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Microalgae Cultivation and Biomass Quantification in a Bench-Scale Photobioreactor with Corrosive Flue Gases

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Cover Letter

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JoVE Editor

Dear JoVE Editor,

We seek to submit our original methods paper, entitled “Microalgae cultivation and biomass quantification in a bench-scale photobioreactor with corrosive flue gases”, to *JoVE* for publication.

The subject matter of this manuscript specifically aligns with the goal of *JoVE,* as a detailed protocol for microalgal cultivation and quantification. We are working to improve the biomass productivity of microalgal cultivation systems with corrosive flue gases and wish to share our research protocol with the *JoVE* audience. Our work illustrates the preparation and use of a bench-scale photobioreactor for microalgal cultivation, including the safe use of corrosive gas inputs, and details relevant biomass measurements and biomass productivity calculations.

This manuscript is original work and all authors agree to submit the manuscript to *JoVE*.

Thank you for taking the time to consider our work.

Sincerely,

Hannah Molitor

TITLE:

Microalgae Cultivation and Biomass Quantification in a Bench-Scale Photobioreactor with Corrosive Flue Gases

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KEYWORDS:

microalgae cultivation, photobioreactor, axenic cultures, experimental control, biomass productivity, adapting for corrosive gas use

SUMMARY:

Bench-scale, axenic cultivation facilitates microalgal characterization and productivity optimization before subsequent process scale-up. Photobioreactors provide the necessary control for reliable and reproducible microalgal experiments and can be adapted to safely cultivate microalgae with the corrosive gases (CO_2, SO_2, NO_2) from municipal or industrial combustion emissions.

ABSTRACT:

Photobioreactors are illuminated cultivation systems for experiments on phototrophic microorganisms. These systems provide a sterile environment for microalgal cultivation with temperature, pH, and gas composition and flow rate control. At bench-scale, photobioreactors are advantageous to researchers studying microalgal properties, productivity, and growth optimization. At industrial scales, photobioreactors can maintain product purity and improve production efficiency. The video describes the preparation and use of a bench-scale photobioreactor for microalgal cultivation, including the safe use of corrosive gas inputs, and details relevant biomass measurements and biomass productivity calculations. Specifically, the video illustrates microalgal culture storage and preparation for inoculation, photobioreactor assembly and sterilization, biomass concentration measurements, and a logistic model for microalgal biomass productivity with rate calculations including maximum and overall biomass productivities. Additionally, since there is growing interest in experiments to cultivate microalgae using simulated or real waste gas emissions, the video will cover the photobioreactor equipment adaptations necessary to work with corrosive gases and discuss safe sampling in such scenarios.

INTRODUCTION:

Photobioreactors are useful for controlled experiments and cultivation of purer microalgal products than can be achieved by open ponds. Microalgal cultivation in bench-scale photobioreactors supports the development of fundamental knowledge that may be used for process scale-up. Slight changes to environmental conditions can significantly alter microbiological experiments and confound the results[1]. A sterile process with temperature, pH, and gas sparging control is advantageous for studying microalgal properties and performance under varied conditions. Additionally, the control over input gas concentrations, temperature, shear force from mixing, and medium pH can support diverse species that are otherwise challenging to cultivate. Photobioreactors can be run as a batch process with continuous gas feed and sparging, or as a chemostat flow-through system with continuous gas feed and sparging plus influent and effluent wastewater nutrient inputs. Here, we demonstrate the batch process with continuous gas feed and sparging.

The use of photobioreactors addresses several microalgal cultivation and production challenges. The field generally struggles with concerns of contamination by other microorganisms, efficient substrate utilization (which is especially important in the case of CO_2 mitigation or wastewater treatment)[2], pH control, illumination variability, and biomass productivity[3]. Photobioreactors enable researchers to study a wide range of phototrophs in closely-controlled batch systems, where even slow growing species are protected from predators or competing microorganisms[4]. These batch systems are also better at facilitating greater CO_2 utilization rates and biomass productivity because they are closed systems that are more likely to be in equilibrium with supplied gases. Photobioreactor technology also offers pH control, the lack of which has hindered high biomass productivity in past studies[5]. At bench scale, the level of control offered by photobioreactors is advantageous to researchers. At larger industrial scales, photobioreactors can be used to maintain commercial bioproduct purity and improve production efficiency for nutraceutical, cosmetic, food, or feed applications[6].

