THE PALOS VERDES
ARTS AND SCIENCE JOURNAL

Article

# Fueling the Future: Isolation of Chlorella vulgaris Biomass from Novel Media Structure

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## SUMMARY

**Chlorella vulgaris is a species of unicellular green algae celebrated for its many uses in industry and agriculture. Its potential in the aerospace industry as a multifunctional material is a priority. Biofuel companies have also explored Chlorella lipid content as a viable option for sustainable biofuel production. Current systems of cultivation face challenges associated with growth and harvesting efficiency. A novel solid-media agarose culture was developed, tested, and patented by the author to solve issues stemming from liquid-media culture in zero-gravity conditions. This medium has potential terrestrial utility and can accelerate carbon dioxide sequestration. The hypothesis is that this solid media method of algal growth will evenly distribute light, heat, and nutrients to C. vulgaris, allowing the medium to match the growth of algae in liquid medium. In addition, biomass isolation from the agarose medium will have comparable results to liquid medium. Results support the viability of the solid media system in increasing C. vulgaris population density. Analysis of spectrophotometer data displays consistent growth cycles for both solid and liquid media algae, with solid media outperforming liquid media. Moreover, biomass dry weight results support the feasibility of extraction for both medium types. Future research could focus on the many applications of algae growth in solid medium. In the aerospace industry, a new method of micronutrient solidification could create a reliable method for algae cultivation in astronaut supplementation. Use of the agarose medium on other algae species or even other plant species could revolutionize agricultural practices.**

## INTRODUCTION

Hailed as a “material of the future,” algae has been thrust into the spotlight of scientific inquiry due to its multifaceted nature. Beyond its historical application as a superfood for civilizations, a multitude of novel uses for the protist have been discovered.

Chlorella vulgaris is a species of unicellular green algae with demonstrated potential in a variety of industrial fields. As a nutritional supplement, C. vulgaris holds large amounts of vitamin B12, folate, and dietary fiber (Bito et al., 2020). These properties translate to immunomodulatory and anti-carcinogenic effects, leading algae to outperform some dairy and meat-based products in both sustainability and nutritional content.

As an integral part of bioremediation, algae functions as an effective absorber of agricultural and metallic wastes, taking up orthophosphates, cadmium, lead, and nickel, among others (Manzoor et al., 2019). In the dental industry, C. vulgaris has proven useful in the reduction of lead, tin, and other heavy metal accumulation in patients with long-term fillings (Merino et al., 2019).

Within the last decade, companies searching for cleaner alternatives to petroleum in fuel production have turned to C. vulgaris as an efficient and consistent biomass producer. Pharmaceutical companies have begun research into methods of accelerating algal growth through genetic engineering. New products are consistently developed in the field of algae, where algae dyes, plastics, and cosmetics have become increasingly prevalent in everyday consumer lives. With its myriad applications, algae serves as a “one-size-fits-all” material: an invaluable trait for aerospace travel.

Beginning in 2019, the National Aeronautics and Space Administration (NASA) has investigated the potential of algae

(particularly C. vulgaris) as a multifunctional material and bioregenerative life support system (BLSS) (Fahrion et al., 2021). Chlorella's simple structure favors its utility in a bioregenerative system. In comparison to solar or even nuclear energy, a culture of C. vulgaris can be sustained almost indefinitely, providing a source of food, fuel, polymer production and more. The fact that C. vulgaris is a unicellular organism also works to its benefit, as algae systems can be extremely compact and modular. To explore this concept, NASA collaborated with Dr. Gisela Detrell on a 2019 project: Photobioreactor at the Life Support Rack (PBR@LSR), a project aimed at testing the viability of algae in a photobioreactor (PBR).

While different PBR structures exist, they generally hold algae in an enclosed system that exchanges carbon dioxide, light, and nutrients for oxygen and biomass. In the 2019 NASA study, algae cells suspended in water were circulated around a lighting element, with a mechanical setup of pumps, pressure valves, and carbon dioxide canisters providing contingencies for such a complicated system (Detrell et al., 2019). The requirement of algae to be in a liquid form has been the largest setback to its otherwise high potential, as mechanical complexity reduces spatial and system efficiency, and creates multiple points of potential failure. In realistic aerospace applications, a system leaking liquids would pose a risk to electrical components, living quarters, and air filtration. In the case of PBR@LSR, the photobioreactor system failed following two weeks of growth due to pump system failure (Detrell et al., 2020).

Given the unpredictable nature of liquid algae media based systems, a novel solid media agarose system was developed, tested, and patented by the author. Such a system would be useful in outer space, as its ability to cultivate algae without pumps and other liquid-media requirements would greatly increase reliability. The ability of agarose to shift from liquid to solid with temperature also ensures a flexible material not compromising on the initial benefit of space-efficiency. Through multiple series of experimentation, the solid media system was not only comparable to liquid growth media in growth density and carbon dioxide sequestration, but exceeded the industry standard by upwards of 20 to 30 percent (Lin, 2023). Experimental results not only substantiated the possibility and potential for a new system of algal growth in outer space, but also a method of increasing the consistency of biofuel and bioplastic production to meet consumer demand on Earth.

In this experiment, cultures of C. vulgaris immobilized in the previously developed agarose-based medium are grown in a photobioreactor system at a large scale (2.5 L algae per photobioreactor). This system is compared to current industry standard, liquid-media form of growth, using measurements of population density. Another objective of this experiment is to determine a method of algal isolation from the agarose medium, and to assess the feasibility of extracting algae from the solid media system.

The hypothesis of this project is that the solid media method of algal growth will evenly distribute light, heat, and nutrients to C. vulgaris culture, allowing the medium to match the growth of algae in liquid medium. In addition, biomass isolation from the agarose medium will have comparable results to liquid medium.

If functional, solid media cultivation systems could prove extremely useful for the transportation, production, and sustainability of algae in both terrestrial applications and aerospace missions.

## RESULTS

Two categories of data are collected throughout each two-week experimental period: spectrophotometry data (absorbance) tracking growth during the two weeks, and biomass (dry weight) from extraction following two weeks of growth.

A sample of a spectrophotometry graph is shown in Figure 4. The wavelengths of 430.4 nm, 449.9 nm, 639.8 nm, and 662.0 nm correspond to chlorophyll a (430.4 nm, 662.0 nm) and chlorophyll b (449.9 nm, 639.8 nm). Chlorophyll a and b are found abundantly in photosynthetic organisms such as Chlorella vulgaris, enabling the absorbance values at these wavelengths to be indicators of favorable growth.

