Freshwater algae: bioreactor setup and monitoring protocol

Coral Defense Technical Report #1

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I. Objective

The objective of this report is to describe the methods used for culturing *Scenedesmus obliquus* in an environmental chamber with constant temperature at University of Maryland Center for Environmental Science (UMCES) Horn Point Laboratory (HPL) as part of the Coral Defense program. In addition, methods for conducting routine pH measurements and cell counts on algae are described.

From March 2019 to March 2020, two types of algal bioreactors were used: 1) a custom-built horizontal bioreactor system, and 2) a glass bottle bioreactor system. While daily care differed between the two systems, the same data was collected and analyzed from both.

II. Algal stock cultures

Scenedesmus obliquus is a green microalga that can be found either single celled or in colonial arrays (Preston et al. 1964). A strain of *S. obliquus* (*S. obliquus* Back River 1) was isolated from the Back River by Dr. Feng Chen and colleagues of UMCES Institute of Marine and Environmental Technology (Wang et al. 2016) and was provided by Dr. Chen for these experiments. Stock cultures of *S. obliquus* used for inoculating experiments were fed with BG11 and kept in a controlled environmental chamber in the HPL Oyster Hatchery. Stir bars and aeration kept the culture suspended and allowed for air exchange.

A set of algae batch cultures used to scale up production were kept in 500 mL Erlenmeyer flasks under ambient conditions in the oyster hatchery algae laboratory. Algal cultures were swirled daily and fed once a week with ~ 0.5 mL of BG11 media (stock solution). J. Trommatter oversaw algae husbandry and maintained backup cultures.

III. Environmental Chamber

An environmental chamber in the HPL Coastal Sciences building (Fig. 1) was set to maintain a constant temperature of 25 °C and a 24-hour light cycle was provided by 2 LED Strip Lights (Metalux) installed 15 cm above algae bioreactors. Each light strip measured 6.4 cm wide by 119.4 cm long and generated 4000 lumens. Light levels ranged from 346 +/- 6 mmol m^{-2} s^{-1} near the top of the glass bottle bioreactors to 258 +/- 2 mmol m^{-2} s^{-1} near the bottom (measurements made with a Walz PAR Sensor adjacent to 1-L bottle). The light spectra underneath the two light strips showed peaks at 452 and 589 nm when measured with a hyperspectral 2π radiometer (TRIOS RAMSES) adjacent to 1-L glass bottle. (Fig. 2).

Fig. 1. Photograph of environmental chamber taken on 3/17/2020 by E. North.

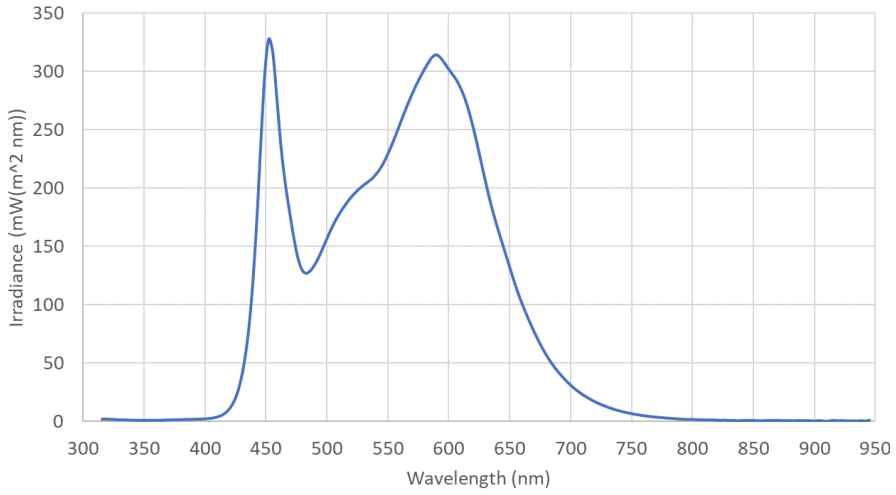

Fig. 2. Light spectrum of the LED strip lights within the environmental chamber. Measurements taken by G. Silsbe.

