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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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KEYWORDS:

Environment, outdoor microalgae cultivation, raceway ponds, carbon capture, carbon utilization, industrial flue gas, *Chlorella sorokiniana*.

SUMMARY:

A protocol is described to utilize the carbon dioxide in natural gas power plant flue gas to cultivate microalgae in open raceway ponds. Flue gas injection is controlled with a pH sensor, and microalgae growth is monitored with real-time measurements of optical density.

ABSTRACT:

In the United States, 35% of the total carbon dioxide (CO_2) emissions come from the electrical power industry, of which 30% represent natural gas electricity generation Microalgae can biofix CO_2 10 to 15 times faster than plants and convert algal biomass to products of interest, such as biofuels. Thus, this study presents a protocol that demonstrates the potential synergies of microalgae cultivation with a natural gas power plant situated in the southwestern United States in a hot semi-arid climate. State-of-the-art technologies are used to enhance carbon capture and utilization via the green algal species *Chlorella sorokiniana*, which can be further processed into biofuel. We describe a protocol involving a semi-automated open raceway pond and discuss the results of its performance when it was tested at the Tucson Electric Power plant, in Tucson, Arizona. Flue gas was used as the main carbon source to control pH, and *Chlorella sorokiniana* was cultivated. An optimized medium was used to grow the algae. The amount of CO_2 added to the system as a function of time was closely monitored. Additionally, other physicochemical factors affecting algal growth rate, biomass productivity, and carbon fixation were monitored, including optical density, dissolved oxygen (DO), electroconductivity (EC), and air and pond

temperatures. The results indicate that a microalgae yield of up to 0.385 g/L ash-free dry weight is attainable, with a lipid content of 24%. Leveraging synergistic opportunities between CO_2 emitters and algal farmers can provide the resources required to increase carbon capture while supporting the sustainable production of algal biofuels and bioproducts.

INTRODUCTION:

Global warming is one of the most important environmental issue that the world faces today[1]. Studies suggest that the major cause is the increase in greenhouse gas (GHG) emissions, mainly CO_2, in the atmosphere due to human activities [2–7]. In the U.S., the largest density of CO_2 emissions originates mainly from fossil fuel combustion in the energy sector, specifically electric power generation plants [3, 7–9]. Thus, carbon capture and utilization (CCU) technologies have emerged as one of the major strategies to reduce GHG emissions [2, 7, 10]. These include biological systems that utilize sunlight to convert CO_2 and water via photosynthesis, in the presence of nutrients, to biomass. The use of microalgae has been proposed due to the fast growth rate, high CO_2 fixation ability, and high production capacity. Additionally, microalgae have broad bioenergy potential because the biomass can be converted into products of interest, such as biofuels that can replace fossil fuels [7, 9–12].

Microalgae can grow and achieve biological conversion in a variety of cultivation systems or reactors, including open raceway ponds and closed photobioreactors[13–19]. Researchers have studied the advantages and limitations that determine the success of the bioprocess in both cultivation systems, under either indoor or outdoor conditions[5, 6, 16, 20–25]. Open raceway ponds are the most common cultivation systems for carbon capture and utilization in situations where flue gas can be distributed directly from the stack. This type of cultivation system is relatively inexpensive, is easy to scale up, has low energy cost, and has low energy requirements for mixing. Additionally, these systems can easily be co-located with the power plant to make the CCU process more efficient. However, there are some drawbacks that need to be considered, such as the limitation in CO_2 gas/liquid mass transfer. Although there are limitations, open raceway ponds have been proposed as the most suitable system for outdoor microalgal biofuel production[5, 9, 11, 16, 20].

In this article, we detail a method for microalgae cultivation in open raceway ponds that combines carbon capture from the flue gas of a natural gas power plant. The method consists of a semi-automated system that controls flue gas injection based on the culture pH; the system monitors and records the *Chlorella sorokiniana* culture status in real-time using optical density, dissolved oxygen (DO), electroconductivity (EC), and air and pond temperature sensors. Algal biomass and flue gas injection data are collected by a data logger every 10 min at the Tucson Electric Power facility. Algae strain maintenance, scale up, quality control measurements, and biomass characterization (e.g., correlation between optical density, g/L, and lipid content) are performed in a laboratory setting at the University of Arizona. A previous protocol outlined a method for optimizing flue gas settings to promote microalgae growth in photobioreactors via computer simulation[26]. The protocol presented here is unique in that it utilizes open raceway ponds and is designed to be implemented on-site at a natural gas power plant in order to make direct use of the flue gas produced. Additionally, real-time optical density measurements are part

of the protocol. The system as described is optimized for a hot semiarid climate (Köppen BSh), which exhibits low precipitation, significant variability in precipitation from year to year, low relative humidity, high evaporation rates, clear skies, and intense solar radiation[27].

PROTOCOL:

1. Growth system: outdoor open raceway pond settings

1.1. Set up the open raceway ponds close to the flue gas source (containing 8–10% CO_2). Ensure water and electricity are available at the pond reactor location and that the reactor is not in the shade the majority of the day (**Figure 1**).

1.2. Capture flue gas during the post-combustion process using a 0.95 cm fuel hose, a few meters before the flue gas enters the stack to be discharged into the atmosphere (**Figure 2**).

1.3. Remove water from the flue gas using a 20 L water trap and a condenser (coil length ~12 m) between the stack and the compressor (**Figure 2**).

NOTE: Flue gas typically contains approximately 9–13.8% water[28]. In addition, the condenser and pipeline cool the flue gas[16].

1.4. Connect the following sensors to a datalogger to monitor algal growth: (1) a real-time optical density sensor[29], which measures absorbance at two wavelengths—650 and 750 nm—and can detect a maximum algal cell concentration of 1.05 g/L; (2) a DO sensor; (3) air and pond thermocouples; (4) a pH sensor; and (5) an EC sensor.

NOTE: Additionally, the pH and EC sensors are connected to a transmitter. The datalogger unit configuration is shown in **Figure 3**.

1.5. Ensure that all components of the algal growth system are calibrated and properly working before inoculation.

2. pH control system

2.1. Manage flue gas injection by using a compressor, a control valve system, and the data logger program, as shown in **Figure 2** and **Figure 3** (**Supplementary material A–D**).

2.2. Use a tube to direct the flue gas from the control valve to the bottom of the raceway pond through a stone diffuser.

2.3. Inject the flue gas into the growth system based on pH. When the pH value is greater than 8.05, the system will inject flue gas, whereas when the pH is less than 8.00, the system will stop the flue gas injection in periods of no growth. The flow rate is measured in standard liters per minute (SLPM).

NOTE: In the control valve, the inlet flue gas pressure is limited to a maximum of 50 psi.

3. Algae selection and strain maintenance (light and temperature)

NOTE: The green algae *Chlorella sorokiniana* DOE 1412 was isolated by Juergen Polle (Brooklyn College)[30, 31] and selected by the National Alliance or Advanced Biofuels and Bioproducts (NAABB); its selection was based on the previous strain characterization studies performed by Huesemann et al.[32, 33] . Their research regarding algal screening, biomass productivity, and climate-simulated culturing (e.g., temperature and light) in the Southwest region when using outdoor open raceway ponds informed the method used in this project.

3.1. Maintain cultures at room temperature (25 °C) using a 12 h/12 h light/dark cycle.

3.2. Keep light intensity at 200 µM/m^2/s for culture maintenance grown on plates, and in small liquid cultures (50 mL to 500 mL).

3.3. Keep light intensity for scale up grown in liquid cultures 50 mL to 500 mL at 400 µM/m^2/s, and liquid cultures 5 L to 20 L at 600–800 µM/m^2/s.

4. Scale up and quality control

4.1. Prepare the BG11 culture medium using deionized water and the following salts, for macronutrients, in g/L: 1.5 $NaNO_3$, 0.04 K_2HPO_4, 0.075 $MgSO_4*H_2O$, 0.036 $CaCl_2*H_2O$, 0.006 $(NH_4)_5Fe(C_6H_4O_7)_2$, 0.006 $Na_2EDTA*2H_2O$, 0.02 Na_2CO_3; add 1 mL/L of trace element solution, which contains the following micronutrients in g/L: 2.86 H_3BO_3, 1.81 $MnCl_2*4H_2O$, 0.22 $ZnSO_4*7H_2O$, 0.39 $Na_2MoO_4*2H_2O$, 0.079 $CuSO_4*5H_2O$, 0.0494 $Co(NO_3)_2*6H_2O$.

NOTE: For plate inoculation and/or long-term storage, add 7.5 g/L of Bacto agar; for culture inoculation, no addition of agar is needed. Sterilize culture medium in the autoclave for 21 min at 121 °C.

4.2. Pour the BG11 medium with agar into Petri dishes in a sterile laminar flow hood or biosafety cabinet. Once plates are firm and cool, pipette 500 µL from a re-suspended frozen algal stock culture and add Ampicillin (100 µg/mL); incubate the algal plates in a shaker table (120 rpm) for 1 to 2 weeks.