A sample of the calculations for data

compilation and results graphing are displayed in Figures 8 and 9, respectively. Absorbance data values for the four wavelengths over the experimental period are collected, tabulated, and organized in terms of time period and population density change.

The change in population density for liquid media is calculated using the equation =(((fL+(iL-iS))-(iL1+(iL-iS)))/(iL1+(iL-iS)))*100, where iL1 represents the first value of liquid absorbance, fL represents the second value of liquid absorbance, iL represents the initial value of liquid absorbance, and iS represents the initial value of solid (agarose) absorbance. The equation is based on the general percent change formula ((f-i)/(i))*100, but altered because solid media absorbance begins higher than liquid media absorbance. To limit overrepresentation of changes in liquid media population density, the difference between liquid and solid media is set in the equation. To achieve this function in a digital spreadsheet, the equation =(((B16+($C$15-$B$15))-(B15 ($C$15-$B$15)))/(B15+($C$15-$B$15)))*100 is used (dollar signs represent unchanging values). As the liquid media population density changes are balanced to meet the solid media population density changes, the equation for solid media is simply the percent change formula: ((f-i)/(i))*100. In the digital spreadsheet, the equation =((C5-C4)/C4)*100 is utilized.

The values of liquid and solid media population density change for all ten sets of experimentation are categorized into four population change sections based on wavelengths: 430.4 nm, 449.9 nm, 639.8 nm, and 662.0 nm. Graphs for percent change in population density of liquid media algae, solid media algae, and the difference between both types are created; images of the twelve graphs (organized into 430.4 nm, 449.9 nm, 639.8 nm, and 662.0 nm, respectively) are included in Figures 10, 11, 12, and 13. Error bars are calculated and included in the graphs.

Figures 10, 11, 12, and 13 display consistent growth patterns for liquid and solid media over the experimental period. The liquid media culture displays growth during the first week, peaking at days 8-10 with 6.30% density increase. In comparison, the solid media culture peaks at growth during days 6-8 with an average increase of 33.02%. Once the period 8-10 has passed, both cultures display decreases in growth productivity, with liquid media growth decreasing to -6.54% and solid media growth decreasing to 20.15%. Solid media population density is also found to increase to a greater degree than liquid media population density during the beginning of the growth cycle, with a 31.86% increased growth percentage compared to liquid media. As population absorbance approaches the maximum of the absorbance software (3.000), the precision of absorbance readings decreases. In the case of solid media agarose, this restriction leads to a seeming decrease in population productivity during the period of days 12-14.

The second category of data collection is biomass dry weight. Once data values are obtained from the scale, they are tabulated (Figure 14). These values are averaged for two sets of data (20 data points), where the average weight of liquid media isolations is found to be 87.0 mg and the average weight of solid media isolations is found to be 87.7 mg. Using the relative change formula, the percent difference between both data points is 0.80%.

## DISCUSSION

The objectives of this project are to observe the growth of Chlorella vulgaris in an experimental medium, determine a method of isolating algal biomass from the medium, and quantify algal biomass for growth productivity and potential application in industry.

Analysis of spectrophotometer data displays consistent growth cycles for both solid and liquid media algae over each two-week experimental period. Both media types display growth trends similar to the general life cycle of algae, with seven days of growth, followed by population decrease (due to a carrying capacity being reached). While both systems display reductions in population density change during the second week, the solid media system has a longer period of growth (up to 33.63% growth during days 12-14) versus the liquid media system (up to 6.40% growth during days 6-8). In the error percentage calculations (Figures 15.1, 15.2, 15.3, 15.4), both cultures are found to have rates of error below 10%, besides two outliers of 15.09% and 15.42% for solid media at wavelengths 662.0 nm and 639.8 nm,

respectively. As shown in Figure 16, the up to 34.58% average difference between solid and liquid media growth suggests the utility of agarose medium in enhancing algal growth.

A novel method of algal extraction is necessary to functionally isolate algal cells from a solid medium. Various isolation methods have been tested, with vacuum filtration showing promise as a potential method of extraction (Figure 5). The use of a preheating procedure for proper agarose passage through filter paper allowed for the collection of algae on 0.22 um filter papers. In the experiment, solid media algae was heated to 45 degrees Celsius and water was heated to 60 degrees Celsius for proper passthrough. Further experimentation with temperature variability to allow survival of algae cells can be explored.

With the data collected by vacuum filtration, the average mass of isolated liquid media algae (filter included) is 87.0 mg, compared to the solid media mass of 87.7 mg. This difference is represented by a change of 0.80%. With the included error percentage of 9.07% (Figure 17), this difference is not significant.

Experimentation is conducted on a small sample size, with further 1:9 dilution to prevent membrane fouling (the changed properties of filters during filtration) and pore size complications, common issues in filtration systems (Erkan et al., 2018). Additional testing on pore size and scraping methods can further refine the filtration method and allow subsequent utility of the method on a large scale.

While filter-based methods show potential, current limitations exist. Other isolation techniques have been considered and tested. Methods such as centrifugation or gravity-assisted isolations would not work for a solid, agarose-based material. A potential solution arises in the use of flocculants: namely, aluminum sulfate and chitosan (Machado et al., 2024). The ability of flocculants to isolate up to 90% of an algal culture in ten minutes, as well as structural stability to 220 degrees Celsius, may prove the key to effective isolation and quantification of the experimental agarose medium (Grząbka-Zasadzińska et al., 2017). In the near future, the environmentally-friendly flocculation agent chitosan will be tested for use in algal isolation.

Throughout experimentation, precautions were taken to ensure experimental accuracy. All materials were sterilized and acid washed before and after use, only one culture of algae was handled with a clean pair of gloves to prevent contamination, and a mask, gloves, and goggles were used consistently. During the growth period, petri dishes were held in a plexiglass storage container with a sliding door system to prevent contamination. Data collection was conducted three times for each spectrophotometric set, with a total 1680 data points. Data collection on biomass was conducted using a precise milligram scale and measured in an enclosed shell, and 20 data points were collected. Following experimentation, error analysis was conducted.

The hypothesis of the experiment was proven correct. The agarose-based algae medium not only supports algal growth, but surpasses liquid media growth over a two-week period. Biomass isolation shows comparability between algae of solid and liquid media types.

