UFS Winter 2014 Experiment Protocol:

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Standard Operating Procedure (SOP)

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1. Introduction

1.1 The overall objective of the UFS is to generate a robust inter-testbed dataset in algal biomass and lipid productivity and yield for a *Nannochloropsis oceanica* Cellana strain KA32 and *Chlorella vulgaris* ASU strain LRB-AZ-1201 as a function of different regional, and environmental conditions among sites by controlling non-geographical-related variables including production systems, production process and protocols, system scale, and algae strain.

1.2 A total of 6 x 1,000 L (nominal volume at 25 cm depth) identical raceway pond simulators, paddle wheels, and data logging (e.g., YSI 5200 monitoring and control system with probes for pH, temperature, oxidative reduction potential (ORP), dissolved oxygen, conductivity/salinity probes, and a PAR sensor connected to one YSI unit) have been installed at each site. The six ponds allow experiments to be conducted in triplicate for up to two experimental variables in a simultaneous, randomized design. In addition to the outdoor cultivation system harmonization, we have installed at each site an identical system for cultivation of indoor seed culture. This coupled with consistent cultivation management will allow for uniform production of starting culture across the sites in terms of volume of seed available and quality.

2. Scope

2.1 The Unified Field Study (UFS) for winter 2014 is a direct comparison of three harvests per week with one dilution rate vs. one harvest per week with one dilution rate using KA32 (*Nannochloropsis oceanica*) and LRB-AZ-1201 (*Chlorella vulgaris*).

2.2 **Objectives:**

- 2.2.1 Conduct coordinated and controlled experiments at five testbeds with both strains for up to six weeks per strain.
- 2.2.2 Collect the following seasonal data for one selected algal strains across six testbeds:
  - 2.2.2.1 Baseline cultivation data for 1 harvest per week vs. 3 harvests per week
  - 2.2.2.2 Feasibility of outdoor production for selected strains
  - 2.2.2.3 Biomass productivity
  - 2.2.2.4 FAME productivity and FAME profile
  - 2.2.2.5 Projected Biomass and FAME yield (from harvested biomass)
  - 2.2.2.6 Proximate composition (protein, carbohydrate, ash, moisture and FAME)
  - 2.2.2.7 Collect, share and report data to the industry to inform TEA, LCA and resource assessment/location screening analysis as well as production methodologies.

3. Reagents, Materials, and Apparatus Needed

3.1 **Materials for Pond Inoculation, Dilutions and Monitoring:**

- 3.1.1 Sterilized barrel (or equivalent container for transfer of up to 210 L of indoor seed culture)
- 3.1.2 ATP^3 ponds
- 3.1.3 YSI units with sensors (See YSI manual for proper calibration/maintenance procedures)
- 3.1.4 Functional weather station
- 3.1.5 Aeration/mixing apparatus for barrel
- 3.1.6 Sampling vials with labels
- 3.1.7 Label printer and labels (for biochemical composition, harvest, and molecular sample tracking)
- 3.1.8 Graduated cylinders for inoculum and nutrient addition
- 3.1.9 Lab supplies (e.g. AFDW filters, reagents for nutrient analysis, etc.)
- 3.1.10 Sodium hypochlorite solution (6%, 10% or 12%)

3.1.11 Sodium thiosulfate
3.1.12 Chlorine test kit or test strips from local pool supply or aquarium store
3.1.13 PAR meter with a LI-COR sensor
3.1.14 Sea salt (e.g., Oceanic™ Sea Salt Mix) for Cal Poly, GT, ASU; or natural seawater (Florida Algae, Cellana).

4. ES&H Considerations and Hazards

4.1 Standard laboratory personal protective equipment shall be worn. Lab coat, nitrile or latex gloves and eye protection should be worn while working with chemicals. Special care should be given when handling sodium nitrate because it is a strong oxidizer. Contact with other materials may cause fire. Harmful if swallowed or inhaled. May cause irritation to skin, eyes and respiratory tract.

4.2 Outdoor PPE determined by each sites EH&S policies. Care should be taken when working around ponds and any electrical equipment, connections. Use caution working around the ponds, including keeping all fingers and equipment away from the paddlewheels.

5. Procedures

5.1 Indoor Seed Production

5.1.1 Indoor seed production must be maintained in (2) columns of each strain during the length of the experiment in order to provide seed for scale up between experiment runs. See "**Indoor Seed Production in Columns and Panels"**.