4.3. Use a sterile loop to select a single algal colony from a culture plate and inoculate it in a 50 mL tube containing sterile growth medium in a clean biosafety cabinet. Grow the small liquid culture on a shaker table (120 rpm) for one week.

4.4. Transfer 50 mL of algae culture (linear growth phase, $OD_{750nm} \geq 1$) into a 1 L flask with 500 mL liquid medium. Each flask is fitted with a rubber stopper and stainless-steel tubing to provide aeration. Filter the air using 0.2 µm air sterilization filters. Let the culture grow for one to two

weeks. Monitor cell density using a spectrophotometer (OD_{750nm}).

4.5. Place the 500 mL liquid medium into a 10 L carboy containing 8 L of non-sterile culture medium and inject a mixture of 5% CO_2 and 95% air. Then, cultivate algae under the same conditions as in step 4.4.

4.6. Monitor stock plate and liquid cultures (in steps 4.2–4.5) once a week. Take an aliquot and observe it under the microscope at 10x and 40x magnification to ensure the growth of the desired strain. Cultures are kept until they have been compromised or used for experiments. Discard contaminated cultures.

5. Concentrated medium preparation for open pond cultivation

5.1. To prepare trace elements solution partially fill a 1 L volumetric flask with distilled water (DW). Insert a magnetic stir bar and add the chemicals shown in **Table 1** sequentially. Ensure that each ingredient dissolves before the addition of the next constituent. Remove the magnetic and fill the flask to the 1 L volume mark.

5.2. Partially fill a 1 L glass bottle with DW and insert the magnetic stir bar. Place the container on the top of a magnetic stirrer plate and add the chemicals for the reactor's final volume, adding them sequentially, ensuring each fully dissolves. **Table 2** lists the chemicals to prepare 1 L of medium, so multiply all the values by the reactor's final volume. Fill the glass bottle to 1 L.

6. Outdoor open raceway pond inoculation

6.1. Thoroughly clean the reactor using 30% bleach before each inoculation and after harvesting. It is recommended to leave the bleach overnight. Rinse the reactor well to remove all bleach.

6.2. Calibrate all the sensors before algae inoculation according to their corresponding calibration procedure.

6.3. Dilute the concentrated media (in step 5) using the water source by filling the raceway pond up to 80%.

6.4. Inoculate the reactor using a 10 L carboy filled with algae (linear growth phase $OD_{750nm} \geq 2$) and bring it to its the final volume.

6.5. Acclimate microalgae by partially shading the raceway pond with wooden pallets for ~ 3 days (**Figure 4**), once the exponential phase has passed, as an adaptation strategy to avoid photoinhibition. This period will also provide time, so the microalgae can adapt the stress condition caused by the direct injection of flue gas.

7. Batch growth experiment at the generating station

7.1. Inspect and record any day to day variations including water evaporation, paddlewheel motor, sensor functionality, and anything out of the ordinary.

7.2. Drain and inspect the compressor and water trap every day to remove any excess of water to minimize corrosion since flue gas is highly corrosive[34].

7.3. Configure the data logger to scan each sensor measurements every 10 s and to store the average data every 10 min. These include DO, pH, EC, real-time optical density as well as air and reactor temperature.

8. Discrete sampling and monitoring

8.1. Make sure water level remains constant at the reactor's final volume otherwise the optical density measurement will be affected.

8.2. After replenishing water in the reactor, take a 5 mL sample for cell mass measurements by optical density (540, 680, and 750 nm) using an ultraviolet-visible spectrophotometer. Repeat the process daily.

8.3. Take a 500 mL sample three times per week for microscope observations and biomass concentration based on ash-free dry weight (AFDW).

8.3.1. Perform microscope observations with 10x and 40x objective lenses. Additionally, these microscope magnifications are used a as part of algal quality control in step 4.6.

8.3.2. Use 400 mL of the sample in step 8.3 for AFDW

8.3.2.1. Set each 0.7 µm pore size glass microfiber filter in an aluminum foil tray and pre-treat each aluminum foil tray/filter using a furnace for 4 h at 540 °C.

8.3.2.2. Label each aluminum foil tray using a #2 pencil, record its weight (A), and place it in the vacuum filter apparatus.

8.3.2.3. Stir the algae sample vigorously before measuring out a volume to be filtered. Filter enough algae sample to give a pre/post ash weight difference of between 8 and 16 mg. Pick a weight difference to use throughout the course of the experiment and keep this value constant.

8.3.2.4. Place each filter containing the algae sample in its foil tray in the oven at 105 °C for at least 12 h.

8.3.2.5. Remove the foil tray/filter from the drying oven and place it in a glass desiccator to prevent water uptake. Record each foil tray/filter weight (B).

8.3.2.6. Place the foil tray/filter in the 540 °C muffle furnace for 4 h.

8.3.2.7. Turn off the muffle furnace, cool down foil trays/filters, place them into the desiccator, and record each foil tray/filter weight (C).

8.3.2.8. Calculate AFDW using gravimetrical analysis:

% AFDW= C – A × 100 / B

8.4. Hold 2 L of algae before harvesting for microwave assisted extraction (MAE) lipid extraction analysis using solvents.

8.4.1. Centrifuge the algae sample at relative centrifugal force (RFC) of 4,400 × *g* for 15 min. Take the algae pellet and dry it using an oven at 80 °C for at least 24 h.

8.4.2. Grind the algae sample and weigh the algal powder (recommended biomass ranges from 0.3 g to 0.5 g).

8.4.3. Add the algae powder (dry algal biomass) into the microwave accelerated reaction system (MARS) Express vessels, add 10 mL of chloroform:methanol (2:1, v/v) solvent solution under the hood, close the vessels, and let stand overnight.

8.4.4. Place the vessels into the MARS machine using the solvent sensor for 60 min at 70 °C and 800 W of power.

8.4.5. Take vessels out of MARS and let them cool down under the hood.

8.4.6. Use a funnel and glass wool to separate the liquid part which contains chloroform, methanol, and lipids by transferring each liquid sample to a pre-weighed glass test tube and keep the solids (biomass free of lipids) for other analyses.

8.4.7. Take the test tubes containing the lipids to the nitrogen evaporator, remove them once the liquid has been evaporated, and then leave the tubes overnight under the hood to ensure complete dryness.

8.4.8. Calculate lipid content (wt. %) using gravimetric analysis:

Lipid content (wt. %) = Dry biomass of lipids × 100/ Dry Algal mass

9. Algal harvesting and crop rotation

9.1. Harvest 75% of the total algae culture volume when the culture is close to reaching stationary phase. Take 2–5 L of culture to perform biomass productivity analysis in the laboratory. Process and convert the rest of the algae into the desired algal products.

9.2. Re-grow the open raceway pond by using the 25% algae remaining as inoculum. Add water up to 80% of the total reactor's volume, add the concentrated media, and then finish filling up to the reactor's final volume if necessary.

9.3. Cultivate the appropriate algae strain according to the season, based on temperature and light intensity conditions.

10. Data management

10.1. Record data in the data logger and collect for analysis as in step 7.3.

10.2. Consider saving raw and analyzed data in the Regional Algal Feedstock Testbed (RAFT) share drive. RAFT project collaborators assign their data to simulate and model algal productivity and validate outdoor cultivation.

REPRESENTATIVE RESULTS:

Prior experimental results from our lab indicate that microalgae cultivation using a semi-automated open raceway pond can be coupled with carbon capture processes. To better understand the synergy between these two processes (**Figure 2**), we developed a protocol and tailored it for cultivating the green algal species *Chlorella sorokiniana* under outdoor conditions in a hot semiarid climate. Natural gas flue gas was obtained from an industrial power generation station. This protocol uses various technologies to assess algal biomass productivity: (1) algae growth using a real-time optical density sensor (**Figure 5**); (2) algae growth with respect to flue gas on-off pulse injections into the culture as a function of pH (**Figure 6** and **Figure 7**); and (3) algae growth correlations with environmental parameters such as temperature, dissolved oxygen, and electroconductivity (**Figure 8** and **Figure 9**).

We test a real-time optical density sensor that monitors algae growth and physiological dynamics. This sensor allowed us to establish, via lab correlation, the corresponding ash free dry weight biomass (g/L). **Figure 5** shows a comparison between the sensor and laboratory measurements. Both readings show similar trends, increasing as a function of time. However, the in-situ sensor readings can track the day/night algae growth cycle. Said cycle shows that the optical density values increase during the day but decrease at night during respiration, indicating a change in biomass productivity. The integration of the real-time optical density sensor makes it possible to make effective management decisions about the overall algal production system.

We deploy a semi-automated on-off flue gas pulse injection system, which is represented in **Figure 6** by a 24 h flue gas injection cycle measured during a particularly warm fall season in Tucson, AZ. As shown in **Figure 6**, flue gas was injected from approximately 8 am to 6 pm (diurnal period) but was not injected between 6 pm and 8 am (nocturnal period). 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. **Figure 7** presents the cumulative flue gas injected (L) during this algal batch. In this case, 6,564 L of flue gas, corresponding to 538 L CO_2, were used to grown 0.29 g of algal biomass. The graph shows that as the algal growth rate

increased, more flue gas (CO_2) was required (**Figure 6**). The experimental results have confirmed that the on-off flue gas pulse injection system is effective at facilitating carbon capture and utilization through microalgae cultivation.