Results support the viability of the solid media system in increasing Chlorella vulgaris population density in multiple industries. The ability of the medium to support increased and prolonged growth cycles could be extremely useful in the aerospace industry, where a quick-growing algal gel could provide astronauts with a sustainable and bioregenerative source of food. On Earth, solid media systems would be much easier to transport, in addition to their benefits to consumer markets. The increased presence of algae in consumer products could be supported by a faster growing material, decreasing costs as sustainability becomes much easier to achieve. As a solution to humanity's existential problems, solid-media algae could sequester large volumes of carbon dioxide over long periods of time, alleviating the climate crisis and creating a source of industrial biomass.

Future experimentation could focus on the many applications of algae growth in solid medium. In the aerospace industry, a new method of micronutrient solidification could create a reliable growth method for algae cultivation in astronaut supplementation. The use of the agarose medium on other algae species (such as Medakomo Hakoo) or even

other plant species could revolutionize agricultural cultivation. Experimentation with the limits of the solid medium isolation properties could yield higher efficiency in biomass collection.

With all its applications, algae holds substantial promise in today's emphasis on sustainability. Fueled by an experimental medium, the future of cleaner, greener energy can be realized.

## MATERIALS AND METHODS

**Materials:**

50 mL Chlorella vulgaris from Algae Research Supply, distilled water, 1 Vernier GoDirect SpectroVis Plus Spectrophotometer, 151 3.5 mL standard disposable cuvettes, Benchmark Scientific low melting point agarose, 11 incandescent light bulbs, lamp covers and clamps, 8 Commercial Electric 305x305x23 mm color-changing LED panels, plexiglass plating (the shell of the photobioreactor system), 2 air pumps, plastic tubing (for algae culture growth), 6 plexiglass rods, 6 3000 mL glass jars, 7 1000 mL beakers, 7 500 mL beakers, 1 100 mL beaker, 1 50 mL beaker, 5 100 mL plastic beakers, 3 250 mL Erlenmeyer flasks, vacuum pump, 100 60 mm diameter, 0.22 um pore size filter papers, 1 300 mL Buchner funnel, 1 500 mL vacuum filtration flask, plastic tubing (for vacuum filtration), 32 245x245x25 mm petri dishes, 40 1 mL pipettes, 20 10 mL syringes, pestle and mortar, 600 watt microwave, weighing scale, 100 weigh boats, 5 mL micropipette, 50 5 mL micropipette tips, temperature resistant glove, thermometer, temperature controlled refrigerator, hot plate and stirrer, 5 stir bars, chemical wipes, metallic forceps, Guillard's F/2 micronutrient (45 mL Part A, 45 mL Part B).

Methods:

Throughout the course of the experiment, a steady culture of Chlorella vulgaris is maintained for experimental use. An image of the algae culture setup is shown in Figure 1. A beginning solution of 50 mL C. vulgaris is inoculated into a 450 mL micronutrient solution, creating a 1:9 inoculation ratio. This micronutrient solution is composed of 450 mL fresh water and 0.25 mL each of Guillard's F/2 micronutrients A and B. A fluorescent bulb is used as a source of light and heat (25 degrees Celsius being the optimal temperature for growth). The algae is grown with constant lighting in a 24-hour cycle. Air tubing attached to plexiglass rods are used to remove gasses produced by the algae and to circulate the algae; the rate of 100 rpm is approximated to stimulate algal growth. Micronutrients are added every third and seventh day in a week (Tuesday, Saturday) at a concentration of 0.5 mL each of parts A and B per 1000 mL algae solution, allowing for further growth. Every seven days, each culture is split into two parts, and micronutrient solution and water is added to replenish the lost volume and allow for further algal growth. This process is repeated over the course of a month, resulting in a minimum of 12 L algae at any given time.

Four photobioreactor cores have been designed and built for use in experimentation; the design of a core is shown in Figure 2. The fabrication process includes the creation of a hard acrylic box with sliding doors. Rack rails are affixed to the sides of the box at 8.31 cm intervals, and acrylic racks are placed on the rails to hold petri dishes. Light panels are attached to the sides of the photobioreactor to provide algae cultures the conditions for growth. The purpose of the photobioreactors is to house the algae in a stable environment throughout the course of experimentation. An image of a functioning core with algae is displayed in Figure 3.

Utilizing the algae grown in the aforementioned process, 10 experimental algae plates are created for use in two of the photobioreactor cores, including 5 liquid media plates and 5 solid media plates. 2.5 L of algae culture is utilized for each experimental set.

Each liquid media algae plate is made by creating a 500 mL, 1:1 solution composed of 250 mL of C. vulgaris culture, and 250 mL micronutrient solution (0.25 mL of each micronutrients A and B incorporated into 250 mL freshwater). This solution is poured into a petri dish.

Each solid media algae plate is made with 250 mL of C. vulgaris culture and 250 mL agarose micronutrient solution composed of a 2% agarose solution and 0.125 mL each of micronutrients A and B. The agarose micronutrient solution is created by adding 5 g

of low melting point agarose powder to 250 mL water. This solution is heated in a 600-watt microwave oven in 30-second intervals until homogenous. The resulting solution is allowed to cool. Once the agarose solution reaches a temperature of 45 degrees Celsius, 0.125 mL of each micronutrients A and B are added. When the mixture reaches a temperature of 38.7 degrees Celsius, the algae solution is incorporated, and the resulting mixture is poured into a petri dish.

The 10 petri dishes are placed in the two photobioreactor cores, with one core containing five liquid media algae plates, and one core containing five solid algae plates. These algae plates are grown with a constant (24-hour) light system.

The two cores are grown in an open system, with 20 mL micronutrient solution (20 mL freshwater with 0.26 mL each micronutrients A and B) added every 2 days. This system is utilized to simulate field application, where oxygen and carbon dioxide levels are not restricted by a complete seal.

Spectrophotometric measurements are performed every 2 days throughout the 14-day experimental timeframe. A sample of each culture is taken; this is achieved by using a pipette and agitating the liquid in the liquid media petri dish before placing a sample within a cuvette, and homogenizing a portion of the solid media algae that is cut from the dish to place within a second cuvette. Homogenization of the solid media algae is conducted in a plastic beaker using a pestle. Cuvettes are loaded into a Vernier spectrophotometer and measured for light absorbance. Spectrophotometry is repeated three times for each sample to ensure experimental consistency. Absorbance values at the wavelengths 430.4 nm and 662.0 nm (corresponding to chlorophyll a) and 449.9 nm and 639.8 nm (corresponding to chlorophyll b) are recorded.

