5-2013

Novel method for sequential batch recovery of biomass from a helical photobioreactor

Matthew Hurst
University of Arkansas, Fayetteville

Novel Method for Sequential Batch Recovery of Biomass from Helical Photobioreactor

An Undergraduate Honors College Thesis

in the

Ralph E. Martin Department of Chemical Engineering
College of Engineering
University of Arkansas
Fayetteville, AR

by

Matthew Hurst

April 23, 2013

This thesis is approved.

Thesis Advisor

Bob Beitle Date: 4/23/13

Dr. Bob Beitle, Ph.D.

Thesis Committee

Tammy Rechtin Date: 4/23/13

Dr. Tammy Lutz-Rechtin, Ph.D.

Abstract

This study developed and evaluated a novel method to recover the microalgae species *Arthrospira platensis* from a closed helical photo bioreactor system. A recovery apparatus was designed and shown to increase recovery by 40% via periodic online recovery runs during exponential growth phase of biomass. Considerations for the apparatus design included the low shear tolerance of the algae and the necessity for a closed system in order to monitor CO_2 consumption. The recovery method draws on methods used for large scale recovery as reported in literature by Vonshak and Shimmatsu [1], [2]. This work seeks to improve reactor biomass yield for generating sugar extracts from *A. platensis* which may be used subsequently as a feedstock to generate recombinant proteins from *E. coli*.

1 Contents

2 Introduction

2.1 Commercial Importance

Arthrospira platensis, or Spirulina as it is known commercially, is a widely cultivated species of filamentous cyanobacteria recovered not only as a source of nutrition but also for use as a feedstock for subsequent conversion to high value biomedical and commercial products [3], [4]. Spirulina was first historically reported as a food source as far back as 1300 AD by the Aztecs and today it is widely used as a dietary supplement [5], [6]. Spirulina was first isolated as a species in 1827 [7]. It was rediscovered and first cultivated commercially on a large scale in the late 1970s [2]. Several compounds such as unsaturated fatty acids, pigments, vitamins and polysaccharides may be derived from Spirulina but must be produced in a closed system to prevent contamination and ensure product integrity [4], [5], [8]. Spirulina may be grown using a photobioreactor system which consists of a vessel stage where culture media can be contained along with the algae and a photo-stage possessing a relatively high surface area to volume ratio [9].

2.2 Recovery

Spirulina grows in the form of left-handed open helix trichomes from which its name is derived. The trichomes can reach lengths up to a few millimeters and can range from 5-6 µm in diameter [10], [11]. Fragmentation of the Spirulina trichomes will occur if there is an excessive amount of shear exerted on the growth system used to cultivate Spirulina. Such fragmentation which can potentially result in cell damage has been observed to inhibit or stop growth altogether; this damage can also effect the quality products derived from Spirulina downstream. Petit et al. mentions that fragmentation will not stop growth if cell damage does not take place [12]. Recovery methods take advantage of Spirulina's filamentous structure and tendency to form conglomerate like mats at large scale [1]. Current large scale production and recovery methods of edible Spirulina involve growth in raceway ponds and recovery with a combination of inclined gravity and vibrating screen staged filtration followed by vacuum filter steps [1], [2]. Filter presses operated under vacuum have also been used effectively to recovery Spirulina

on a large scale [2]. Several small scale recovery methods described in literature include microfiltration and ultrafiltration as a means for “development of auto-regenerative biological life support systems for men in space” [12]. Petit et al. and Rossi et al. discuss use of inorganic and organic type membranes for the recovery of Spirulina [12], [13].

2.3 System Advantages

Several advantages apply specifically to the system chosen for cultivation of the algae. Of particular importance is the ubiquitous use of Spirulina as a health food and dietary supplement. Two US companies, Earthrise Farms of California and Cyanotech Corporation of Hawaii, produce Spirulina with a “Generally Recognized as Safe” status under FDA policy [4]. The toxicology of Spirulina is well studied and several sources as indicated by Belay et al. show it to be safe, non-toxic, and even beneficial for human consumption [4]. Cultivation of the algae in a closed system allows for controlled growth and prevents any potential contamination from occurring [9]. Important growth parameters including pH, temperature, and feed rate of a carbon source may be monitored and controlled to maximize growth. Spirulina is a photosynthetic cyanobacteria that uses sunlight to convert carbon absorbed from solution as bicarbonate ion HCO_3^- to sugars and other metabolites necessary for growth [14].

2.4 Local Importance

Two important characteristics distinguish this work: 1) the nature of downstream biomass use, and 2) uniqueness of the recovery method. First, Spirulina recovered using the method developed by this study will be processed downstream to recover sugar extracts for subsequent conversion to recombinant proteins by genetically modified *E. coli*. T. Rechtin shows in unpublished preliminary results an increased yield of recombinant protein using sugars extracted from U. lactuca derived media as opposed to yeast extract [15].

Finally, the current bench top method for recovering Spirulina is accomplished by pouring contents of the reactor taken at the end of a growth run across across a 280 μm stainless steel mesh screen. Reactor dry weight concentration of Spirulina ranged from

0.4-1.0 g/L during recovery runs. The biomass collected on the screen is then scraped and rinsed with deionized water and then centrifuged for storage and downstream processing. The novel method in this paper draws on large scale methods previously stated, and adds a uniquely designed filter holder that maintains air tight recovery of the algae during continual reactor operation.

3 Materials and Methods

It was necessary to determine an appropriate pump size and type to act as a means of fluid transport for the recovery apparatus. In addition to pump selection the mesh size for the filter used to separate Spirulina needed to allow for a fraction of the Spirulina to return to the reactor to permit continued growth. A flow through dead-end filtration scheme was chosen to keep the apparatus design simple. A tangential flow separation scheme was considered in the initial design phase of the filter holder, this consideration is discussed in section 5.

3.1 Concentration Measurements

Throughout experimentation it was necessary to measure the concentration of the Spirulina in solution in order to evaluate results. While optical absorbance is a commonly used method it was decided that dry weight sample measurements would give a more reliable estimate of algae concentration in solution due to changes in chlorophyll content. Samples of 20 or 30 mL volumes were extracted using an automatic pipetteman and filtered with VMR glass microfiber filter no. 696 (diameter: 4.7 cm) then dried and weighed with a Mettler Toledo MJ33 scale. Microfiber filters were chosen as they have an average opening size of 1 µm which should capture even individual cells. The concentration was then calculated by dividing the mass of Spirulina on the filter by the volume of the sample taken. A detailed explanation of the procedure is provided in Appendix Section 9.2.2.

3.2 Pump Selection

Three pumps were tested on their tendency to shear the algae during operation.