5.2 Pond Set Up/YSI Set Up

5.2.1 Paddlewheel speed = 20 Hz (approximately 7.45 rpm)
5.2.2 pH = 7.9 for *Nannochloropsis,* 8.0 for *Chlorella*
5.2.3 Depth = 25 cm (approximately 40 L per cm, this may vary slightly by site)
5.2.4 Salinity = 35 ppt for *Nannochloropsis,* 0 ppt for *Chlorella*

5.3 Pond Preparation

5.3.1 Wash all ponds and paddlewheels thoroughly and perform a surface sterilization with a 16:1 solution of water:household bleach (Chlorox brand or equivalent without scent). Rinse thoroughly.
5.3.2 Flush all water lines and filters prior to collecting water for cultivation purposes for at least 5 minutes.
5.3.3 Type of water used is strain and site dependent. For *Nannochloropsis* fill ponds to 24 cm with either seawater or artificial SW (add amount of salt sufficient to achieve 35 ppt, approximately 34.6 kg/pond), verify salinity once dissolved and add more salt as necessary to achieve the 35 ppt. For *Chlorella* fill ponds to 24 cm with freshwater.
5.3.4 Sterilize culture water in the pond (system and water sterilization) with sodium hypochlorite (bleach). Use 1 ml 12% bleach solution/L water, 1.6 ml 10% bleach solution/L water, 2 ml 6% bleach solution/L water to achieve a 100 ppm (100 mg/L) chlorine concentration for at least 12 hours.

5.4 Pond Inoculation

5.4.1 Check ponds for dechlorination, if chlorine residual remains dechlorinate with 0.005 ml/L culture water of sodium thiosulfate stock solution (dissolve 500 g sodium thiosulfate in 1 L DI

or milli-Q water). Verify dechlorination is complete with a chlorine test kit after 10 minutes. If dechlorination not complete add 0.005 ml/L sodium thiosulfate stock solution, wait 10 minutes and test again with the chlorine test kit. Repeat this process until dechlorination is complete. No more than 0.2 ml/L in total should be added to the pond.

5.4.2 For *Nannochloropsis* use modified f/2 media stocks (see **"Modified f/2 Media"**) and add 1 ml N:P stock solution/L seawater (1 L N:P stock solution) to achieve a N concentration of ~70 mg/L (70 ppm) N-nitrate, as well as 1 ml trace solution/L seawater (1 L trace stock solution). See Table 3.

5.4.3 For *Chlorella* use modified BG-11 media stocks (see **"Modified BG-11 Media"**) and add 1 ml N:P stock solution/L culture water (1 L N:P stock solution) to achieve a N concentration of ~70 mg/L (70 ppm) N-nitrate, as well as 0.25 ml/L culture water (250 ml) of each additional stock solution (Mg, Ca, Fe, carbonate and trace). See Table 4.

5.4.4 Using microscopy, check that all panels are healthy. Combine healthy panels (ideally 14) into the sterilized barrel and aerate to mix (total approximate inoculum volume = 210 L). Sample the combined inoculum for OD, AFDW, and nutrient analysis. In addition, collect a 1L combined sample and retain for biochemical analysis and a 50 mL sample for molecular fingerprinting (see 5.7.6 for procedure).

5.4.5 Check pH, salinity and temperature of inoculum at time of transfer.

5.4.6 Starting concentration in the ponds is a minimum of 0.05 g/L AFDW. If all 14 panels are brought forward as seed for the ponds, they would need to be on average a minimum of 1.5 g/L AFDW per panel in order to achieve the necessary amount of biomass to inoculate all 6 ponds with some margin for transfer loss and sampling in step 5.6.4. If less than 14 panels are healthy, a higher density from the panels is needed for inoculation. There must be a minimum of 300 g AFDW basis of biomass from all panels combined (cumulative biomass) post sampling to inoculate all six ponds at the minimum starting density.

5.4.7 Add the seed culture to each of the ponds to achieve a minimum density of 0.05 g/L AFDW at the desired volume/depth. Top off pond volume with fresh water (for both *Nannochloropsis* and *Chlorella*) to 25 cm. Allow sufficient time to mix (at least 10 min), and take time zero (T0) sample for OD, AFDW and nutrients.

