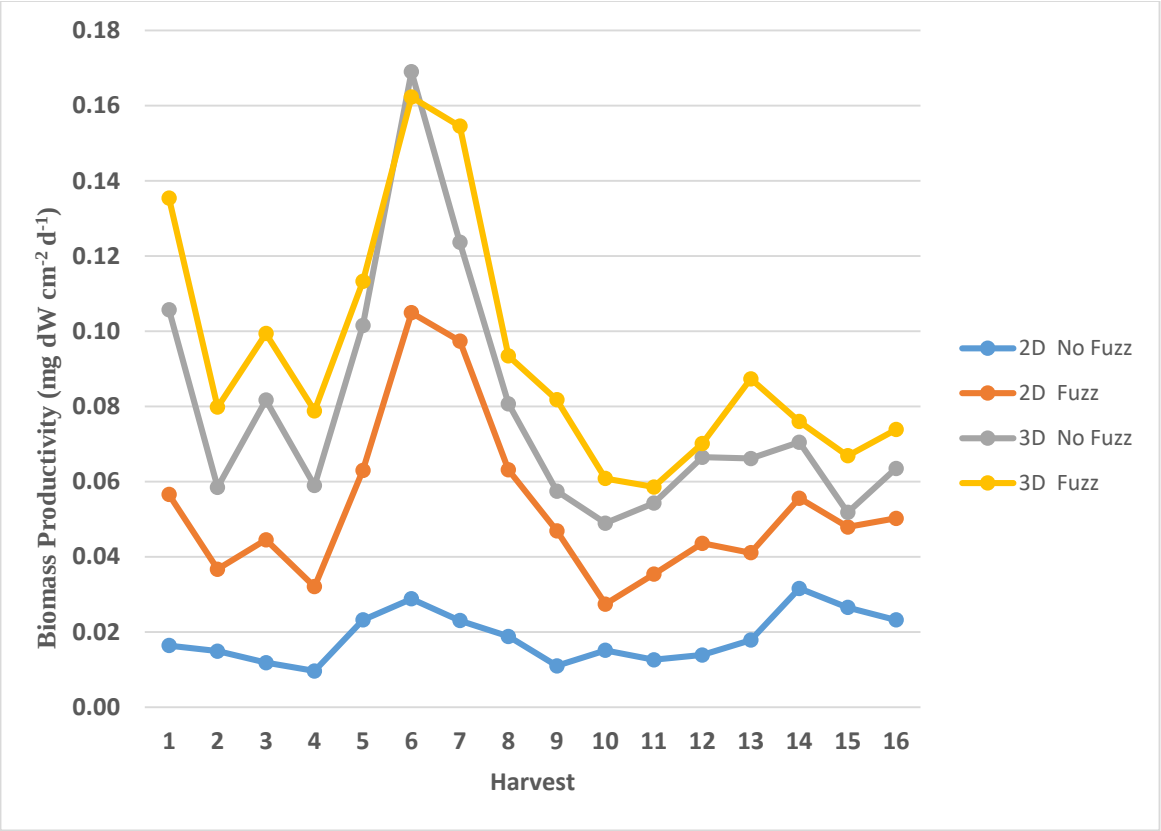


Figure 39: Algal Biomass Productivity (mg dW cm^{-2} d^{-1}) versus substratum base roughness level for both substrate treatment levels.

As in the previous experiment, an analyses of underlying ANOVA assumptions were performed to determine a suitable approach to use in analyzing the data statistically. The analyses included test of normality and analyses of residual plots. Based on the results of these analyses, parametric statistical tests were employed after checking conformity of ANOVA assumptions.

A two-way ANOVA analyses and a pairwise comparison analyses were performed using Minitab 18. All the plots and histograms for residual analyses on harvested algal biomass are presented in Figure 40.

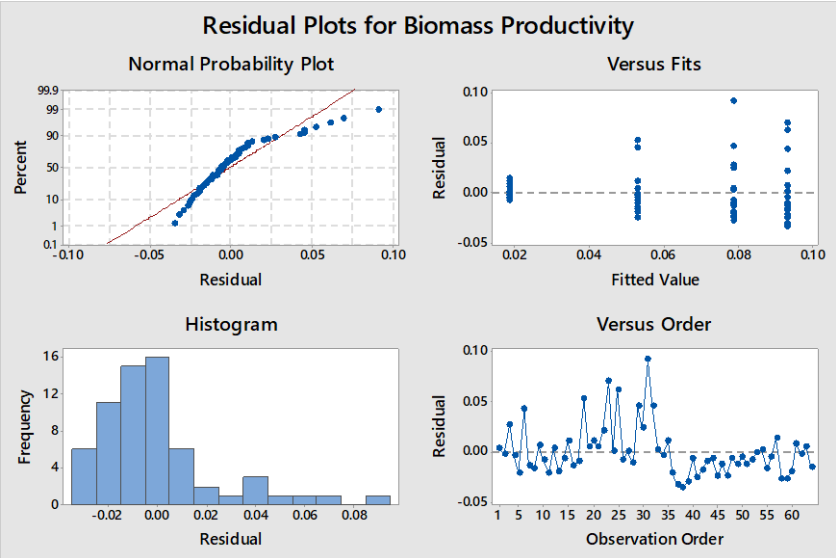


Figure 40: Residual plots for algal biomass productivity (mg dW cm^{-2} d^{-1}) for experiment 3.

As found before, the algal biomass productivity on the 3D substrata was greater than the productivity observed on the 2D counterparts. Also, the algal productivity increased when the fuzzy base attribute was introduced to both 2D and 3D substratum. However, a more pronounced increase was observed on the 2D substratum than on the 3D substratum.

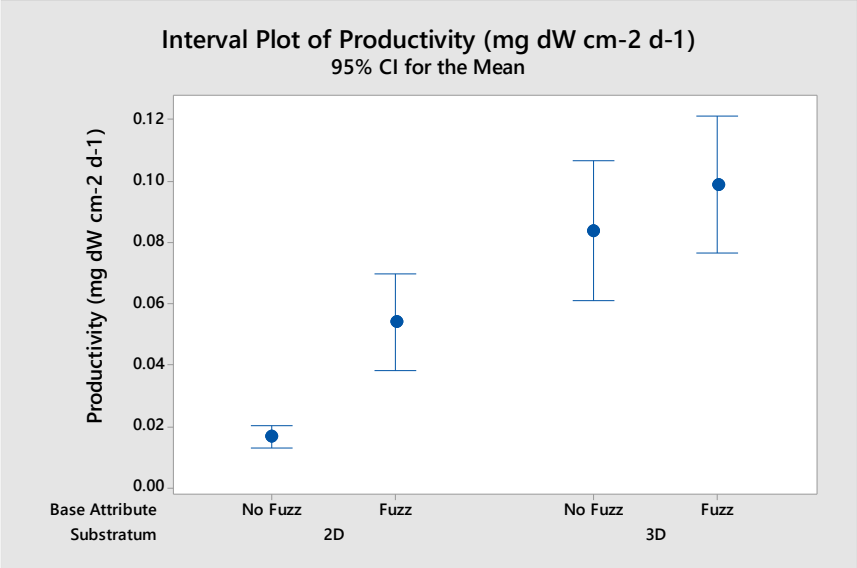


Figure 41: Interaction plot for algal biomass productivity (mg dW cm^{-2} d^{-1}) versus substratum base roughness levels for both substratum levels.

Across all treatments, the highest productivity was observed at the 3D substratum with a fuzzy base treatment combination (Treatment A) with the mean productivity value being (0.09 ± 0.03 mg dW cm^{-2} d^{-1}). The lowest productivity was observed consistently at the 2D substratum having no fuzzy base treatment (Treatment D) with the mean productivity value being (0.02 ± 0.01 mg dW cm^{-2} d^{-1}) [Figure 41]. The result of ANOVA testing showed that the main effect of substratum type was significant ($F[1,60] = 62.7$, $p < 0.001$). In addition, the main effect of substratum base roughness was significant ($F[1,60] = 14.8$, $p < 0.001$). However, the interaction effect of substratum type and substratum base attribute was not significant ($F[1,60] = 2.4$, $p = 0.126$). Table 7 shows the results of the ANOVA analyses.

Table 7: Summary of two-way ANOVA for algal biomass productivity for experiment 3.

Factor Information

Factor	Type	Levels	Values
Substratum	Fixed	2	2D, 3D
Base Roughness	Fixed	2	No Fuzz, Fuzz

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Substratum	1	0.040336	0.040336	62.73	0.000
Base Roughness	1	0.009549	0.009549	14.85	0.000
Substratum*Base Roughness	1	0.001547	0.001547	2.41	0.126
Error	60	0.038583	0.000643		
Total	63	0.090015			

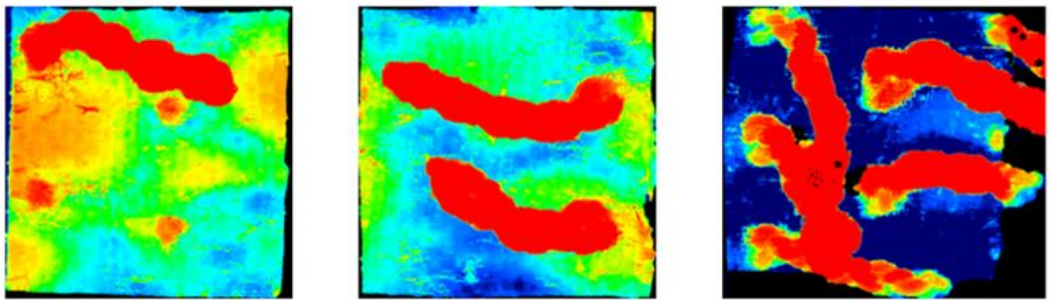
Pairwise comparison analyses showed that there was no significant difference between the results observed for both three dimensional substrata irrespective of the substratum base roughness (p-value = 0.209). However, all other pairwise comparisons were significantly different (p-value $\leq$ 0.02).

7. Discussion

The results obtained from the three experiments strongly appeared to indicate a significant difference in algal biomass productivity among the different treatment levels used, supporting the hypothesis that the physical characteristics of the features in three dimensional substratum play an important role in algal biomass productivity. This may be attributed to the effect of these features on algal growth limiting factors. As mentioned previously, several factors may affect the growth behavior of algae on substratum surfaces. These factors include light intensity, nutrient concentration, nutrient transport, flow velocities, and substratum area [18-20], among others. In an effort to understand the findings in these experiments, further analyses of self-shading and surface area were performed on 9 cm^2 samples of the three variations of three dimensional substrata (Treatments B, C and D) used in experiment 1 (Figure 42). For these analyses, each of the 3 samples were scanned using a VR-3000 One-shot Measuring Macroscope (Keyence, Itasca, IL) and isometric height images and heat topographical maps were taken. Figures 43 and 44 show the height images and isometric height images respectively for the 3 samples.

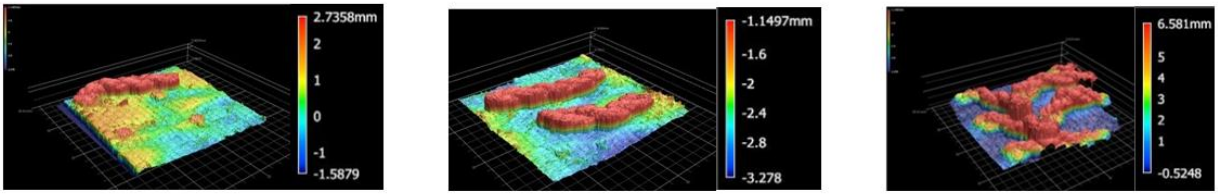
(a) (b) (c)

Figure 42: Photographs of 9 cm^2 samples of (a) Treatment B, (b) Treatment C and (c) Treatment D 3D substratum types used in experiment 1



(a) (b) (c)

Figure 43: Heat topographical map of substratum treatments.



(a) (b) (c)

Figure 44: Isometric height images of substratum treatments.

These images and heat maps provided estimates for the cross sectional area, surface area and volume of the fibers shown in Figures 43 and 44. Estimates obtained from the images were used to estimate upper and lower bounds for the surface area of an individual substratum fiber on the 3D substratum. Also, based on the values obtained, the surface area of the entire growing area for each of the three variations of the 3D substratum treatment levels used in the three experiments were calculated. Figure 45 shows a cross-sectional view of one of the substratum fibers. Each fiber is composed of 60 nylon threads that are extruded into a trilobal shape, spun into a continuous yarn and bent into a loop prior to stitching to the substrate. Thus, a cross-section of a fiber (such as the one in Figure 45) shows 120 cross-sections of the individual trilobal threads.

The yellow markings show a trace of the perimeter of individual threads within a single yarn and the values in red represent the area of each highlighted thread. To calculate the upper limit for surface area of each substratum fiber, the substratum fiber is considered to be made up of a collection of a number of stand-alone threads, such that the total possible surface area of each substratum fiber is expressed as the summation of the surface area of all the 60 threads making up a substratum fiber. Similarly, the lower bound for the substratum fiber surface area is expressed as the surface area of the substratum fiber, when assumed as one cylindrical feature. Results of this analyses showed that the lower and upper bounds for the surface area of each substratum fiber are 1.89 cm^2 and 20.15cm^2 respectively. However, in practice, it might be reasonable to assume that the surface that is more likely to experience colonization would be located in the outer surface of the fiber, although some organisms might attach to the interstitial spaces within the fiber. Surface area measurements obtained from measuring the fibers in Figures 43 and 44 showed that the surface area of each substratum fiber was on average 3.18 cm^2, which is close to the theoretical lower bound of the fiber's surface area.

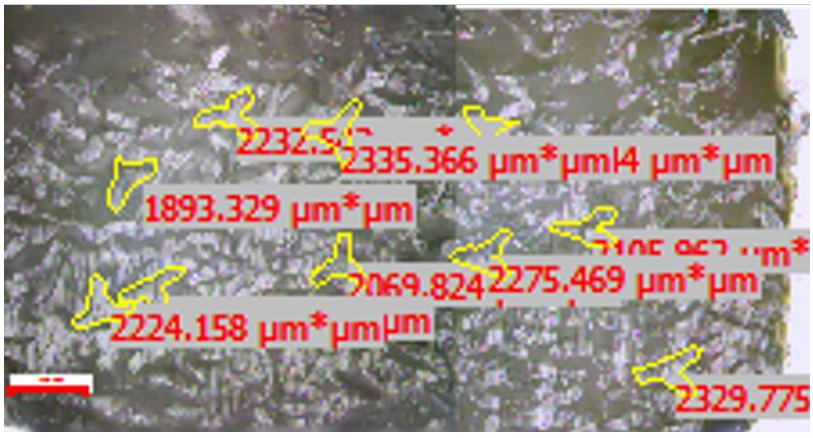


Figure 45: A cross sectional view of a looped fiber with 120 trilobal threads in a single substratum fiber

Shading percentages and surface areas of the three different substratum types were measured using data obtained from Figures 43 and 44. The shading percentages were calculated as the proportion of the base protected by the fibers (as measured by the orthogonal 2D projection of the fibers onto the substratum), while the substratum's surface area was calculated as the total surface area (base area plus substratum fiber area). For the base surface area, an estimation for the increase in surface area due to the "fuzzy base" is not considered because this feature is common among the three substratum treatments. Table 8 gives a summary of the shading percentages and the surface area measurements for the three substratum treatments levels of three dimensional substratum used in experiment 1. Detailed calculations for the shading percentages, substrate surface, and lower and upper limits for fiber surface areas are presented in Appendix VII.

