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Title: Coupling Carbon Capture from a Power Plant with Semi-automated Open Raceway Ponds for Microalgae Cultivation

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **Y, 16 mi**

Protocol Length
Number of Shots: **42**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Margarita Acedo**: This semiautomated raceway cultivation system, controlled by a pH sensor, can directly capture flue gas from power plants for microalgae cultivation and can monitor microalgae growth with real-time measurements **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Kimberly Ogden**: This reactor system allows the direct capture and usage of carbon from industrial flue gas to grow microalgae in on-site, semiautomated, open pond raceway systems **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Juan R. Gonzalez**: The system can be used to cultivate alternative algae species and to capture carbon from any power plant **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time – We recorded this*

Protocol

2. Outdoor Open Raceway Pond Setup

2.1. To set up an open raceway pond, attach a 0.95-centimeter fuel hose to capture the flue gas during the post-combustion process a few meters before the flue gas enters the stack to be discharged into the atmosphere **[1]**.

2.1.1. WIDE: Talent attaching gas to setup

2.2. Place a 20-liter water trap and an approximately 12-meter condenser between the stack and the compressor to remove water from the flue gas **[1]**.

2.2.1. Talent placing trap and/or condenser into position

2.3. To monitor algal growth, connect a real-time optical density sensor that measures the absorbance at 650 and 750 nanometers **[1]**, a dissolved oxygen sensor **[2]**, air and pond thermocouples **[3]**, a pH sensor **[4]**, and an electroconductivity sensor to a datalogger **[5]**.

2.3.1. Talent connecting optical density sensor to datalogger
2.3.2. Talent connecting DO sensor
2.3.3. Talent connecting thermocouple(s)
2.3.4. Talent connecting pH sensor
2.3.5. Talent connecting EC sensor

3. pH Control System Setup

3.1. To set up a pH control system, connect a compressor and control valve system to the data logger program to manage the flue gas injection **[1]** and use a tube to direct the flue gas from the control valve through a stone diffuser to the bottom of the raceway pond **[2]**.

3.1.1. WIDE: Talent connecting compressor and/or system to data logger *Videographer: Important/difficult step*
3.1.2. Talent connecting tube *Videographer: Important/difficult step* - Use shot 2.3.1

3.2. Then set the system to inject flue gas when the pH value is greater than 8.05 **[1]** and to stop the flue gas injection when the pH is less than 8.00 **[2-TXT]**.

3.2.1. Talent setting system – Use shot 5.2.3

3.2.2. Talent stopping flue gas injection **TEXT: Flow rate measured in SLPM**

4. Algae Strain Maintenance

4.1. To set up an algae culture system, set the culture room to 25 degrees Celsius and a 12-hour light-12-hour dark cycle **[1]** and use deionized water salts and macro- and micronutrients to prepare BG11 (B-G-eleven) culture medium **[2-TXT]**.

4.1.1. WIDE: Talent setting temp and/or light/dark cycle NOTE: This and next shot together
4.1.2. Talent adding salts to water **TEXT: See text for all medium preparation details**

4.2. Use a sterile loop to select a single algal colony from a culture plate **[1]** and inoculate the algae in a 50-milliliter tube containing sterile growth medium in a clean biosafety cabinet **[2]**.

4.2.1. Shot of colonies, then colony being selected
4.2.2. Talent adding colony to tube

4.3. Grow the small liquid culture on a shaker table at 120 revolutions per minute for one week **[1]**.

4.3.1. Shot of tube on shaker

4.4. At the end of the incubation, transfer the entire volume of algae culture into 500 milliliters of liquid culture in a 1-liter flask **[1-TXT]** and close the flask with a rubber stopper fitted with stainless-steel tubing to provide aeration **[2]**.

4.4.1. Talent adding culture to flask **TEXT: Day 7: linear growth phase, $OD_{750nm} \geq 1$** NOTE: This and next shot together
4.4.2. Talent placing stopped into flask

4.5. Filter the air with 0.20-micron air sterilization filters **[1]** and allow the culture to grow for another 1-2 weeks **[2]**, using a spectrophotometer to monitor the cell density every day **[3]**.

4.5.1. Talent placing filter(s) NOTE: This and next shot together
4.5.2. Talent placing flask for culture
4.5.3. Talent adding sample to spectrophotometer Author NOTE: A & B Taking sample

4.6. At the end of the culture period, add 500 milliliters of the liquid algae culture to 8 liters of non-sterile culture medium in a 10-liter carboy **[1]** and inject a mixture of 5% carbon dioxide and 95% air into the carboy **[2]**.

4.6.1. Talent adding medium to carboy *Videographer: Important step*
4.6.2. Talent adding injecting CO2 and air to carboy *Videographer: Important step*

4.7. Monitor the stock plate and liquid cultures under a light microscope at the 10- and 40x magnifications once a week to ensure growth of the strain of interest **[1-TXT]**.

4.7.1. Talent at microscope, checking growth OR LAB MEDIA: To be provided by Authors: Image(s) of 10x and/or 40x magnification of algae **TEXT: Discard contaminated cultures** NOTE: Author will upload image by October 5th

5. Outdoor Open Raceway Pond Inoculation

5.1. To inoculate an outdoor open raceway pond with algae, first thoroughly clean the reactor overnight with 30% bleach **[1]**.

5.1.1. WIDE: Talent adding bleach to reactor **TEXT: Bleach before each inoculation and after each harvest**

5.2. Rinse the reactor the next morning until all of the bleach has been removed **[1]** and calibrate all of the sensors **[2]** according to their corresponding calibration procedures **[3-4]**.