5.5 **Pond Sampling Plan (see Table 2)**

5.5.1 Monday - Friday at Sunrise ± 30 min. record depth and return depth to T0 level with freshwater (i.e. replace evaporative loss). Flush water lines and filters 5 – 10 minutes prior to water use.

5.5.2 After pond has mixed for 10 minutes post-evaporative loss replacement; sample for:

5.5.2.1 OD Monday through Friday AM.

5.5.2.2 AFDW, nutrients and microscopy on Monday, Wednesday and Friday AM.

5.5.2.3 Manually check pH, temperature, conductivity/salinity daily Monday through Friday AM and PM. This can be done post-morning sampling.

5.5.3 Once during each experimental run, determine % shading of pond at three points during the day: AM, mid-day and PM.

5.5.3.1 Take a picture of a pond from an elevated position (e.g. a ladder). Note the date and time of day when photo is taken.

5.5.4 At the start and end of each experimental cycle, sites will sample 50 ml (in a plastic sample tube wrapped in foil) of their source water at the pond for ICP-OES testing to determine water

quality (store refrigerated at 4 C in the dark) until sent to ASU on ice. A consistent tube source is mandatory for the entire run (the same manufacturer and product lot).

5.5.4.1 Water chemistry samples should also be taken from the culture pond at the end of an experimental run, as well as at pond crash event. This sample must be filtered to remove biomass prior to storage.

5.5.5 Prior to harvest, take a time harvest (TH) or time final (TF) sample for OD, AFDW, nutrient analysis, and 1-2 L (volume centrifuged determined by pond density, must have 300 mg cumulative dried biomass) mass balance sample for proximate composition: lipid/FAME, carbohydrates, and protein analysis. Ideally – harvest/dilution operations will be completed in the morning immediately following the morning sampling (Sunrise ± 30 min.) thus allowing for that morning's samples to be utilized as time harvest sample (TH) prior to harvest/dilution and precluding the necessity for an additional sample draw. If for some reason the harvest/dilution operation cannot be completed within 1 hour of the morning sampling, a separate TH sample draw must occur. After harvest/dilution operation is complete and ponds have been brought back to T0 starting depth, nutrients added, and allowed to mix for 10 minutes, a post-harvest (PH) sample should be drawn for OD/AFDW and nutrient measurements.

5.5.6 Molecular fingerprinting will be done on inoculum for ponds, weekly during experiment runs, and additional sampling of all ponds upon pond health decrease or crash. Sample by taking a 50 ml sample, spin to pellet, add RNALater based on AFDW estimation from an OD at 750 nm. Freeze at 4 °C for 24 hour, and then move to -20 °C. When requested samples are to ship with icepacks to Sandia National Laboratory via overnight freight.

5.5.6.1 RNALater amount based on biomass in sample.

Table 1: AFDW (based on OD):RNA Later

AFDW (g/L) based on OD	Milliliters of RNALater
0.13	0.625
0.25	1.25
0.38	1.88
0.5	2.5
0.75	3.75
1	5
1.25	6.25
1.5	7.5