IV. Culture Methods and Measurements

Two types of bioreactors were used to maintain non-axenic cultures of *S. obliquus* (Back River 1) algae: horizontal bioreactors and glass bottle bioreactors.

a. Horizontal Bioreactors

Cultures were grown in custom-built horizontal acrylic bioreactors with built-in passive filtration (Fig. 3). These systems were used in the environmental chamber for experiments conducted from May 2019 to September 2019.

System

The custom-built horizontal bioreactors for freshwater algae were composed of two main sections of 750 ml (for algae culture) and 250 ml or 70 ml (for high-pH filtrate of long and short bioreactors, respectively) (Fig. 4). The sections were separated by a 1 µm pore size Whatman Nuclepore Track-Etch membrane of 142 mm diameter, allowing passage for high-pH filtrate.

Both versions of reactors were equipped with independent lines for media and air inputs and output lines for sampling from algae and high pH chambers. Reactors also were fitted with magnetic stir bars and air stones, and placed on a magnetic stirring plate. Each bioreactor was

Key design aspects of horizontal bioreactors

- Cylinder diameter was 127 mm
- Long chambers: Algae section was 6”, filtrate section was 2”
- Short chambers: Algae section was 6”, filtrate section was 10 mm
- Sections separated by 1 micron 142 mm polycarbonate membrane
- Ports were 1/4” diameter with #28 threads
- Fittings for ports were compatible with 3/16th tubing

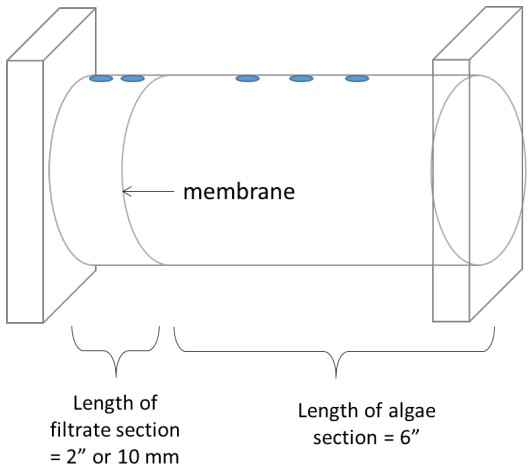

Fig. 3. Schematic and key elements of the horizontal bioreactors. Designed by G. Silsbe and E. North.

coupled to a 2 L Pyrex glass bottle containing BG11 media. Air for each bioreactor was supplied by a Pawfly MA-60 Aquarium Pump (1.8 L/min flow rate). The whole system containing the bioreactor, media bottle, air pump and magnetic stir plate (HYCC MX-3K, 4.8" diameter) was on custom-built plywood stands inside a plastic bin (Fig. 5).

Media and filtrate

Media for the algal cultures was created by mixing 20 ml of 50x BG-11 culture media solution (Sigma-Aldrich Cyanobacteria BG-11 Freshwater Solution) with 1 L of deionized (DI) water. The BG-11 media was pumped into the algae chamber of the bioreactor using an auto-dosing pump (Jebao DP-4 4-channel) at a rate of 18 ml/hr for 24 hrs per day. As BG-11 media was delivered to the algae section of the bioreactor, 18 ml/hr of high-pH filtrate was simultaneously pumped out from the high-pH filtrate section into glass bottles (Pyrex) for collection.

To maintain the horizontal bioreactors, the membrane was changed every 1-2 weeks when the bioreactor was not filtering well (when fluid levels were much lower in the filtrate section than in the algae section). In addition, the algal culture was split to reduce cell concentrations to help with passive filtration. J. Veenhof maintained the algae cultures with assistance from Francesca Galasso.