A control volume of 2 L was circulated through each pump and 1 mL samples were extracted to be photographed under light microscope. Analysis of the sample images was performed using open source software ImageJ. Average relative trichome length was determined as a benchmark to measure effects of fragmentation of the Spirulina due to shear exerted on the algae through the pump. The samples were photographed under 10x objective magnification using a Amscope T490-10MA at with a MT500 10 MP or under 10x objective magnification using an Olympus BH2 with a iPhone 4S 10 MP through the viewing lense of the microsope. The sampling processing procedure is listed in Appendix B. Each pump is shown in Figure 1 below.

Figure 1. Pumps used from left to right: Triflo centrifugal, NCRST LT-1 variable speed centrifugal, and Cole Parmer 7017-20 peristaltic.

3.3 Mesh Size Determination

3.3.1 Dead-End Filtration Experiments

Initial recovery experiments were performed using a Millipore P141 high pressure filter with various stainless steel mesh sizes inserted. The mesh opening sizes varied from 300-800 µm. A 2 L volume of biomass grown on bench top air agitated open vessels at concentrations ranging from 0.8-1.0 g/L dry weight was passed across the mesh inside the holder. Dry weight concentrations of the filtrate were taken to determine amount recovered. Four opening sizes were compared to determine ideal size for recovery: 120, 280, 500, and 800. Three-hundred micron mesh is used in large scale industrial recovery applications by Earthrise Nutritionals [1]. The average trichome length was evaluated using ImageJ. The application has a measuring tool which will give the length

of any object inside the image in pixels. The relative length of each trichome in an image was recorded. At 10x magnification some trichomes were longer or outside of the image boundaries. This issue is dealt with in the conclusions and future work sections below.

3.3.2 Hollow Fiber Membrane Experiment

A Koch Romicon PM50 1” diameter polysulfone hollow-fiber membrane with a MWCO of 50kDa was used in a tangential flow mode for single pass and multi pass recovery runs with a 2 L sample. Dry-weight concentrations of the filtrate were taken and samples were photographed under microscope at 10x magnification to evaluate harvest yield and size distribution.

3.4 Algae Growth

Active culture of the Spirulina strain UTEX 1926 was obtained from the Algae Culture Collection of the University of Texas in Austin. Approximately 10 mL of the algae at minimal concentration was cultured on bench top in an open air agitated 2 L vessel in a modified Zarrouk media containing (per liter): 18.0 g $NaHCO_3$, 2.5 g $NaNO_3$, 0.5 g K_2HPO_4, 1 g K_2SO_4, 1 g NaCl, 0.04 g $CaCl_2$, 0.08 g $Na_2EDTA•2H_2O$, 0.2 g $MgSO_4•7H_2O$, and 0.01 g, $FeSO_4•7H_2O$ [16]. In addition the media contained 1 mL of a nutrients solution with the composition (per liter): 2.86 g H_3BO_3, 0.02 g $(NH_4)6Mo_7O_{24}$, 1.8 g $MnCl_2•4H_2O$, 0.08 g $Cu_2SO_4•5H_2O$, and 0.22 g $ZnSO_4•7H_2O$. Initial cultivation in the bench top vessel was carried out until sufficient biomass was generated to allow for seeding of the helical photobioreactor at a concentration of 0.04 g/L dry weight.

3.5 Bioreactor System

An airlift system was devised by T. Rechtin based on Vonshak to circulate Spirulina within the reactor and through the photo-stage of the system [1], [15]. The closed 2.8 L reactor system consists of a 2-L glass fermentation vessel attached by 0.25 ID masterflex peroxide cured silicone tubing to a 10 inch long helical glass coil (0.5 in. I.D. tubing, 6.3 in. coil diameter) which holds approx. 650 ml of liquid. To control illumination intensity, the glass coil is place horizontally on a photostage of a bank of 24” cool 20-W

white fluorescent lights. Circulation of the system is obtained by an airlift system with an air pump (*AIR 3000*) to ensure a flow rate of 1.0 L min^{-1} and bubble size of less than 2 mm. The output in placed into the bottom of the fermentation vessel to promote circulation and removal of soluble inhibitory O_2 gas produced by algae growth. Additional circulation can be added by placing a magnetic stir bar into the fermentation vessel. The temperature of the system is maintained in the fermentation vessel by use of an *Autonics* thermocouple attached to a heating element. A nonfouling pH probe (*Cole-Parmer*) is located in-line between the fermentation vessel and photostage. The pH is maintained by use of a customized CAT 1000 pH controller with a solenoid valve for regulation of CO_2 gas that is gently bubbled into the reactor for acidifying the media and as a source of carbon for biomass growth. CO_2 gas concentrations and rates were determined by an in-line *Alltech* Digital Flow Check meter and use of a chart recorder (*Pharmacia* LKB-Rec102). A pressure release value is placed on top of the closed fermentation vessel to prevent pressure and gas buildup. To prevent potential contamination of cultures, all potential atmospheric gas exchange points in the system have a 0.2 micron PVDF filter. A process flow diagram of the system is shown in Figure 2 below.

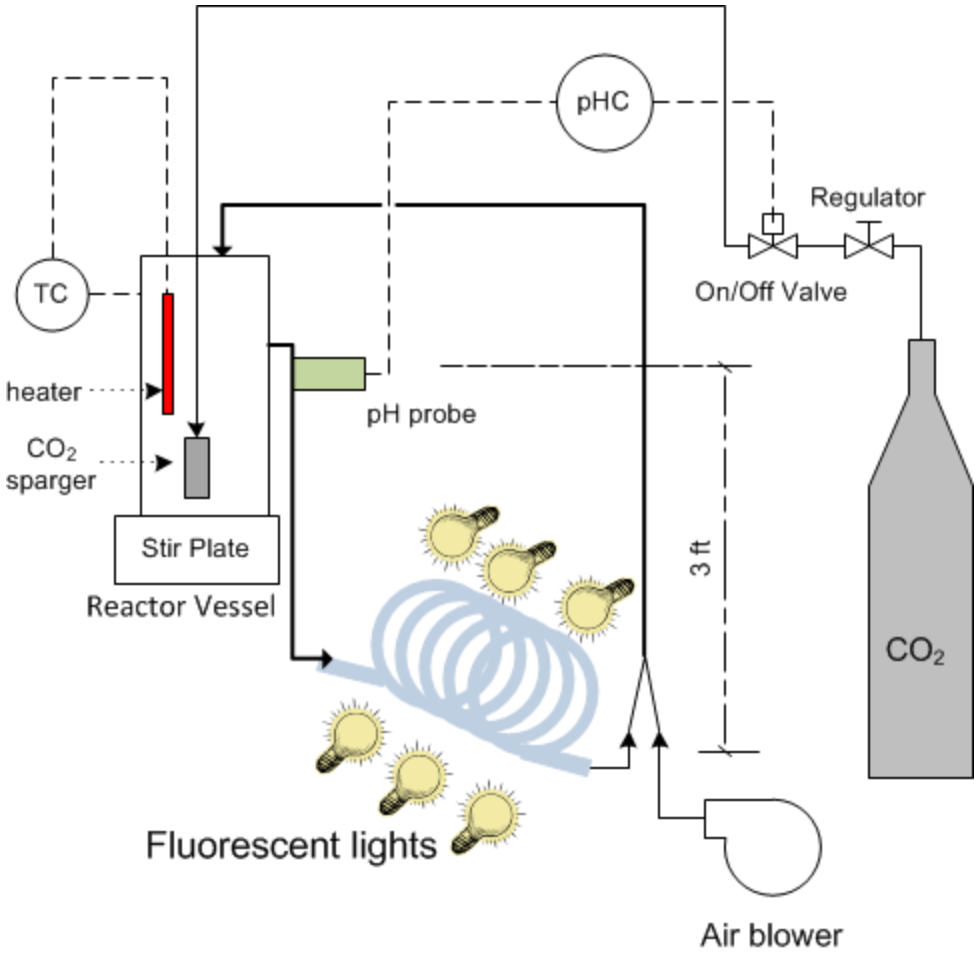

Figure 2. Airlift bioreactor system.