Microalgae are of great interest for biosequestration of CO_2 because they can rapidly fix CO_2 as biomass carbon. However, most anthropogenic sources of CO_2 are contaminated with other corrosive and toxic gases or contaminants (NOx, SOx, CO, Hg), depending on combustion process fuel source. Growing interest in sustainable CO_2 sequestration has prompted development of photobioreactor technologies to treat CO_2-rich emissions, such as those from coal-fired power plants (**Table 1**). Unfortunately, there is inherent risk of human and environmental exposure to the corrosive and toxic contaminants during research and scale-up processes. As such, describing the safe assembly and operation of bioreactors using corrosive gases is necessary and instructive.

This method is for the use of a 2 L bench-scale photobioreactor for the growth of microalgae under carefully controlled experimental conditions. The protocol describes microalgal storage, inoculum preparation, and photobioreactor setup and sterilization. Beyond basic operation, this work describes microalgal biomass measurements and biomass productivity calculations, and adaptation of the equipment for microalgal cultivation with corrosive gases. The protocol described below is appropriate for researchers seeking to exert greater experimental control, optimize microalgal growth conditions, or axenically culture a range of phototrophic microbes. This method does not describe appropriate materials for cultivation of microbes that produce or

consume flammable gases (e.g. CH_4, H_2, etc.)[7].

PROTOCOL:

1. Safe use and sampling of a photobioreactor sparged with corrosive gases

NOTE: This method does not describe appropriate procedures for safe sampling of microalgal cultures that produce or consume highly flammable gases.

1.1. Manage toxic gas as a risk to human health.

NOTE: Per the University of Iowa's Chemical Hygiene Plan, the authors worked with the University Fire Safety Coordinator and University Environmental Health & Safety Industrial Hygiene Officer to develop a safety protocol for working with the toxic gases.

1.2. Set up a toxic gas monitoring system with sensors for each of the toxic gases in use. Calibrate the sensors according to manufacturer instructions. Bump test (check for sensor and alarm functionality with calibration gases) frequently, according to manufacturer instructions. Locate the gas monitor just outside the fume hood.

1.3. Prior to beginning any corrosive gas experiments, notify nearby personnel of the risk and alarm system. Also, notify the appropriate local emergency responders. Post signs on laboratory entrances specifying which hazardous gases are in use.

1.3.1. Instruct all nearby personnel to evacuate if toxic gas is detected. Notify laboratory supervisors and emergency response personnel.

NOTE: In a power outage, the gas-regulating tower will stop gas flow when it loses power. However, if the room heating, ventilation, and air conditioning (HVAC) system or fume hood go down without a power outage, this will result in leaking toxic gas.

1.4. Model the possible accumulated concentration of toxic gases in the room if the fume hood were to fail using the American Industrial Hygiene Association's (AIHA) mathematical modeling spreadsheet IH MOD[8] for each gas.

1.4.1. Obtain the room supply/exhaust air rate, Q, in $m^3\ min^{-1}$ from building HVAC maintenance personnel or HVAC technician. Calculate the volume, V, of the laboratory (L x W x H) in m^3. Calculate the contaminant emission rate, G, of each type of toxic gas in mg min^{-1}, using Equation 1 adapted from the ideal gas law:

$$G = \frac{PQ_{gas}}{RT} * MW * \frac{10^3\ mg}{g} \quad (1)$$

where P is the fraction of pressure exerted by the toxic gas at 1 atm (ppm gas/10^6 ppm), Q_{gas} is

the flow rate of the gas in L min^{-1}, *R* is the universal gas constant (0.082057 $L \cdot atm \cdot mol^{-1} \cdot K^{-1}$), *T* is temperature in K, and *MW* is the gas' molecular weight in mol g^{-1}.