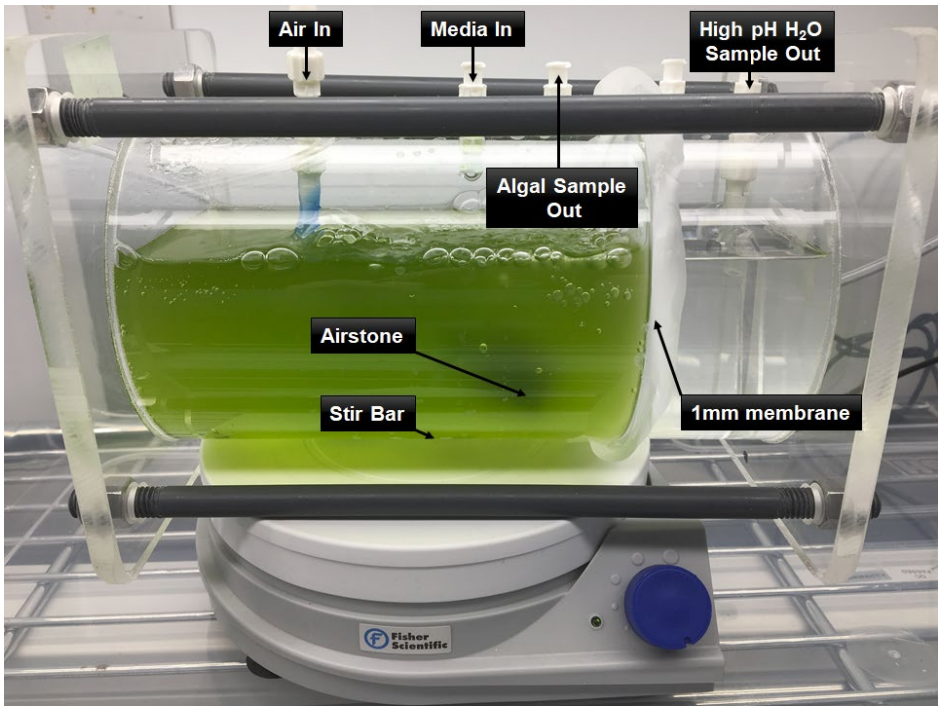

Fig. 4. Custom-built acrylic horizontal bioreactors with passive filtration. Photo taken on 4/18/2019 by J. Veenhof.