A sample absorbance graph from a spectrophotometer is shown in Figure 4. By obtaining the absorbance values for pigments found most abundantly in C. vulgaris, growth data is traced and graphed over time.

The above experimental process is conducted two times (ten total datasets) to validate experimental results.

Biomass isolation is conducted once the two-week experimental period has concluded; the process utilizes vacuum filtration to isolate and allow for the quantification of algal biomass. A picture of the isolation setup is shown in Figure 5.

For solid media algae plates, approximately 100 mL algae volume is excised and placed within a 250 mL beaker. The mixture is heated to 45 degrees Celsius to melt the agarose, of which 10 mL agarose solution is removed and placed in another 250 mL beaker. 90 mL distilled water is added to create 100 mL dilute solid media solution. This dilute solution is placed on a hot plate to maintain the temperature.

For liquid media algae plates, 10 mL solution is removed and placed in a 250 mL beaker. 90 mL distilled water is added to bring total volume to 100 mL dilute liquid media solution. This dilute solution is placed on a hot plate to maintain the temperature.

To prepare the vacuum filtration setup, 1000 mL water is heated in the microwave until it reaches a temperature of 80 degrees Celsius. A buchner funnel is connected to a vacuum filtration flask, clamped in place, and connected by air tubing to a vacuum pump. One disc of 60 mm diameter, 0.22 micrometer filter paper is then weighed and placed on the buchner funnel.

To begin the isolation process, 200 mL heated water (60 degrees Celsius) is added to the buchner funnel. This process both prepares the filter paper for liquids to pass through and enables the solid medium solution to pass through filtration without solidifying. The heated water is emptied from the filtration flask, and the vacuum filtration setup is reassembled. 10 mL of the dilute solution (either liquid media or solid media) is then added, and allowed to flow through the filter paper. Next, 200 mL heated water is added to the buchner funnel to account for residual algae on the sides of the funnel. An image of isolated algae is shown in Figure 6.

Once algae is collected on the filter paper, the vacuum is released, forceps are used to collect the filter paper, and the filter paper is placed on a chemical wipe (which assists in moisture removal). The filter paper is sealed in a box and dried over the course of 24 hours. The sample is then weighed in a precise milligram scale. Data on biomass dry weight are collected for analysis. An image of the

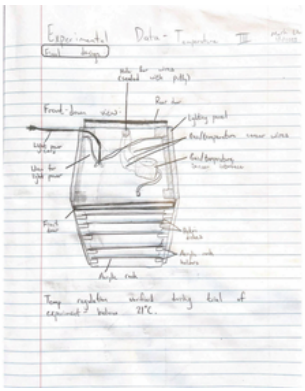
scale setup is shown in Figure 7.

## REFERENCES


- Bito, T., Okumura, E., Fujishima, M., & Watanabe, F. (2020, August 20). Potential of Chlorella as a Dietary Supplement to Promote Human Health. NLM. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7551956/
- Detrell, G., Helisch, H., Keppler, J., & Martin, J. (2019, July). PBR@LSR: the Algae-based Photobioreactor Experiment at the ISS -Configuration and Operations. Research Gate. https://www.researchgate.net/publication/334364229_PBRLSR_the_Algae-based_Photobioreactor_Experiment_at_the_ISS_-Configuration_and_Operations
- Detrell, G., Helisch, H., Keppler, J., & Martin, J. (2020, July). (PDF) PBR@LSR: The Algae-based photobioreactor ... Research Gate. https://www.researchgate.net/publication/344427196_PBRLSR_the_Algae-based_Photobioreactor_Experiment_at_the_ISS_-_Operations_and_Results
- Erkan, H. S., Turan, N. B., & Engin, G. Ö. (2018, March 27). Membrane bioreactors for wastewater treatment. Comprehensive Analytical Chemistry. https://www.sciencedirect.com/science/article/abs/pii/S0166526X18300035
- Fahrion, J., Mastroleo, F., Dussap, C.-G., & Leys, N. (2021, June 29). Use of Photobioreactors in Regenerative Life Support Systems for Human Space Exploration. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8281973/
- Grząbka-Zasadzińska, A., Amietszajew, T., & Borysiak, S. (2017, March 21). Thermal and mechanical properties of chitosan nanocomposites with cellulose modified in ionic liquids - journal of thermal analysis and Calorimetry. SpringerLink. https://link.springer.com/article/10.1007/s10973-017-6295-3#:~:text=Chitosan%20exhibits%20a%20high%20sensitivity,220%20%C2%B0C%20%5B37%5D
- Lin, M. (2023, August 22). Highly scalable and practical method for immobilizing and efficiently culturing algae for various applications. https://patents.google.com/patent/US11732235B1/en
- Machado, C. A., Esteves, A. F., & Pires, J. C. M. (2024, January 10). Chlorella vulgaris harvesting: Chemical flocculation with chitosan, aluminum sulfate, and ferric sulfate. MDPI. https://www.mdpi.com/2076-3417/14/2/598#:~:text=Inorganic%20flocculants%20were%20very%20effective,vulgaris
- Manzoor, F., Karbassi, A. R., & Golzary, A. (2019, July). Heavy metal contaminants removal from wastewater by using chlorella ... Heavy Metal Contaminants Removal from wastewater by using Chlorella vulgaris: A Review. https://www.researchgate.net/publication/334641350_Heavy_Metal_Contaminants_Removal_from_wastewater_by_using_Chlorella_vulgaris_A_Review
- Merino, J. J., Parmigiani-Izquierdo, J. M., Gasca, A. T., & Cabaña-Muñoz, M. E. (2019, April 16). The Long-Term Algae Extract (Chlorella and Fucus sp) and Aminosulphurate Supplementation Modulate SOD-1 Activity and Decrease Heavy Metals (Hg++, Sn) Levels in Patients with Long-Term Dental Titanium Implants and Amalgam Fillings Restorations. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6523211/
- Torres-Tiji, Y., Fields, F. J., & Mayfield, S. P. (2020, March 16). Microalgae as a future food source. Biotechnology Advances. https://www.sciencedirect.com/science/article/abs/pii/S0734975020300331?via%3Dihub