1.4.2. Use the values for *V*, *Q*, and *G* for each gas (calculated in step 1.4.1) in the "Well-Mixed Room Model With option to cease generation and model room purge" algorithm in the IH MOD spreadsheet to calculate the accumulated room gas concentrations for each gas over a 1440 min (24 h) simulation period. Compare these values to the exposure limits (**Table 2**)[9].

2. Preparation of the microalgal inoculum

2.1. Prepare the *Scenedesmus obliquus* inoculum, or other microalgal species selected for the photobioreactor, prior to the start of the experiment by transferring it from storage, whether cryopreserved or cultured on agar media.

2.1.1. Add 30−50 mL of sterile microalgal growth medium (triple-nitrogen Bold's basal medium [3N-BBM], **Table 3**) to a sterile (150−250 mL) shake flask capped with a foam stopper.

NOTE: Unless the flask is sparged, only one-fifth of the shake flask volume should be occupied by liquid media.

2.1.2. Use a biosafety cabinet to maintain sterility when transferring cells to a slant or shake flask. For cultures on agar, use a sterile loop to transfer microalgae from its agar plate or slant to the shake flask. For cryopreserved cultures, gradually thaw the cryopreserved sample and rinse away the cryoprotectant according to the chosen protocol[10], then add the cells to the shake flask.

2.1.3. Culture cells in 3N-BBM at 20−25 °C under 16 h:8 h light:dark and shaking at 115−130 rpm.

2.1.4. Track microalgal growth over time using optical density (OD) measurements (as in sections 5 and 6). Allow the microalgae to reach its exponential growth phase (2−4 days) before transferring cells to the photobioreactor.

NOTE: Depending on the goal of the experiment, the cells may be rinsed of culture medium (this study) and/or concentrated with multiple centrifugation steps prior to inoculation of the bioreactor.

3. Setup and operation of photobioreactor

3.1. Use the photobioreactor (**Figure 1**) to control temperature, pH, stirring rate, gas flow rates, and input solution flow rates.

NOTE: The photobioreactor may be used to control the flow of up to four different input solutions, commonly acid, base, antifoam, and substrate.

3.1.1. Prepare 100 mL each of 1 N NaOH and 1 N HCl and add each to a 250 mL input solution bottle. Use secondary containment for these solutions.

3.1.2. Store metered input solutions in autoclavable capped bottles equipped with dip tubes and a vent tube with a sterile inline air filter. Connect the dip tubes to the photobioreactor's four input ports using autoclavable tubing and, during photobioreactor operation, submerse the dip tubes in the input solutions. Pass the 1.6 mm inside dimeter (ID) autoclavable tubing between the input solutions and their ports through separate peristaltic pumps which may be controlled either by manually selected flow rates or by feedback from pH and foam-level probes (in the case of acid, base, and antifoam solutions).

3.2. Calibrate the photobioreactor pH meter prior to autoclaving.

3.2.1. Connect the pH probe to the photobioreactor control tower by fitting the probe to the connecting line and twisting to lock. Use pH 4 and pH 7 buffers to calibrate the pH meter. Wait until values have stabilized before accepting the pH meter value.

3.2.2. Disconnect the pH probe from the pH meter cord that connects the probe to the control tower.

3.2.3. Surface sterilize with 70% ethanol or autoclave the probe with the reactor. To autoclave, cap the pH probe tightly with aluminum foil.

NOTE: If the probe is autoclaved, there is a risk of corrosion of the probe interior from steam damage. This capping method does not completely guarantee damage prevention.

3.2.4. Add 10 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer to the culture medium to better control pH.

3.3. Insert and screw closed the cold finger and exhaust condenser on the photobioreactor headplate.

3.4. Insert the inoculation port and screw tightly in place. Add a length of autoclavable tubing to the section of inoculation port above the photobioreactor headplate. Prior to autoclaving the bioreactor, clamp the tubing closed with an autoclavable hose clamp.