3.6 Recovery Apparatus

The recovery apparatus consisted of a closed loop from the reactor to the peristaltic pump, through filters and back to the reactor. The pump generated a pressure head across the filters of approximately 2 psi during normal operation for a recovery run. Three identical filter holders were designed and constructed with the assistance of Mr. George Fordyce. Figure 3 below shows photographs of the constructed filter holder with steel mesh inserted. Coarse and Fine mesh of opening sizes 120 µm and 280 µm were used during recovery runs. The recovery area for each filter is 155 cm^2.

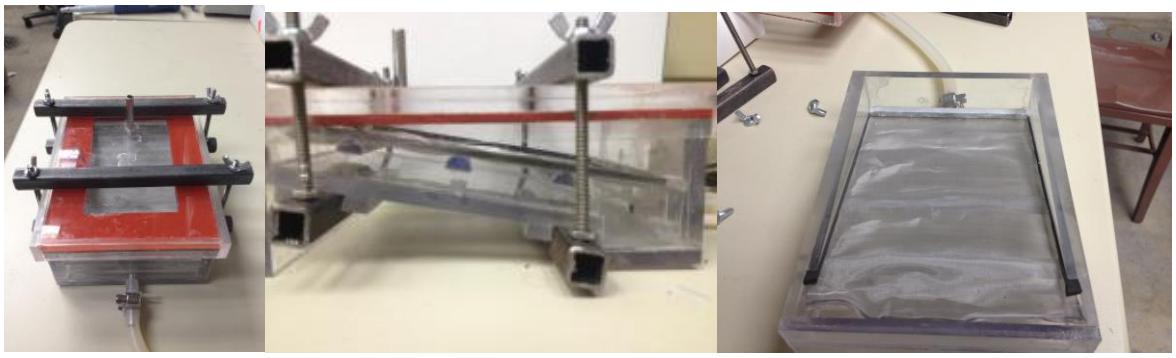

Figure 3. Photograph of filter holder, from left to right: with clamps sealed, side view, lid removed mesh exposed.

The inlet to the filter holder is located at the top on the lid. Biomass enters through the top and encounters an inclined screen. The screen was inclined to facilitate accumulation of biomass starting at the bottom to prevent obstruction of the inlet flow. The resulting filtrate of biomass remains on the screen while the remainder of liquid media passes through the screen along with trichomes with length smaller than that of the mesh opening size. A diagram of the harvest loop is shown next in Figure 4:

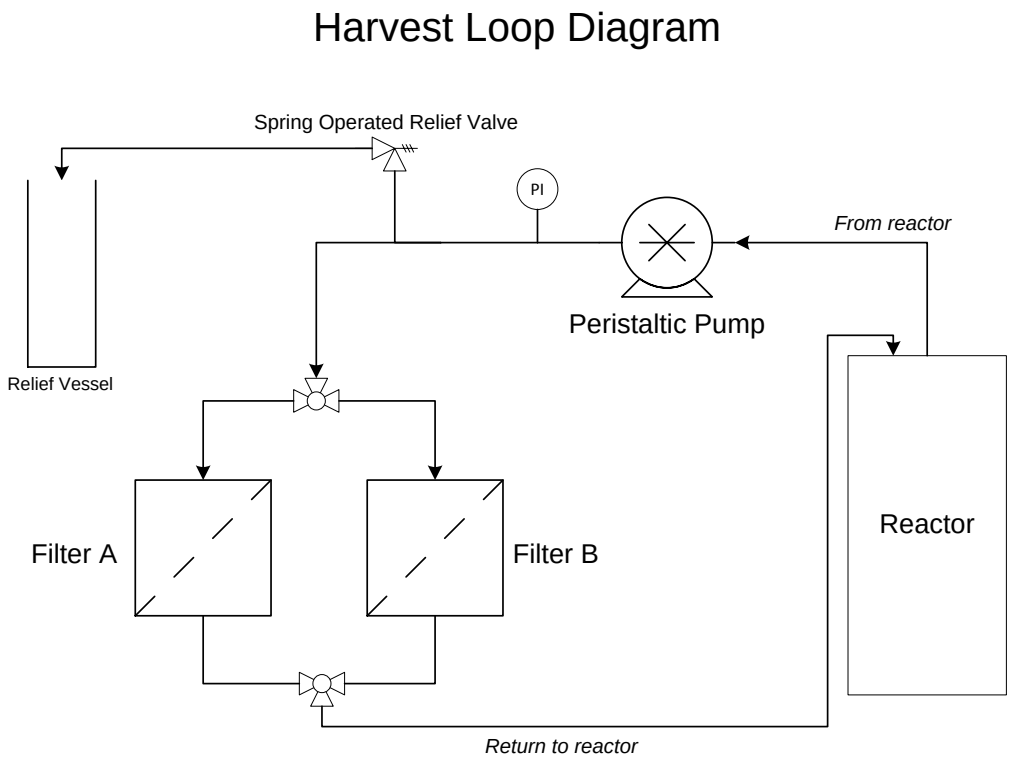

Figure 4. Recovery apparatus diagram.

Recovery runs were performed using the Cole Parmer peristaltic pump at a flow rate of 4 mL/s to accomplish three full cycles of the total system volume of 2.8 L within 35-40 minutes. Figure 5 shows the recovery apparatus during a run in the lab. The photobioreactor system is out the frame to the right.

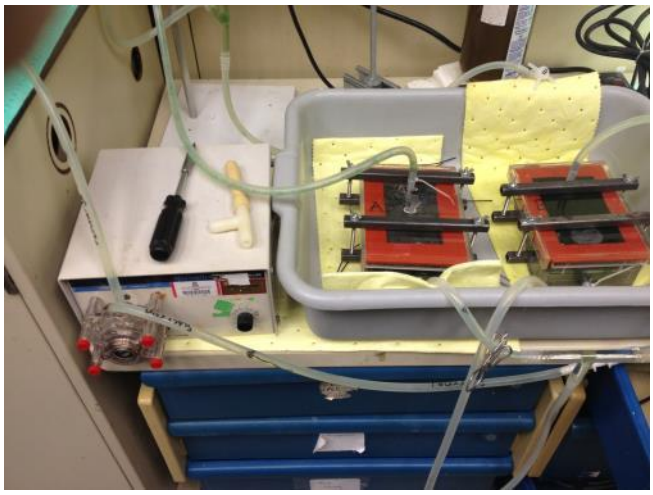

Figure 5. Photograph of recovery apparatus including filters A, B, and masterflex peristaltic pump.