Table 8: Substratum surface area and shading percentages for each substratum treatment level.

Substratum Treatment Level	Number of fibers (per 9 cm^2)	Substrate Surface Area (per 9 cm^2)	Substrate Total Surface Area (cm^2)	Area of base protected by fibers (per 9 cm^2)	Percentage Shading (%)
Treatment B (Low)	1	11.40	2929.00	0.97	10.78
Treatment C (Medium)	2	15.10	3885.00	2.26	25.11
Treatment D (High)	6	22.70	5866.00	4.27	47.44

7.1 Substratum Effective Surface Area

The results of experiment 1 showed a significant effect of substratum fiber density for the substratum types used. One of the factors that may account for this difference is the effective surface area of the substratum. The effective surface area of the substratum has been shown to

enhance algal biomass productivity (especially in nutrient-deficient water), as well as, encouraging biodiversity in both two dimensional and three dimensional substratum [17, 81]. Clearly, with increased surface area, the opportunity for cell attachment and colonization is increased, thereby providing more locations for algal cells to settle.

Figure 46 shows that of the three dimensional substratum treatments used, Treatment C had the highest daily mean productivity of algal biomass. The results show that total algal biomass productivity was significantly more abundant as fiber density increased from low (Treatment Level B) with a substratum effective surface area of 2929 cm^2 to medium (Treatment Level C) with a substratum effective surface area of 3885 cm^2, suggesting that productivity was enhanced by an increase in substratum effective surface area. However, as the substratum effective surface area is increased to 5866 cm^2 for the high fiber density 3D substratum (Treatment Level D), there is a significant drop in algal biomass productivity.

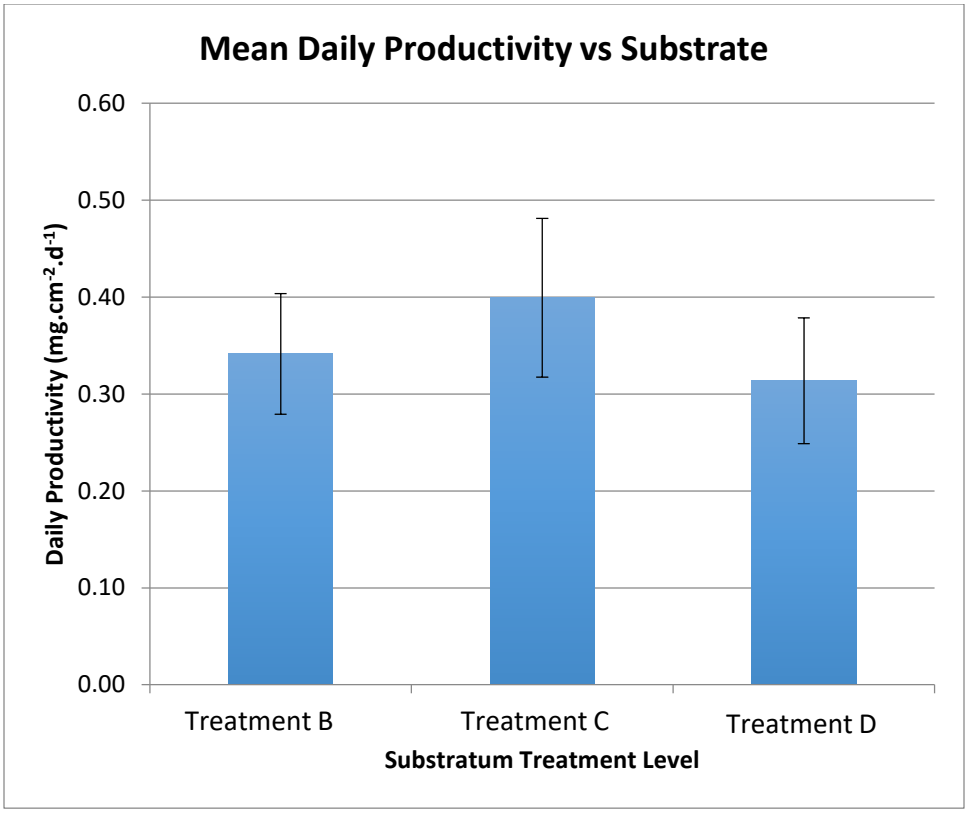


Figure 46: Mean algal biomass productivity versus substratum treatment level

A possible explanation for the observed relationship between effective surface area of the substratum and algal biomass productivity may be as a result of how substratum surface area is increased in the three dimensional substratum. For a two dimensional substratum, it is easy to understand why an increase in substratum surface area may positively impact algal biomass productivity. This is because an increase in the substratum surface area in two dimensional substrata does provide more area for algal cell attachment and colonization, though this increase does not negatively impact limiting factors (such as light intensity, photosynthesis, flow dynamics) that are vital for growth processes. However, when employing three dimensional substrata, the effective surface area is augmented by increasing the number of substratum fibers per unit area. It is possible that, as the substratum surface area continues to increase, the

corresponding algal attachment and subsequent growth and productivity may be hindered by certain limitations introduced by self-induced conditions form enhanced growth or by conditions deriving from the increased number of substratum fibers. For instance, light incidence and nutrient transportation may be adversely affected by the vertical component of the substratum surface area.

Figure 47 shows that the contribution to algal biomass productivity made by each unit of substratum surface area decreases as the substratum effective surface area increased. As the substratum effective surface area is initially increased from Treatment Level B (2929 cm^2) to Treatment Level C (3885 cm^2), although there is a decrease in the contribution to algal biomass productivity made by each unit of substratum surface area, the increase in substratum effective surface area more than compensates for this drop and results in an overall gain in total algal biomass productivity. However, beyond a critical point, the drop in algal biomass productivity per unit substratum surface area is so significant that further increase in substratum effective surface area does not compensate for the loss in algal biomass productivity per unit substratum surface area. As illustrated by the results of experiment 1, with further increase in substratum effective surface area of Treatment Level D (5866 cm^2) leading to a decrease in total algal biomass productivity. This suggests that there may be an optimum substratum fiber density level that results in optimal substratum effective surface area, and that an increase in substratum effective surface area beyond such an optimal point may result in reduced total algal biomass productivity.

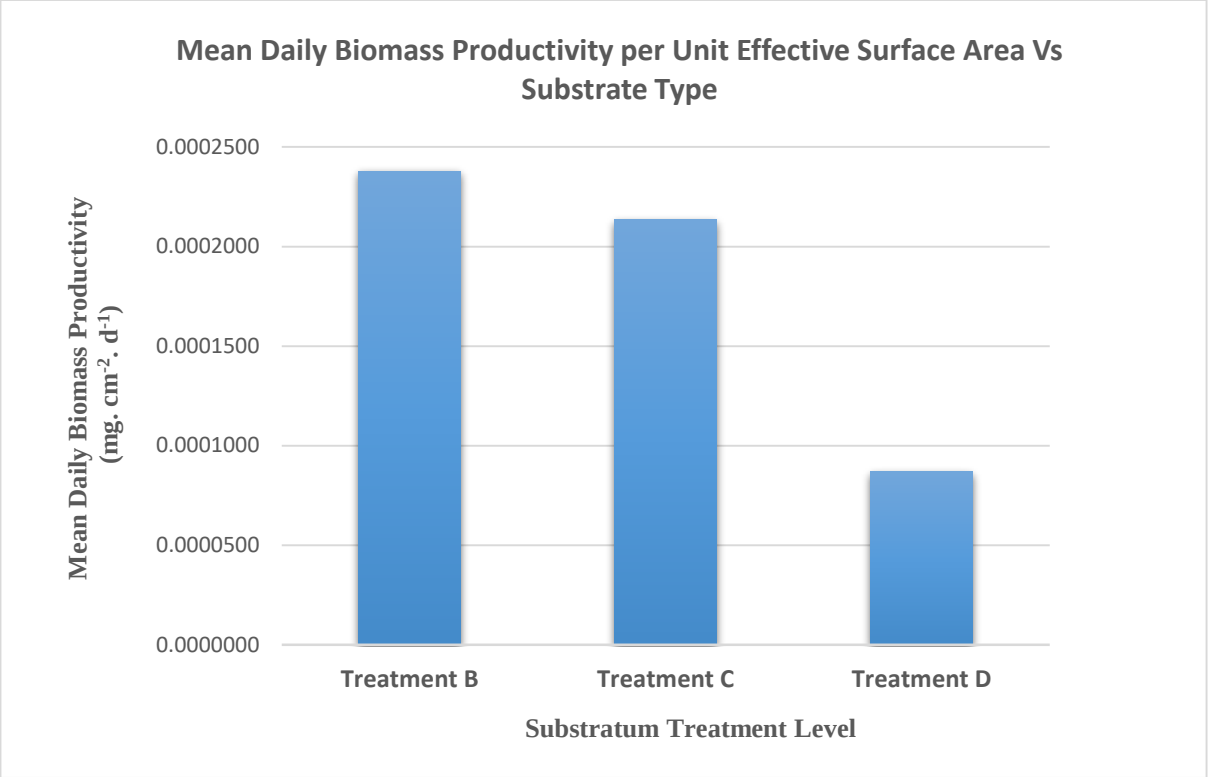


Figure 47: Algal biomass productivity per unit substratum effective surface area versus substratum treatment level of increasing fiber density.

7.2 Light Intensity

Light is considered a vital requirement for algal growth as it is critical for the photosynthetic process [98]. As such, an insufficient supply of light will certainly affect algal growth. One possible way light limitation can be experienced by algae is through self-shading [108]. Gosselain et al. [19] pointed out that in cases involving macrophytes, a sharp decrease is experienced in the intensity of light with respect to depth due to the tendency of submerged macrophytes to form thick subsurface canopies that monopolize incident light. In this study, the colonized substratum fibers seem to exhibit characteristics similar to macrophytes. Due to the flexible nature of their structure, the substratum fibers have a tendency to have similar

effects on incident light, thereby limiting the light intensity available to the shaded base. It is hypothesized that this effect becomes more pronounced as the density of the substratum fibers increases and as the algae grow, thereby significantly limiting the light available for algae growing in areas shaded from the light source. It is possible that although the effect of self-shading does not necessarily reduce the surface area available for cell attachment, the light may be distributed in ways that fail to optimize overall algal productivity, especially for high shading percentages. Self-shadowing estimation analyses performed on the three dimensional substratum surfaces suggest that the percentage of the base shaded as a result of the substratum fibers increases as the density of the substratum fibers increases. This is typical as the relationship between the incident light on the substratum surface and fiber density would suggest that at low fiber density level, the substratum surface would enjoy sufficient supply of light. As the fiber density is increased, the substratum surface area would increase and provide greater opportunity for algal cell attachment, but a limit is reach where the supply of light to the substratum begins to decrease with further increase in fiber density. As such, the relationship between incident light and the level of fiber density can be represented as a curve that first increases and then begins to diminish after a certain point.

A comparison of the productivities of the low fiber density 3D substratum (Treatment Level B) and the medium fiber density 3D substratum (Treatment Level C), suggests that although the medium density 3D substratum (Treatment Level C) has a higher shading percentage (25%) than the low density 3D substratum (Treatment Level B) with a shading percentage of (11%), both substratum surfaces were exposed to sufficient light for algal growth. However, as the shading percentage increased due to increased substratum fiber density as in the high fiber density 3D substratum (Treatment Level D) with a shading percentage of (47%), a point is

reached where the impact of self-shading adversely affects light availability to the growing algae. Overall algal biomass productivity is negatively impacted as a result. Figure 48 illustrates the relationship between algal biomass productivity and light shading. It is important to note that the light shading numbers are an estimate as incident light value were not directly measured during the experiment as a result of inadequate measurement apparatus to take such measurements during the experimental period.

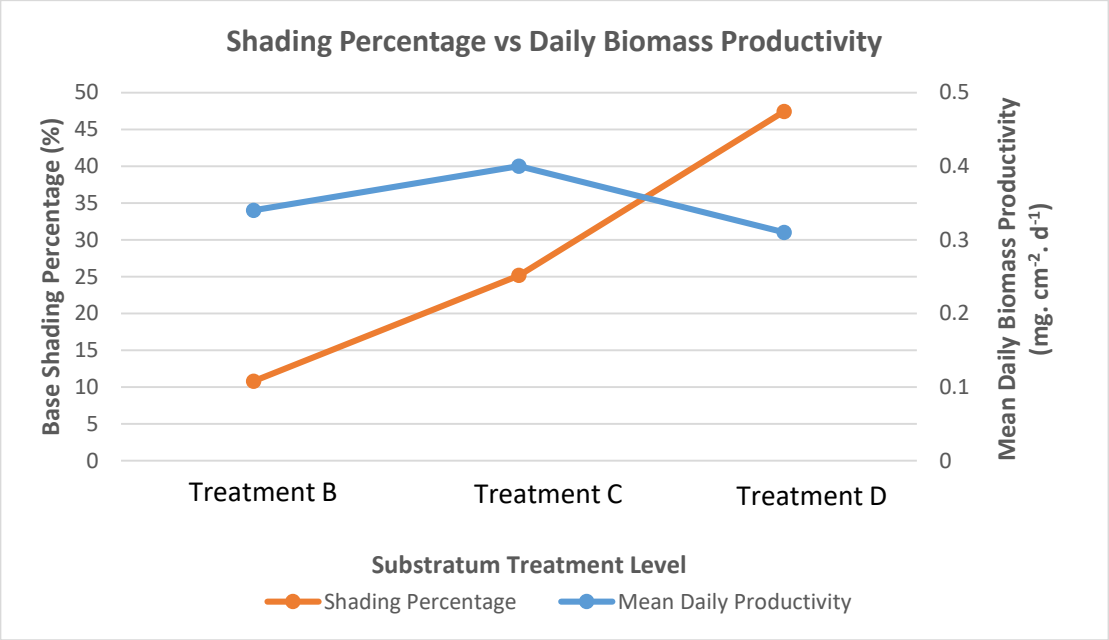


Figure 48: Algal biomass productivity versus shading percentage for different 3D substratum treatment levels.

7.3 Flow Dynamics

Velocity flow is crucial for algal growth in water bodies because the velocity flow regulates nutrient transport to algae. Algae's growth process could be affected by nutrient limitation due to alteration in hydrodynamic conditions in aquatic systems [109]. Velocity flow and the

associated near-bed velocity has been shown to have the ability to significantly affect periphyton biomass, structure and processes [110, 111]. These effects can be pronounced especially in vegetated water bodies, where submerged aquatic vegetation is known to have the ability to drastically influence the transport of nutrients and dissolved oxygen in aquatic systems. Biggs et al [88] showed that higher velocity can positively affect biomass accrual via greater mass transfer of limiting nutrients, but negatively impact biomass accrual because of increased sloughing in response to greater form drag and friction. Studies conducted by Hondzo and Wang [20] suggested that the growth of the freshwater periphyton is minimal in a stagnant fluid. Zhu et al. [112] points out that such stagnant fluid conditions (backwater phenomenon) are significant in water bodies with high vegetation density. Flow velocity studies indicate that when boundary layer flow encounters vegetative drag, the flow experiences a mixing region in the physical water column, where water flow is redirected to an upper region of rapid exchange, as well as, a lower region where the fluid decelerates and water renewal is limited [113, 114]. Based on observation of the experiments, it is hypothesized that each substratum treatment level experienced different flow characteristics as a result of alterations in the hydrodynamic conditions triggered by the substratum fiber density and possible vegetative drag introduced by the substratum features. It is further hypothesized that the higher fiber density 3D substratum (Treatment Level D) in experiment 1 may have experienced significant nutrient limitation in some part of the substratum that resulted in an overall reduced algal biomass productivity.