We measure and monitor other physicochemical parameters to establish a correlation between them and algal growth and productivity (**Figure 8** and **Figure 9**). The environmental parameters measured were dissolved oxygen, electroconductivity (EC), and both air and pond temperatures. As expected, all the parameters, except EC, displayed similar trends that were highly correlated with solar radiation. The results indicate that these environmental variables had the most significant impact on algal growth and are used for algal biomass modeling[35]. The EC did not change significantly during the batch process. Thus, it did not provide any relevant information regarding algal growth. For cultivation of *Chlorella sorokiniana* using non-saline water, EC measurements can be omitted.

FIGURE AND TABLE LEGENDS:

Figure 1: Pilot site location at Tucson Electric Power for coupling carbon capture from power plant and semi-automatized open-pond reactors for microalgae cultivation. The two locations are represented by: 1) Algae Site U3 (unit 3) and 2) Algae Site U4 (unit 4) photo credit: Jose Manuel Cisneros Vazquez.

Figure 1: Process flow chart for coupling carbon capture and semi-automatized open raceway ponds for microalgae cultivation in a hot semiarid climate. (A) Open Raceway Paddlewheel design; (B) Real experiment facility; (C) Process: coupling carbon capture and microalgae cultivation modified from Van Den Hende[28]. Legends: T = Temperature; DO = Dissolved oxygen; OD = Optical density; EC = Electrical conductivity; Data Logger.

Figure 3: Schematic representation of sensors set up. (**A**) Representation of the overall outdoor open-pond sensors set up, in which CV1 and CV2 are the control valves, DL: Data logger, T1 and T2 are the transmitters. (**B**) Representation of a control valve. (**C**) Representation of the sensors' connection to the datalogger; dark blue circle: real-time optical density, orange triangle: pH and EC, black triangle: thermocouples, red triangle: dissolved oxygen, light blue: control valve. (**D**) pH and EC transmitter.

Figure 4: Algae under the acclimation process. Microalgae acclimation strategy using wooden pallets during the exponential phase.

Figure 5: Representation of algae growth monitoring. (**A**) Graph for AFDW biomass concentration (g/L) vs. time of laboratory measurements; (**B**) Graph for correlation between optical density sensor and laboratory measurements at 650 nm; and (**C**) graph for real-time optical density sensor vs time for an experimental batch.

Figure 6: Graph for on/off flue gas pulse injection as a fuction of pH. The dattalogger was set up to start flue gas injection (controlled valve on) at pH = 8.05 and to end flue gas injection (controlled valve off) at pH = 8.00.

Figure 7: Graph for algal growth (g/L), amount of flue gas injected, and amount of CO_2 injected as a function of time.

Figure 8: Representation of temperatures monitoring. Legends: solid yellow line = raceway pond reactor temperature; solid grey line = air temperature; and dashed blue line = AZMET Station temperature represented by.

Figure 9: Monitoring of algae growth parameters. Legends: orange solid line = solar radiation; grey solid line = electroconductivy (EC); and yellow solid line = dissolved oxygen (DO).

Table 1: Trace elements solution recipe.

Table 2: Optimized media recipe for 1 L.

DISCUSSION:

In this study, we demonstrate that synergistically coupling flue gas carbon capture and microalgae cultivation is possible in a hot semi-arid climate. The experimental protocol for the semi-automated raceway pond system integrates state-of-the-art technology to monitor relevant parameters in real time that correlate to algal growth when using flue gas as a carbon source. The proposed protocol is intended to reduce uncertainty in algal cultivation, which is one of the main drawbacks of raceway ponds[20, 21, 36]. In our experience, the protocol's most critical steps involve the pH control system and an effective method to inoculate the system (**Figure 2**). The pH control system delivers flue gas/CO_2, and represents a strategy to optimize efficiency in CO_2 capture and utilization (**Figure 3**)[37]. This controlled system has been proven to be more efficient than a continuous injection system for the microalgae cultivation process because it reduces outgassing while delivering enough flue gas to attain the maximum algal growth rate[20, 37]. When the flue gas injection is based on pH, a key factor for algal cultivation is selecting an adequate pH value for the microalgae species before inoculating the raceway pond[38, 39]. Qiu et al.[40] found that a pH value of 8 is the best for the freshwater species *Chlorella sorokiniania* when considering cell growth and lipid production[40]. Moreover, Molina Grima et al. [41] recommend a pH below 8 to reduce nitrogen loss and achieve better nitrogen uptake by the microalgae/biomass[41]. However, Yuvraj et al. [42] suggest that pH is not an appropriate method to evaluate the CO_2 content in the water because of the effect of nitrogen fertilization on the medium's acidity[42]. Our results show that pH can be effectively used to manage CO_2 injection for the system presented here (**Figure 6**); our flue gas injection management, which kept the culture at pH 8, resulted in high biomass yields and replicability (**Figure 7**).

After inoculation, the algae must acclimate to the system to avoid photoinhibition and to adjust to the high temperature of the raceway media. In this hot semi-arid climate, we have observed algal photoinhibition due to high solar radiation[39, 43, 44] (**Figure 9**). This effect can not only delay but also inhibit microalgae inoculation during the exponential phase[32, 35, 45–47]. To reduce the impact of acclimation on the microalgae, we designed a successful and feasible strategy consisting of partially shading the raceway pond with wooden pallets. This strategy allows the microalgae to be exposed repeatedly but for short periods of time to the solar conditions.

Another stress factor is the high temperature of the flue gas and the ambient air[33, 48] (**Figure 8**). The flue gas temperature is quite high at the post-combustion stage[10, 48, 49]. Utilizing the flue gas by directly injecting it from the dispatched pipeline into the raceway pond can contribute to further increasing the medium's temperature. Hence, a condenser followed by a water trap located before the compressor will not only reduce the heat transfer but also the amount of water reaching the compressor (**Figure 2**). We found that both devices were necessary to reduce the compressor failure rate. Additionally, humidity, flue gas temperature, and the corrosive nature of the flue gas must be considered when estimating the compressor's life cycle and maintenance. Furthermore, high temperatures cause higher evaporation rates.

This protocol is subject to some limitations. According to **Figure 6**, the control valve was not able to inject enough flue gas when photosynthesis was at its peak. This effect can be attributed to low mass transfer from the gaseous to the liquid phase due to the reactor design[5, 16, 50, 51]. Mendoza et al. [36, 52] and I. Godos et al. [16] stated that raceway ponds have a poor gas/liquid mass transfer, which represents one of the most severe design constraints[16, 36, 52]. Their shallow channel design limits CO_2 mass transfer due to the short interface area between the gas and the culture medium, which causes an increase in CO_2 off-gassing (**Figure 2**). Thus, devices and novel configurations have been proposed to increase the gas/liquid contact time, including sumps, mixing columns, permeable silicone, and sparging-diffusion systems[36, 52, 53]. All these systems have been used in an attempt to enhance CO_2 mass transfer; however, some of these systems also improve nutrient distribution, control pH, and remove excess O_2[5, 24, 36, 52]. Finally, outages are other limitations that can arise when capturing and utilizing real flue gas from a power plant. These outages are not always scheduled. Thus, temporary alternative sources of CO_2 should be considered, for example, relocation or connecting the CO_2 mainline to multiple power units (**Figure 1**).

The ability to produce microalgae with this protocol is supported by our results on algal productivity (**Figure 5**), algal responses to the selected parameters (**Figure 6**, **Figure 8**, **Figure 9**), and successful cultivation of the desired algal species when nurtured by direct flue gas injection. Open reactors are cheaper to operate, and thus, this protocol builds upon their strengths to accelerate commercial-scale deployment of this form of carbon capture and utilization[16, 20, 54–56]. This hot semi-arid region experiences high solar radiation and significant temperature fluctuations year-round (**Figure 8** and **Figure 9**)[57]; hence, it is a prime location to test this sort of protocol. The optical density sensor provided consistent OD readings for our outdoor open system (**Figure 5**); this type of data collection would be impractical using other sensors. Also, the sensors responded well to the significant temperature variations from day to night (**Figure 8**), enabling us to make timely algal productivity decisions[29]. Furthermore, the proposed optimized medium has the critical advantage of being based on commercial fertilizer and readily available nutrient sources[58] (**Table 1** and **2**); this medium can be easily produced in-house or could be sourced upon request from agricultural liquid fertilizer companies[58]. Finally, the semi-automated protocol was tested in an additional natural gas power plant. The results of that confirmation study are not presented in this paper. In that confirmation study, the protocol was successful despite the extreme weather conditions in Tucson and the exceptionally hot temperatures at the generation station due to the reactor's location within the power plant layout. Therefore,

protocol replicability has been examined for Tucson's environment when natural gas is used as fuel to produce electricity.