Once a recovery run is completed the recovery loop is drained to the reactor system and the filter holders are isolated with valves and clamps as required to keep the reactor system closed. The lids of the filters are held in place by simple clamps which must be removed before biomass may be extracted from the filter surface. The mesh is removed and all biomass caught by the filter is rinsed into a temporary holding vessel using deionized water. The resulting filtrate volume is measured using a graduated cylinder and a dry wt. concentration is recorded. Prior to and following the recovery run dry weight concentrations for the reactor system are also recorded to determine a theoretical yield which is compared to actual yield of biomass recovered by the filters.

4 Results and Discussion

4.1 Pump Selection

Pump selection describes the methods used to evaluate the effects of shear stress on the algae as it is being circulated by the pumps. The peristaltic pump proved to be ideal as it had the longest effective operation time and smallest amount of overall trichome

length reduction at 8%. The before and after columns show average relative trichome length using ImageJ for analysis. The hollow fiber shows length reduction using the hollow fiber membrane as a means of separation with the peristaltic pump.

Table 1. Pump performance summary and shear comparison.

Pump Description	flow rate	Max Shear Time	Before*	After*	% size reduction
Tri-Flo Centrifugal**	2500 mL/s	5 minutes	1200	20	99+%
Hollow Fiber w/ peristaltic	6 mL/s	2 hours	1800	150	92%
NCRST high***	40 mL/s	30 minutes	780	390	50%
NCRST low***	6 mL/s	24 hours	823	574	30%
Peristaltic***	6 mL/s	72 hours	1040	960	8%

*Indicates average relative trichome length in pixels before and after shear test as determined by the measure tool ImageJ from images taken at 10x magnification.
**fixed speed pump
***variable speed pumps

4.1.1 Tri-Flo Centrifugal

A shear evaluation was performed using a 2L sample of Spirulina in media solution at ~2 g/L dry weight concentration. The sample was circulated through pump 1 for 5 minutes. Flow rate was 2500 mL/s. Excessive fragmentation was observed at such a high flow rate. Average relative trichome length was reduced to around 1-2 turns. This pump essentially destroyed the trichome structure.

4.1.2 NCSRT LT-1 variable speed centrifugal

Two shear evaluations were performed using this pump. A low flow evaluation was performed at 6 mL/s for 15 min and another high flow evaluation was performed at 40 mL/s, for 5 min. A second low flow evaluation was performed at 6 mL/s for 180 min. Most before and after photographs showed some fragmentation but results are inconclusive as to the exact amount of fragmentation that took place using this pump. Inconsistencies in the method to analyze trichome length were encountered but average length reduction is shown above in Table 1.

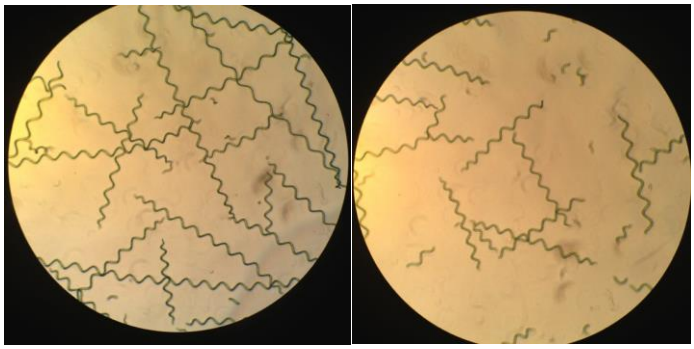

Figure 6. Before (left) and after (right) photographs of Spirulina under 10x magnification after a 30 minute run at 40 mL/s.

4.1.3 Masterflex Peristaltic pump

The peristaltic pump had little effect on fragmentation of spirulina even at maxium flow rate of 6 mL/s after 24 hours of circulation of a 2 L volume of biomass with approximate concentration of 0.8 g/L. The overall average length reduction was measured to be approximately 8% as shown in Table 1.

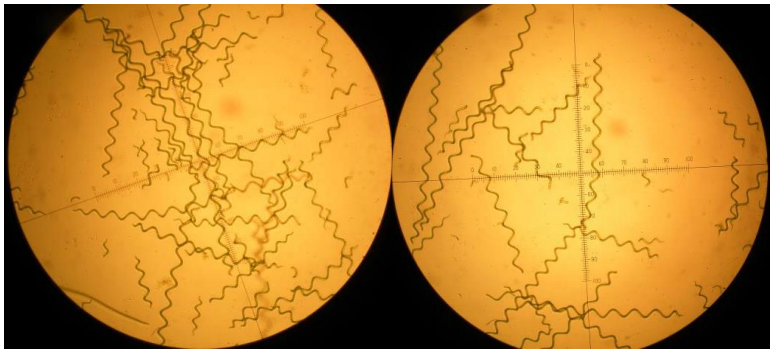

Figure 7. Before (left) and after (right) photographs of Spirulina under 10x magnification for a 24 hr run at 6 mL/s.

4.2 Mesh Size Determination

The overall results for the mesh size determination indicated the largest effective size for recovering the majority of biomass from solution is approximately 300 µm. Section 4.3 details effectiveness of recovery for 280 µm and 120 µm mesh size in the bioreactor system.

4.2.1 Dead-end filtration

Dead-end filtration experiments showed that mesh sizes above 300 µm recovered negligible amounts of algae.

Table 2. Mesh Opening Size Determination Data, using various mesh sized through Millipore filter holder**.**

size [µm]	50	100	120	280	500	800
initial conc. [g/L]	-	-	0.15	0.15	0.25	0.31
after filtration [g/L]	-	-	0.05	0.05	0.25	0.31
% recovery	-	-	66.7	66.7	0.0	0.0

The Millipore dead end filter holder was used to validate mesh size initially. Figure 8 shows the results of filtration runs with the Millipore filter holder for its default insert and a 280 µm filter. The default insert has an approximate opening size of 500 µm.