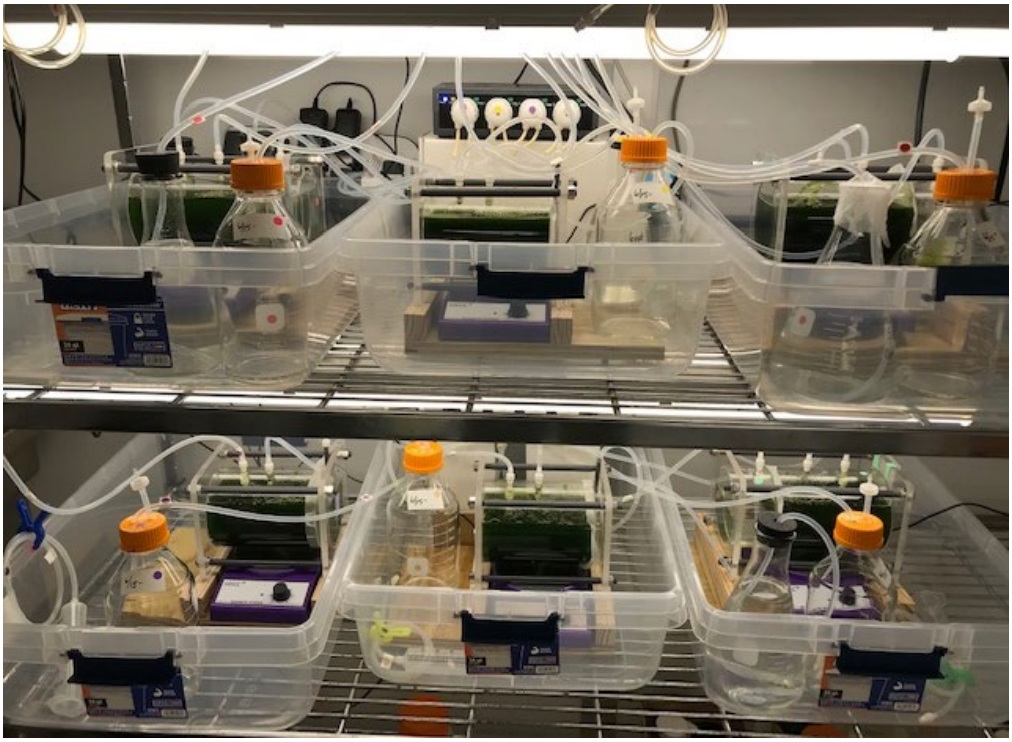

Fig. 5. Horizontal bioreactor systems. Each system rested on a magnetic stir plate (purple) and had media pumped in (from flasks) and high-pH filtrate pumped out (into 1-L pyrex bottles) using Jebao autodosing pumps. The Jebao pump on the top shelf, a black box with 4 peristaltic pump heads, can be seen in the upper center of the photo. The Jebao pump on the lower shelf was blocked from sight by the top shelf. System set-up by E. North. Photo taken on 6/15/2019 by E. North.

Sample collection

Environmental conditions of the algal culture in the bioreactor were monitored by measuring pH, temperature and cell concentrations three times per week. Samples of ~15 mL were collected from the algae and high pH chambers and transferred into individual 20-mL scintillation vials. All water samples were collected through the sampling ports of each chamber using a Pasteur pipette (when the Jebao pump system was not operational), or by pouring straight from bottles or flasks that contained media or filtrate (when the Jebao pump system was functioning). Measurements were taken in the environmental chamber immediately after sample collection, with the probe rinsed between each measurement with DI water. Samples were collected by J. Veenhof with assistance from F. Galasso.

pH measurements

Two pH meters, a Hanna pH sensor (Model HI5221-01) and a NeuLog pH Logger Sensor (Model NUL-206) were used to record pH and temperature readings. The Hanna pH meter was calibrated in the environmental chamber using three calibration points (7.00, 10.00 and 12.00) provided by Ricca 1550-16 buffer reference standards. pH readings were recorded from sampled water obtained from both the algae culture and high pH chambers. pH measurements were conducted by J. Veenhof with assistance from F. Galasso.

Cell concentrations

Cell concentrations were calculated by counting cells on a Levy hemocytometer. First, samples from the algae bioreactors were diluted with DI water. For most of the cell counts for the horizontal bioreactors, dilution ratios ranged from 1:100 to 1:300 depending upon the concentration of cells in the culture. After dilution, the hemocytometer chamber was filled by capillary action with well-mixed algal suspension using a Pasteur pipette. The hemocytometer was carefully transferred to the stage of a compound microscope, where counts were done using the 4x or 10x objectives. On each half of the slide, all cells in the four outer corner squares were counted (for a total of 8 squares). Cells that touched the top and left Neubauer lines were counted and cells on the bottom and right lines were omitted. The slide was rinsed with DI water, dried with a KimWipe, and the process was repeated two more times (for a total of 3 slides for each sample). Cell concentrations were calculated for each half of a slide and averaged, then these concentrations were averaged across the three times the slide was counted for the final cell concentration of each sample. Cell counts were conducted by J. Veenhof and F. Galasso with training provided by J. Trommatter.

b. Glass Bottle Bioreactors

In September 2019, acrylic chambers were replaced with 1-L and 2-L autoclaved Pyrex bottles until the experiments ended in March 2020 due to COVID-19. Separation of algae from filtrate was either conducted in the laboratory with 142 mm GF/F filter apparatus or was performed in-line as part of the bioreactor system using a filter cassette that was made from two 10-mm ends of the horizontal bioreactors. With these in-line cassettes, the membranes were easier to change. In addition, there were fewer non-*Scenedesmus* organisms because the bottles could be autoclaved. In October 2019, one glass bottle bioreactor with algae and one with DI water (a control) were in use. Two more glass bottle bioreactors were added in December 2019, for a total of four systems which functioned until March 2020 when experiments were ended. In November 2019, an inline filter cassette was added to the algae systems, and the blank was replaced with an algal culture. In February-March 2020, the in-line filter was removed except during the Carbon Trace II experiment from February 10-14 when one system included an in-line cassette.