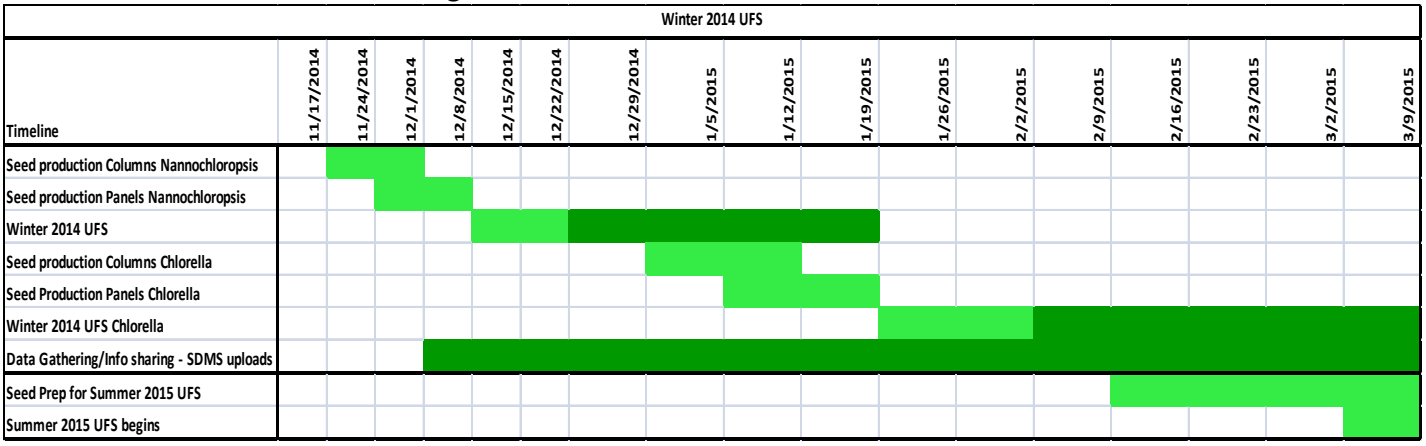
Table 2: Sample Table

Samples	Schedule
OD@ 750nm	Inoculum, sunrise* (+ 30 min) M – F, pre and post-harvest dilutions, triplicates
DW/AFDW	Inoculum, sunrise* (+ 30 min) M, W, F, pre and post-harvest dilutions, triplicates
Mass Balance (lipid/FAME, carbohydrates, starch, protein)	Inoculum, during initial scale up, day 7, TH of scale up ponds (prior to the first harvest or dilution occurring) – samples MUST be taken at same time as AFDW/OD (Sunrise* + 30 min), TH once each week during

	experimental treatments (Wednesdays), TF (at complete pond harvest)
Nutrients	Inoculum, sunrise* (+ 30 min) M, W, F, pre and post harvest dilutions, triplicates
Weather data	Real time (no less than hourly)
In-situ YSI sensors	Real time (15 min sampling frequency)
Microscopic exam	Inoculum, sunrise* (+ 30 min) M, W, F, duplicate (both on one slide)
Molecular fingerprinting	Inoculum, weekly during experiment run, and additional sampling of all ponds upon pond health decrease or crash.
Manual checks (pH, temp, salinity, depth with paddlewheel off)	M - F; AM – PM
Manual check (PAR)	Every other week
% Shading	Once during experimental run; AM, Mid-day, PM
Water chemistry, culture water	The end of the experimental run, and at pond crash
Water chemistry, incoming water	Beginning and end of experiment. ICPMS testing, triplicates

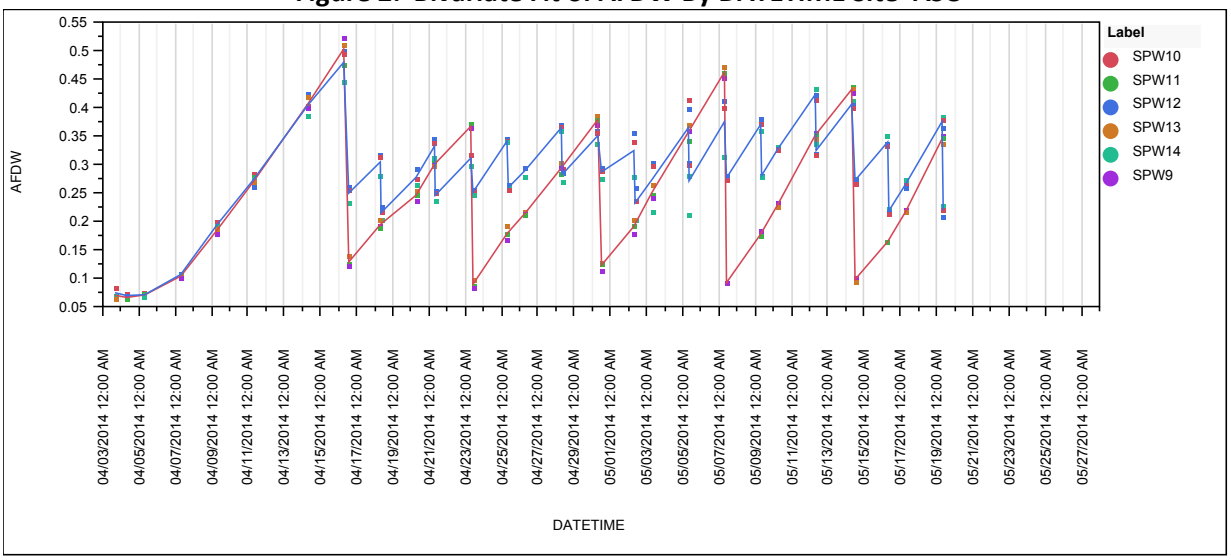
*Sunrise sample time range per site: DO NOT sample in the dark, the sun must be up.
Florida Algae = 7:30 am (± 30 min);
Cal Poly = 6:30 am (± 30 min);
ASU = Spring, Summer 6:15 am (± 30 min), Winter, Fall 7:30 am (± 30 min);
GT = 7:15 am (± 30 min);
Cellana = 7:30 am (± 30 min).