7.4 Nutrient Concentration

As expected based on Ye et al. [101] modified version of Monod model, nutrient concentration played a significant role in algal biomass productivity. In experiment 2, it was observed that

the productivity of algal biomass was greater under high nutrient conditions than under low nutrient conditions. Both two dimensional and three dimensional substratum treatment levels experienced greater productivities under high nutrient concentration than both two dimensional and three dimensional substratum treatment levels under the low nutrient conditions. Also, it was observed that the introduction of three dimensional substratum resulted in significant improvement in algal biomass productivity under both nutrient conditions. However, this improvement was more pronounced under low nutrient conditions than under high nutrient conditions. This is consistent with the model. Under low nutrient concentration, the nutrient concentration is a limiting factor. As such, there is competition for limited available nutrient in the system. The maximum specific growth rate of algal communities growing on the different substratum treatment levels would then be a function of the substratum type on which the algal communities grow. Under high nutrient concentrations, the nutrient concentration is no longer a limiting factor. As such, the maximum specific growth rate of algal communities growing on different substratum types is not impeded and all the different algal communities can attain their maximum growth rates. This results in a less pronounced difference in algal biomass productivity under high nutrient concentrations.

It was observed that the introduction of three dimensional substrata in place of the two dimensional substratum resulted in a 174% improvement in algal biomass productivity under low nutrient conditions, while an 89% improvement was observed under high nutrient conditions. This is consistent with the finding by Ahn et al. [81] that in nutrient deficient water bodies, having more surface area impacts algal biomass productivity positively.

7.5 Substratum Structure

In both experiments 2 and 3, greater algal biomass productivity was observed on the three dimensional substratum treatment levels than on the two dimensional substratum treatment levels. A unique feature of the three dimensional substrata used is that fibers extending in the vertical direction were introduced. The introduction of these fibers on three dimensional substratum provided opportunities for algal cell attachment at different water levels and a more rigid structure to support growing algal communities.

In experiment 3, it is observed that for both three dimensional substrata having fibers in the vertical direction, the algal biomass productivity is high even when the substratum base is smooth. This suggests that the introduction of substratum fibers to substratum, even in cases where the base is smooth, plays a significant role in improving algal biomass productivity. It was observed that the introduction of substratum fibers to the smooth two-dimensional substratum resulted in a 322% increase in algal biomass productivity, while the introduction of substratum fibers to the rough two dimensional substratum caused the productivity to increase by 76%.

Findings from experiment 3 show that the productivity of cultivated algal biomass could be significantly improved by introducing fibers extending in the vertical direction to the substratum base, as well as, improving the roughness of the substratum base to support algal attachment and retention.

7.6 Limitations

As with most experimental studies, several limitations were encountered during the course of the three experiments. In the calculations and discussions involving self-shading on the substratum base due to the presence of the fibers, it was assumed that the fibers were stationary. This implied that the fibers maintained a rigid structure or posture all through the experimental period. However, this is practically not the case. In practice, the fibers are flexible and do move especially as the water flows along. As a result, the shading percentages would be different depending on the positions of the fibers at any given time during the experiments and cannot be assumed to be fixed all through the experiments.

Apart from the positional change of the fibers, the light shading effect is equally dynamic due to changes in the system as the algae grow. Light shading percentages were calculated at the beginning of the experiment. This means that the contribution to self-shading made by the algae themselves were not taken into account. Also, the fact that the algae's contribution to light shading changes continuously as the algae grow was not considered. It is possible that the results obtained may be considerably different if these considerations were incorporated.

Another area of limitation was the two-dimensional and three-dimensional substrata used for the experiments. As mentioned earlier, these substrata were obtained from two different companies and as such were different. A unique difference between the two substrata were their colors. The two-dimensional substrata were black, while the three-dimensional substrata were white. Being that light intensity is an important factor for algal growth, the color of the substratum may have an effect on how light is absorbed or reflected on the two types of substrata used. As such, this may introduce an unfair basis when comparing the two different substrata.

Additionally, the two substrata had differences in terms of how their designs affect the available surface area for algal attachment and colonization. Although the surface area of the two-dimensional substrata was not measured for surface area analyses, it is clear that the three-dimensional substrata design enhances surface area maximization as every part of the real estate can support algal attachment. However, for the two-dimensional substrata design, a significant portion of the real estate cannot support algal attachment since there were portions of the real estate that were open. Based on the results of experiment 1, where the three levels of the three-dimensional substrata showed better algal biomass productivity than the two-dimensional substrata, it is difficult to tell how much of this difference in productivity is the result of the design of the two-dimensional substrata.

Finally, the substratum was modified in some cases to meet experimental design needs. In such instances, it is possible that unintended features may have been introduced. For example, in experiment 3, the two-dimensional substrata were achieved by cutting off the fibers. However, part of the fiber's stems or genes were left behind. It is likely that the two-dimensional substrata did not exhibit the exact characteristics that a typical two-dimensional substratum should exhibit. Similarly, with respect to the roughness, it was assumed that by scraping off the "fuzzy" base of the substratum, a reduction in surface roughness occurred. In practice, the reverse may be the case.

7.7 Economic Impact

Economic consideration is usually a key limitation when considering algal growth systems. As mentioned previously, the ATS is an attractive system for algal cultivation because of the potential to also use it for waste water remediation. This makes it possible to offset some of the cost of the system with the gains achieved through waste water remediation efforts. Some of the cost

associated with the ATS include biomass harvesting and drying costs, as well as the cost of substratum material.

An interesting observation from the results of experiment 1 is that the greatest productivity was observed in Treatment C, where the fiber density was reduced from the original 0.67 stitches per square cm to 0.22 stitches per square cm, a 67% reduction in fiber material requirement. This finding suggests that a more effective and productive substratum for algal cultivation can be obtained at a reduced production cost.

This cost saving potential becomes attractive in practical settings where algal cultivation typically requires hectares of land for operational purposes. It is also possible that further exploration of three-dimensional substratum features could uncover other potential cost saving opportunities that could make the substratum more attractive.

8. Conclusion and Future Work

The use of three dimensional substrata as growth surfaces for algal cultivation has been investigated, with a goal of exploring how the features of three dimensional substratum affect algal biomass yield. The effectiveness of three dimensional substrata for algal biomass cultivation was explored under different nutrient concentrations. Also, the importance of the substratum base roughness for three dimensional substrata was examined.

Results showed that three dimensional substratum proved to be an effective substratum for supporting algal attachment, colonization and total yield. Overall, the results and findings obtained from the experiments were very significant. This shows that three-dimensional substrata would positively impact the productivity of cultivated algae. Some features of three dimensional substrata may be responsible for the algal yield observed on the different levels of substratum treatments. Specifically, substratum features such as fiber density, fiber height and substratum base roughness were investigated. Fiber density was shown to have the greatest effect on algal biomass productivity, increasing productivity by over 300%. The effect of substratum base roughness was also shown to be significant, especially for two dimensional substrata, with rougher surfaces showing an increase of 180% over two dimensional with smooth surfaces. However, for three dimensional substrata, the effect of substratum roughness was less drastic, with algal productivity experiencing a modest 20% improvement. Unlike fiber density and substratum base roughness, fiber height was not a significant feature.

As suggested by the modified version of the Monod model presented by Ye et al [101], the maximum specific growth rate of algal communities growing on three dimensional substrata may vary depending on the features of the substratum as seen in the experiments where different substratum treatment levels show different algal biomass productivity under similar

environmental conditions. The interaction of these substratum features with limiting factors may also have an influence on the maximum specific growth rate of the algal communities. For example, algal biomass productivity increased as fiber density of three dimensional substrata was increased; however, this gain in productivity reaches a maximum and begins to decrease as the fiber density continues to increase. This behavior may be due to how the fiber density feature interacts with limiting factors (such as light intensity, flow dynamics and nutrient concentrations) that impact algal biomass productivity. Investigation of three dimensional substrata under different nutrient concentrations showed that three dimensional substrata significantly increases algal biomass productivity, especially under low nutrient conditions. This observation suggests that the use of three dimensional substrata significantly boosts algal biomass productivity, irrespective of the nutrient concentration and may be prove to be a boost in applications such as waste water remediation.

It is clear that three dimensional substrata have a lot of potential for improving algal biomass productivity in cultivation system. Further exploration of three dimensional substrata is warranted to investigate other features of three dimensional substrata, such as fiber diameter and fiber arrangement, to uncover how they may influence productivity. In addition, models could be developed to incorporate three dimensional substratum features to help predict algal biomass growth dynamics under different three dimensional substrata characteristics.

An investigation into the relationship between three-dimensional substrata and light intensity would be an interesting study. How does the light intensity level change with respect to time as water flows through the bioreactor system? How does this dynamic behavior change as the algae's growth rate changes over time? Would this dynamic behavior influence the interval between

harvests? Is there an optimal harvest interval for different levels of fiber density? Finding answers to these questions would contribute to the understanding of algal growth dynamics.

Also, understanding the effect of three-dimensional substratum color on light intensity level would be helpful. In this study, both black and white substrata were used. Which color would best support algal productivity? Does the color even make a difference for three-dimensional substratum? Additionally, comparisons between two-dimensional and three-dimensional substratum would be more accurate if both substrata are of the same color.

Finally, a comprehensive economic analyses that highlights the cost saving potentials of three-dimensional substratum would be helpful. Economic analyses could include the improvements in productivity as a result of using three-dimensional substratum and the costs savings generated from reduction in material requirements.

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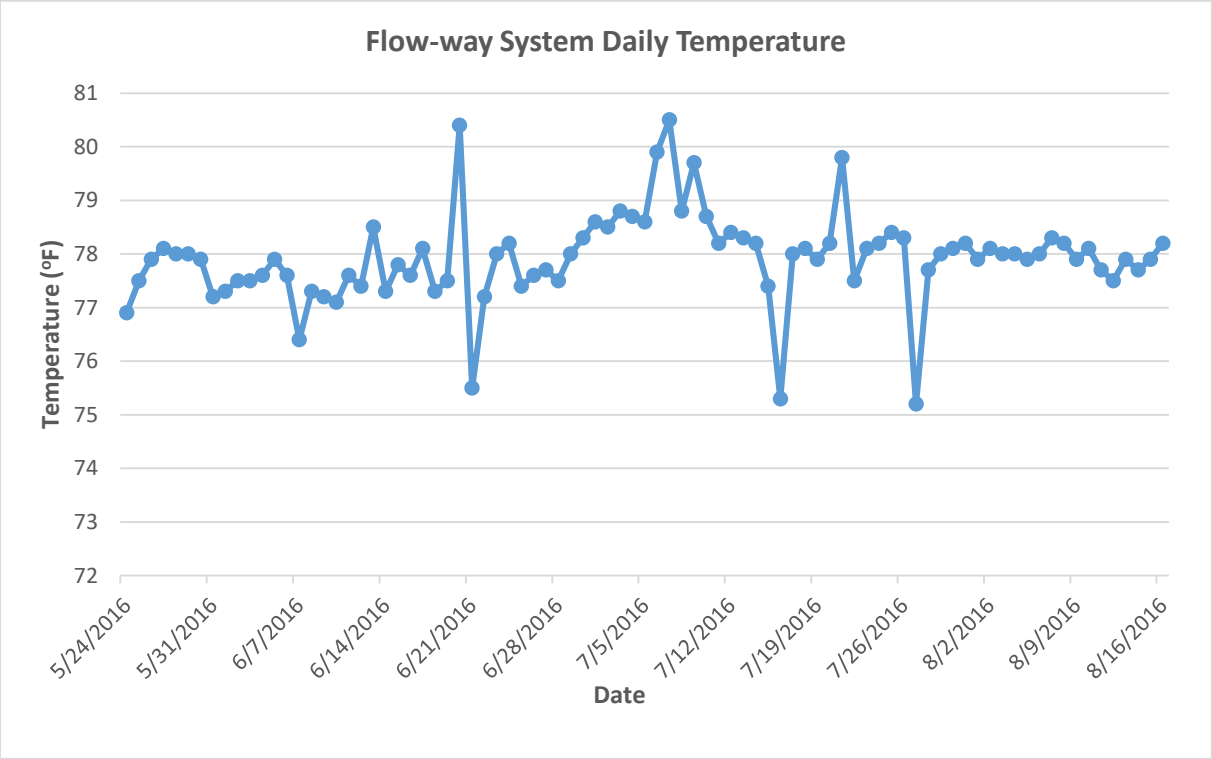
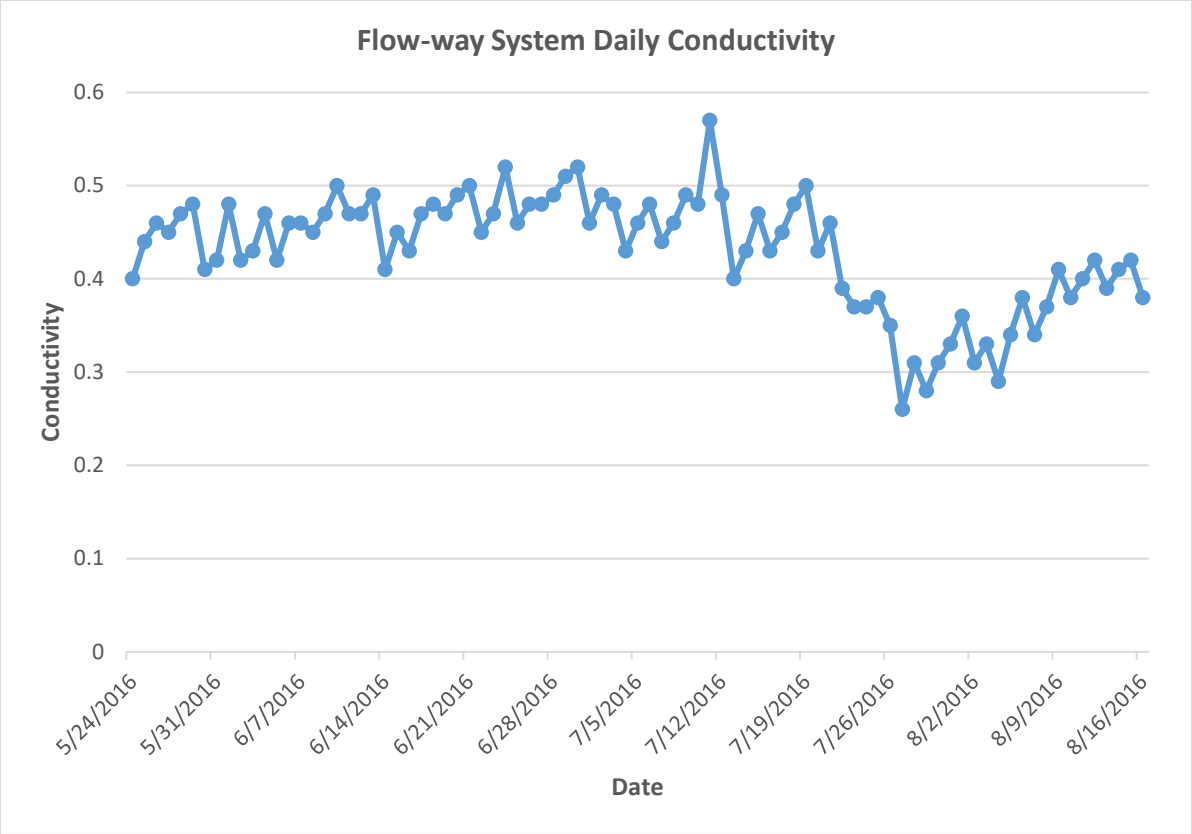
Appendix I: Log data from bioreactor (Experiment 1)