## APPENDIX

**Figure 1** Algae culture and experimental setup.



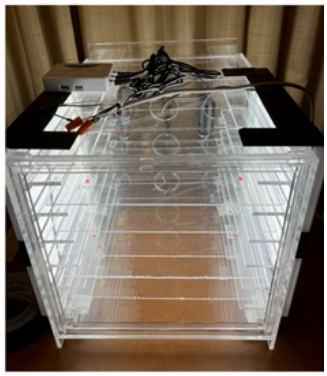


**Figure 2** Design and image of photobioreactor core.

**Figure 3** Functioning photobioreactor core.

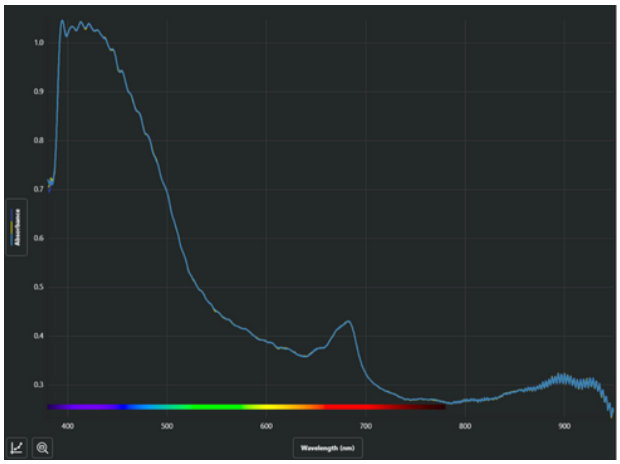


**Figure 4** Sample of Chlorella vulgaris light absorbance graph generated by Vernier spectrophotometer (Experiment 1.1 Day 2 - Algae culture in solid media).

**Figure 5** Vacuum filtration setup

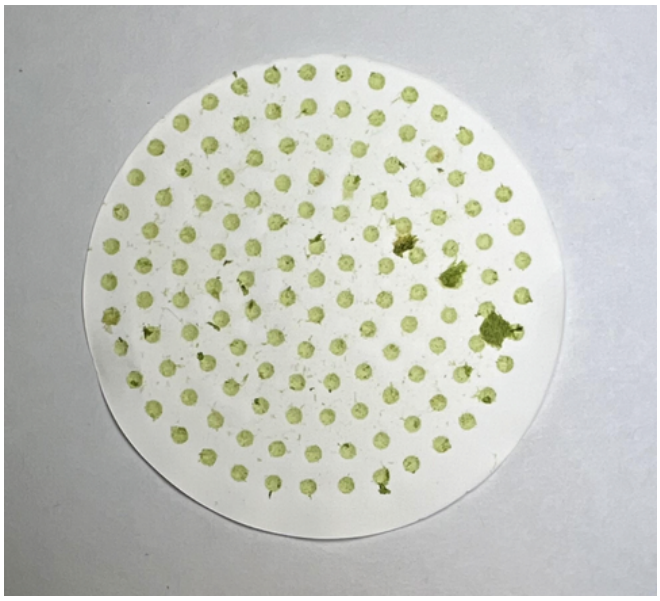


**Figure 6** Isolated algae on filter paper (Experiment 2.3, Algae culture in solid media)

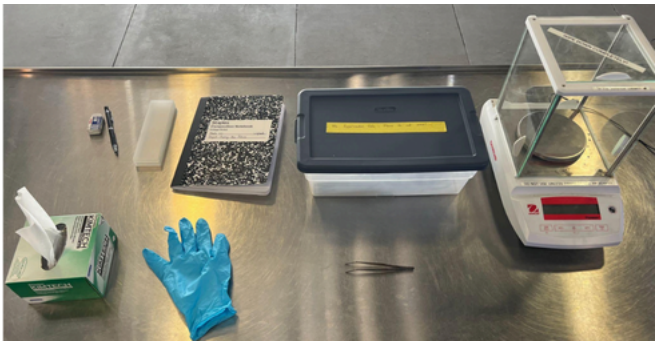
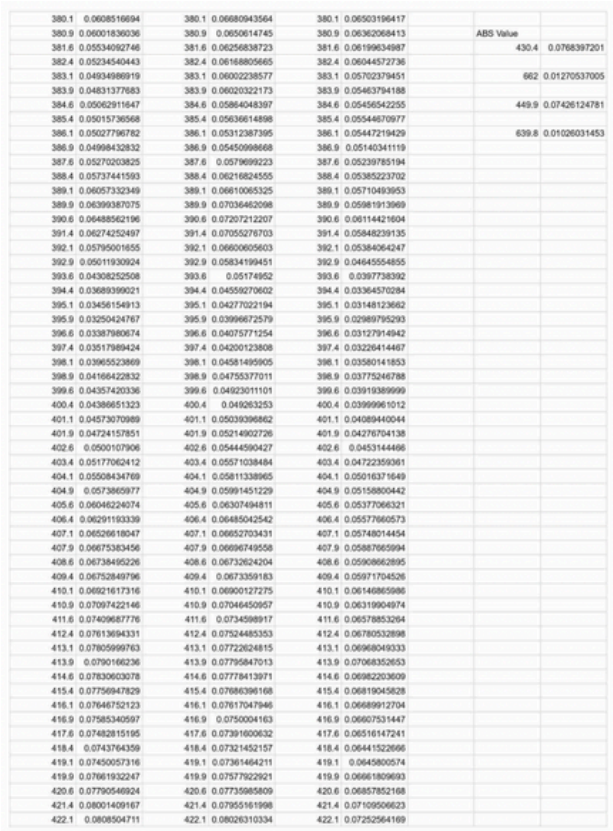