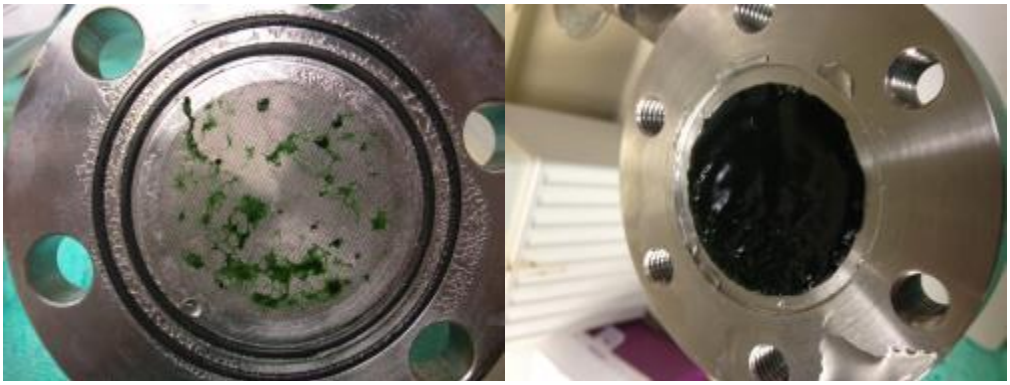

Figure 8. Dead end filtration results for factory insert (little biomass recovered) and 280 µm mesh insert.

4.2.2 Hollow Fiber membrane

The results for trichome length reduction using a hollow fiber membrane are tabulated in Table 2 above. Photographs in the figure below show severe reduction in trichome length and possible cell damage during the recovery run.

Figure 9. Hollow fiber membrane tangential flow filtration samples from left to right: before, single pass, multi pass.

4.3 Recovery

4.3.1 Trial Runs

Five trial runs were performed using the recovery apparatus to evaluate performance. To simulate a growth run the photobioreactor was seeded at concentrations ranging from 0.3 g/L – 1.8 g/L as shown below in Table 3. In each trial run the reactor was seeded with an initial concentration. The first two runs used a coarse filter of mesh size 280 µm. Runs 3, 4 and 5 were performed in 2 stages, the first stage used 280 µm mesh and the second stage used 120 µm. The fine mesh was incorporated after the coarse mesh ceased to reduce dry weight concentration past 0.05 g/L on average. Results are tabulated below:

Table 3. Trial recovery run data.

run		1	2	3	4	5
initial using coarse	**[g/L]**	0.63	1.76	0.3	0.65	0.85
after coarse mesh	**[g/L]**	0.08	0.06	0.04	0.04	0.06
final using fine	**[g/L]**			0.01	0.01	0.02
Dry Wt.						
coarse % yield	%	87%	97%	87%	94%	93%
fine % yield	%			83%	75%	67%
coarse dwt mass	**g**	1.76	4.93	0.84	1.82	2.38
fine dwt mass	**g**			0.11	0.11	0.17
Filtrate						
coarse volume	**L**		0.973	0.915	0.439	0.495
fine volume	**L**				0.463	0.537
coarse conc.	**g/L**		2.23	0.65	2.23	3.6
fine conc.	**g/L**				0.25	0.24
coarse mass	**g**		2.17	0.59	0.98	1.78
fine mass	**g**				0.12	0.13
coarse % yield	%		44%	71%	54%	75%
fine % yield	%				103%	77%

The results in Table 4 show the effectiveness of the recovery apparatus.
Dry weight concentration indicates a 91% average yield for total recovery of all mass from the system. The amount of biomass recovered was calculated theoretically from before and after concentrations measurements. Filtrate mass was also determined to compare with theoretical mass recovered. Filtrate mass calculations showed that mass was lost during the recovery procedure. This may be attributed to error in the measurement technique and small amounts of mass lost through rinsing and removal of biomass from the filter holder. Typically dry weight concentration measurements may vary up to 10% due to technique.

4.3.2 Recovery Run

The results for an online recovery run presented positive potential for increased biomass yield compared to a control growth run without periodic recovery. The reactor was seeded at a concentration of 0.04 g/L for both a control and recovery runs. The algae was allowed to grow undisturbed during the control run. During the recovery run periodic recovery cycles were performed every 4-5 days. The recovery points were taken as the reactor approached a concentration of approximately 0.4 g/L to ensure exponential growth phase was maintained. Figure 10 presents growth data for a control run and then a recovery run. Note that for each square/triangle pair a recovery was performed. The control run was performed without recovery.

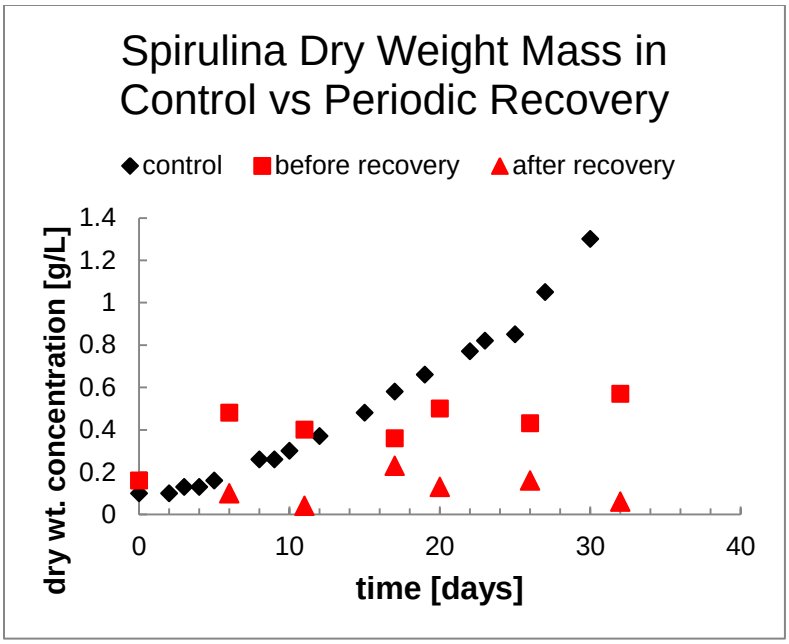

Figure 10. Growth data for recovery run (red) and control growth run (black). Concentration was determined according to procedure 8.2.2 in appendix B.

Figure 10 shows the growth data for two separate reactor runs. The data in black represents a control growth run during which no recovery was performed. The red data shows concentrations before a recovery point (squares) and after a recover point (triangles) during a recovery run. It is shown below that cumulative mass recovered from the reactor for the recovery run after 30 days was 33% greater than actual mass present in the control run (see Figure 11). In error it must be noted that media composition was diluted mistakenly by a factor of 5 for the recovery run and trial runs. This would suggest that even better biomass yield results would have been obtained had the media been correctly formulated. The control run media composition was corrected as per compositions listed in section 2.3. The cumulative mass yield per recovery point is shown below in Figure 11.