Figure 1: Schedule for Winter UFS Run 2014

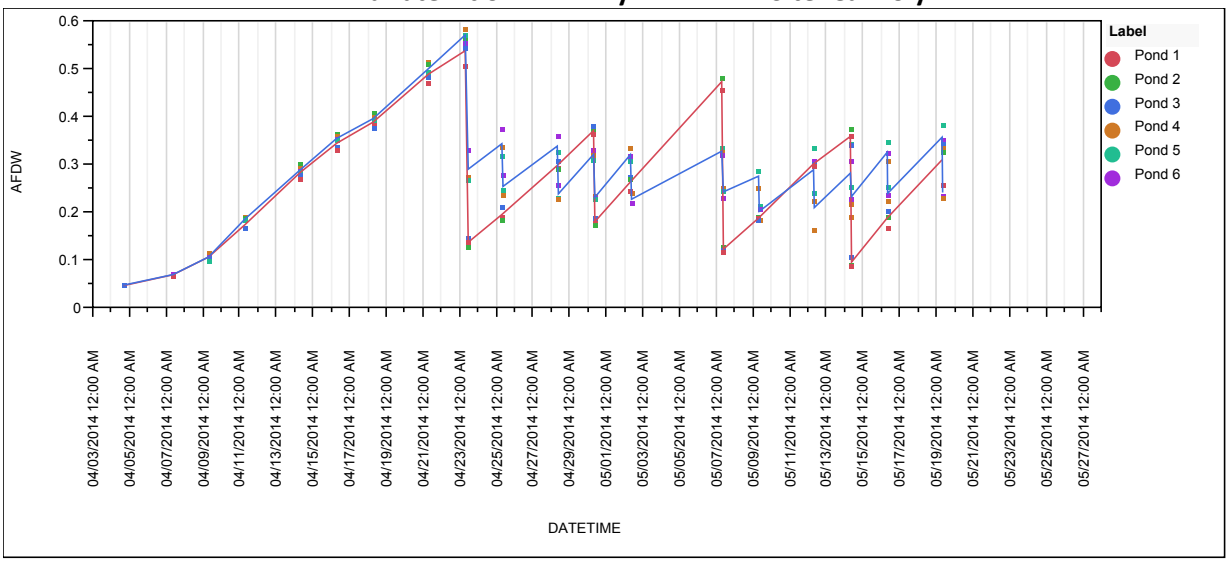


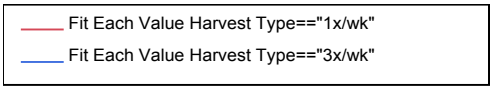
5.5.7 Density curves (AFDW vs. time) for two sites (ASU and Cal Poly) during the Spring 2014 UFS run are shown in Figure 2. Harvests occurred every Monday, Wednesday and Friday for three harvest per week treatments (3X) and once a week on either Monday, Wednesday or Friday for one harvest per week treatments (1X). Time to reach harvest density varied by site. Harvest volumes were increased in response to density increases at ASU, while this scenario did not occur at Cal Poly. Additionally if ponds were lost during experimental runs, ponds were re-set from remaining healthy ponds.

Figure 2: Bivariate Fit of AFDW By DATETIME Site=ASU



Bivariate Fit of AFDW By DATETIME Site=Cal Poly





5.6 Initiation of Experimental Treatments

5.6.1 All sites will conduct harvesting operations on M-W-F only. Given historical growth rates, it is estimated that ponds will take approximately one to two weeks to reach harvest density of 0.5 g/L.

5.6.2 One harvest per week treatment ponds will receive 75% by volume harvest once per week (equivalent to a dilution rate of 0.11 per day) for both strains. This treatment will be run

every season and serve as a control treatment across seasons as dilution rates on three harvests per week ponds will vary.