**Figure 7** Scale setup for biomass measurement



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|---|---|---|---|---|---|---|---|
| 380.1 | 0.0608516694 | 380.1 | 0.06680943564 | 380.1 | 0.06503196417 | | |
| 380.9 | 0.0600183603 | 380.9 | 0.0650614745 | 380.9 | 0.06362068413 | ABS Value | |
| 381.6 | 0.0553409274 | 381.6 | 0.06256838723 | 381.6 | 0.06199634987 | 430.4 | 0.0768397201 |
| 382.4 | 0.0523454044 | 382.4 | 0.06168805665 | 382.4 | 0.06044572736 | | |
| 383.1 | 0.0493498691 | 383.1 | 0.06002238577 | 383.1 | 0.05702379451 | 662 | 0.01270537005 |
| 383.9 | 0.0483137768 | 383.9 | 0.0602032217 | 383.9 | 0.05463794188 | | |
| 384.6 | 0.0506291164 | 384.6 | 0.05864048397 | 384.6 | 0.05456542255 | 449.9 | 0.07426124781 |
| 385.4 | 0.0501573656 | 385.4 | 0.05636614898 | 385.4 | 0.05544670977 | | |
| 386.1 | 0.0502779678 | 386.1 | 0.05312387395 | 386.1 | 0.05447219429 | 639.8 | 0.01026031453 |
| 386.9 | 0.0499843283 | 386.9 | 0.05450998668 | 386.9 | 0.05140341119 | | |
| 387.6 | 0.0527020382 | 387.6 | 0.0579699223 | 387.6 | 0.05239785194 | | |
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| 389.9 | 0.0639938707 | 389.9 | 0.07036462098 | 389.9 | 0.05981913969 | | |
| 390.6 | 0.0648856219 | 390.6 | 0.07207212207 | 390.6 | 0.06114421604 | | |
| 391.4 | 0.0627425249 | 391.4 | 0.07055276703 | 391.4 | 0.05848239135 | | |
| 392.1 | 0.0579500165 | 392.1 | 0.06600605603 | 392.1 | 0.05384064247 | | |
| 392.9 | 0.0501193092 | 392.9 | 0.05834199451 | 392.9 | 0.04645554855 | | |
| 393.6 | 0.0430825250 | 393.6 | 0.05174952 | 393.6 | 0.0397738392 | | |
| 394.4 | 0.0368939902 | 394.4 | 0.04559270602 | 394.4 | 0.03364570284 | | |
| 395.1 | 0.0345615491 | 395.1 | 0.04277022194 | 395.1 | 0.03148123662 | | |
| 395.9 | 0.0325042476 | 395.9 | 0.03996672579 | 395.9 | 0.02989795293 | | |
| 396.6 | 0.0338798067 | 396.6 | 0.04075771254 | 396.6 | 0.03127914942 | | |
| 397.4 | 0.0351798942 | 397.4 | 0.04200123808 | 397.4 | 0.03226414467 | | |
| 398.1 | 0.0396552386 | 398.1 | 0.04581495905 | 398.1 | 0.03580141853 | | |
| 398.9 | 0.0416642283 | 398.9 | 0.04755377011 | 398.9 | 0.03775246788 | | |
| 399.6 | 0.0435742033 | 399.6 | 0.04923011101 | 399.6 | 0.03919389999 | | |
| 400.4 | 0.0438665132 | 400.4 | 0.049263253 | 400.4 | 0.03999961012 | | |
| 401.1 | 0.0457307098 | 401.1 | 0.05039396862 | 401.1 | 0.04089440044 | | |
| 401.9 | 0.0472415785 | 401.9 | 0.05214902726 | 401.9 | 0.04276704138 | | |
| 402.6 | 0.0500107906 | 402.6 | 0.05444590427 | 402.6 | 0.0453144466 | | |
| 403.4 | 0.0517706241 | 403.4 | 0.05571038484 | 403.4 | 0.04722359361 | | |
| 404.1 | 0.0550843476 | 404.1 | 0.05811338965 | 404.1 | 0.05016371649 | | |
| 404.9 | 0.057386597 | 404.9 | 0.05991451229 | 404.9 | 0.05158800442 | | |
| 405.6 | 0.0604622407 | 405.6 | 0.06307494811 | 405.6 | 0.05377066321 | | |
| 406.4 | 0.0629119333 | 406.4 | 0.06485042542 | 406.4 | 0.05577660573 | | |
| 407.1 | 0.0652661804 | 407.1 | 0.06652703431 | 407.1 | 0.05748014454 | | |
| 407.9 | 0.0667538345 | 407.9 | 0.06696749558 | 407.9 | 0.05887665994 | | |
| 408.6 | 0.0673849522 | 408.6 | 0.06732624204 | 408.6 | 0.05908662895 | | |
| 409.4 | 0.0675284979 | 409.4 | 0.0673359183 | 409.4 | 0.05971704526 | | |
| 410.1 | 0.0692161731 | 410.1 | 0.06900127275 | 410.1 | 0.06146865986 | | |
| 410.9 | 0.0709742214 | 410.9 | 0.07046450957 | 410.9 | 0.06319904974 | | |
| 411.6 | 0.0740968777 | 411.6 | 0.0734598917 | 411.6 | 0.06578853264 | | |
| 412.4 | 0.0761369433 | 412.4 | 0.07524485353 | 412.4 | 0.06780532898 | | |
| 413.1 | 0.0780599976 | 413.1 | 0.07722624815 | 413.1 | 0.06968049333 | | |
| 413.9 | 0.079016623 | 413.9 | 0.07795847013 | 413.9 | 0.07068352653 | | |
| 414.6 | 0.0783060307 | 414.6 | 0.07778413971 | 414.6 | 0.06982203609 | | |
| 415.4 | 0.0775694782 | 415.4 | 0.07686396168 | 415.4 | 0.06819045828 | | |
| 416.1 | 0.0764675212 | 416.1 | 0.07617047946 | 416.1 | 0.06689912704 | | |
| 416.9 | 0.0758534059 | 416.9 | 0.0750004163 | 416.9 | 0.06607531447 | | |
| 417.6 | 0.0748281519 | 417.6 | 0.07391600632 | 417.6 | 0.06516147241 | | |
| 418.4 | 0.074376435 | 418.4 | 0.07321452157 | 418.4 | 0.06441522666 | | |
| 419.1 | 0.0745005731 | 419.1 | 0.07361464211 | 419.1 | 0.0645800574 | | |
| 419.9 | 0.0766193224 | 419.9 | 0.07577922921 | 419.9 | 0.06661809693 | | |
| 420.6 | 0.0779054692 | 420.6 | 0.07735985809 | 420.6 | 0.06857852168 | | |
| 421.4 | 0.0800140916 | 421.4 | 0.07955161998 | 421.4 | 0.07109506623 | | |
| 422.1 | 0.080850471 | 422.1 | 0.08026310334 | 422.1 | 0.07252564169 | | |