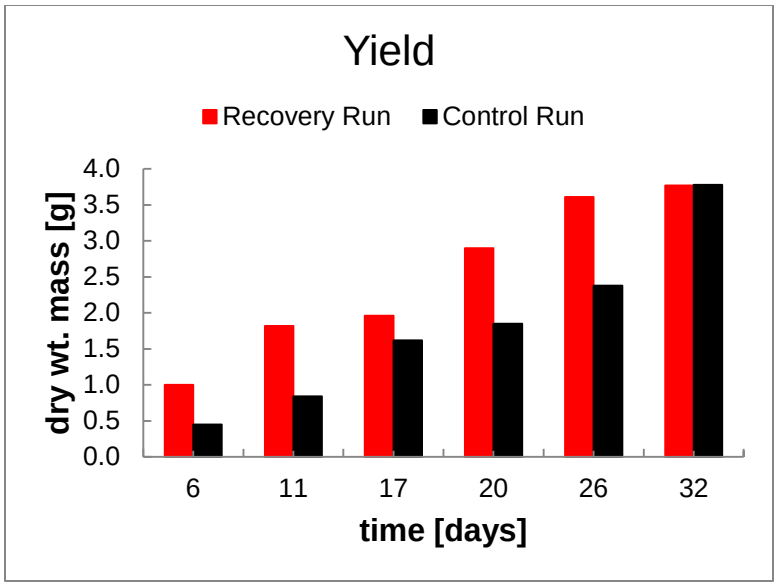

Figure 11. Biomass yield curve showing the mass recovered using the online recovery apparatus in red and theoretical mass present according to dry weight concentration for the control run in black.

Figure 12 shows the percent increase in the mass recovered during the recovery run as compared to the calculated mass present in the system during the control run. Again the average increase in mass recovered was about 1/3 more for 30 days of continuous growth.

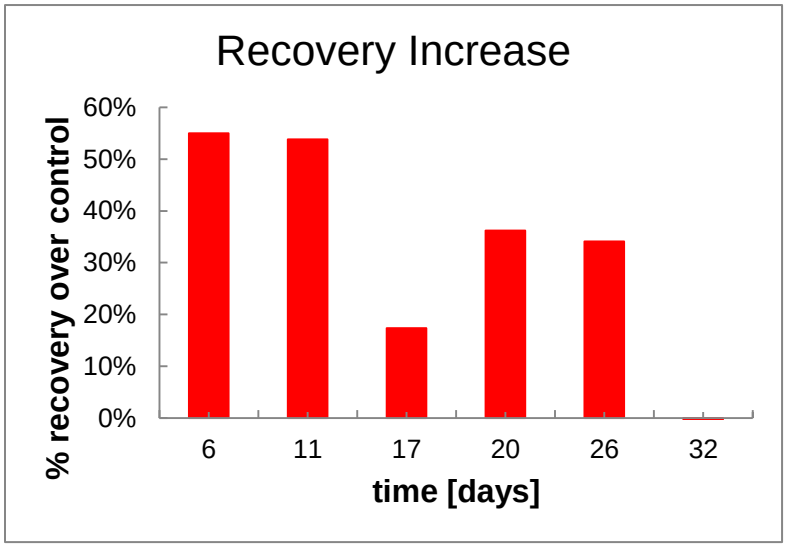

Figure 12. Percent recovery increase over theoretical yield for control run

Figure 13 shows two example photographs of the filter holder after a recover point. The photograph to the right is from a late recovery point where the biomass concentration in the reactor began to drop off.

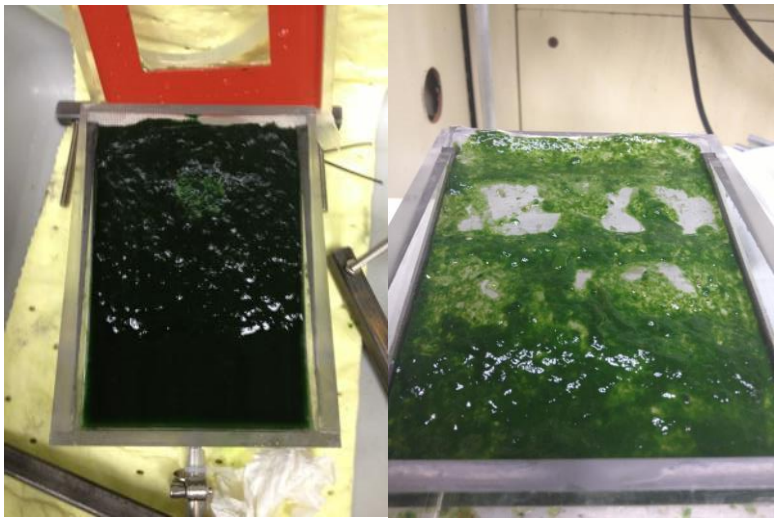

Figure 13. Photographs of Spirulina after two individual recovery points during online growth.

4.4 Morphology

Spirulina is know to change morphologically from a helical or spiral shape to a straight form during laboratory cultivation; such changes in the structure are noted in literature [20]. Prolonged bench top cultivation in repeated sequential use of micro-cultures of the UTEX 1926 strain resulted in the formation of the straight form of Spirulina. More experimentation needs to be performed to determine the effects of morphology during growth. Observations suggest that when the Spirulina trichomes morph to a straight form their growth is inhibited as compared to the original spiral-like form seen in the fresh UTEX 1926 strain. The straight form may be observed in Figure 9.

5 Conclusions

This project summarizes two years of work towards the development of a closed recovery apparatus. Spirulina's sensitivity to excessive shear must be considered in devising a method recovery. For a lab scale recovery apparatus the best mode of fluid transport is a peristaltic type low flow rate pump. Stainless steel mesh with an opening

size of 120 μm provides an ideal filter mechanism for retaining most biomass but permitting enough to pass to maintain growth in the reactor. The apparatus was designed, built, and tested successfully to show potential for 40% increased yield of biomass using periodic recoveries during exponential growth phase. There is still room for improvement as discussed below and it is desired to test the apparatus with system carbon monitoring to permit evaluation of the system using a material balance.

6 Future Work and Improvements

A recovery run needs to be performed with the correct media for growth to validate and show improvement in overall yield of biomass.

A note on image analysis: an effort was made to develop a script using Matlab and its image processing suite which would automate the procedure of determining relative trichome length. The program was successfully run for one image but several factors prevented the program from being an effective means of analysis: 1) photographs taken using the Amscope typically did not provide a uniform background gradient, this prevents the image processing tools in matlab from being able to count objects uniformly, 2) under 10x magnification a significant fraction of trichomes extended to a length longer than that permitted by the frame being analyzed in the first place and such a discrepancy would skew any relative trichome length analysis, and 3) before such an analysis method could be used effectively a procedure needs to be developed to provide uniform sample distributions.

All three issues would need to be addressed in future work however if effective sampling and imaging procedures could be developed, the Matlab script would be a powerful means to measure size distribution the Spirulina trichomes. Such data would provide insight into growth rates and how average trichome length effects Spirulina growth.