3.5. Attach tubing capped with sterile filters to any unused photobioreactor ports.

3.6. Attach acid and base input solutions to the photobioreactor input ports via autoclavable tubing. Add 1.5 L of culture medium.

3.7. Autoclave the reactor and associated input solutions for 30−45 min according to working volume.

NOTE: If the culture medium is adversely affected by autoclaving, add the media after autoclaving under sterile conditions in a laminar flow hood. The protocol can be paused here.

CAUTION: Reactor will be hot after removing from autoclave.

3.8. Attach the impeller motor to the impeller shaft and tighten the fitting.

3.9. Arrange LED light panels symmetrically outside the bioreactor according to illumination requirements.

3.9.1. Prior to autoclaving, measure and record the light intensity with a photometer. Place the illuminance sensor inside the photobioreactor vessel and face the sensor toward the light source.

3.10. Connect up to two gas cylinders to supply the simulated coal-fired power plant emissions (**Table 1**) to the microalgae in the photobioreactor.

NOTE: The gas concentrations used in this study approximated those of the University of Iowa power plant.

3.10.1. Assemble the connections between the gas cylinder, gas regulating tower, and photobioreactor sparging ring. Attach appropriate regulators capable of 20 psi outlet pressure to the gas cylinders. Attach 6 mm ID pressure resistant tubing to the regulator outlet hose barb and secure with a hose clamp. Attach the other end of the pressure resistant tubing to the gas regulating tower gas inlet using a hose barb to 6 mm stem quick connect fitting secured with a hose clamp. Connect 3.2 mm ID tubing to the gas-regulating tower gas outlet using another 6 mm quick connect fitting and connect the other end of the outlet tubing to the sparging ring port at the photobioreactor headplate.

NOTE: For a second input gas, repeat step 3.9.1, but use a T-shaped hose barb to consolidate the two input gas lines to a single section of tube connected to the sparging ring port.

3.10.2. Set the outlet pressure to 20 psi on each gas regulator and use the bioreactor interface to set experimental gas flow rates.

NOTE: Calculate and report the volume of air under standard conditions per volume of liquid per minute (vvm); divide the volumetric gas flow rate by the culture volume. Report in units of per miniute.

3.11. When sparging with more than one gas cylinder, confirm the supplied CO_2 concentration to the photobioreactor with a CO_2 sensor.

3.11.1. Connect a software (e.g., GasLab) compatible CO_2 sensor to the USB port of a computer. Download the most recent software that corresponds to the CO_2 sensor model. Open the software and input the sensor model, measurement time interval, and duration of measurement data logging.

3.11.2. Place the CO_2 sensor and combined gas flow tube (prior to connecting the tube with the

bioreactor) in a 100−250 mL, capped, vented vessel (external to the bioreactor).

NOTE: During the experiment, the headspace CO_2 concentrations may be measured from one of the venting tubes on the photobioreactor headplate.

3.11.3. Start the CO_2 concentration measurements on the user interface and wait for the measurements to equilibrate.

3.11.4. Use the photobioreactor user interface to adjust the gas flow rates from each tank until the desired total flow rate (0.1 L min^{-1}) and CO_2 concentration (12%) is achieved.

3.12. On the photobioreactor user interface, use the STIRR function to set the impeller rotation rate. Ensure that the mixing rate is rapid enough for the culture medium to assimilate the sparged gas bubbles.

NOTE: Certain microalgal species have weak cell structures and will be damaged or ruptured by high shear force.

4. Adapting the photobioreactor and experimental setup for toxic gas use

CAUTION: The corrosive gases in real or simulated flue gas are corrosive and toxic. These gases pose serious risk if inhaled.

NOTE: This method does not describe appropriate materials for safe cultivation of microbes that produce or consume highly flammable gases (i.e., methane, hydrogen, etc.).