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water addition (g)	Nutrient addition (ml)	Comments
5/24/2016	8.85	76.90	0.40	24.00	0.00	24.00	
5/25/2016	8.48	77.50	0.44	22.00	2.00	2.00	
5/26/2016	8.56	77.90	0.46	20.50	3.50	3.50	
5/27/2016	8.55	78.10	0.45	21.00	3.00	3.00	
5/28/2016	8.72	78.00	0.47	21.00	3.00	3.00	
5/29/2016	8.89	78.00	0.48	22.00	2.00	2.00	
5/30/2016	8.64	77.90	0.41	21.00	3.00	3.00	
5/31/2016	8.79	77.20	0.42	21.00	3.00	3.00	Harvest 1
6/1/2016	8.63	77.30	0.48	21.50	2.50	2.50	
6/2/2016	8.43	77.50	0.42	20.00	4.00	4.00	
6/3/2016	8.54	77.50	0.43	21.00	3.00	3.00	
6/4/2016	8.75	77.60	0.47	21.00	3.00	3.00	
6/5/2016	8.63	77.90	0.42	22.50	1.50	1.50	
6/6/2016	8.73	77.60	0.46	21.00	3.00	3.00	
6/7/2016	8.78	76.40	0.46	21.00	3.00	3.00	Harvest 2
6/8/2016	8.41	77.30	0.45	21.00	3.00	3.00	
6/9/2016	8.46	77.20	0.47	22.00	2.00	2.00	
6/10/2016	8.55	77.10	0.50	20.50	3.50	3.50	
6/11/2016	8.41	77.60	0.47	21.00	3.00	3.00	
6/12/2016	8.66	77.40	0.47	21.00	3.00	3.00	
6/13/2016	8.84	78.50	0.49	21.00	3.00	3.00	
6/14/2016	8.85	77.30	0.41	21.50	2.50	2.50	Harvest 3
6/15/2016	8.52	77.80	0.45	20.00	4.00	4.00	
6/16/2016	8.49	77.60	0.43	21.00	3.00	3.00	
6/17/2016	8.57	78.10	0.47	21.00	3.00	3.00	
6/18/2016	8.65	77.30	0.48	21.50	2.50	2.50	
6/19/2016	8.58	77.50	0.47	21.00	3.00	3.00	
6/20/2016	8.72	80.40	0.49	22.00	2.00	2.00	
6/21/2016	8.41	75.50	0.50	21.00	3.00	3.00	Harvest 4
6/22/2016	8.32	77.20	0.45	21.00	3.00	3.00	
6/23/2016	8.47	78.00	0.47	22.50	1.50	1.50	
6/24/2016	8.59	78.20	0.52	21.00	3.00	3.00	
6/25/2016	8.46	77.40	0.46	21.00	3.00	3.00	
6/26/2016	8.54	77.60	0.48	20.00	4.00	4.00	
6/27/2016	8.65	77.70	0.48	22.50	1.50	1.50	
6/28/2016	8.81	77.50	0.49	22.00	2.00	2.00	Harvest 5
6/29/2016	8.45	78.00	0.51	22.00	2.00	2.00	
6/30/2016	8.52	78.30	0.52	22.00	2.00	2.00	

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water addition (g)	Nutrient addition (ml)	Comments
7/1/2016	8.39	78.60	0.46	21.00	3.00	3.00	
7/2/2016	8.42	78.50	0.49	21.50	2.50	2.50	
7/3/2016	8.59	78.80	0.48	21.00	3.00	3.00	
7/4/2016	8.41	78.70	0.43	21.00	3.00	3.00	
7/5/2016	8.67	78.60	0.46	22.50	1.50	1.50	Harvest 6
7/6/2016	8.39	79.90	0.48	22.00	2.00	2.00	
7/7/2016	8.25	80.50	0.44	21.00	3.00	3.00	
7/8/2016	8.41	78.80	0.46	22.50	1.50	1.50	
7/9/2016	8.39	79.70	0.49	22.00	2.00	2.00	
7/10/2016	8.46	78.70	0.48	22.00	2.00	2.00	
7/11/2016	8.64	78.20	0.57	20.00	4.00	4.00	
7/12/2016	8.67	78.40	0.49	21.50	2.50	2.50	Harvest 7
7/13/2016	8.35	78.30	0.40	21.00	3.00	3.00	
7/14/2016	8.18	78.20	0.43	21.50	2.50	2.50	
7/15/2016	8.30	77.40	0.47	21.00	3.00	3.00	
7/16/2016	8.21	75.30	0.43	21.00	3.00	3.00	
7/17/2016	8.37	78.00	0.45	21.00	3.00	3.00	
7/18/2016	8.51	78.10	0.48	21.50	2.50	2.50	
7/19/2016	8.79	77.90	0.50	21.50	2.50	2.50	Harvest 8
7/20/2016	8.32	78.20	0.43	22.00	2.00	2.00	
7/21/2016	8.35	79.80	0.46	21.00	3.00	3.00	
7/22/2016	8.41	77.50	0.39	22.00	2.00	2.00	
7/23/2016	8.27	78.10	0.37	21.50	2.50	2.50	
7/24/2016	8.31	78.20	0.37	21.00	3.00	3.00	
7/25/2016	8.26	78.40	0.38	21.50	2.50	2.50	
7/26/2016	8.29	78.30	0.35	22.00	2.00	2.00	Harvest 9
7/27/2016	7.97	75.20	0.26	21.00	3.00	3.00	
7/28/2016	8.27	77.70	0.31	21.50	2.50	2.50	
7/29/2016	8.25	78.00	0.28	21.00	3.00	3.00	
7/30/2016	8.36	78.10	0.31	22.00	2.00	2.00	
7/31/2016	8.41	78.20	0.33	23.00	1.00	1.00	

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water addition (g)	Nutrient addition (ml)	Comments
8/1/2016	8.44	77.90	0.36	22.00	2.00	2.00	
8/2/2016	8.27	78.10	0.31	22.50	1.50	1.50	Harvest 10
8/3/2016	8.13	78.00	0.33	20.00	4.00	4.00	
8/4/2016	7.89	78.00	0.29	21.00	3.00	3.00	
8/5/2016	7.97	77.90	0.34	21.00	3.00	3.00	
8/6/2016	8.34	78.00	0.38	21.00	3.00	3.00	
8/7/2016	8.37	78.30	0.34	21.50	2.50	2.50	
8/8/2016	8.35	78.20	0.37	22.00	2.00	2.00	
8/9/2016	8.42	77.90	0.41	21.00	3.00	3.00	Harvest 11
8/10/2016	8.19	78.10	0.38	21.00	3.00	3.00	
8/11/2016	8.31	77.70	0.40	21.50	2.50	2.50	
8/12/2016	8.27	77.50	0.42	23.00	1.00	1.00	
8/13/2016	8.13	77.90	0.39	21.00	3.00	3.00	
8/14/2016	8.38	77.70	0.41	21.50	2.50	2.50	
8/15/2016	8.55	77.90	0.42	22.00	2.00	2.00	
8/16/2016	8.27	78.20	0.38	21.00	3.00	3.00	Harvest 12

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5/24/2016
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6/14/2016
6/21/2016
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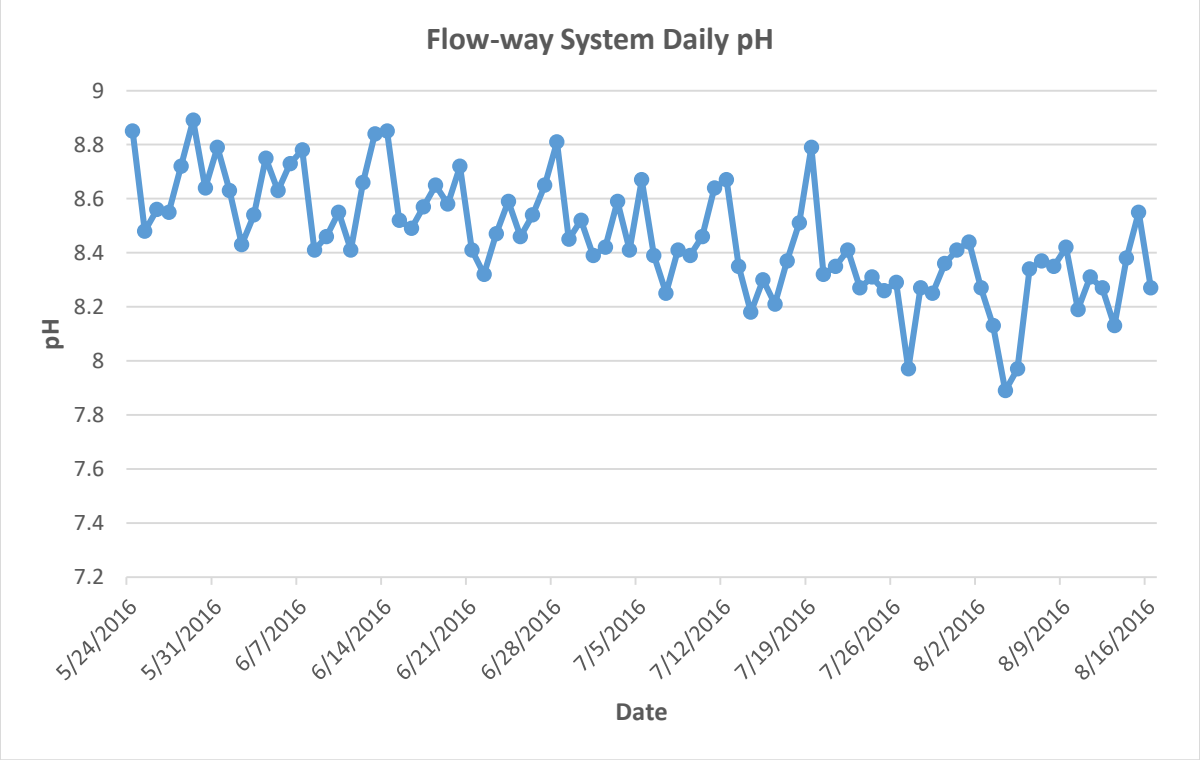
7/12/2016
7/19/2016
7/26/2016
8/2/2016
8/9/2016
8/16/2016
Date


Flow-way System Daily pH
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8.4
8.2
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7.6
7.4
7.2
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5/31/2016
6/7/2016
6/14/2016
6/21/2016
6/28/2016
7/5/2016
7/12/2016
7/19/2016
7/26/2016
8/2/2016
8/9/2016
8/16/2016
Date

Appendix II: Raw data of all harvests (Experiment 1)

Harvests	Substratum Treatment Level	Weight Before Drying (g)	Weight After Drying (g)	Total Biomass (g)	Daily Biomass (mg cm^{-2} d^{-1})
1	Treatment A	5.994	9.353	3.359	0.207
	Treatment B	5.783	12.179	6.396	0.393
	Treatment C	5.914	12.832	6.918	0.426
	Treatment D	5.985	11.597	5.612	0.345
2	Treatment A	6.002	9.951	3.949	0.243
	Treatment B	6.001	11.892	5.891	0.362
	Treatment C	6.072	13.366	7.294	0.449
	Treatment D	5.900	11.934	6.034	0.371
3	Treatment A	5.028	9.721	4.693	0.289
	Treatment B	4.965	11.747	6.782	0.417
	Treatment C	5.132	13.925	8.793	0.541
	Treatment D	4.976	10.216	5.240	0.322
4	Treatment A	5.753	8.632	2.879	0.177
	Treatment B	5.759	10.855	5.096	0.313
	Treatment C	5.923	13.087	7.164	0.441
	Treatment D	5.894	12.540	6.646	0.409
5	Treatment A	5.844	8.974	3.130	0.193
	Treatment B	5.891	11.643	5.752	0.354
	Treatment C	5.907	10.973	5.066	0.312
	Treatment D	5.879	12.832	6.953	0.428
6	Treatment A	5.602	7.008	1.406	0.086
	Treatment B	5.665	12.943	7.278	0.448
	Treatment C	5.633	14.195	8.562	0.527
	Treatment D	5.635	10.351	4.716	0.290
7	Treatment A	5.344	9.667	4.323	0.266
	Treatment B	5.336	11.552	6.216	0.382
	Treatment C	5.38	12.398	7.018	0.432
	Treatment D	5.428	10.321	4.893	0.301
8	Treatment A	5.293	9.042	3.749	0.231
	Treatment B	5.304	9.891	4.587	0.282
	Treatment C	5.316	10.649	5.333	0.328
	Treatment D	5.424	10.129	4.705	0.289

Harvests	Substratum Treatment Level	Weight Before Drying (g)	Weight After Drying (g)	Total Biomass (g)	Daily Biomass (mg cm^{-2} d^{-1})
9	Treatment A	5.303	7.186	1.883	0.116
	Treatment B	5.344	10.473	5.129	0.315
	Treatment C	5.361	10.324	4.963	0.305
	Treatment D	5.316	9.44	4.124	0.254
10	Treatment A	5.295	7.882	2.587	0.159
	Treatment B	5.365	9.443	4.078	0.251
	Treatment C	5.345	10.611	5.266	0.324
	Treatment D	5.334	8.748	3.414	0.210
11	Treatment A	5.702	8.24	2.538	0.156
	Treatment B	5.675	9.887	4.212	0.259
	Treatment C	5.722	11.321	5.599	0.344
	Treatment D	5.746	9.834	4.088	0.251
12	Treatment A	5.94	8.799	2.859	0.176
	Treatment B	5.956	11.138	5.182	0.319
	Treatment C	5.931	11.853	5.922	0.364
	Treatment D	5.871	10.656	4.785	0.294