The following steps are recommended to further develop this protocol and to improve and enhance the automation of the processes involved. The first recommendation is to make the flue gas injection a completely variable-rate process, thus improving CO_2 and pH management; the current program fully opens the injection valve when the pH falls below 8 and closes it when the pH reaches 8 again. Improving the way CO_2 is injected is also necessary. The aim is to reduce the size of the CO_2 bubbles, i.e., to generate microbubbles to enhance CO_2 diffusion in the medium without resorting to injecting flue gas at higher pressure. Using improved injectors, thus reducing operational energy costs, is deemed necessary in a commercial application of the protocol. The inclusion of predictive tools based on the weather forecast and current microalgae status for controlling the flue gas and fertilizer, mainly N, to improve N use efficiency, is also recommended. The use of computational fluid dynamic modeling is considered a vital tool in developing the proposed protocol further; modeling can help optimize the design, configuration, and operation of all the hardware involved in the monitoring and management of the microalgae. Another area that could be explored in the future is the application of environmental DNA (eDNA) and real-time PCR techniques to monitor the health and composition of the microalgae crop. Water samples could be analyzed, and the results would indicate whether the objective microalgae are the predominant species in the medium or whether it is competing or has being replaced by a different organism.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure

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Figure

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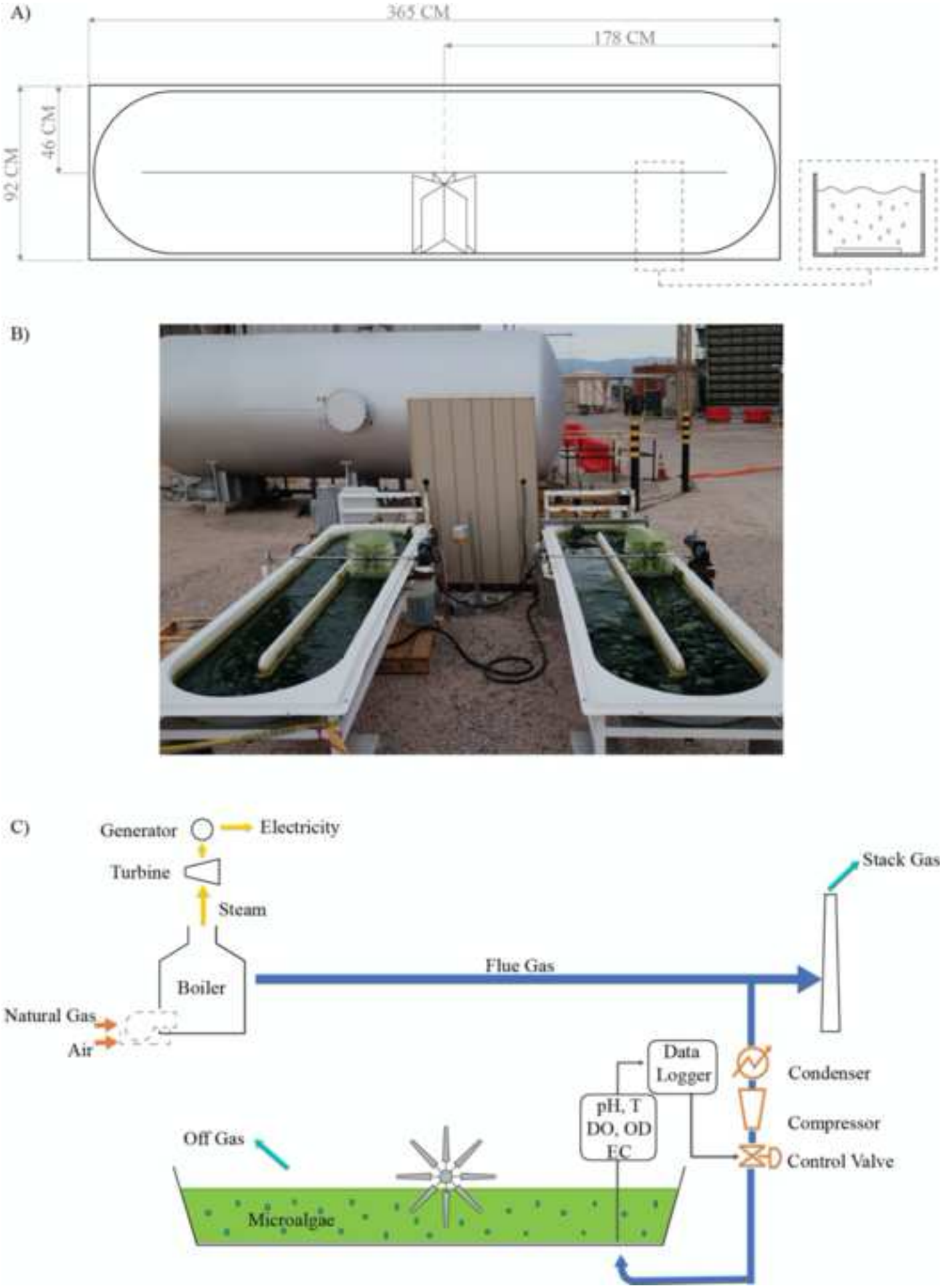

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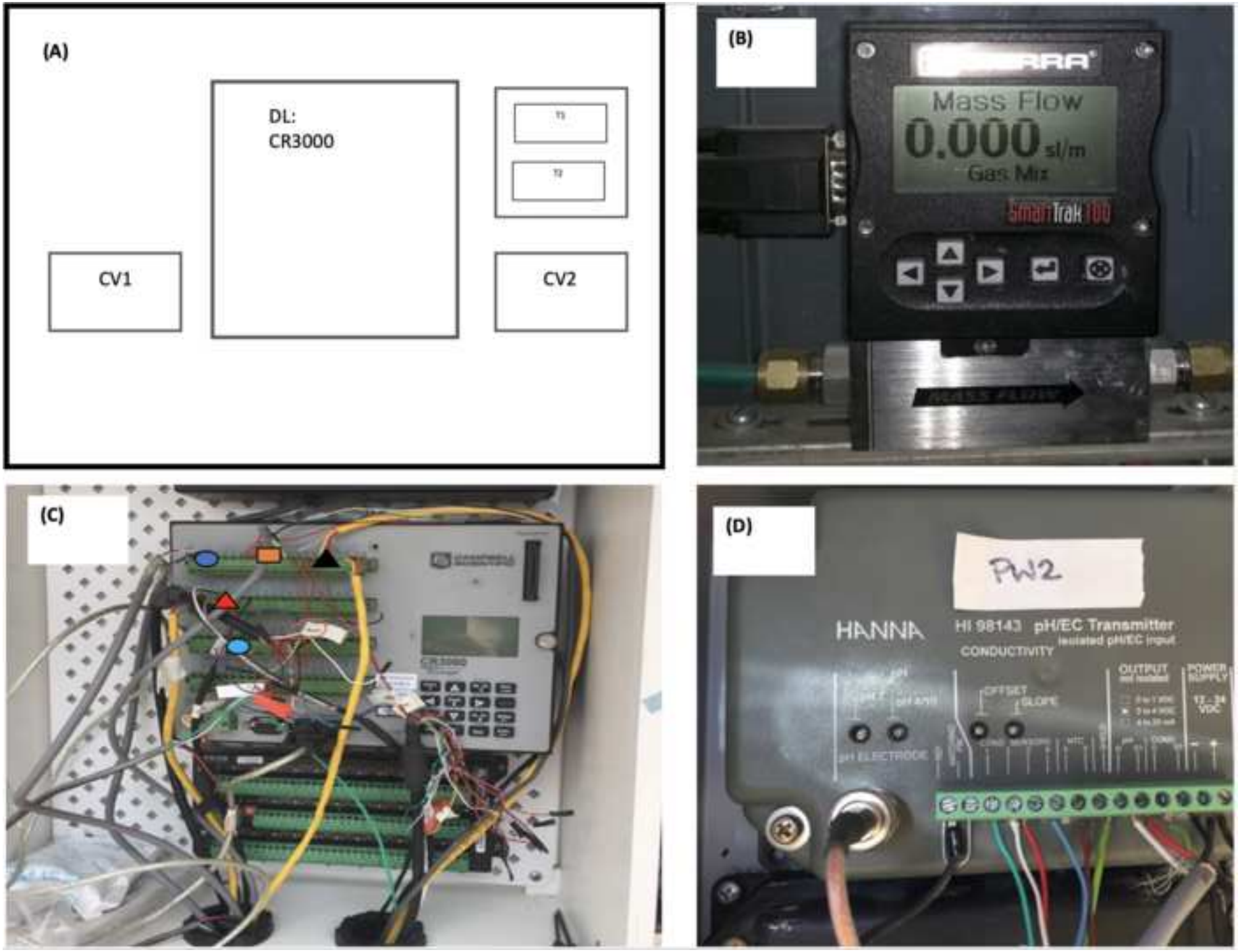