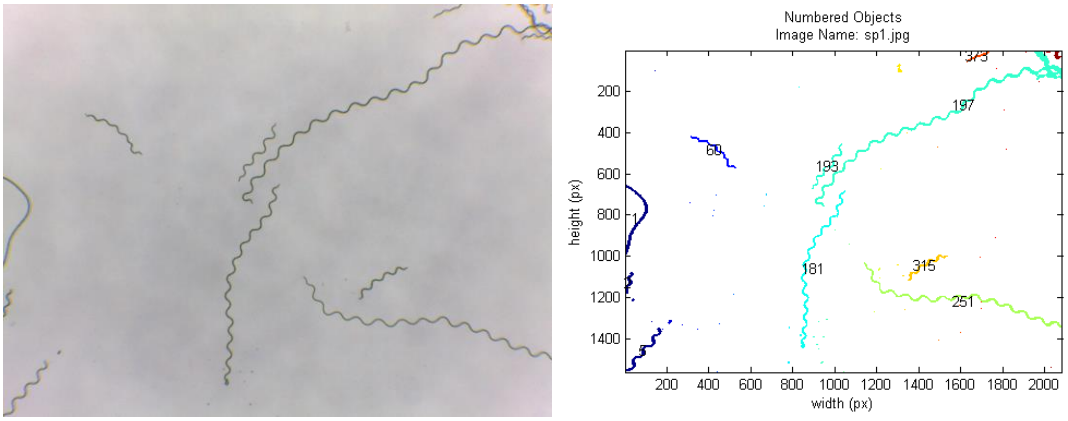

Figure 14. Photgraph of Spirulina under 10x magnification (left) and the same image after processing with Matlab (right).

7 Acknowledgments

First, an acknowledgement of my friend and graduate Jonathan Powell's recommendation to seek out undergraduate research in Dr. Beitle's lab in the first place should not go without saying. I need to thank Tom Potts and the Hestekin research group for the use of their Tri-flo pump and dead-end filter holder. The access to these pieces of equipment allowed for validation and proper design of all the other equipment used to make the recovery system. I owe another thank-you to Dr. Richard Ulrich for his patience in allowing me to use the light microscope camera system located in his office. I would also like to recognize the amazing craftsmanship and machining expertise of Mr. George Fordyce. Without his help the recovery apparatus and in particular the filter holders would have just been a nice idea written down on a piece of paper. His practical knowledge and skills allowed me to make the recovery loop apparatus a reality and I cannot express my gratitude for his help enough. I would like to personally thank Dr. Tammy Rechtin for her invaluable experience and guidance in the planning and execution of this project. Without her help this project would not have been possible. Her backgrounds in Biochemistry and lab experience were essential for maintaining and cultivating the Spirulina as well as in the assembly of the bioreactor system in the first place. Finally I need to thank my advisor Dr. Bob Beitle for his help in putting together the framework for this project including his guidance in applying for and receiving an undergraduate honors college research grant to fund the project.

8 Bibliography

[1] A. Vonshak and A. Richmond, “Mass production of the blue-green Alga Spirulina: An overview,” *Biomass*, vol. 15, no. 4, pp. 233–247, 1988.

[2] H. Shimamatsu, “Mass production of Spirulina, an edible microalga,” *Hydrobiologia*, vol. 512, no. 1–3, pp. 39–44, Jan. 2004.

[3] C. Bergeron, D. J. Carrier, and S. Ramaswamy, Eds., *Biorefinery Co-Products: Phytochemicals, Primary Metabolites and Value-Added Biomass Processing*, 1st ed. Wiley, 2012.

[4] A. Belay and M. E. Gershwin, Eds., *Spirulina in Human Nutrition and Health*. CRC Press Taylor & Francis Group, 2008.

[5] Z. Khan, P. Bhadouria, and P. S. Bisen, “Nutritional and Therapeutic Potential of Spirulina,” *Current Pharmaceutical Biotechnology*, vol. 6, no. 5, p. 7, 2005.

[6] W. V Farrar, “Tecuitlatl; a glimpse of Aztec food technology,” *Nature*, vol. 211, pp. 341–342, 1966.

[7] P. J. F. Turpin, “Spirulina oscillarioide,” *Dictionnaire des sciencesnaturelles*, vol. 50, pp. 309–310, 1827.

[8] C. Bergeron, D. J. Carrier, and S. Ramaswamy, Eds., *Biorefinery Co-Products: Phytochemicals, Primary Metabolites and Value-Added Biomass Processing*, 1st ed. Wiley, 2012.

[9] D. Soletto, L. Binaghi, L. Ferrari, A. Lodi, J. C. M. Carvalho, M. Zilli, and A. Converti, “ScienceDirect - Biochemical Engineering Journal : Effects of carbon dioxide feeding rate and light intensity on the fed-batch pulse-feeding cultivation of Spirulina platensis in helical photobioreactor,” *Biochemical Engineering Journal*, vol. 39, no. 2, pp. 369–375, Apr. 2008.

[10] A. Richmond, *Handbook of microalgal culture: biotechnology and applied phycology*. Wiley-Blackwell, 2008.

[11] S. Spiller, G. Denbeaux, G. Jones, and A. L. Pearson, “Fine structure of Cyanobacteria , Spirulina platensis and Spirulina subsalsa , as viewed by x-ray microscope , XM-1 , beamline 6 . 1 . 2 .,” *Materials Science*, pp. 2–4.

[12] I. P. N. Rossi, P. Jaouen, P. Legintilhomme, “Harvesting of Cyanobacterium Arthrospira Platensis using Organic Filtration Membranes.pdf,” *Food and Bioproducts Processing*, vol. 82, no. C3, pp. 244–250, 2004.

[13] N. Rossi, I. Petit, P. Jaouen, P. Legentilhomme, and M. Derouiniot, “Harvesting of Cyanobacterium Arthrospira platensis Using Inorganic Filtration Membranes,” *Separation Science and Technology*, vol. 40, no. 15, pp. 3033–3050, Nov. 2005.

[14] G. Cogne, J.-B. Gros, and C.-G. Dussap, “Identification of a metabolic network structure representative of Arthrospira (spirulina) platensis metabolism.,” *Biotechnology and bioengineering*, vol. 84, no. 6, pp. 667–76, Dec. 2003.

[15] T. M. Rechtin, M. Hurst, T. Potts, J. McLaughlin, P. May, J. Hestekin, and R. Beitle, “Production of Recombinant Protein in Escherichia Coli Cultured in Extract from Waste Product Algae, Ulva Lactuca.”

[16] C. Zarrouk, *Contribution a L’etude D'une Cianophycee: Influence de Divers Facteurs Physiques Et Chimiques Sur la Croissance Et la Photosynthese de Spirulina Maxima (Setch. Et Garndner) Geitler*. Faculte des Sciences, Universite de Paris, 1966.

[17] E. Molina, J. Fernández, F. G. Acién, and Y. Chisti, “Tubular photobioreactor design for algal cultures.,” *Journal of Biotechnology*, vol. 92, no. 2, pp. 113–131, 2001.

[18] M. Borowitzka, “Commercial production of microalgae: ponds, tanks, tubes and fermenters,” *Journal of Biotechnology*, vol. 70, no. 1–3, pp. 313–321, 1999.