5.6.3 Three harvests per week treatment ponds will receive 50% by volume harvest (equivalent to a dilution rate of 0.214 per day) for both strains. Dilution rates will be based on site environmental limitations and will be adjusted in real time to avoid over or under dilution of ponds.

5.6.4 Inoculate ponds as per section 5.6.

Table 3: *Nannochloropsis* Pond Inoculation and Harvest Table

	Initial Pond Depth: cm	Pond Harvest: cm to remove and replace	Final Depth: cm	N:P Solution: Volume per Pond f/2*	Trace Solution: Volume per Pond f/2*
Pond Inoculation	24 cm Without Inoculum	N/A	25 cm with Inoculum	1 L	1 L
Harvest 1X per Week Treatment Ponds (75%)	25 cm	18.75 cm	25 cm	600 ml	750 ml
Harvest 3X per Week Treatment Ponds (50%)	25 cm	12.5 cm	25 cm	300 ml	500 ml

Table 4: *Chlorella* Pond Inoculation and Harvest Table

	Initial Pond Depth: cm	Pond Harvest: cm to remove and replace	Final Depth: cm	N:P Vol. per Pond BG-11*	Mg, Ca, Fe, and Carbonate Sln. per Pond BG-11*	Trace Sln. Per pond BG-11
Pond Inoculation	24 cm Without Inoculum	N/A	25 cm with Inoculum	1 L	250 mL	250 ml
Harvest 1X per Week Treatment Ponds (75%)	25 cm	18.75 cm	25 cm	600 ml	250 mL	188 ml
Harvest 3X per Week Treatment Ponds (50%)	25 cm	12.5 cm	25 cm	300 ml	250 ml	125 ml

*Nutrient addition volumes may be adjusted during experimentation based on nutrient assays. All ponds must remain above 30 ppm N (30 mg/L) at harvest, and 1 Harvest per Week Treatments should not exceed 70 ppm (70 mg/L), and 3 Harvest per Week Treatments should not exceed 50 ppm (50 mg/L) during the course of the harvesting portion of the experiment.

5.6.5 Growth curves and productivity calculations based on OD750 will be plotted daily to monitor the growth of the strain at each site. Productivity will be calculated by AFDW once this number available. This will happen automatically when data is entered daily into the production spreadsheet (see GRAPHS tab of spreadsheet). If ponds are growing faster or slower than our scheduled harvest schedule supports, harvest volume and/or timing of harvests can be adjusted to accommodate. Under no circumstances should a pond be diluted below 0.05 g/L, so dilution volume may have to be reduced. Pond density should never exceed 0.5 g/L, so dilution volume may have to be increased. Additionally, ponds should not be harvested if their biomass is below 0.3 g/L, and this scenario would lead to an evaluation of the harvest rates/dilution rates.

5.6.6 Once one replicate pond density reaches 0.5 g/L (based on OD/AFDW growth curve) or the ponds are two weeks old or one replicate pond stops increasing in density (based on two days of slow growth not caused by an environmental event that slows growth such as rain or cloudy days) whichever comes first, pond treatments will initiate.

5.6.7 Randomly assign treatments to triplicate ponds for both strains.

5.6.7.1 When ponds are ready for harvest, collect and sterilize make up water (either seawater, artificial seawater or fresh water) in a storage tank the day of work prior to the harvest. This translates to every Friday, Tuesday and Thursday collect or prepare the volume of water needed for a dilution, and sterilize with bleach (use 1 ml 12% bleach solution/L culture water, 1.6 ml 10% bleach solution/L culture water, 2 ml 6% bleach solution/L culture water to achieve a 100 ppm (100 mg/L) chlorine concentration) for at least 12 hours. The tank should be mixed when prepared on Tuesday and Thursday to facilitate out-gassing of chlorine overnight, however the tank collected on Friday for Monday's harvests <u>should not</u> be mixed in order to prevent out-gassing of chlorine prior to harvests occurring.

5.6.7.2 Prior to any harvest/dilution, check the tank for chlorine residual and if chlorine residual remains dechlorinate the make-up water using the 500 g/L sodium thiosulfate solution at a maximum of 0.2 ml/L addition in 0.005 ml/L increments. Start with 0.005 ml/L of stock solution and add enough to be certain there is no more chlorine residual, but no more than 0.2 ml/L in total. Wait ten minutes between additions and dechlorination checks. Check for dechlorination prior to using make-up water.