**Figure 8** Sample calculations for data compilation (Page 1/14, Experiment 2.5, Day 6, Algae culture in solid media)

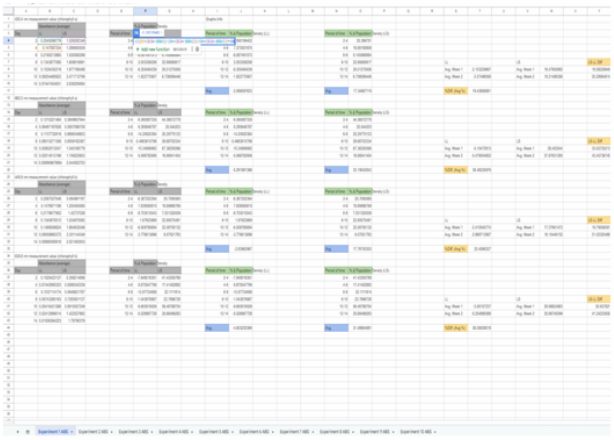

**Figure 9** Sample of calculations for results graphing (Experiment 1.1)

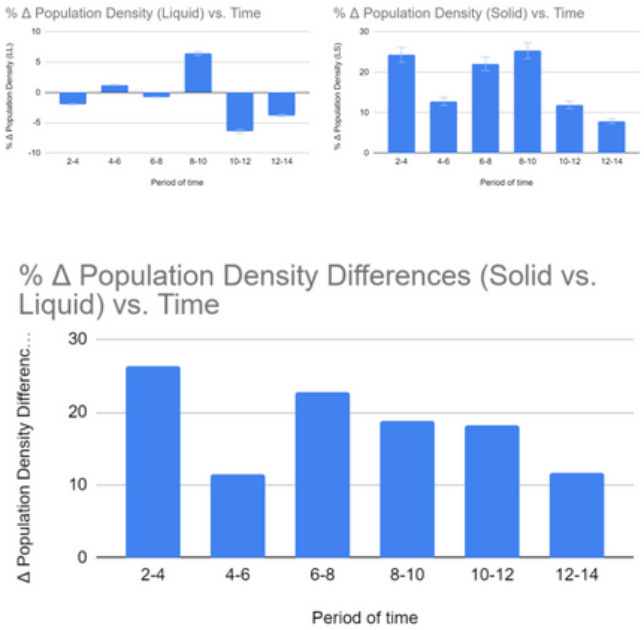


**Figure 10** Bar charts displaying change in population density at 430.4 nm (chlorophyll a): Top left represents liquid media, top right represents solid media, bottom represents comparison between solid and liquid media.

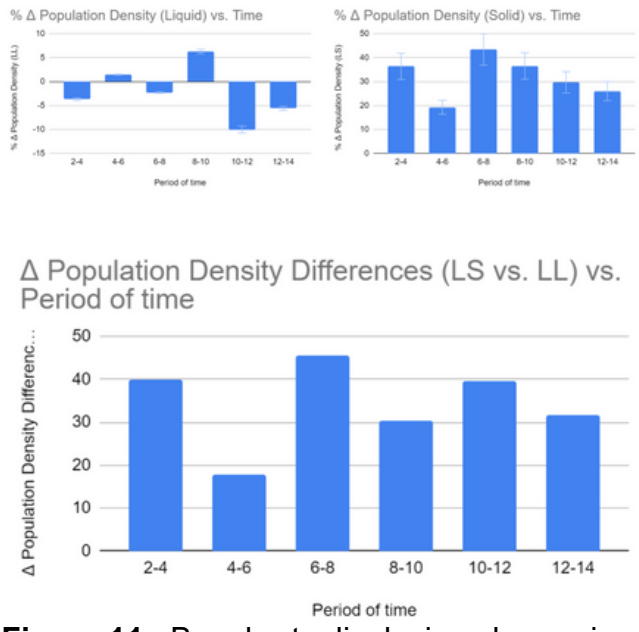


**Figure 11** Bar charts displaying change in population density at 662.0 nm (chlorophyll a): Top left represents liquid media, top right represents solid media, bottom represents comparison between solid and liquid media.

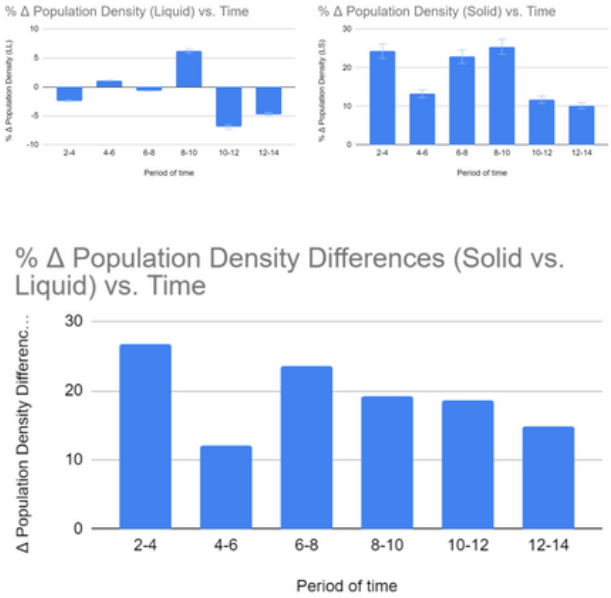


**Figure 12** Bar charts displaying change in population density at 449.9 nm (chlorophyll b): Top left represents liquid media, top right represents solid media, bottom represents comparison between solid and liquid media.

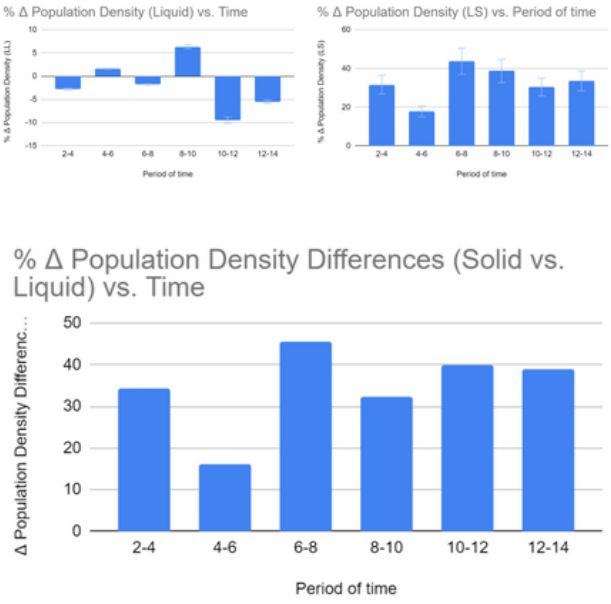


**Figure 13** Bar charts displaying change in population density at 639.8 nm (chlorophyll b): Top left represents liquid media, top right represents solid media, bottom represents comparison between solid and liquid media.