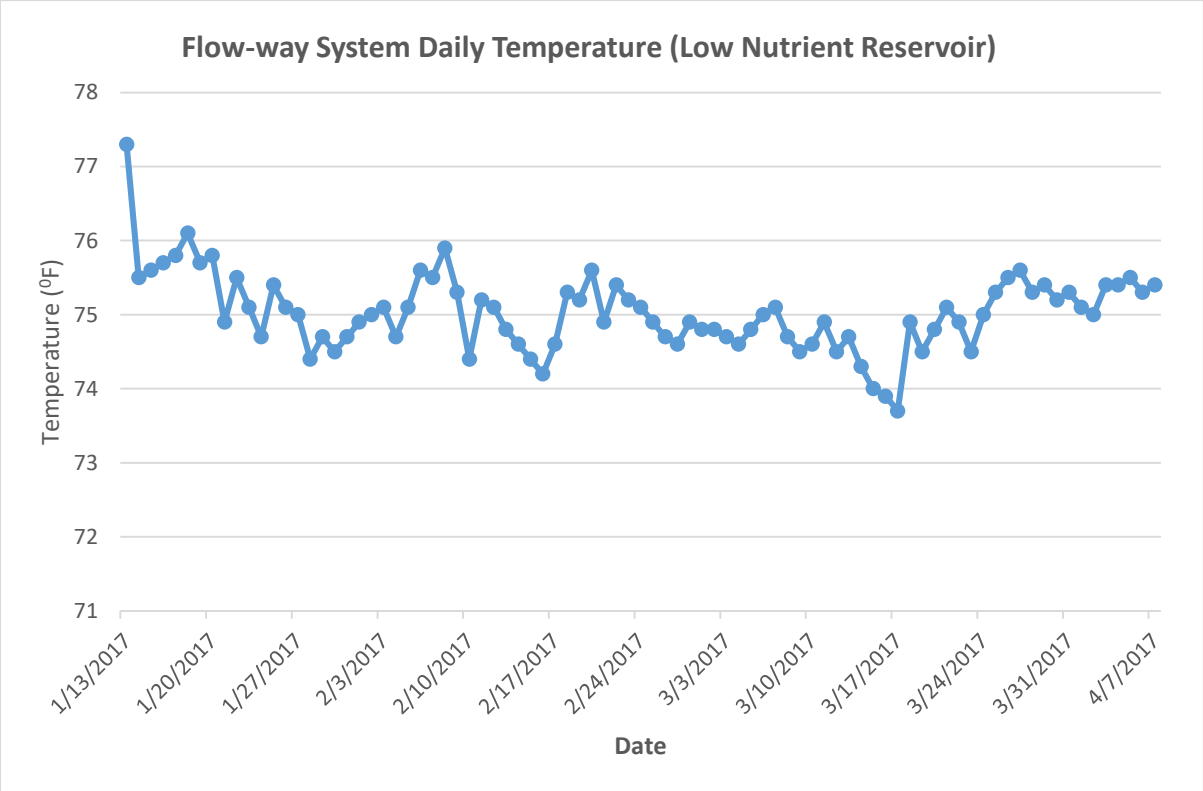
Appendix III: Log data from bioreactor (Experiment 2)

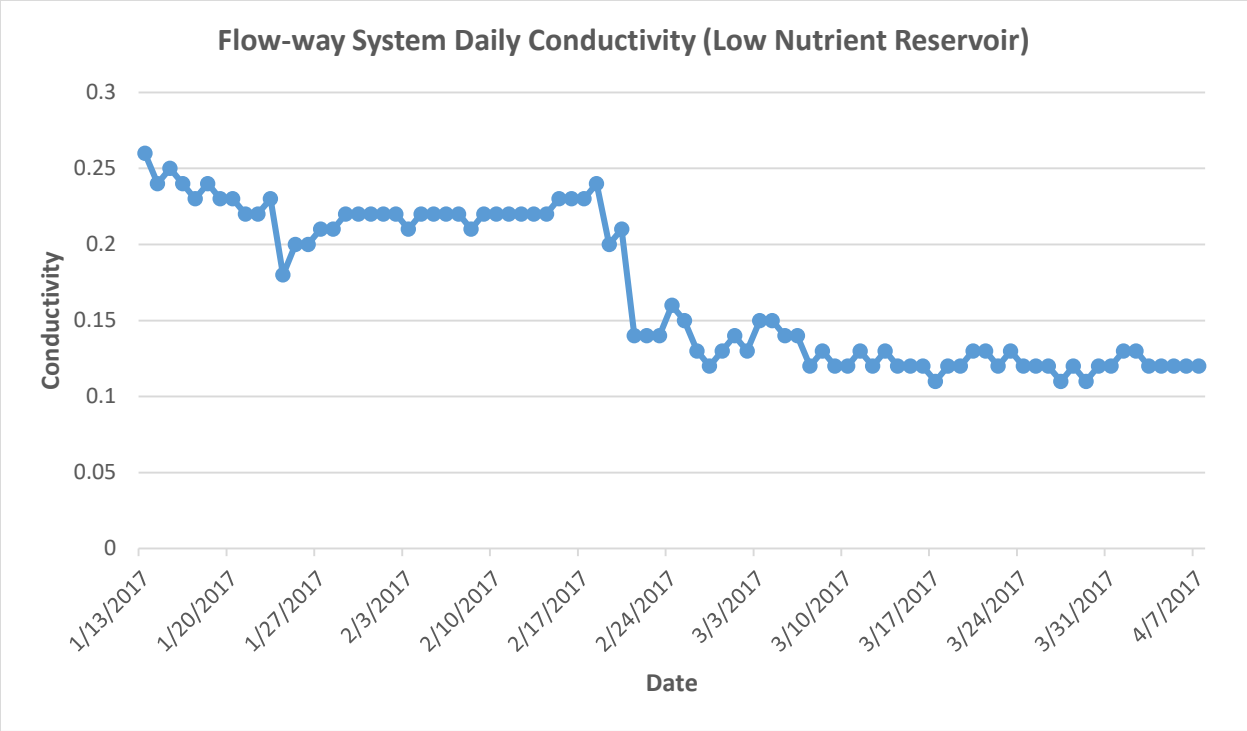
Low Nutrient Treatment

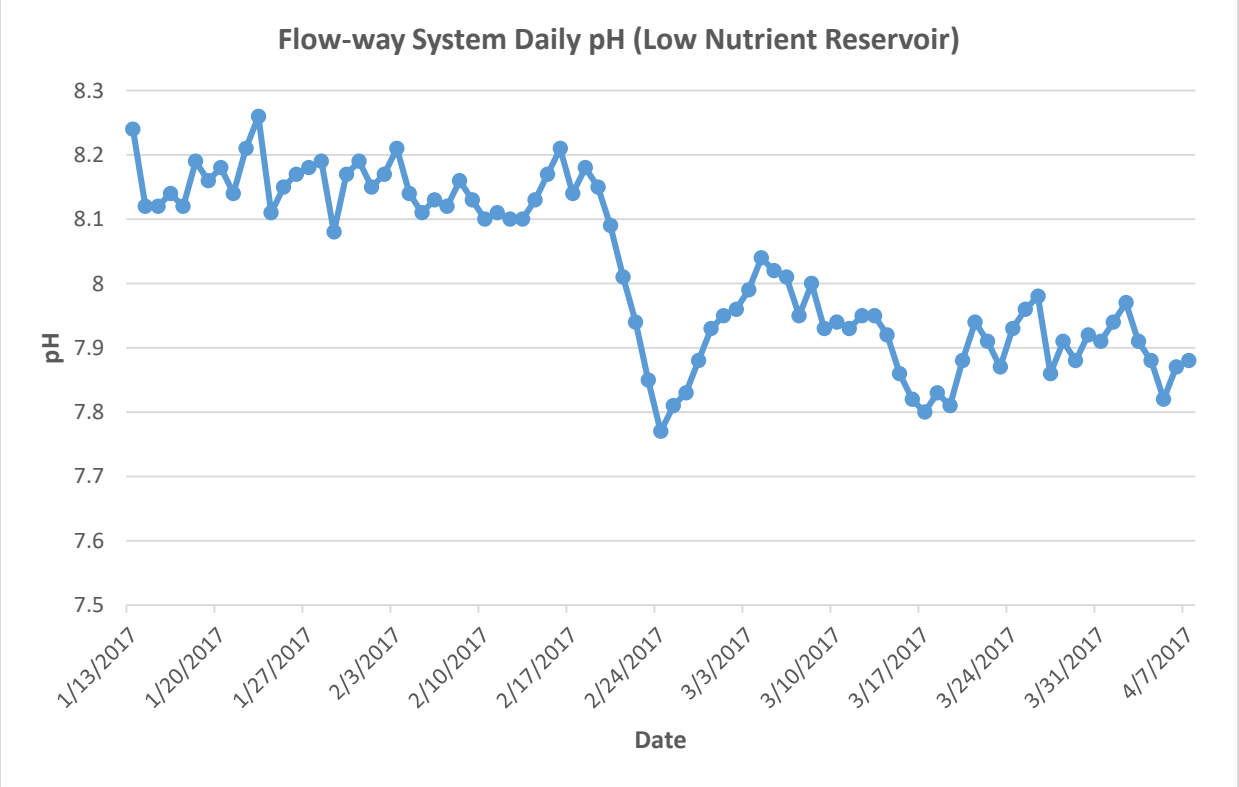
Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
1/14/2017	8.12	75.50	0.24	10.00	0.00	0.00	1.00	
1/15/2017	8.12	75.60	0.25	8.00	2.00	5.00	0.50	
1/16/2017	8.14	75.70	0.24	9.00	1.00	5.00	0.50	
1/17/2017	8.12	75.80	0.23	9.00	1.00	5.00	0.50	
1/18/2017	8.19	76.10	0.24	8.00	2.00	5.00	0.50	
1/19/2017	8.16	75.70	0.23	8.00	2.00	5.00	0.50	
1/20/2017	8.18	75.80	0.23	8.00	2.00	5.00	0.50	Harvest 1
1/21/2017	8.14	74.90	0.22	8.00	2.00	5.00	0.50	
1/22/2017	8.21	75.50	0.22	7.00	3.00	5.00	0.50	
1/23/2017	8.26	75.10	0.23	8.00	2.00	5.00	0.50	
1/24/2017	8.11	74.70	0.18	8.00	2.00	5.00	0.50	
1/25/2017	8.15	75.40	0.20	8.00	2.00	5.00	0.50	
1/26/2017	8.17	75.10	0.20	9.00	1.00	5.00	0.50	
1/27/2017	8.18	75.00	0.21	9.00	1.00	5.00	0.50	Harvest 2
1/28/2017	8.19	74.40	0.21	8.00	2.00	5.00	0.50	
1/29/2017	8.08	74.70	0.22	8.00	2.00	5.00	0.50	
1/30/2017	8.17	74.50	0.22	8.00	2.00	5.00	0.50	
1/31/2017	8.19	74.70	0.22	8.00	2.00	5.00	0.50	
2/1/2017	8.15	74.90	0.22	8.00	2.00	5.00	0.50	
2/2/2017	8.17	75.00	0.22	8.00	2.00	5.00	0.50	
2/3/2017	8.21	75.10	0.21	9.00	1.00	5.00	0.50	Harvest 3
2/4/2017	8.14	74.70	0.22	9.00	1.00	5.00	0.50	
2/5/2017	8.11	75.10	0.22	9.00	1.00	5.00	0.50	
2/6/2017	8.13	75.60	0.22	8.00	2.00	5.00	0.50	
2/7/2017	8.12	75.50	0.22	8.00	2.00	5.00	0.50	
2/8/2017	8.16	75.90	0.21	8.00	2.00	5.00	0.50	
2/9/2017	8.13	75.30	0.22	8.00	2.00	5.00	0.50	
2/10/2017	8.10	74.40	0.22	8.00	2.00	5.00	0.50	Harvest 4
2/11/2017	8.11	75.20	0.22	8.00	2.00	5.00	0.50	
2/12/2017	8.10	75.10	0.22	8.00	2.00	5.00	0.50	
2/13/2017	8.10	74.80	0.22	8.00	2.00	5.00	0.50	
2/14/2017	8.13	74.60	0.22	9.00	1.00	5.00	0.50	
2/15/2017	8.17	74.40	0.23	8.00	2.00	5.00	0.50	
2/16/2017	8.21	74.20	0.23	8.00	2.00	5.00	0.50	
2/17/2017	8.14	74.60	0.23	8.00	2.00	5.00	0.50	Harvest 5

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
2/18/2017	8.18	75.30	0.24	8.00	2.00	5.00	0.50	
2/19/2017	8.15	75.20	0.20	8.00	2.00	5.00	0.50	
2/20/2017	8.09	75.60	0.21	9.00	1.00	5.00	0.50	
2/21/2017	8.01	74.90	0.14	8.00	2.00	5.00	0.50	
2/22/2017	7.94	75.40	0.14	8.00	2.00	5.00	0.50	
2/23/2017	7.85	75.20	0.14	8.00	2.00	5.00	0.50	
2/24/2017	7.77	75.10	0.16	9.00	1.00	5.00	0.50	Harvest 6
2/25/2017	7.81	74.90	0.15	8.00	2.00	5.00	0.50	
2/26/2017	7.83	74.70	0.13	8.00	2.00	5.00	0.50	
2/27/2017	7.88	74.60	0.12	8.00	2.00	5.00	0.50	
2/28/2017	7.93	74.90	0.13	8.00	2.00	5.00	0.50	
3/1/2017	7.95	74.80	0.14	8.00	2.00	5.00	0.50	
3/2/2017	7.96	74.80	0.13	9.00	1.00	5.00	0.50	
3/3/2017	7.99	74.70	0.15	8.00	2.00	5.00	0.50	Harvest 7
3/4/2017	8.04	74.60	0.15	8.00	2.00	5.00	0.50	
3/5/2017	8.02	74.80	0.14	8.00	2.00	5.00	0.50	
3/6/2017	8.01	75.00	0.14	8.00	2.00	5.00	0.50	
3/7/2017	7.95	75.10	0.12	7.00	3.00	5.00	0.50	
3/8/2017	8.00	74.70	0.13	7.00	3.00	5.00	0.50	
3/9/2017	7.93	74.50	0.12	8.00	2.00	5.00	0.50	
3/10/2017	7.94	74.60	0.12	8.00	2.00	5.00	0.50	Harvest 8
3/11/2017	7.93	74.90	0.13	8.00	2.00	5.00	0.50	
3/12/2017	7.95	74.50	0.12	8.00	2.00	5.00	0.50	
3/13/2017	7.95	74.70	0.13	8.00	2.00	5.00	0.50	
3/14/2017	7.92	74.30	0.12	8.00	2.00	5.00	0.50	
3/15/2017	7.86	74.00	0.12	8.00	2.00	5.00	0.50	
3/16/2017	7.82	73.90	0.12	8.00	2.00	5.00	0.50	
3/17/2017	7.80	73.70	0.11	7.00	3.00	5.00	0.50	Harvest 9
3/18/2017	7.83	74.90	0.12	8.00	2.00	5.00	0.50	
3/19/2017	7.81	74.50	0.12	7.00	3.00	5.00	0.50	
3/20/2017	7.88	74.80	0.13	8.00	2.00	5.00	0.50	
3/21/2017	7.94	75.10	0.13	7.00	3.00	5.00	0.50	
3/22/2017	7.91	74.90	0.12	7.00	3.00	5.00	0.50	
3/23/2017	7.87	74.50	0.13	8.00	2.00	5.00	0.50	
3/24/2017	7.93	75.00	0.12	8.00	2.00	5.00	0.50	Harvest 10

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
3/25/2017	7.96	75.30	0.12	8.00	2.00	5.00	0.50	
3/26/2017	7.98	75.50	0.12	7.00	3.00	5.00	0.50	
3/27/2017	7.86	75.60	0.11	7.00	3.00	5.00	0.50	
3/28/2017	7.91	75.30	0.12	7.00	3.00	5.00	0.50	
3/29/2017	7.88	75.40	0.11	8.00	2.00	5.00	0.50	
3/30/2017	7.92	75.20	0.12	8.00	2.00	5.00	0.50	
3/31/2017	7.91	75.30	0.12	8.00	2.00	5.00	0.50	Harvest 11
4/1/2017	7.94	75.10	0.13	7.00	3.00	5.00	0.50	
4/2/2017	7.97	75.00	0.13	8.00	2.00	5.00	0.50	
4/3/2017	7.91	75.40	0.12	8.00	2.00	5.00	0.50	
4/4/2017	7.88	75.40	0.12	7.00	3.00	5.00	0.50	
4/5/2017	7.82	75.50	0.12	8.00	2.00	5.00	0.50	
4/6/2017	7.87	75.30	0.12	8.00	2.00	5.00	0.50	
4/7/2017	7.88	75.40	0.12	8.00	2.00	5.00	0.50	Harvest 12