[19] A. Converti, A. Lodi, A. Del Borghi, and C. Solisio, “Cultivation of Spirulina platensis in a combined airlift-tubular reactor system,” *Biochemical Engineering Journal*, vol. 32, no. 1, pp. 13–18, Nov. 2006.

[20] Z. P. Wang and Y. Zhao, “Morphological Reversion of Spirulina (Arthrospira) Platensis (Cyanophyta): From Linear To Helical,” *Journal of Phycology*, vol. 41, no. 3, pp. 622–628, May 2005.

9 Appendices

9.1 Appendix A – Filter Holder Diagrams

Filter Holder Schematic Figures A.1 – 3

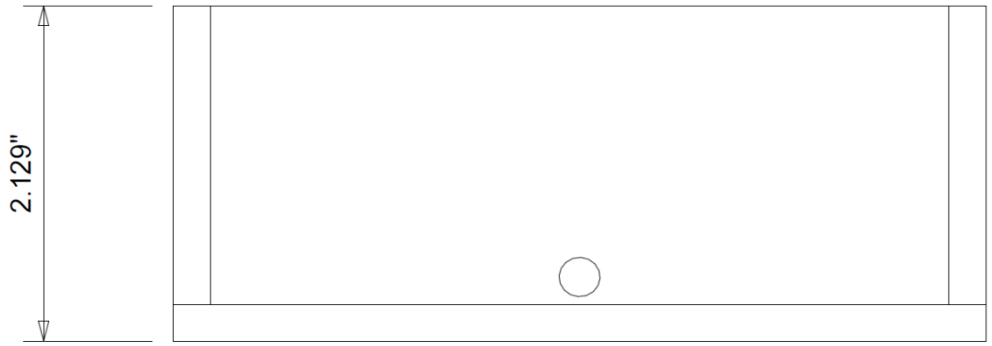

Figure A.1 Front View

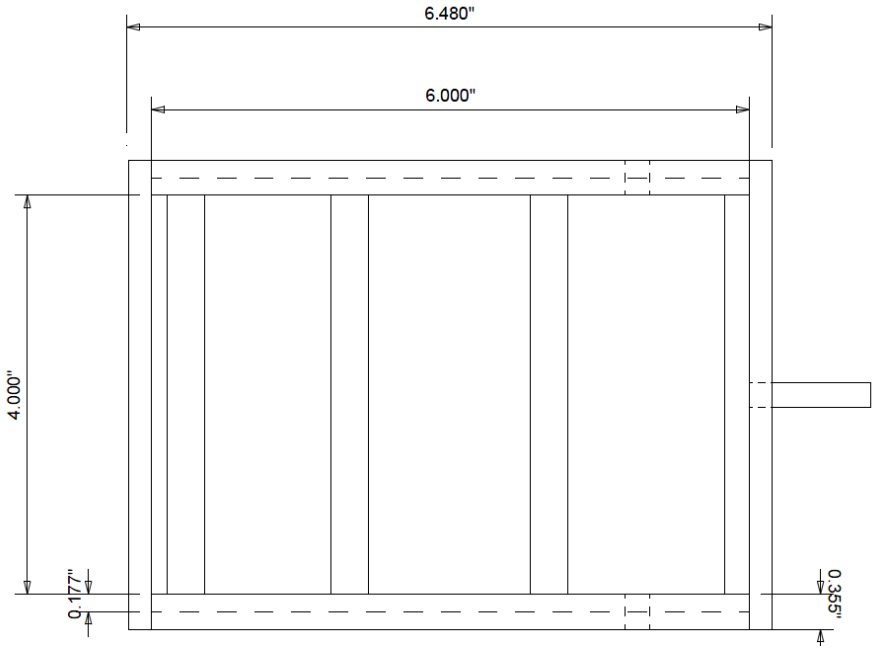

Figure A.2 Top View

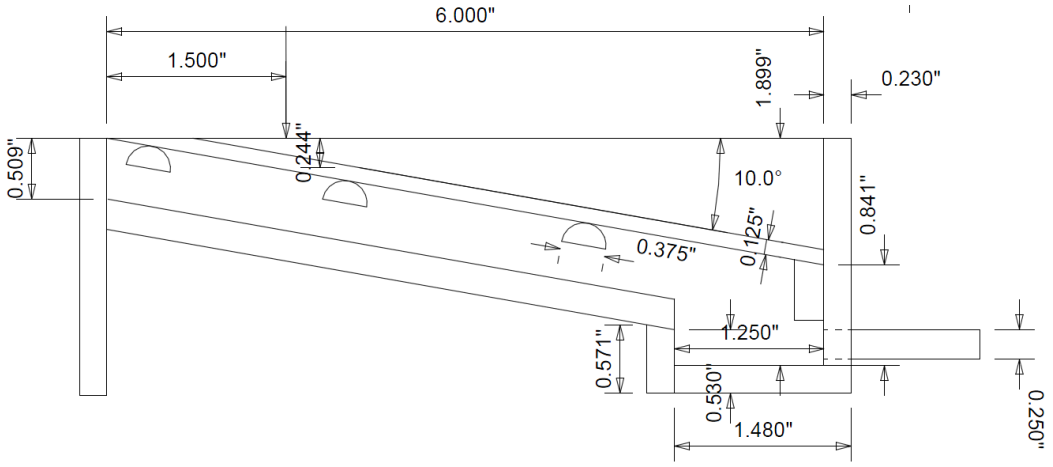

Figure A.3 Side View

9.2 Appendix B – Procedures

Various experimental procedures are listed below.

9.2.1 Sample extraction for microscopy.

1. Extract 1mL sample using autopipetteman and transfer to 15mL centrifuge tube
2. Extract 10 µL and deposit onto microscope glass slide with coverslip.
3. Repeat step 2 for a total of two individual samples to be photographed on the slide
4. Place under light microscope at 10x magnification
5. Take 3 photographs of each sample

*9.2.2 Pre-harvest Concentration**

1. Agitate reactor with stir bar to mix up any clumps that settled out in bottom of reactor
2. Find tare weight for glass fiber filter and load into filter apparatus
3. Extract 30 mL and add to glass filter vessel
4. Remove liquid from sample using vacuum flask
5. Rinse sample with deionized water
6. Dry sample
7. Record final weight.

9.2.3 Post-harvest Concentration

*Use same procedure for post-harvest dry-weight concentration measurement

9.2.4 Harvest Cycle

1. Load filter holder with steel mesh and seal.
2. Activate peristaltic pump and adjust flow setting to 3.5.
3. Run pump for 30 minutes.
4. Turn off pump.
5. Close off reactor feed and return lines.

6. Remove mesh from filter holder with harvested spirulina.
7. Rinse spirulina into open vessel with deionized water.
8. Record volume of filtrate.
9. Ensure filtrate is mixed well.

9.2.5 Filtrate Concentration

Perform a dry weight concentration measurement using a 5 mL volume instead of 30mL as specified in the Pre-harvest dry weight concentration measurement procedure