| Set 1 | | | Set 2 | |
|---|---|---|---|---|
| LL (mg) | LS (mg) | | LL (mg) | LS (mg) |
| 95 | 70 | | 83 | 95 |
| 100 | 75 | | 95 | 78 |
| 87 | 98 | | 98 | 108 |
| 80 | 96 | | 73 | 93 |
| 82 | 91 | | 77 | 73 |
| AVE | AVE | | AVE | AVE |
| 88.8 | 86 | | 85.2 | 89.4 |
| | | | | |
| | AVE LL | | AVE LS | |
| | 87 | | 87.7 | |
| | | | | |
| | | % Difference | | |
| | | 0.8045977011 | | |

**Figure 14** Biomass dry weight table for Experiments 1 and 2.

| Comparison (LS vs. LL) | |
|---|---|
| Period of time | Δ Population Density Differences (LS vs. LL) |
| 2-4 | 34.42429471 |
| 4-6 | 16.15648616 |
| 6-8 | 45.56946967 |
| 8-10 | 32.39853022 |
| 10-12 | 39.81458086 |
| 12-14 | 39.10851081 |
| | |
| Avg. % Δ Diff | 34.57864541 |

**Figure 16** Table displaying absorbance differences between solid media algae and liquid media algae at 639.8 nm.

Mean Data

| Period of time | % Δ Population Density (LL) | AveDev | Period of time | % Δ Population Density (LS) | AveDev |
|---|---|---|---|---|---|
| 2-4 | -1.962929342 | 2.720105836 | 2-4 | 24.30028524 | 8.706365395 |
| 4-6 | 1.23400829 | 5.199381021 | 4-6 | 12.78358282 | 5.951014652 |
| 6-8 | -0.77775444452 | 5.500957251 | 6-8 | 22.05531549 | 10.0371853 |
| 8-10 | 6.395608162 | 7.015529339 | 8-10 | 25.30065694 | 7.926512698 |
| 10-12 | -6.366267571 | 6.615392256 | 10-12 | 11.89705764 | 6.289788996 |
| 12-14 | -3.803046684 | 4.22965998 | 12-14 | 7.841985336 | 7.045461696 |
| | | Error Bar % | | | Error Bar % |
| | | 5.21358428 | | | 7.659388122 |

**Figure 15.1** Error calculations for absorbance at 430.4 nm.

Mean Data

| Period of time | % Δ Population Density (LL) | AveDev | Period of time | % Δ Population Density (LS) | AveDev |
|---|---|---|---|---|---|
| 2-4 | -3.673482467 | 3.111034783 | 2-4 | 36.36372319 | 12.71220549 |
| 4-6 | 1.497292144 | 4.954733504 | 4-6 | 19.29273475 | 5.59923298 |
| 6-8 | -2.309382632 | 8.323210055 | 6-8 | 43.41538393 | 17.92122121 |
| 8-10 | 6.29531919 | 10.64689861 | 8-10 | 36.53499178 | 9.009674981 |
| 10-12 | -10.0067531 | 9.806476926 | 10-12 | 29.67618682 | 18.06964737 |
| 12-14 | -5.571729003 | 6.377797601 | 12-14 | 25.99425907 | 27.21715179 |
| | | Error Bar % | | | Error Bar % |
| | | 7.203358579 | | | 15.08818897 |

**Figure 15.2** Error calculations for absorbance at 662.0 nm

Mean Data

| Period of time | % Δ Population Density (LL) | AveDev | Period of time | % Δ Population Density (LS) | AveDev |
|---|---|---|---|---|---|
| 2-4 | -2.404959454 | 2.964560347 | 2-4 | 24.31167665 | 8.289558734 |
| 4-6 | 1.101832649 | 5.668461185 | 4-6 | 13.24793282 | 5.796868724 |
| 6-8 | -0.7058119032 | 5.820838591 | 6-8 | 22.85089581 | 9.789689309 |
| 8-10 | 6.164830507 | 7.379172992 | 8-10 | 25.41605027 | 8.032433295 |
| 10-12 | -6.894368512 | 6.869727214 | 10-12 | 11.7136732 | 6.521734278 |
| 12-14 | -4.708556998 | 4.349778333 | 12-14 | 10.11362006 | 7.836603104 |
| | | Error Bar % | | | Error Bar % |
| | | 5.508756444 | | | 7.708144574 |

**Figure 15.3** Error calculations for absorbance at 449.9 nm

Mean Data

| Period of time | % Δ Population Density (LL) | AveDev | Period of time | % Δ Population Density (LS) | AveDev |
|---|---|---|---|---|---|
| 2-4 | -2.792723029 | 2.802915512 | 2-4 | 31.63157168 | 11.35821741 |
| 4-6 | 1.586934132 | 4.899228185 | 4-6 | 17.7434203 | 5.053798757 |
| 6-8 | -1.800061527 | 7.882062447 | 6-8 | 43.76940814 | 17.42783556 |
| 8-10 | 6.335257508 | 10.54326187 | 8-10 | 38.73378773 | 9.936643672 |
| 10-12 | -9.469523927 | 9.596468147 | 10-12 | 30.34505693 | 18.63714978 |
| 12-14 | -5.475471315 | 6.824427143 | 12-14 | 33.6330395 | 30.10972406 |
| | | Error Bar % | | | Error Bar % |
| | | 7.091393884 | | | 15.42056154 |

**Figure 15.4** Error calculations for absorbance at 639.8 nm

| Percent error | | | |
|---|---|---|---|
| 6.526315789 | 22.85714286 | 2.65060241 | 5.894736842 |
| 12.75 | 20 | 9.736842105 | 12.82051282 |
| 2.931034483 | 5.357142857 | 15 | 15.87962963 |
| 4.5 | 5.208333333 | 7.397260274 | 8.458781362 |
| 4.146341463 | 2.747252747 | 5.324675325 | 11.23287671 |
| AVE | AVE | AVE | AVE |
| 6.170738347 | 11.23397436 | 8.021876023 | 10.85730747 |
| | | | |
| | AVE % Error (LL) | AVE % Error (LS) | |
| | 7.096307185 | 11.04564092 | |
| | | | |
| | %Error | | |
| | 9.070974051 | | |

**Figure 17** Error calculations for biomass dry weight measurements.