Flow-way System Daily Conductivity (Low Nutrient Reservoir)
Conductivity
0.3
0.25
0.2
0.15
0.1
0.05
0
1/13/2017
1/20/2017
1/27/2017
2/3/2017
2/10/2017
2/17/2017
2/24/2017
3/3/2017
3/10/2017
3/17/2017
3/24/2017
3/31/2017
4/7/2017
Date

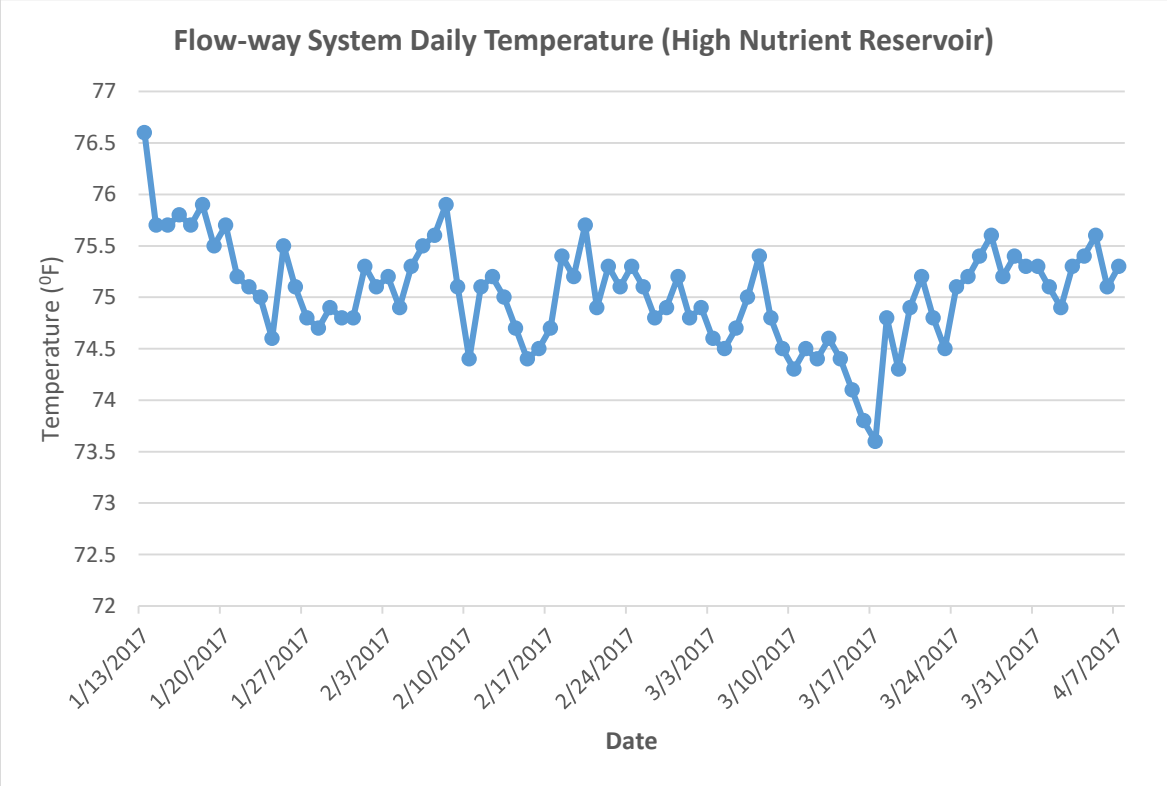

Flow-way System Daily pH (Low Nutrient Reservoir)
pH
8.3
8.2
8.1
8
7.9
7.8
7.7
7.6
7.5
1/13/2017
1/20/2017
1/27/2017
2/3/2017
2/10/2017
2/17/2017
2/24/2017
3/3/2017
3/10/2017
3/17/2017
3/24/2017
3/31/2017
4/7/2017
Date

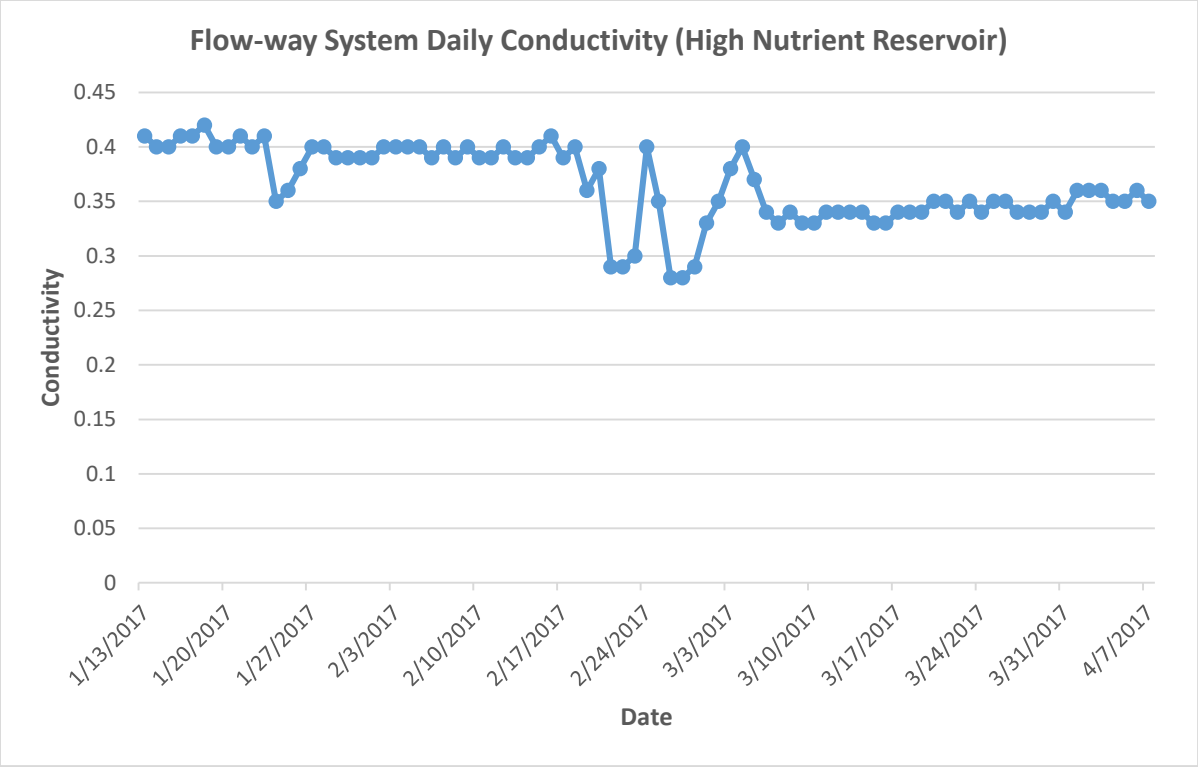
High Nutrient Treatment

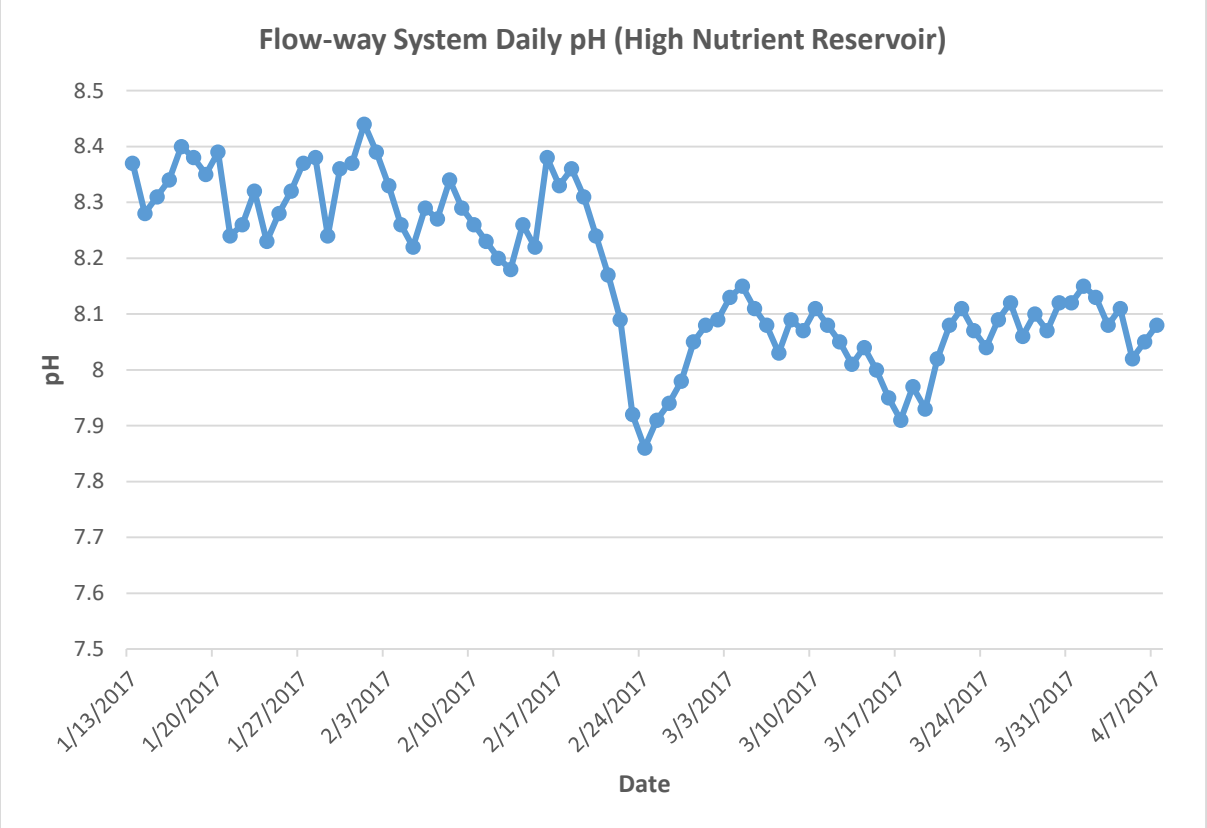
Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
1/14/2017	8.28	75.70	0.41	10.00	0.00	0.00	10.00	
1/15/2017	8.31	75.70	0.40	8.00	2.00	5.00	5.00	
1/16/2017	8.34	75.80	0.40	9.00	1.00	5.00	5.00	
1/17/2017	8.40	75.70	0.41	8.00	2.00	5.00	5.00	
1/18/2017	8.38	75.90	0.41	8.00	2.00	5.00	5.00	
1/19/2017	8.35	75.50	0.42	8.00	2.00	5.00	5.00	
1/20/2017	8.39	75.70	0.40	9.00	1.00	5.00	5.00	Harvest 1
1/21/2017	8.24	75.20	0.40	8.00	2.00	5.00	5.00	
1/22/2017	8.26	75.10	0.41	8.00	2.00	5.00	5.00	
1/23/2017	8.32	75.00	0.40	8.00	2.00	5.00	5.00	
1/24/2017	8.23	74.60	0.41	8.00	2.00	5.00	5.00	
1/25/2017	8.28	75.50	0.35	7.00	3.00	5.00	5.00	
1/26/2017	8.32	75.10	0.36	9.00	1.00	5.00	5.00	
1/27/2017	8.37	74.80	0.38	8.00	2.00	5.00	5.00	Harvest 2
1/28/2017	8.38	74.70	0.40	8.00	2.00	5.00	5.00	
1/29/2017	8.24	74.90	0.40	8.00	2.00	5.00	5.00	
1/30/2017	8.36	74.80	0.39	7.00	3.00	5.00	5.00	
1/31/2017	8.37	74.80	0.39	8.00	2.00	5.00	5.00	
2/1/2017	8.44	75.30	0.39	8.00	2.00	5.00	5.00	
2/2/2017	8.39	75.10	0.39	8.00	2.00	5.00	5.00	
2/3/2017	8.33	75.20	0.40	8.00	2.00	5.00	5.00	Harvest 3
2/4/2017	8.26	74.90	0.40	9.00	1.00	5.00	5.00	
2/5/2017	8.22	75.30	0.40	9.00	1.00	5.00	5.00	
2/6/2017	8.29	75.50	0.40	8.00	2.00	5.00	5.00	
2/7/2017	8.27	75.60	0.39	8.00	2.00	5.00	5.00	
2/8/2017	8.34	75.90	0.40	8.00	2.00	5.00	5.00	
2/9/2017	8.29	75.10	0.39	9.00	1.00	5.00	5.00	
2/10/2017	8.26	74.40	0.40	8.00	2.00	5.00	5.00	Harvest 4
2/11/2017	8.23	75.10	0.39	8.00	2.00	5.00	5.00	
2/12/2017	8.20	75.20	0.39	8.00	2.00	5.00	5.00	
2/13/2017	8.18	75.00	0.40	7.00	3.00	5.00	5.00	
2/14/2017	8.26	74.70	0.39	9.00	1.00	5.00	5.00	
2/15/2017	8.22	74.40	0.39	9.00	1.00	5.00	5.00	
2/16/2017	8.38	74.50	0.40	8.00	2.00	5.00	5.00	
2/17/2017	8.33	74.70	0.41	8.00	2.00	5.00	5.00	Harvest 5

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
2/18/2017	8.36	75.40	0.39	8.00	2.00	5.00	5.00	
2/19/2017	8.31	75.20	0.40	9.00	1.00	5.00	5.00	
2/20/2017	8.24	75.70	0.36	8.00	2.00	5.00	5.00	
2/21/2017	8.17	74.90	0.38	8.00	2.00	5.00	5.00	
2/22/2017	8.09	75.30	0.29	8.00	2.00	5.00	5.00	
2/23/2017	7.92	75.10	0.29	8.00	2.00	5.00	5.00	
2/24/2017	7.86	75.30	0.30	7.00	3.00	5.00	5.00	Harvest 6
2/25/2017	7.91	75.10	0.40	9.00	1.00	5.00	5.00	
2/26/2017	7.94	74.80	0.35	8.00	2.00	5.00	5.00	
2/27/2017	7.98	74.90	0.28	8.00	2.00	5.00	5.00	
2/28/2017	8.05	75.20	0.28	8.00	2.00	5.00	5.00	
3/1/2017	8.08	74.80	0.29	7.00	3.00	5.00	5.00	
3/2/2017	8.09	74.90	0.33	8.00	2.00	5.00	5.00	
3/3/2017	8.13	74.60	0.35	8.00	2.00	5.00	5.00	Harvest 7
3/4/2017	8.15	74.50	0.38	8.00	2.00	5.00	5.00	
3/5/2017	8.11	74.70	0.40	8.00	2.00	5.00	5.00	
3/6/2017	8.08	75.00	0.37	7.00	3.00	5.00	5.00	
3/7/2017	8.03	75.40	0.34	8.00	2.00	5.00	5.00	
3/8/2017	8.09	74.80	0.33	8.00	2.00	5.00	5.00	
3/9/2017	8.07	74.50	0.34	8.00	2.00	5.00	5.00	
3/10/2017	8.11	74.30	0.33	8.00	2.00	5.00	5.00	Harvest 8
3/11/2017	8.08	74.50	0.33	9.00	1.00	5.00	5.00	
3/12/2017	8.05	74.40	0.34	9.00	1.00	5.00	5.00	
3/13/2017	8.01	74.60	0.34	8.00	2.00	5.00	5.00	
3/14/2017	8.04	74.40	0.34	8.00	2.00	5.00	5.00	
3/15/2017	8.00	74.10	0.34	8.00	2.00	5.00	5.00	
3/16/2017	7.95	73.80	0.33	9.00	1.00	5.00	5.00	
3/17/2017	7.91	73.60	0.33	8.00	2.00	5.00	5.00	Harvest 9
3/18/2017	7.97	74.80	0.34	8.00	2.00	5.00	5.00	
3/19/2017	7.93	74.30	0.34	8.00	2.00	5.00	5.00	
3/20/2017	8.02	74.90	0.34	8.00	2.00	5.00	5.00	
3/21/2017	8.08	75.20	0.35	7.00	3.00	5.00	5.00	
3/22/2017	8.11	74.80	0.35	9.00	1.00	5.00	5.00	
3/23/2017	8.07	74.50	0.34	8.00	2.00	5.00	5.00	
3/24/2017	8.04	75.10	0.35	8.00	2.00	5.00	5.00	Harvest 10