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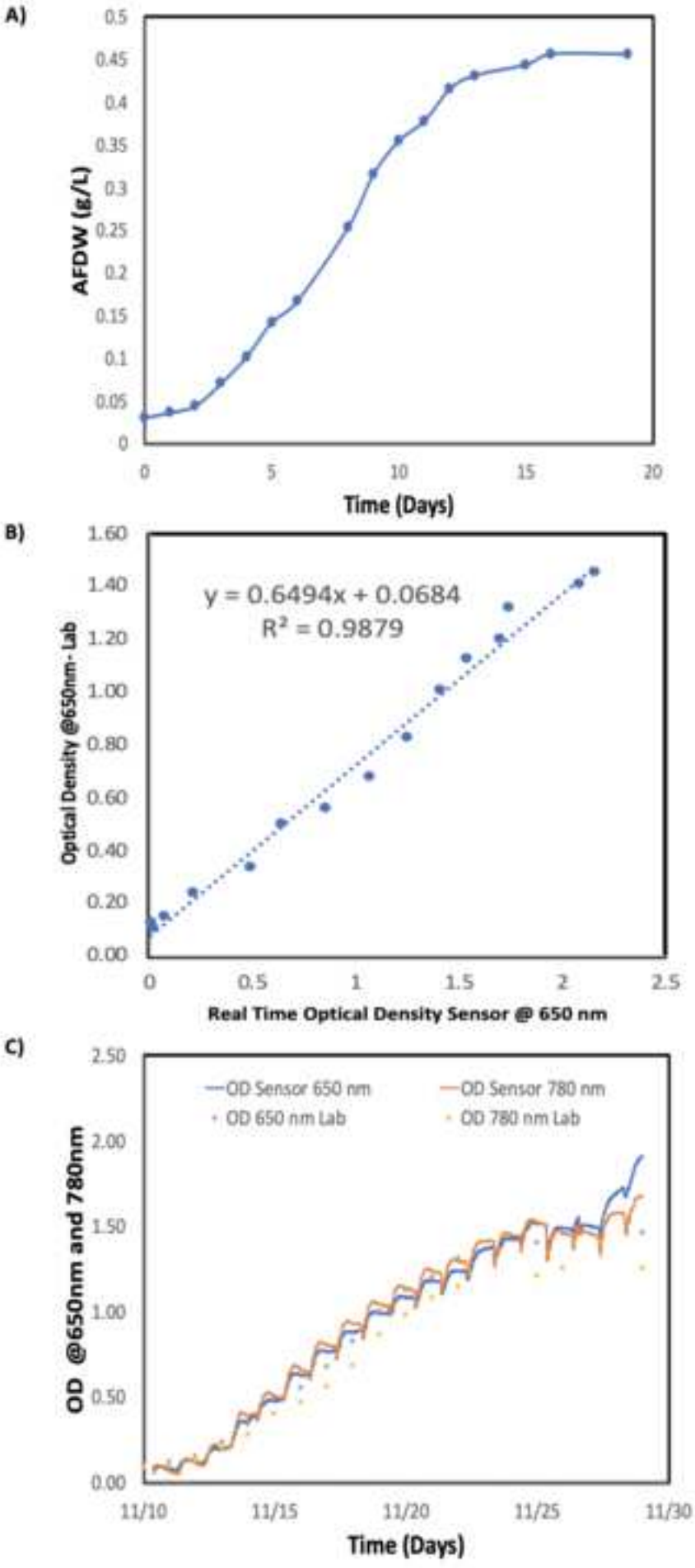

Figure

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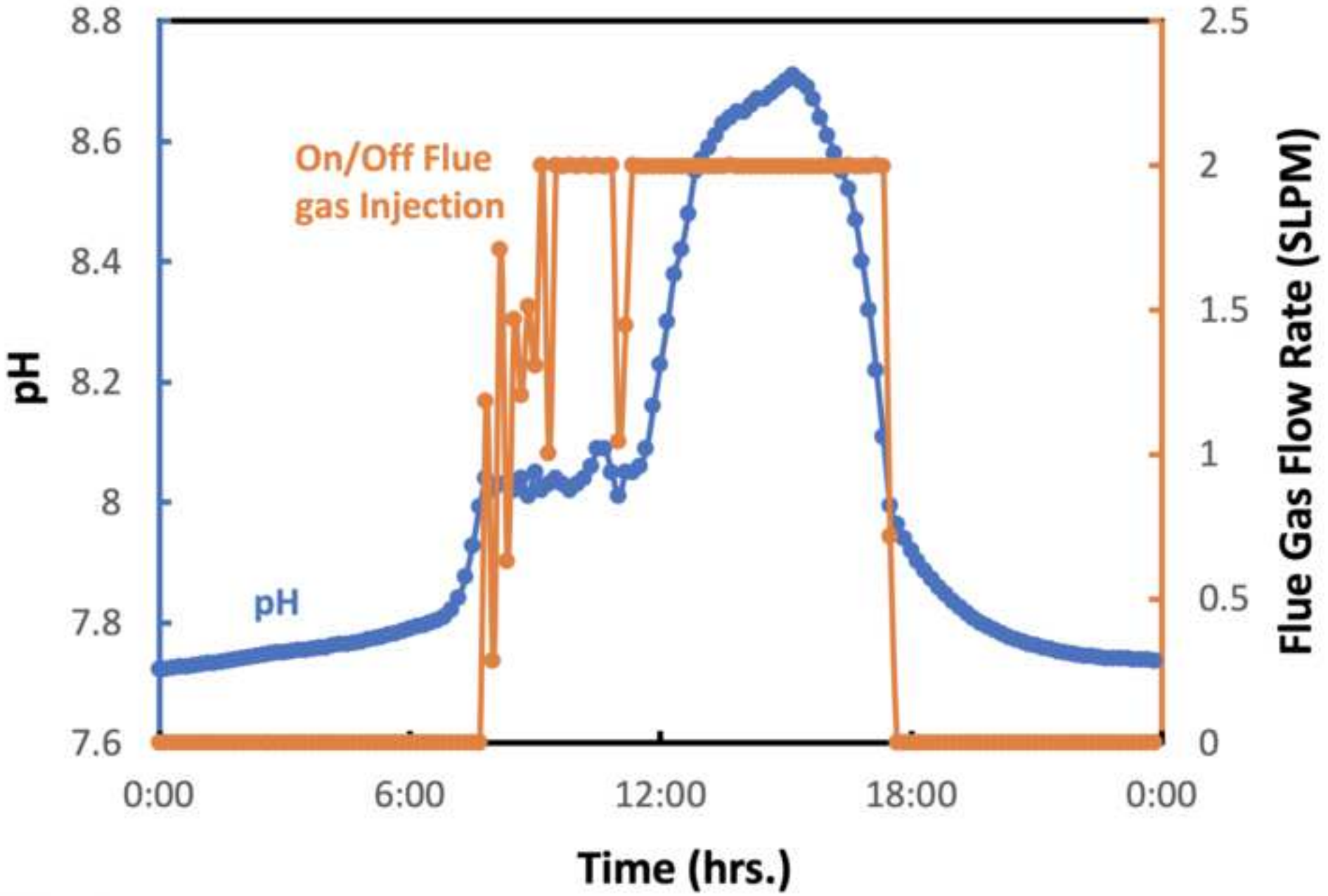