5.6.7.3 Take a TH pond sample (OD, AFDW, nutrient analysis, molecular finger printing and mass balance (lipid/FAME, carbs, and protein) from each pond, sampling time must correlate with an AFDW sample (try to do dilutions within one hour of morning samples to avoid a second set of AFDW samples on a harvest day) in order to determine total biomass harvested. Volume centrifuged for mass balance determined by pond density, must have 300 mg cumulative dried biomass. A TH sample is required at first harvest, and then only once per week on Wednesday. On other harvest days the TH sample only requires nutrients and AFDW measurements.

5.6.7.4 Turn the paddlewheel off when measuring depth, but leave the paddlewheel on while actually harvesting/draining ponds. Remove desired volume from ponds as per treatment assignment and dispose of according to site regulations. Be sure depth measurements are

made with the ruler resting on the bottom of the pond – do not rely on a ruler that has been fixed permanently to the center board.

5.6.7.5 Replace the media in the pond by filling the pond with sterilized make up water. Flush water lines for at least 5 minutes prior to using water.

5.6.7.6 For *Nannochloropsis* use modified f/2 media recipes (see **"Modified f/2 Media"**). Add macronutrients for full pond volume: nitrate and phosphate solution at 1 ml/L or 1 L; and add micronutrients (trace solution) for replacement media only at 1 ml/L of trace stock solution. Ponds should not be allowed to fall below 30 ppm (30 mg/L) N as nitrate at harvest.

5.6.7.7 For *Chlorella* use modified BG-11 media recipes (see **"Modified BG-11 Media"**). Add macronutrients for full pond volume: nitrate and phosphate solution at 1 ml/L or 1 L; magnesium, calcium, ferric citrate and carbonate solutions at 0.25 ml/L or 250 ml, and add micronutrients (trace solution) for replacement media only at 0.25 ml/L. Ponds should not be allowed to fall below 30 ppm (30 mg/L) N as nitrate at harvest.

5.6.7.8 Take a PH (post-harvest) sample from each pond (OD, AFDW, and nutrient analysis) after nutrients are well mixed/dissolved.

5.6.7.9 If a pond is not healthy enough to continue to operate, it can be harvested completely (requiring a TF sample and a water quality sample) and re-inoculated from a healthy pond, ideally a pond from the same treatment. This requires the pond to be cleaned, filled with sterilized culture water and the re-inoculated as per treatment.

6. Data Processing

6.1 Production Spreadsheet

6.1.1 Lab and operational data to be entered daily into production spreadsheet.

6.1.2 YSI data to be entered into the spreadsheet at least weekly.

6.1.3 Weather data to be entered into the spreadsheet at least bi-weekly.

6.1.4 Unique sample IDs for analytical grab samples must be generated for each sample taken via the sample label printing system.