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
3/25/2017	8.09	75.20	0.34	8.00	2.00	5.00	5.00	
3/26/2017	8.12	75.40	0.35	7.00	3.00	5.00	5.00	
3/27/2017	8.06	75.60	0.35	7.00	3.00	5.00	5.00	
3/28/2017	8.10	75.20	0.34	8.00	2.00	5.00	5.00	
3/29/2017	8.07	75.40	0.34	7.00	3.00	5.00	5.00	
3/30/2017	8.12	75.30	0.34	7.00	3.00	5.00	5.00	
3/31/2017	8.12	75.30	0.35	8.00	2.00	5.00	5.00	Harvest 11
4/1/2017	8.15	75.10	0.34	8.00	2.00	5.00	5.00	
4/2/2017	8.13	74.90	0.36	8.00	2.00	5.00	5.00	
4/3/2017	8.08	75.30	0.36	7.00	3.00	5.00	5.00	
4/4/2017	8.11	75.40	0.36	8.00	2.00	5.00	5.00	
4/5/2017	8.02	75.60	0.35	8.00	2.00	5.00	5.00	
4/6/2017	8.05	75.10	0.35	8.00	2.00	5.00	5.00	
4/7/2017	8.08	75.30	0.36	7.00	3.00	5.00	5.00	Harvest 12




Flow-way System Daily Conductivity (High Nutrient Reservoir)
Conductivity
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
1/13/2017
1/20/2017
1/27/2017
2/3/2017
2/10/2017
2/17/2017
2/24/2017
3/3/2017
3/10/2017
3/17/2017
3/24/2017
3/31/2017
4/7/2017
Date


Flow-way System Daily pH (High Nutrient Reservoir)
pH
8.5
8.4
8.3
8.2
8.1
8
7.9
7.8
7.7
7.6
7.5
1/13/2017
1/20/2017
1/27/2017
2/3/2017
2/10/2017
2/17/2017
2/24/2017
3/3/2017
3/10/2017
3/17/2017
3/24/2017
3/31/2017
4/7/2017
Date

Appendix IV: Raw data of all harvests (Experiment 2)

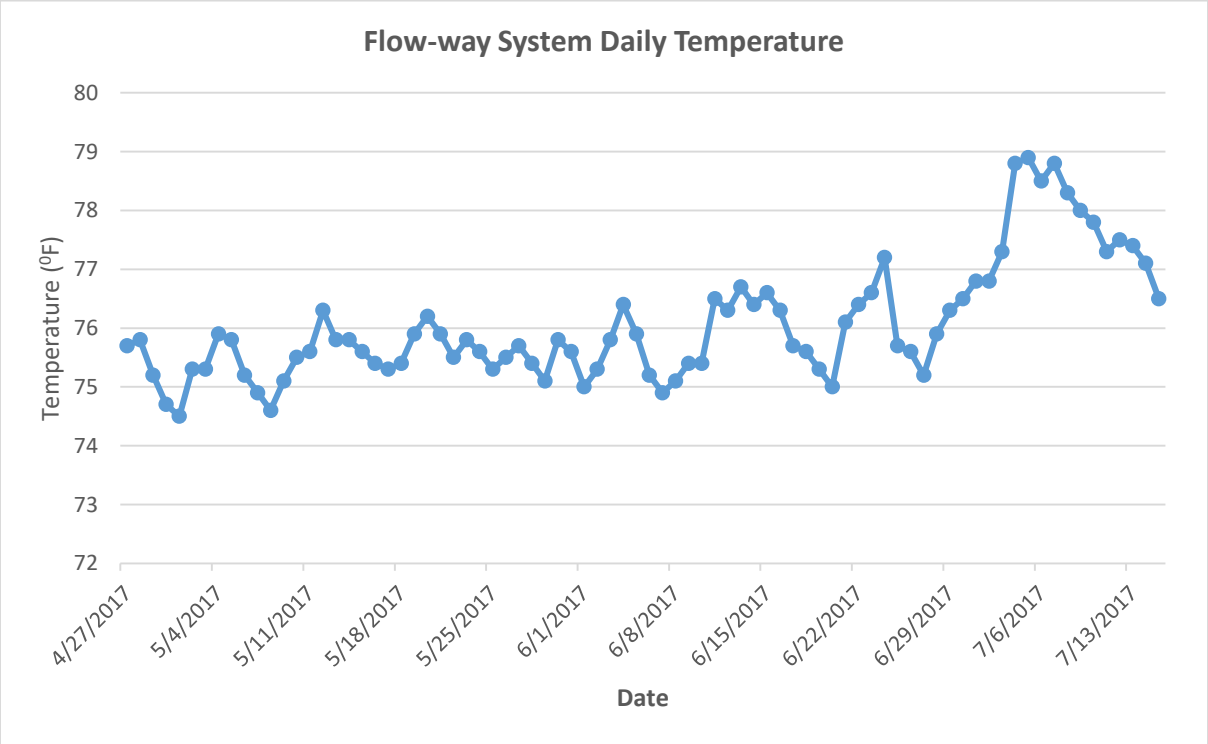
Harvests	Nutrient Treatment Level	Substratum Treatment Level	Weight Before Drying (g)	Weight After Drying (g)	Total Biomass 1 (g)	Total Biomass 1 (mg cm^{-2} d^{-1})	Total Biomass 2 (mg cm^{-2} d^{-1})	Daily Biomass (mg cm^{-2} d^{-1})
1	Low	2D	5.429	5.849	0.420	0.065	0.002	0.067
		3D	5.515	6.258	0.743	0.115	0.007	0.122
	High	2D	5.509	6.766	1.257	0.194	0.005	0.199
		3D	5.475	8.189	2.714	0.418	0.009	0.427
2	Low	2D	5.452	5.865	0.413	0.064	0.003	0.067
		3D	5.461	6.389	0.928	0.143	0.005	0.148
	High	2D	5.522	6.956	1.434	0.221	0.007	0.228
		3D	5.476	8.320	2.844	0.439	0.011	0.450
3	Low	2D	5.426	5.742	0.316	0.049	0.002	0.051
		3D	5.471	6.479	1.007	0.155	0.006	0.161
	High	2D	5.442	7.138	1.695	0.261	0.008	0.269
		3D	5.483	8.891	3.409	0.526	0.010	0.536
4	Low	2D	5.611	5.970	0.359	0.055	0.002	0.057
		3D	5.638	6.515	0.877	0.135	0.008	0.143
	High	2D	5.682	7.424	1.742	0.269	0.008	0.277
		3D	5.633	9.059	3.426	0.528	0.012	0.540
5	Low	2D	5.625	5.976	0.351	0.054	0.003	0.057
		3D	5.639	6.722	1.083	0.167	0.005	0.172
	High	2D	5.651	7.167	1.516	0.234	0.006	0.240
		3D	5.601	8.804	3.204	0.494	0.009	0.503
6	Low	2D	5.522	5.851	0.328	0.051	0.004	0.055
		3D	5.513	6.349	0.836	0.129	0.007	0.136
	High	2D	5.498	6.825	1.327	0.205	0.005	0.210
		3D	5.515	7.908	2.393	0.369	0.011	0.380
7	Low	2D	5.526	5.863	0.337	0.052	0.003	0.055
		3D	5.595	6.625	1.030	0.159	0.008	0.167
	High	2D	5.655	7.868	2.213	0.341	0.005	0.346
		3D	5.520	9.346	3.826	0.590	0.009	0.599
8	Low	2D	5.550	5.857	0.307	0.047	0.004	0.051
		3D	5.603	6.449	0.845	0.130	0.006	0.136
	High	2D	5.570	6.752	1.182	0.182	0.007	0.189
		3D	5.592	7.400	1.807	0.279	0.012	0.291
9	Low	2D	5.624	6.058	0.434	0.067	0.003	0.070
		3D	5.609	6.795	1.186	0.183	0.005	0.188
	High	2D	5.619	7.499	1.880	0.290	0.008	0.298
		3D	5.639	9.095	3.456	0.533	0.011	0.544
10	Low	2D	5.628	5.979	0.351	0.054	0.002	0.056
		3D	5.618	6.972	1.354	0.209	0.008	0.217
	High	2D	5.645	6.882	1.237	0.191	0.007	0.198
		3D	5.618	8.218	2.600	0.401	0.009	0.410
11	Low	2D	5.637	5.944	0.307	0.047	0.003	0.050
		3D	5.645	6.457	0.813	0.125	0.007	0.132
	High	2D	5.692	7.060	1.368	0.211	0.006	0.217
		3D	5.622	7.782	2.160	0.333	0.011	0.344
12	Low	2D	5.492	5.772	0.281	0.043	0.004	0.047
		3D	5.494	6.298	0.804	0.124	0.005	0.129
	High	2D	5.478	6.775	1.297	0.200	0.007	0.207
		3D	5.489	7.948	2.459	0.379	0.013	0.392

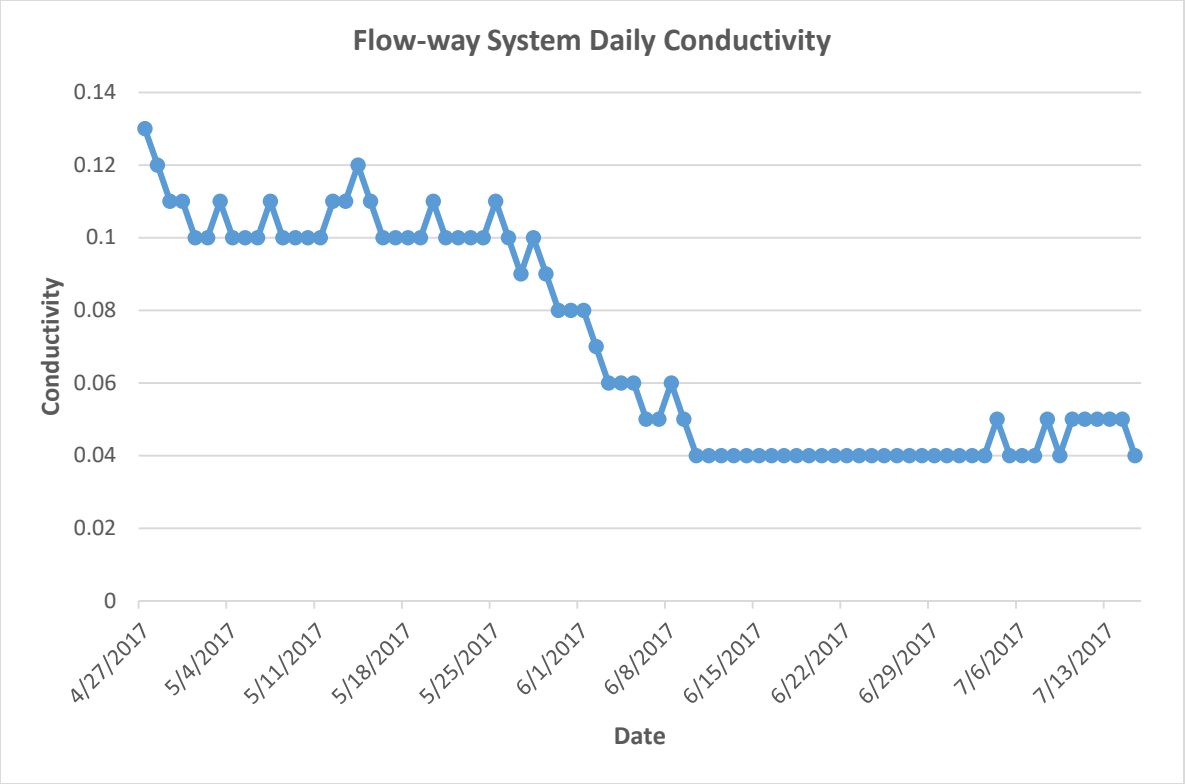
Appendix V: Log data from bioreactor (Experiment 3)

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
4/27/2017	7.65	75.70	0.13	12.00	3.00	5.00	0.50	
4/28/2017	7.65	75.80	0.12	13.00	2.00	5.00	0.50	
4/29/2017	7.63	75.20	0.11	13.00	2.00	5.00	0.50	
4/30/2017	7.64	74.70	0.11	13.00	2.00	5.00	0.50	
5/1/2017	7.58	74.50	0.10	13.00	2.00	5.00	0.50	Harvest 1
5/2/2017	7.62	75.30	0.10	13.00	2.00	5.00	0.50	
5/3/2017	7.68	75.30	0.11	12.00	3.00	5.00	0.50	
5/4/2017	7.71	75.90	0.10	12.00	3.00	5.00	0.50	
5/5/2017	7.74	75.80	0.10	13.00	2.00	5.00	0.50	
5/6/2017	7.78	75.20	0.10	13.00	2.00	5.00	0.50	Harvest 2
5/7/2017	7.72	74.90	0.11	13.00	2.00	5.00	0.50	
5/8/2017	7.67	74.60	0.10	13.00	2.00	5.00	0.50	
5/9/2017	7.61	75.10	0.10	13.00	2.00	5.00	0.50	
5/10/2017	7.63	75.50	0.10	12.00	3.00	5.00	0.50	
5/11/2017	7.58	75.60	0.10	13.00	2.00	5.00	0.50	Harvest 3
5/12/2017	7.54	76.30	0.11	13.00	2.00	5.00	0.50	
5/13/2017	7.59	75.80	0.11	13.00	2.00	5.00	0.50	
5/14/2017	7.62	75.80	0.12	12.00	3.00	5.00	0.50	
5/15/2017	7.65	75.60	0.11	12.00	3.00	5.00	0.50	
5/16/2017								Harvest 4
5/17/2017								
5/18/2017	7.64	75.40	0.10	13.00	2.00	5.00	0.50	
5/19/2017	7.58	75.90	0.10	13.00	2.00	5.00	0.50	
5/20/2017	7.60	76.20	0.11	12.00	3.00	5.00	0.50	
5/21/2017	7.61	75.90	0.10	12.00	3.00	5.00	0.50	Harvest 5
5/22/2017	7.61	75.50	0.10	13.00	2.00	5.00	0.50	
5/23/2017	7.56	75.80	0.10	13.00	2.00	5.00	0.50	
5/24/2017	7.49	75.60	0.10	13.00	2.00	5.00	0.50	
5/25/2017	7.47	75.30	0.11	13.00	2.00	5.00	0.50	
5/26/2017	7.43	75.50	0.10	13.00	2.00	5.00	0.50	Harvest 6
5/27/2017	7.41	75.70	0.09	13.00	2.00	5.00	0.50	
5/28/2017	7.46	75.40	0.10	12.00	3.00	5.00	0.50	
5/29/2017	7.52	75.10	0.09	13.00	2.00	5.00	0.50	
5/30/2017	7.48	75.80	0.08	13.00	2.00	5.00	0.50	
5/31/2017	7.50	75.60	0.08	13.00	2.00	5.00	0.50	Harvest 7