5.2.1. Talent rinsing reactor
5.2.2. Talent calibrating sensor *Videographer: Important/difficult step*
5.2.3. Added: Inject CO2
5.2.4. Added: Real time OD Sensor

5.3. Fill the raceway pond up to 80% with water to dilute the concentrated medium **[1]** and inoculate the pond with the 10-liter carboy of algae culture **[2-TXT]**.

5.3.1. Talent filling pond with water *Videographer: Important step*
5.3.2. Talent adding carboy to pond *Videographer: Important step* **TEXT: linear growth phase $OD_{750nm} \geq 2$**

5.4. Bring the pond to its final volume **[1]**.

5.4.1. Talent adding water to raceway *Videographer: Important step*

5.5. Then partially shade the raceway pond with wooden pallets for about 3 days as an adaptation strategy to avoid photoinhibition to allow the microalgae to acclimate to the culture system **[1]**.

5.5.1. Talent placing pallet *Videographer: Important step*

6. Batch Growth Experiment

6.1. To perform a batch growth experiment, inspect and record any day to day variations, including water evaporation **[1]**, paddlewheel motor and sensor functionality, or anything else out of the ordinary **[2]**.

6.1.1. WIDE: Talent inspecting and recording observations
6.1.2. Shot of evaporation or other issue as an example of what to monitor for

6.2. Drain and inspect the compressor and water trap daily **[1]** to allow the removal of any excess water and to minimize flue gas corrosion **[2]**.

6.2.1. Talent opening compressor and/or water trap
6.2.2. Water being drained

6.3. Configure the data logger to scan each sensor measurement every 10 seconds and to store the average sensor and air and reactor temperature data every 10 minutes **[1]**.

6.3.1. Talent configuring data logger

7. Discrete Sampling and Monitoring and Algal Harvesting and Crop Rotation

7.1. Make sure that the water level remains constant at the reactor's final volume to avoid affecting the optical density measurement **[1]**.

7.1.1. WIDE: Talent checking/replenishing water level Use shot 5.3.1

7.2. After replenishing the water in the reactor, use an ultraviolet-visible spectrophotometer to acquire a 5-milliliter sample for cell mass measurements by optical density **[1-TXT]**.

7.2.1. Talent taking sample *Videographer: Important step* **TEXT: Measure cell mass daily**

7.3. Check the quality of the algae culture three times a week by light microscopy **[1]**. Use shot 4.7.1

7.3.1. Talent at microscope, checking sample quality

7.4. When the culture is close to reaching stationary phase, harvest 75% of the total algae culture volume **[1]** and use 2-5 liters of the culture suspension to perform biomass productivity analysis in the laboratory **[2]**.

7.4.1. Shot of algae at close to stationary phase, then algae being harvested
7.4.2. Talent adding 2-5 L of culture suspension to container for analysis

7.5. Then process and convert the rest of the algae into the desired algal products **[1-TXT]**.

7.5.1. Representative shot of Talent processing rest of algae sample **TEXT: Regularly collect data logger readouts for analysis**

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

3.1., 4.6., 5.2.-5.5., 7.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.1., 5.2.

Results

8. Results: Representative Algae Growth Monitoring

8.1. Here a comparison between the sensor and laboratory measurements can be observed **[1]**.

8.1.1. LAB MEDIA: Figure 5B

8.2. Both readings show similar trends, with the data increasing as a function of time **[1]**.

8.2.1. LAB MEDIA: Figure 5B *Video Editor: please emphasize dotted line*

8.3. Optical density values increase during the day **[1]** but decrease at night during respiration, indicating a change in biomass productivity **[2]**.

8.3.1. LAB MEDIA: Figure 5C *Video Editor: please emphasize data peaks*
8.3.2. LAB MEDIA: Figure 5C *Video Editor: please emphasize inverted peaks*

8.4. Thus, the integration of a real-time optical density sensor makes it possible to make effective management decisions about the overall algal production system **[1]**.

8.4.1. LAB MEDIA: Figure 5C

8.5. In this analysis, flue gas was injected from approximately 8 am to 6 pm **[1]** but was not injected between 6 pm and 8 am **[2]**.

8.5.1. LAB MEDIA: Figure 6 *Video Editor: please emphasize data line from 6:00 to 18:00*
8.5.2. LAB MEDIA: Figure 6 *Video Editor: please emphasize data line from 0:00 to 6:00 and 18:00-0:00*

8.6. This day-night cycle reflects the daily sunlight exposure and the lack of light during the night, and consequently, the activation of photosynthesis or photorespiration, respectively **[1]**.

8.6.1. LAB MEDIA: Figure 6

8.7. As illustrated in this Figure **[1]**, as the algal growth rate increases **[2]**, more flue gas is required **[3]**, confirming that the on-off flue gas pulse injection system is effective at facilitating carbon capture and utilization through microalgae cultivation **[4]**.

8.7.1. LAB MEDIA: Figure 7
8.7.2. LAB MEDIA: Figure 7 *Video Editor: please emphasize blue data line*
8.7.3. LAB MEDIA: Figure 7 *Video Editor: please emphasize orange data line*
8.7.4. LAB MEDIA: Figure 7

8.8. Other physicochemical parameters can also be used to establish a correlation between the parameters and algal growth and productivity **[1]**.

8.8.1. LAB MEDIA: Figures 8 and 9

Conclusion

9. Conclusion Interview Statements

9.1. **Kasi M. Kielhbaugh**: It is important to make sure that the pH system is properly set up and that all of the sensors are calibrated before inoculating the raceway ponds with microalgae **[1]**.

9.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.1., 5.2., 5.3.)

9.2. **Kimberly Ogden**: Other methods that can be performed with the raceway pond-produced microalgae biomass include lipid, carbohydrate, or pigment extraction, ash-free dry weight measurement, and PCR quality monitoring **[1]**.

9.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time – We recorded this*