6.1.5 Data to be checked and verified in the spreadsheet at least once per week and prior to uploading into the SDMS.

6.1.6 Upload data to SDMS at least once per week.

6.2 Analytical Samples

6.2.1 Process mass balance samples when sample number is sufficient and time permits.

6.2.2 Enter analytical sample data into analytical spreadsheet.

6.2.3 Unique sample IDs for analytical grab samples must be entered into the spreadsheet.

6.2.4 Data to be checked and verified in the spreadsheet prior to uploading into the SDMS.

6.2.5 Upload data to SDMS.

END

Table 5: Modified f/2 Media

f/2 nitrate							
Columns Stock							
Chemical	**Molecular Weight (g)**	**Molecular Weight of N (g)**	**Amount to be added (g)**	**Final Volume (L)**	**Molarity**	**Molarity of N-NO3**	**mg/L N-NO3**
NaNO3	84.995	14.007	85.000	1.000	1.000	1.000	14007.573
		Molecular Weight P (g)				**Molarity of P-P04**	**mg/L P-PO4**
NaH2PO4	119.980	30.974	7.500	1.000	0.063	0.063	1936.179
						15.998	N:P ratio
Target ppm in Columns							
	Amount of stock to be added (L)			**Final volume (L)**	**Final ppm (N-NO3) or (P-PO4)**		
NaNO3	0.009			0.800	155.834		
NaH2PO4	0.009			0.800	21.540		
Panels Stock (Same as Column Stock)							
Chemical	**Molecular Weight (g)**	**Molecular Weight of N (g)**	**Amount to be added (g)**	**Final Volume (L)**	**Molarity**	**Molarity of N-NO3**	**mg/L N-NO3**
NaNO3	84.995	14.007	85.000	1.000	1.000	1.000	14007.573
		Molecular Weight P (g)				**Molarity of P-P04**	**mg/L P-PO4**
NaH2PO4	119.980	30.974	7.500	1.000	0.063	0.063	1936.179
						15.998	N:P ratio
Target ppm in Panels							
	Amount of stock to be added (L)			**Final volume (L)**	**Final ppm (N-NO3) or (P-PO4)**		
NaNO3	0.133			15.000	124.200		
NaH2PO4	0.133			15.000	17.167		
Pond Stock Sodium Nitrate							
Chemical	**Molecular Weight (g)**	**Molecular Weight of N (g)**	**Amount to be added (g)**	**Final Volume (L)**	**Molarity**	**Molarity of N-NO3**	**mg/L N-NO3**
NaNO3	84.995	14.007	6375.000	15.000	5.000	5.000	70037.867
		Molecular Weight P (g)				**Molarity of P-P04**	**mg/L P-PO4**
NaH2PO4	119.980	30.974	562.500	15.000	0.313	0.313	9680.895
						15.998	N:P ratio
Target ppm in Ponds							
	Amount of stock to be added (L)			**Final volume (L)**	**Final ppm (N-NO3) or (P-PO4)**		
NaNO3	1.000			1000.000	70.038		
NaH2PO4	1.000			1000.000	9.681		
Adding 1 mL/L of stock with raise ponds by:							
N-NO3 ppm			70.038				
P-PO4 ppm			9.681				

Table 6: Modified BG-11 Media

BG-11							
Columns Stock							
Chemical	**Molecular Weight (g)**	**Molecular Weight of N (g)**	**Amount to be added (g)**	**Final Volume (L)**	**Molarity**	**Molarity of N-NO3**	**mg/L N-NO3**
NaNO3	84.995	14.007	600.000	2.000	3.530	3.530	49438.494
		Molecular Weight P (g)				**Molarity of P-P04**	**mg/L P-PO4**
K2HPO4	174.200	30.974	320.000	2.000	0.918	0.918	28448.863
						3.843	N:P ratio
Target ppm in Columns							
	Amount of stock to be added (L)			**Final volume (L)**	**Final ppm (N-NO3) or (P-PO4)**		
NaNO3	0.004			0.800	247.192		
K2HPO4	0.0002			0.800	7.112		
Panels Stock (Same as Column Stock)							
Chemical	**Molecular Weight (g)**	**Molecular Weight of N (g)**	**Amount to be added (g)**	**Final Volume (L)**	**Molarity**	**Molarity of N-NO3**	**mg/L N-NO3**
NaNO3	84.995	14.007	600.000	2.000	3.530	3.530	49438.494
		Molecular Weight P (g)				**Molarity of P-P04**	**mg/L P-PO4**
K2HPO4	174.200	30.974	320.000	2.000	0.918	0.918	28448.863
						3.843	N:P ratio
Target ppm in Panels							
	Amount of stock to be added (L)			**Final volume (L)**	**Final ppm (N-NO3) or (P-PO4)**		
NaNO3	0.075			15.000	247.192		
K2HPO4	0.004			15.000	7.112		
Pond Stock							
Chemical	**Molecular Weight (g)**	**Molecular Weight of N (g)**	**Amount to be added (g)**	**Final Volume (L)**	**Molarity**	**Molarity of N-NO3**	**mg/L N-NO3**
NaNO3	84.995	14.007	6375.000	15.000	5.000	5.000	70037.867
		Molecular Weight P (g)				**Molarity of P-P04**	**mg/L P-PO4**
K2HPO4	174.200	30.974	650.000	15.000	0.249	0.249	7704.900
						20.101	N:P ratio
Target ppm in Ponds							
	Amount of stock to be added (L)			**Final volume (L)**	**Final ppm (N-NO3) or (P-PO4)**		
NaNO3	1.000			1000.000	70.038		
K2HPO4	1.000			1000.000	7.705		
Adding 1 mL/L of stock with raise ponds by:							
N-NO3 ppm			70.038				
P-PO4 ppm			7.705				