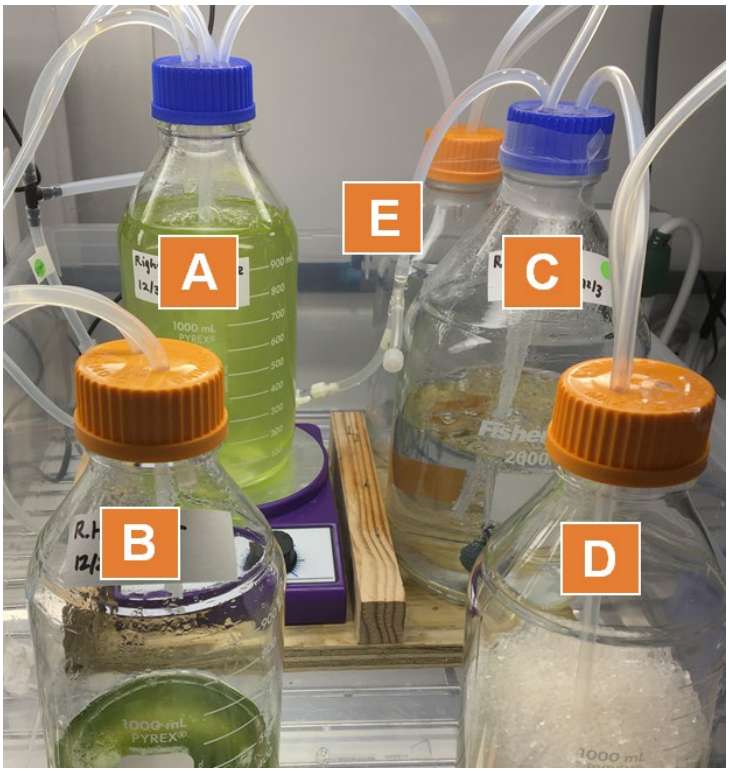

Fig. 6. Glass bottle bioreactor system set up: A) algae culture bottle, B) algae product bottle, C) BG-11 media bottle, D) silica beads bottle, and E) dripleg bottle. Fluid was pumped from the media botte into the algae culture bottle and from the algae culture bottle into the algae product bottle. Air flowed from the Pawfly aquarium pump through an air stone in the algae culture bottle, then into the dripleg bottle, then through an air stone in the BG-11 media bottle, through silica beads bottle, and into the 5-gal carboy with a CO_2 sensor, and finally back into the environmental chamber. The dripleg (E) served to reduce contamination of the media by collecting water droplets containing algae. System set-up by E. North with input from J. Veenhof, G. Silsbe, Yi-Ying Lee, and Alen Place. Photo taken on 12/3/2019 by J. Veenhof.