Figure

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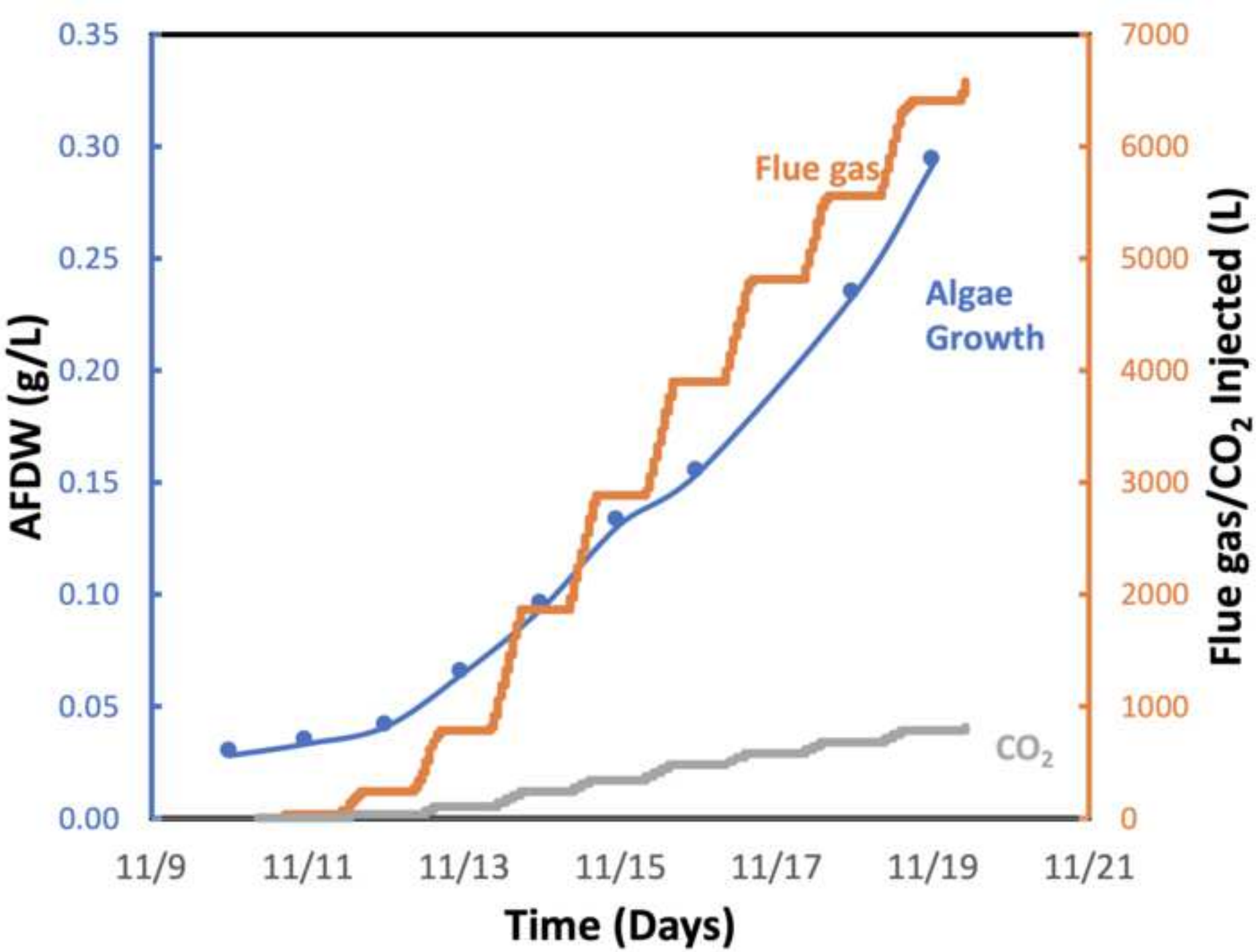

Figure

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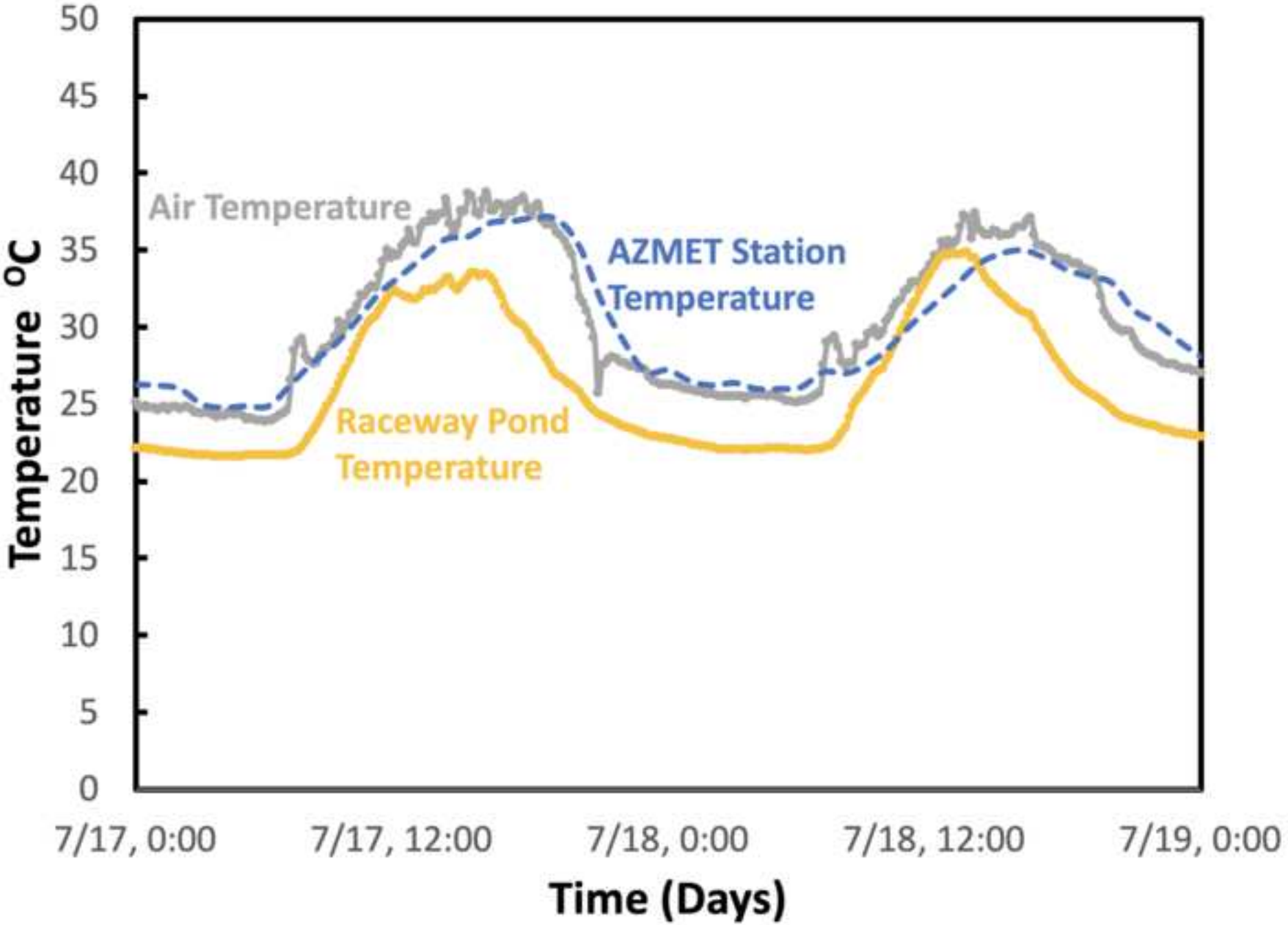

Figure

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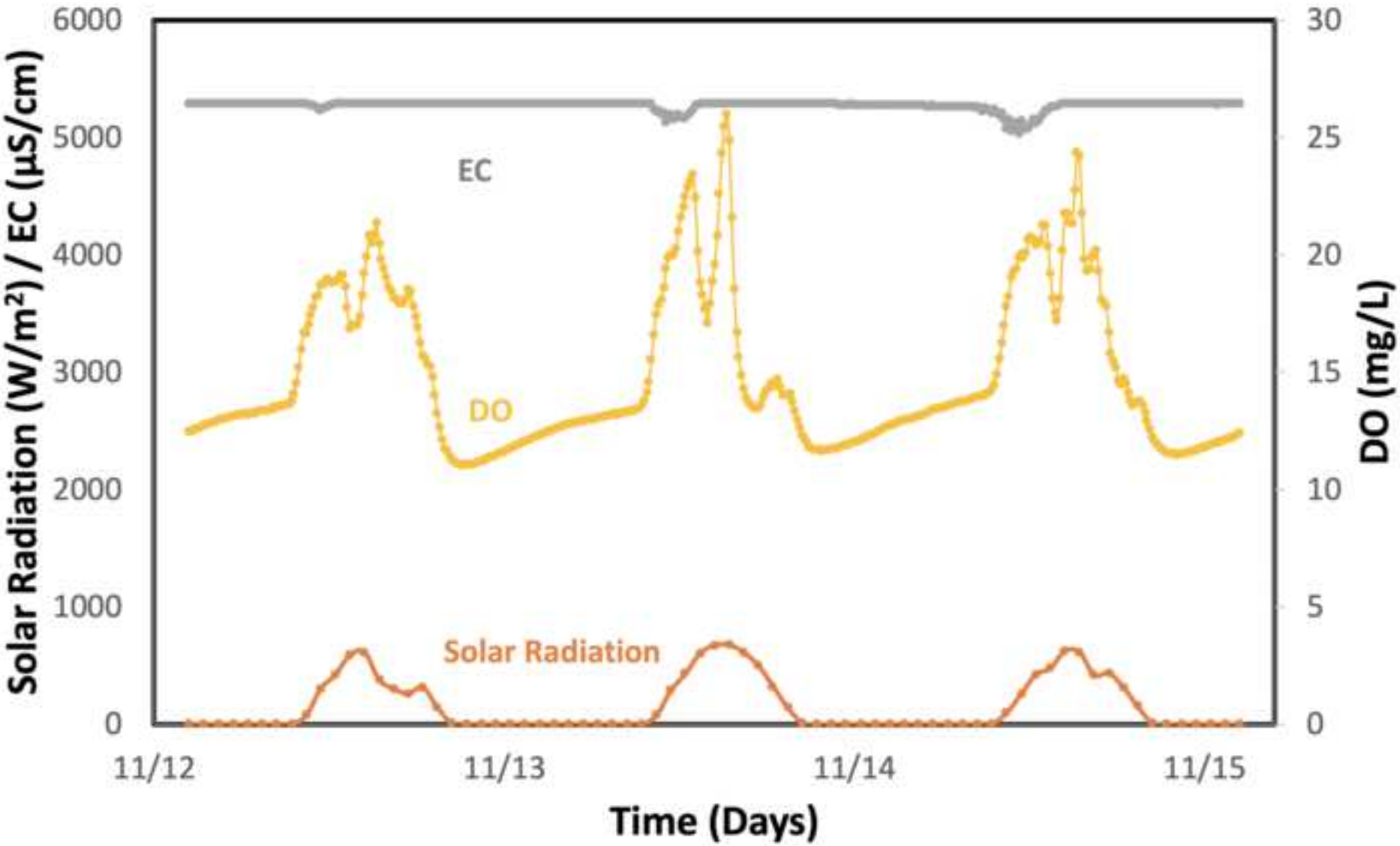