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
6/1/2017	7.46	75.00	0.08	13.00	2.00	5.00	0.50	
6/2/2017	7.51	75.30	0.07	13.00	2.00	5.00	0.50	
6/3/2017	7.55	75.80	0.06	14.00	1.00	5.00	0.50	
6/4/2017	7.59	76.40	0.06	13.00	2.00	5.00	0.50	
6/5/2017	7.65	75.90	0.06	13.00	2.00	5.00	0.50	Harvest 8
6/6/2017	7.71	75.20	0.05	13.00	2.00	5.00	0.50	
6/7/2017	7.78	74.90	0.05	12.00	3.00	5.00	0.50	
6/8/2017	7.83	75.10	0.06	12.00	3.00	5.00	0.50	
6/9/2017	7.87	75.40	0.05	13.00	2.00	5.00	0.50	
6/10/2017	7.91	75.40	0.04	13.00	2.00	5.00	0.50	Harvest 9
6/11/2017	7.43	76.50	0.04	13.00	2.00	5.00	0.50	
6/12/2017	7.12	76.30	0.04	13.00	2.00	5.00	0.50	
6/13/2017	7.05	76.70	0.04	14.00	1.00	5.00	0.50	
6/14/2017	6.98	76.40	0.04	13.00	2.00	5.00	0.50	
6/15/2017	6.94	76.60	0.04	13.00	2.00	5.00	0.50	Harvest 10
6/16/2017	6.99	76.30	0.04	13.00	2.00	5.00	0.50	
6/17/2017	6.78	75.70	0.04	12.00	3.00	5.00	0.50	
6/18/2017	6.75	75.60	0.04	12.00	3.00	5.00	0.50	
6/19/2017	6.82	75.30	0.04	13.00	2.00	5.00	0.50	
6/20/2017	6.68	75.00	0.04	13.00	2.00	5.00	0.50	Harvest 11
6/21/2017	6.83	76.10	0.04	13.00	2.00	5.00	0.50	
6/22/2017	6.85	76.40	0.04	12.00	3.00	5.00	0.50	
6/23/2017	6.85	76.60	0.04	12.00	3.00	5.00	0.50	
6/24/2017	6.77	77.20	0.04	12.00	3.00	5.00	0.50	
6/25/2017	6.79	75.70	0.04	13.00	2.00	5.00	0.50	Harvest 12
6/26/2017	6.74	75.60	0.04	13.00	2.00	5.00	0.50	
6/27/2017	6.81	75.20	0.04	13.00	2.00	5.00	0.50	
6/28/2017	6.72	75.90	0.04	12.00	3.00	5.00	0.50	
6/29/2017	6.60	76.30	0.04	13.00	2.00	5.00	0.50	
6/30/2017	6.49	76.50	0.04	13.00	2.00	5.00	0.50	Harvest 13

Date	pH	Temp (F)	Conductivity (ms/cm)	Total Water (G)	Water removed (g)	Water addition (g)	Nutrient addition (ml)	Comments
7/1/2017	6.52	76.80	0.04	13.00	2.00	5.00	0.50	
7/2/2017	6.58	76.80	0.04	13.00	2.00	5.00	0.50	
7/3/2017	6.63	77.30	0.04	12.00	3.00	5.00	0.50	
7/4/2017	6.66	78.80	0.05	12.00	3.00	5.00	0.50	
7/5/2017	6.71	78.90	0.04	12.00	3.00	5.00	0.50	Harvest 14
7/6/2017	6.67	78.50	0.04	12.00	3.00	5.00	0.50	
7/7/2017	6.63	78.80	0.04	12.00	3.00	5.00	0.50	
7/8/2017	6.67	78.30	0.05	13.00	2.00	5.00	0.50	
7/9/2017	6.93	78.00	0.04	13.00	2.00	5.00	0.50	
7/10/2017	6.90	77.80	0.05	13.00	2.00	5.00	0.50	Harvest 15
7/11/2017	6.85	77.30	0.05	13.00	2.00	5.00	0.50	
7/12/2017	6.81	77.50	0.05	12.00	3.00	5.00	0.50	
7/13/2017	6.84	77.40	0.05	13.00	2.00	5.00	0.50	
7/14/2017	6.79	77.10	0.05	13.00	2.00	5.00	0.50	
7/15/2017	6.81	76.50	0.04	14.00	1.00	5.00	0.50	Harvest 16

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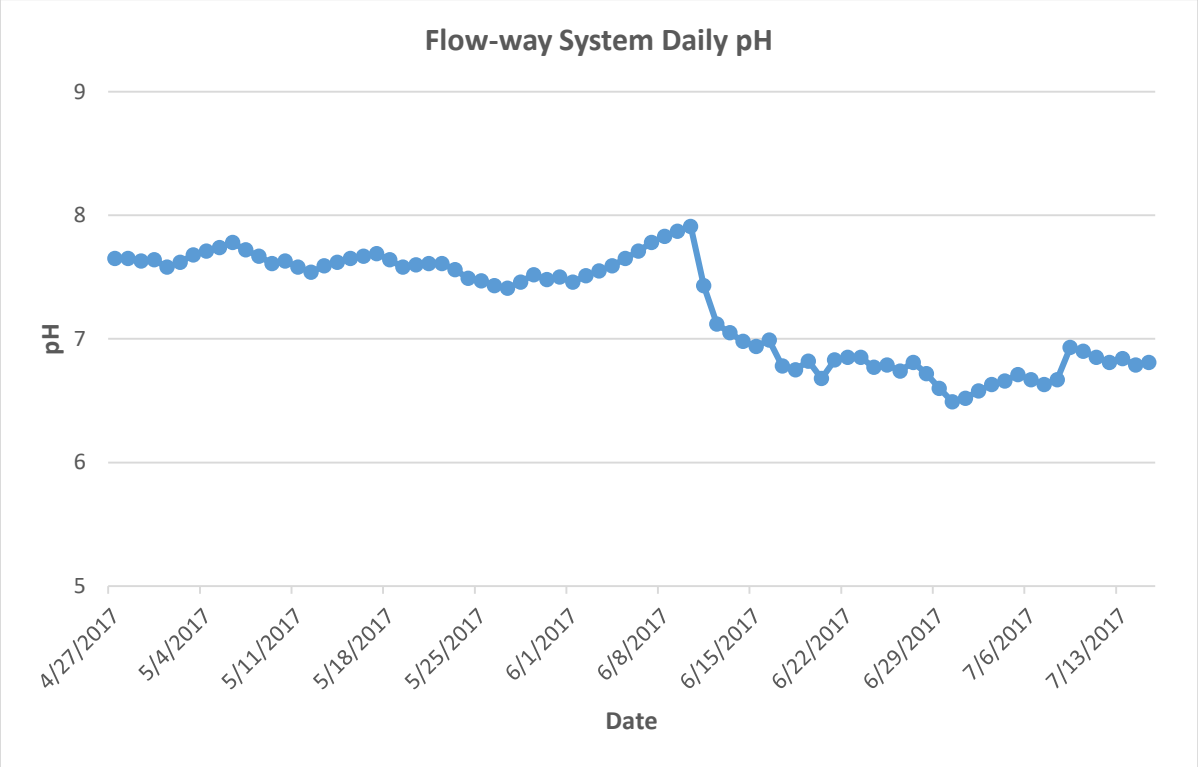
0.1
0.08
0.06
0.04
0.02
0
4/27/2017
5/4/2017
5/11/2017
5/18/2017
5/25/2017
6/1/2017
6/8/2017
6/15/2017
6/22/2017
6/29/2017
7/6/2017
7/13/2017
Date


Flow-way System Daily pH
pH
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8
7
6
5
4/27/2017
5/4/2017
5/11/2017
5/18/2017
5/25/2017
6/1/2017
6/8/2017
6/15/2017
6/22/2017
6/29/2017
7/6/2017
7/13/2017
Date

Appendix VI: Raw data of all harvests (Experiment 3)

Harvests	Substratum Treatment Level	Weight Before Drying (g)	Weight After Drying (g)	Total Biomass (g)	Daily Biomass (mg cm^{-2} d^{-1})
1	Treatment A	5.372	6.250	0.878	0.135
	Treatment B	5.344	6.030	0.686	0.106
	Treatment C	5.306	5.673	0.367	0.057
	Treatment D	5.363	5.469	0.106	0.016
2	Treatment A	5.827	6.345	0.518	0.080
	Treatment B	5.721	6.100	0.379	0.058
	Treatment C	5.698	5.936	0.238	0.037
	Treatment D	5.733	5.830	0.096	0.015
3	Treatment A	5.261	5.906	0.644	0.099
	Treatment B	5.387	5.917	0.530	0.082
	Treatment C	5.363	5.652	0.289	0.045
	Treatment D	5.392	5.469	0.077	0.012
4	Treatment A	5.902	6.413	0.511	0.079
	Treatment B	5.863	6.245	0.382	0.059
	Treatment C	5.805	6.012	0.208	0.032
	Treatment D	5.767	5.829	0.062	0.010
5	Treatment A	5.796	6.531	0.735	0.113
	Treatment B	5.701	6.360	0.659	0.102
	Treatment C	5.798	6.207	0.409	0.063
	Treatment D	5.877	6.027	0.150	0.023
6	Treatment A	5.684	6.737	1.053	0.162
	Treatment B	5.785	6.881	1.096	0.169
	Treatment C	5.913	6.593	0.680	0.105
	Treatment D	5.680	5.867	0.187	0.029
7	Treatment A	5.779	6.782	1.002	0.155
	Treatment B	5.716	6.518	0.802	0.124
	Treatment C	5.764	6.396	0.632	0.097
	Treatment D	5.773	5.923	0.149	0.023
8	Treatment A	5.785	6.391	0.606	0.093
	Treatment B	5.808	6.331	0.523	0.081
	Treatment C	5.942	6.352	0.409	0.063
	Treatment D	5.772	5.894	0.122	0.019

Harvests	Substratum Treatment Level	Weight Before Drying (g)	Weight After Drying (g)	Total Biomass (g)	Daily Biomass (mg cm^{-2} d^{-1})
9	Treatment A	5.828	6.358	0.530	0.082
	Treatment B	5.748	6.121	0.373	0.057
	Treatment C	5.756	6.060	0.304	0.047
	Treatment D	5.668	5.739	0.071	0.011
10	Treatment A	5.805	6.199	0.394	0.061
	Treatment B	5.734	6.052	0.317	0.049
	Treatment C	5.815	5.993	0.178	0.027
	Treatment D	5.755	5.853	0.098	0.015
11	Treatment A	6.010	6.390	0.380	0.059
	Treatment B	5.942	6.294	0.352	0.054
	Treatment C	5.805	6.035	0.229	0.035
	Treatment D	5.545	5.626	0.081	0.013
12	Treatment A	5.572	6.026	0.455	0.070
	Treatment B	5.527	5.958	0.431	0.066
	Treatment C	5.561	5.844	0.283	0.044
	Treatment D	5.567	5.657	0.090	0.014
13	Treatment A	5.537	6.103	0.567	0.087
	Treatment B	5.593	6.022	0.429	0.066
	Treatment C	5.577	5.843	0.266	0.041
	Treatment D	5.529	5.645	0.116	0.018
14	Treatment A	5.495	5.987	0.493	0.076
	Treatment B	5.541	5.999	0.457	0.070
	Treatment C	5.561	5.921	0.360	0.056
	Treatment D	5.550	5.755	0.205	0.032
15	Treatment A	5.655	6.088	0.434	0.067
	Treatment B	5.702	6.038	0.336	0.052
	Treatment C	5.643	5.953	0.311	0.048
	Treatment D	5.652	5.824	0.172	0.027
16	Treatment A	5.568	6.047	0.479	0.074
	Treatment B	5.513	5.925	0.412	0.063
	Treatment C	5.657	5.983	0.326	0.050
	Treatment D	5.529	5.679	0.150	0.023

Appendix VII: Raw data and calculation for Substratum Surface Area and Shading Percentages

Measurements obtained from Heat topographical map and isometric height imaging analyses included

Fiber Cross Sectional Area (CSA in cm^2)

Fiber Surface Area (SA in cm^2)

Circular Equivalent Diameter (cm)

Raw values obtained are given below:

Substratum Treatment	Fiber CSA (cm2)	Fiber SA (cm2)		Fiber Diameter (cm)
Treatment B	0.9712	2.3606		0.1112
Sum	**0.97**	**2.36**	**Average**	**0.11**
Treatment C	1.2046	3.2815		0.1238
	1.0598	2.7956		0.1162
Sum	**2.26**	**6.08**	**Average**	**0.12**
Treatment D	2.4958	8.3575		0.1782
	0.0743	0.1837		0.0308
	0.9282	2.7984		0.1087
	0.7691	2.4506		0.099
Sum	**4.27**	**13.79**	**Average**	**0.10**

Grand Average (Expected Diameter of each fiber)	**0.11**

Percentage Shading

Nominal area of base sample = 9 cm^2

Area of base protected by fibers = Sum of Fiber CSA (in cm^2)

Shading Percentage of Base Sample = (Sum of Fiber CSA/9) * 100%

Substrate Surface Area (per 9 cm^2)

Nominal area of base sample = 9 cm^2

Surface area of fibers on Base Sample = Sum of Fiber SA (in cm^2)

Area of base used up by fibers' diameter = Number of fibers * Area of fiber's base area

Note: fiber's is modeled as a cylinder with an expected diameter of 0.11 cm

Therefore, substrate surface area (per 9 cm^2) = 9 - (Number of fibers * Area of fiber's base area) + Sum of Fiber SA

Appendix VIII: Recipe of F/2 algae food (Guillard and Ryther 1962, Guillard 1975)

Chemical Component	Mass ($gmol^{-1}$)	Final concentration (M)	Final concentration (gL^{-1})
$NaNO_3$	84.98	8.82×10^{-4}	0.075
$NaH_2PO_4 \cdot H_2O$	137.97	3.62×10^{-5}	0.005
$FeCl_3 \cdot 6H_2O$	270.30	1.17×10^{-5}	0.0032
$MnCl_2 \cdot 4H_2O$	197.01	9.10×10^{-7}	1.79×10^{-4}
$ZnSO_4 \cdot 7H_2O$	186.00	7.65×10^{-8}	2.19×10^{-5}
$CoCl_2 \cdot 6H_2O$	237.00	4.20×10^{-8}	9.95×10^{-6}
$CuSO_4 \cdot 5H_2O$	249.00	3.93×10^{-8}	9.79×10^{-6}
$Na_2MoO_4 \cdot 2H_2O$	237.88	2.60×10^{-8}	6.18×10^{-6}
Thiamine · HCl (vitamin B1)	333.27	2.96×10^{-7}	1.00×10^{-4}
Biotin (vitamin H)	242.45	2.05×10^{-9}	5.00×10^{-7}
Cyanocobalamin (vitamin B12)	1355.4	3.69×10^{-10}	5.00×10^{-7}
$Na_2SiO_3 \cdot 9H_2O$	284.04	1.06×10^{-4}	0.030
$Na_2EDTA \cdot 2H_2O$	374.24	1.17×10^{-5}	0.0044