System

Each glass bottle bioreactor system consisted of four 1-L Pyrex bottles and one 2-L Pyrex bottle (Fig. 6). The bottles held the algae culture (A in Fig. 6), the algae product ("harvest fluid") (B in Fig. 6), an empty dripleg bottle (E in Fig. 6) to allow moisture to drop out of the air before bubbling through the BG-11 media bottle (C in Fig. 6), and silica beads (D in Fig. 6) for removing moisture from the air before reaching the CO_2 sensors (in 5-gallon glass carboys in Fig. 7). BG-11 media was in the 2-L bottle. Silicone tubing connected the bottles (HelixMark® Standard Platinum-Cured Silicone Tubing I.D. = 3.18, O.D. = 6.35 mm from VWR). Air for each bioreactor was supplied by a Pawfly MA-60 Aquarium Pump (1.8 L/min flow rate) and an auto-dosing pump (Jebao DP-4 4-channel) was used to control fluid flow. CO_2 sensors were used to monitor the concentration of CO_2 in the air flowing into the algae bioreactor system and out of the system (CO_2 sensors were K30 10,000ppm CO2 Sensors from CO_2 sensor.com). All algae culture bottles were placed directly on top of magnetic stir plates (HYCC MX-3K, 4.8" diameter).

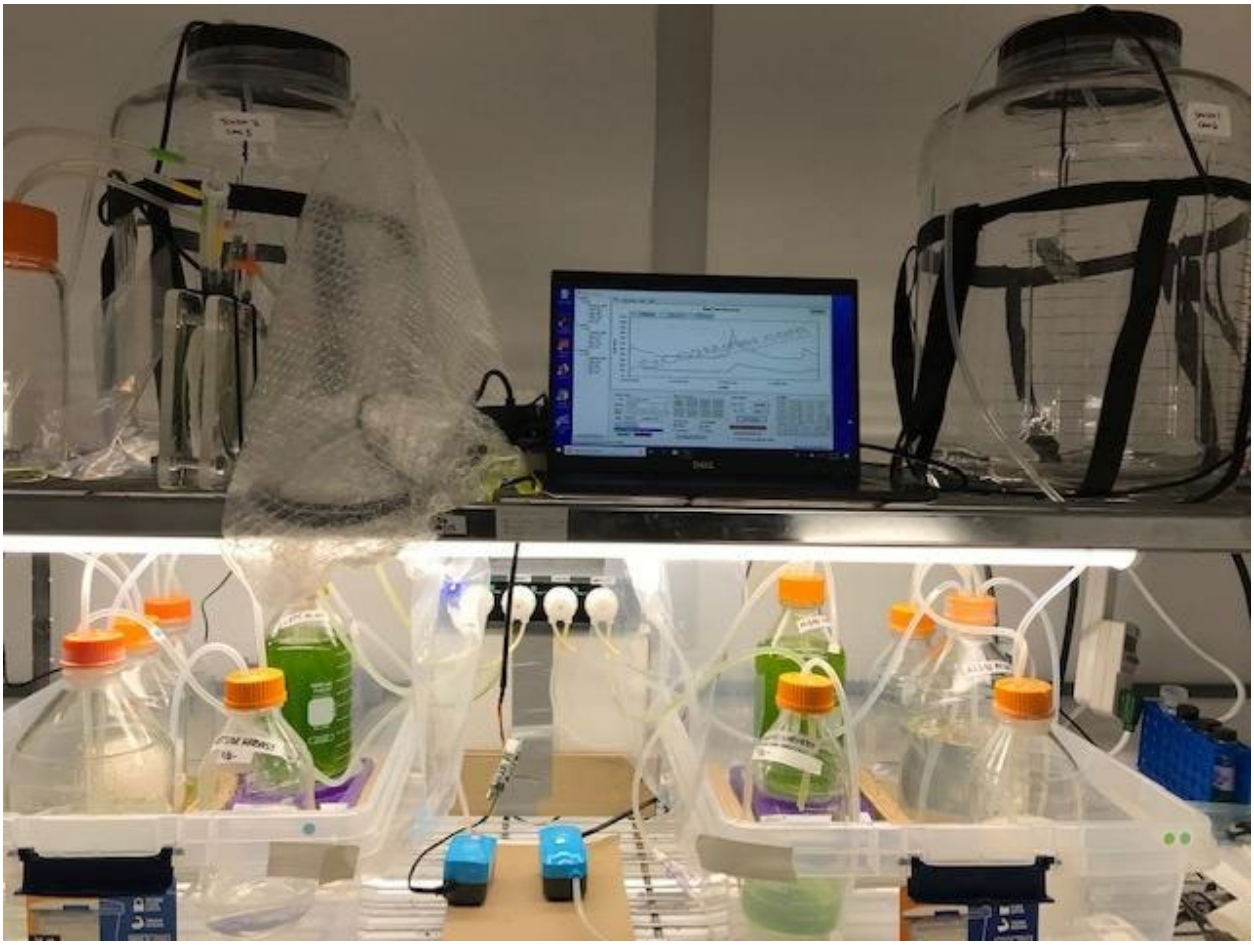

Fig. 7. Two glass bottle bioreactor systems with Pawfly air pumps (blue, center bottom shelf), Jebao autodosing pump (center top of bottom shelf), CO_2 sensor for air inflow (just above the Pawfly pumps), CO_2 sensors for air outflow (in glass carboys on top self) and laptop running GasLab software for the CO_2 sensors. An in-line filtration cassette is on the top shelf on the left. Culture fluid was pumped from the algae culture on the left side into the cassette, then the high-pH filtrate dripped into the algae product bottle on the bottom shelf (note the clear fluid in the product bottle on the left and the algae in the product bottle on the right where there was no in-line filtration cassette). See Fig. 8 for a close-up of the filtration cassette. Photograph taken on 11/9/2019 by E. North.

Media

From November 2019 until January 2020, BG-11 culture media solution 50x (Sigma Aldrich) was diluted to reach a 1:20 (media: DI water) ratio with autoclaved DI water. From January 2020 until the end of the experiment, BG-11 culture media solution 50x (PhytoTech) was used.