Components
H_3BO_3
$MnCl_2 \cdot 4H_2O$
$ZnSO_4 \cdot 7H_2O$
$Na_2MoO_4 \cdot 2H_2O$
$CuSO_4 \cdot 5H_2O$
$Co(NO_3)_2 \cdot 6H_2O$
$NiCl_2 \cdot 6\ H_2O$

Concentration in solution (g/L)
0.00286
0.00181
0.0001373
0.00039
0.000079
0.00005518
0.0001

Components
$(NH_2)_2CO$
$MgSO_4 \cdot 7H_2O$
$NH_4H_2PO_4$
KCl
$FeCl_3$
Trace Metal Solution

Common name
Urea
Magnesium Sulfate
Ammonium Phosphate
Potash
Ferric Citrate (Citraplex)
Volume of 1000x Micros (ml)

Concentration in solution (g/L)
0.1
0.012
0.035
0.175
0.005423
1

Name of Material/ Equipment	Company	Catalog Number
Adjustable speed motor (paddle wheel system	Leeson	174307
Aluminum weight boats	Fisher Scientific	08-732-102
Ammonium Iron (III) $(NH_4)_5[Fe(C_6H_4O_7)_2]$	Fisher Scientific	1185 - 57 - 5
Ammonium Phosphate	Sigma-Aldrich	7722-76-1
Ampicillin sodium salt	Sigma Aldrich	A9518-5G
Autoclave	Amerex Instrument Inc	
Bacto agar	Fisher Scientific	BP1423500
Bleach	Clorox	
Boric Acid (H3BO3)	Fisher Scientific	10043-35-3
Calcium chloride dihydrate (CaCl2*2H2O)	Sigma-Aldrich	10035-04-8
Carboys (20 L)	Nalgene - Thermo Fisher Scientific	2250-0050PK
Centrifuge	Beckman Coulter, Inc	
Chloroform	Sigma-Aldrich	67-66-3
Citraplex 20% Iron	Loveland Products	SDS No. 1000595582 -17-I
Cobalt (II) nitrate hexahydrate (Co(NO3)2*6H2O)	Sigma-Aldrich	10026-22-9
Compressor	Makita	MAC700
Control Valve	Sierra Instruments	SmartTrak 100
Copper (II) Sulfate Pentahydrate (CuSO4*5H2O)	Sigma-Aldrich	7758-99-8
Data Logger: Campbell unit CR3000	Scientific Campbell	CR3000
Dissolvde Oxygen Solution	Campbell Scientific	14055
Dissolved Oxygen probe	Sensorex	
Electroconductivity calibration solution	Ricca Chemical Company	2245 - 32 (R2245000-1A)
Electroconductivity probe sensor	Hanna Instruments	HI3003/D
Ethylenediaminetetraacetic acid disodium salt dihydrate (Na2EDTA*2H2O)	Sigma-Aldrich	6381-92-6
Filters	Fisher Scientific	09-874-48

Flasks	Fisher scientific	09-552-40
Furnace	Hogentogler	Model: F6020C-80
Glass dessicator	VWR International LLC	75871-430
Glass funnel	Fisher Scientific	FB6005865
Laminar flow hood	Fisher Hamilton Safeair	
Magnesium sulfate heptahydrate (MgSO4*7H2O)	Fisher Scientific	10034 - 99 - 8
Methanol	Sigma-Aldrich	67-56-1
Micro bubble Diffuser	Pentair Aquatic Eco-Systems	1PMBD075
Microalgae: Chlorella Sorokiniana	NAABB	DOE 1412
Microoscope		
Microwave assistant extraction	MARS, CEM Corportation	
MnCl2*4H2O	Sigma-Aldrich	13446-34-9
Mortars	Fisher Scientific	FB961B
Nitrogen evaporator	Organomation	
Oven	VWR International LLC	89511-410
Paddle Wheel		
Paddle wheel motor	Leeson	M1135042.00
Pestles	Fisher Scientific	FB961M
pH and EC Transmitter	Hanna Instruments	HI98143
pH calibration solutions	Fisher Scientific	13-643-003
pH probe sensor	Hanna Instruments	HI1006-2005
Pippete tips	Fisher Scientific	1111-2821
Pippetter	Fisher Scientific	13-690-032
Plastic cuvettes	Fisher scientific	14377017
Plates	Fisher scientific	08-757-100D
Potash		
Potassium phosphate dibasic (K2HPO4)	Sigma-Aldrich	7758 -11 - 4
Pyrex reusable Media Storage Bottles	Fisher scientific	06-414-2A
Raceway Pond		
Real Time Optical Density Sensor	University of Arizona	
RS232 Cable	Sabrent	

Shaker Table		
Sodium Carbonate (Na_2CO_3)	Sigma-Aldrich	497-19-8
Sodium molybdate dihydrate ($Na_2MoO_4*2H_2O$)	Sigma-Aldrich	10102-40-6
Sodium nitrate ($NaNO_3$)	Sigma-Aldrich	7631-99-4
Spectophotometer	Fisher Scientific Company	14-385-400
Test tubes	Fisher Scientific	14-961-27
Thermocouples type K	Omega	KMQXL-125G-6
Urea	Sigma-Aldrich	2067-80-3
Vacuum filtration system	Fisher Scientific	XX1514700
Vacuum pump	Grainger	
Zinc sulfate heptahydrate ($ZnSO_4*7H_2O$)	Sigma-Aldrich	7446-20-0

Comments/Description

Lesson 174307.00, type: SCR Voltage; Amps:10
Fisherbrand Aluminum Weighing Dishes
Medium preparation. Ammonium iron(III) citrate
This chemical is used for the optimized medium
This chemical is used for avoiding algae contamination
Hirayama HA300MII
Fisher BioReagents Granulated Agar
Germicidal Bleach, concentrated clorox
Trace Elelements: Boric acid
Medium preparation. Calcium chloride dihydrate

Polypropylene Carboy w/Handles
J2-21
This chemical is used for lipid extraction
https://www.fbn.com/direct/product/Citraplex-20-Iron#product_info

Trace Elements: Cobalt (II) nitrate hexahydrate
This equipment is used for the injection CO2 system
This item needs to be customized for your application. In our case, it was used a 5% CO2 and 95% air mixture.

Trace Elements: Copper (II) Sulfate Pentahydrate
This equipment is used for controlling all the system, motoring and recording data
Dissolved oxygen electrolyte solution DO6002 - Lot No. 211085
DO6400/T Dissolved Oxygen Sensor with Digital Communication

Conductivity Standard, 5000 uS/cm at 25C (2620 ppm TDS as NaCl)
Flow-thru Conductivity Probe - NTC Sensor, DIN Connector, 3m Cable

Medium Preparation: Ethylenediaminetetraacetic acid disodium salt dihydrate
Whatman Binder-Free Glass Microfiber Filters

Pyrex Fernbach Flasks
Thermo Sicentific Thermolyne F6020C - 80 Muffle Furnace
Type 150, 140 mm of diameter
Fisherbrand Reusable Glass Long-Stem Funnels
Fisher Hamilton Stainless Safeair hume hood

Medium Preparation: Magnesium sulfate heptahydrate
Lipid extraction solvent

This equipment is used for the injection CO2 system

Carl Zeiss 4291097
CEM Mars 5 Xtraction 230/60 Microwave Accelerated Reaction System. Model: 907601
Manganese(II) chloride tetrahydrate
Fisherbrand porcelein mortars
N-EVAP 112 Nitrogen Evaporatpr (OA-SYS Heating System)
Forced Air Oven
8-blade horizontal axis propeller. This usually comes as part of the paddlewheel reactor.
Leeson, Model: CM34025Nz10C; 1/4 HP; Volts 90; FR 34; 62 RPM.
Fisherbrand porcelein pestles
Hanna Instruments HI98143-04 pH and EC Transmitter with Galvanic isolated 0-4V.
Thermo Scientific Orion pH Buffer Bottles
Hanna Instruments HI1006-2005 Teflon pH Electrode with matching pin 5m.
1000 ul TipOne graduated blue tip in racks
Eppendorf Reserch plus Variable Adjustable Volume Pipettes: Single-channel
BrandTech BRAND Plastic Cuvettes
Corning Falcon Bacteriological Petri Dishes with Lid
This chemical is used for the optimazed medium preparation. It was bought in a fertilizer local company
Medium Preparation: Potassium phosphate dibasic
1 L and 2 L bottels - PYREX GL45 Screw Caps with Plug Seals
Similar equipment can be bought at https://microbioengineering.com/products
This equipment was design and build by a member of the group
Sabrent USB 2.0 to Serial (9-Pin) DB-9 RS-232 Converter Cable, Prolific Chipset, Hexnuts, [Windows 10/8.1/8/7/VISTA/XP, Mac OS X 10.6 and /

Algae agitation 150 rpm
Sodium carbonate

Medium Preparation: Sodium molybdate dihydrate
Medium Preparation: Sodium nitrate

Thermo Fisher Scientific - 10S UV-Vis GENESTYS Spectrophotometer cylindrical Longpath cell holder; internal reference dectector, Xenon flash
Fisherbrand Disposable Borosilicate Glass Tubes with Plain End (10 ml)

Urea
MilliporeSigma Glass Vacuum Filter Holder, 47 mm. The system includes: Ground glass flask attachment, coarse-frit glass filter support, and fl
Marathon Electric AC Motor Thermally protected G588DX - MOD 5KH36KNA510X. HP 1/4. RPM 1725/1425
Zinc sulfate heptahydrate

Above] 2.5 Feet (CB-DB9P)

ɲ lamp; dual silicon photodiode; 240V, 50 to 60Hz selected automatically.

ask

Dear Dr. DSouza,

Following the editorial revision, we are re-submitting the article “A Best Practices Protocol Coupling Carbon Capture from a Power Plant with Semi-automated Open Pond Raceway Ponds for Microalgae Cultivation” for JoVE. We have endeavored to address all the comments. We uploaded the edited manuscripts with track-change, and please find below line by line the addressed editorial comments.

The manuscript is not under consideration for publication elsewhere and has been approved by all co-authors. The manuscript is original research and discusses findings of it. The authors believe that the manuscript presented will have a high interest to the readers and viewers of JoVE.

Editorial comments line by line:
Line 2 – Trim the tittle. Tittle was changed from “A Best Practice Protocol Coupling Carbon Capture from a Power plant with Semi-automated Open Raceway Ponds for Microalgae Cultivation” to “Coupling Carbon Capture from a Power plant with Semi-automated Open Raceway Ponds for Microalgae Cultivation.”

Line 14, 18-20 – Emails. The University of Arizona has recently updated email format addresses.

Line 33, 35, 37, and 42 – Removed references from the Abstract.

Line 67 -69 – Rephrased weird sentence.

Line 126 – Renamed supplementary file.

Line 138 – Combine steps 3 and 4. Renamed step “Algae selection and strain maintenance (light and temperature)”

Line 142 – Added a new reference for strain isolation.

Line 186, 188, 214, 250, 325 – Renumbered the steps as needed due to changes made before.

Line 189 – Add microscope magnification.

Line 190 – Rephrased

Line 246 – Add an “a” and replaced samples to sample

Line 252 – Rephrased

Line 257 – Named the recording name “(A)”

Line 269 – Named the weight after oven drying “(B)”

Line 274 – Named the weight after furnaced (Ash) "(C)"

Line 278 – Added equation to calculate % AFDW

Line 283 – Changed RPM for Relative centrifugal force. Note: it was a mistake there, we should have said 5000 RPM. The correction was done when changed it to RCF.

Line 325, 387, 394, 411 – Removed CR3000 or Campbell unit

Line 386 – Added superscript reference

Line 455 – Reference. The name was changed for the last name, and superscript reference was added.

Line 457, 458, 485 – Added superscript reference

Figures: Figures order was fixed and added to the manuscript as requested. Note: we accidentally switched figures names in the previous submission for Figures 6 and 7. We resubmitted them as Fig6_Rev2 and Fig7_Rev2. We edited a mistake in Figure 9 and renamed it as Fig9_Rev2.

Tables: The chemical compound formatting was corrected, and tables (1 and 2) were re-submitted in excel files

References: After the abstract's references were removed, the references were updated. Also, the names of the journal were spelled out.

Supplementary Material A. Print out of the program

```
'Declare Variables and Units
Public BattV
Public PTemp_C

Public V650, V780
Public pH_1, EC_1

Public DOmV_1

Public DOppm_1, Temp_1, AirTemp

Public CO2_time, CO2Meter, CO2Accu, CO2Total, portStat, N
Public pHspHigh, pHspLow
Public valveOverRide, valveOpening
Const scanIntvl = 1

Public rTime(9)
Alias rTime(1) = Year
Alias rTime(2) = Month
Alias rTime(3) = DOM
Alias rTime(4) = Hour
Alias rTime(5) = Minute
Alias rTime(6) = Second
Alias rTime(7) = uSecond
Alias rTime(8) = Weekday
Alias rTime(9) = Day_of_Year

'Define Data Tables
DataTable(BathTub,True,-1)
  '10 minutes averaged data is stored in the data table'
  DataInterval(0,10,Min,10)
  Average(1,BattV,FP2,0)
  Average(1,PTemp_C,FP2,0)
  Average(1,DOmV_1,FP2,0)
  Average (1,Temp_1,FP2,False)
  Average(1,pH_1,FP2,0)
  Average(1,EC_1,FP2,0)
  Sample(1,CO2_time,IEEE4)
  Sample(1,CO2Meter,FP2)
  Sample(1,CO2Accu,IEEE4)
  Sample (1,CO2Total,IEEE4)
  Average (1,AirTemp,FP2,False)
  Average (1,V650,FP2,False)
  Average (1,V780,FP2,False)
EndTable

'Main Program
BeginProg
  pHspHigh = 8.02
  pHspLow = 8.00
  valveOverRide =0
  valveOpening =5000
  CO2Accu = 0
  CO2Total = 0
  CO2_time = 0
  'Scan every second'
  Scan(scanIntvl,Sec,1,0)
    'Default Datalogger Battery Voltage measurement BattV
    Battery(BattV)
    'Wiring Panel Temperature measurement PTemp_C
    PanelTemp(PTemp_C,_60Hz)


    'Generic 0-4V Input measurement pH_1
    VoltSe (pH_1,1,mV5000,1,1,0,_60Hz,0.0035,0.0233)
    VoltSe (EC_1,1,mV5000,2,1,0,_60Hz,1.8613,-34.919)
```

```
    'CS511 Dissolved Oxygen Probe measurements DOmV and DOppm_1
    'VoltDiff(DOmV_1,1,mV200,2,False,0,_60Hz,1.0,0.0)
    VoltDiff(DOmV_1,1,mV200,2,False,0,_60Hz,0.297,-0.2377)

    'TypeK thermocouple Water
    TCDiff (Temp_1,1,mV20,4,TypeK,PTemp_C,True ,0,_60Hz,1.0,0)
    'TypeK thermocouple Air
    TCDiff (AirTemp,1,mV20,7,TypeK,PTemp_C,True ,0,_60Hz,1.0,0)

    'OD sensor measurement
    VoltSe (V650,1,mV5000,9,1,0,_60Hz,1.0,0)
    VoltSe (V780,1,mV5000,10,1,0,_60Hz,1.0,0)


    VoltSe (CO2Meter,1,mv5000,5,1,0,_60Hz,0.000406,0.000056) 'CO2 flow rate from the flow meter in SLPM

     If CO2Meter < 0.05 OR CO2Meter = "NAN" Then
      CO2Meter = 0
      EndIf

    CO2Total = CO2Total + CO2Meter * scanIntvl/60 'CO2 accumulative volume in Liters

    If pH_1 >= pHspHigh AND valveOverRide =0 Then                    'Start CO2 injection setpoint
      ExciteCAO (CAO1,valveOpening,False )
      N = N + 1
      CO2_time = CO2_time + scanIntvl/60
      ElseIf pH_1 <= pHspLow AND valveOverRide =0 Then               'Stop CO2 injection setpoint
        ExciteCAO (CAO1,0,False )
        N = 0
        ElseIf valveOverRide = 1 Then
          ExciteCAO (CAO1,valveOpening,False )
        EndIf

    If N <= 7 Then                          'Start accumulate CO2 volume after 7 sec injection started
      CO2Accu = CO2Accu
      Else
      CO2Accu = CO2Accu + CO2Meter * scanIntvl/60      'CO2 accumulative volume in Liters
      EndIf

    'PortGet (portStat,1 )
    'If portStat = 1 Then
       'N = N + 1
       'CO2_time(1) = CO2_time(1) + 1/60        'CO2 injection time in Min
        'Else
        'N = 0
        'EndIf

      'If N <= 7 Then                          'Start accumulate CO2 volume after 7 sec injection started
      'CO2Accu = CO2Accu
      'Else
      'CO2Accu = CO2Accu + CO2Meter * 1/60      'CO2 accumulative volume in Liters
      'EndIf

    'Call Data Tables and Store Data
    CallTable(BathTub)
  NextScan
EndProg
```

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Supplemental Coding Files

Supplementary Material D_Rev.xlsx