BG-11 media was pumped into the algae chamber of the bioreactor using an auto-dosing pump (Jebao DP-4 4-channel) at a rate of 18 ml/hr for 24 hours per day. Just before the BG-11 was delivered, 18 mL of high-pH culture water was pumped out of the culture into the algae product bottle for collection. On 1/13/20, the pumping rates were increased to 30 ml/hr and remained at this level through March 2020. J. Veenhof, C. Sewell, and E. North maintained the algae cultures.

Fig. 8. Close-up of in-line filtration cassette. The 1-L bottle on the left was used for venting and overflow if the 1-micron membrane clogged. Cassette system designed by E. North. Photograph taken on 11/9/2019 by E. North.

Sample Collection

Environmental conditions of the algal culture in the bioreactors were monitored by measuring pH, temperature and cell concentrations three times per week. Using a 60 mL syringe with Luer-Lok tip, ~15 mL samples were removed from algae, high-pH, and harvest bottles through 3-way stopcocks and transferred to clean 20 mL scintillation vials. Measurements were taken in the environmental chamber immediately after sample collection, with the probe rinsed between each with DI water. Samples were collected by J. Veenhof with assistance from Juan Alvarez and Courtney Sewell.

pH measurements

From November 2019 to March 2020, pH measurements were taken with Hanna Multiparameter Meter (Edge® HI2020-01) and Hanna pH/ORP Meter (Edge® HI2002-01). Both were calibrated at chamber temperature to four calibration points (4.00, 7.00, 10.00 & 12.00) using Ricca 1550-16 Buffer Reference Standards. pH measurements were conducted by J. Veenhof with assistance from J. Alvarez and C. Sewell.

Cell concentrations

Cell concentrations were calculated by counting cells on a Levy hemocytometer following the protocol described for the horizontal bioreactors above except that the dilution ratio was fixed at 1:20 (algae: DI water) for all cell concentration measurements. Cell counts were conducted by J. Veenhof and C. Sewell, with oversight provided by J. Trommatter.

V. Acknowledgements

We greatly appreciate funding support from The Bailey Wildlife Foundation. We thank Juan Alvarez, Francesca Galasso, and Courtney Sewell for their excellent assistance in the laboratory, and Jack Seabrease for fabricating the horizontal bioreactors.

VI. Literature Cited

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