4.1. Replace brass, plastic, and standard tubing components with corrosion resistant materials.

4.1.1. Use stainless steel to reliably resist corrosion from the strong acids formed by the reaction between NO_x or SO_x and water. Replace plastic quick connect fittings at the gas inlets and outlets on the gas-regulating tower with stainless steel quick connect fittings. Use stainless steel regulators for gas cylinders including the outlet hose barb rather than brass.

4.1.2. Use polytetrafluoroethylene (PTFE) or natural ethyl vinyl acetate (EVA) tubing to resist corrosion from NO_x and SO_x gases (respectively) on the connections between the gas cylinder to the gas-regulating tower and the gas-regulating tower to the photobioreactor.

4.2. After autoclaving, assemble the photobioreactor and gas cylinders within a walk-in fume hood. Place the photobioreactor on a table inside secondary containment and place gas cylinders in free standing cylinder collars or a cylinder rack.

4.3. After initiating gas flow, use a bubble type liquid leak detector to check for gas leaks in all connections between the gas cylinders and bioreactor. Use a wash bottle filled with a 1:100 (v:v) dilution of dish soap:water to cover the connection with a small stream of soap solution.

NOTE: Leaks will be indicated by bubbling at the connections.

4.4. When initiating the microalgal experiments, begin sparging then adjust pH before inoculation (as in standard photobioreactor experiments).

NOTE: Buffering the culture medium during corrosive gas experiments is highly recommended since the input gases are strongly acidic.

4.5. Inoculate the photobioreactor by aspirating the prepared microalgal inoculum into a sterile syringe, fitting the syringe to the tubing attached to the inoculation port, opening the inoculation tubing clamp, and depressing the syringe.

4.6. Check the gas monitor, gas cylinder pressures, and photobioreactor twice daily (and prior to sampling) for elevated levels of toxic gas or indication of leaks.

4.7. Limit the fume hood sash opening to a width that allows the bioreactor and gas cylinder regulators to be reached. To avoid inhalation exposure risk, ensure that the opening does not expose personnel above the torso region.

4.8. Turn the gas cylinder regulators to the closed position to cease gas flow to the reactor. Close the fume hood sash and allow 5 min for the hood to evacuate the corrosive gases before sampling the photobioreactor culture.

4.9. Sample within the fume hood either by opening a headplate port and using a sterile serological pipet or drawing culture into a syringe through the inoculation/sampling port. Secure the photobioreactor ports prior to opening the gas cylinders and resuming the experiment.

5. Measuring microalgal biomass productivity

5.1. Use a calibration curve to relate microalgal culture OD_{750} measurements to dried microalgal biomass concentrations.

5.1.1. Prepare several (minimum: 4, minimum working volume: 500 mL) flasks with sterile microalgal medium and inoculate with the species of interest (e.g., *S. obliquus* in this study).

5.1.2. Measure the culture OD_{750} until growth is exponential, and promptly sacrificially sample the flasks by filtering known volumes (minimum of 100 mL) of the contents with a 0.45 µm filter membrane of known mass. Use covered aluminum weigh boats or glass vessels to support the biomass and filters as they are dried.

5.1.3. Mass the biomass and filters after drying for approximately 18−24 h in an oven between 80−100 °C. To verify complete drying, measure again after an additional 2−3 h to determine if the mass has stabilized.

5.1.4. Subtract the filter mass from the combined biomass and filter mass to calculate the biomass mass.

5.1.5. Plot the calibration curve as measured OD_{750} against the biomass concentration (mass of biomass divided by the volume of filtered culture) and fit the data with a linear regression.

6. Biomass productivity modeling and rate calculations

6.1. Calculate experiment biomass concentrations from OD_{750} measurements using the calibration curve linear regression (determined in section 5).

6.2. Fit batch microalgal growth data from lag to exponential to stationary phase with a logistic regression (Equation 2) in graphing and statistics software (**Table of Materials**):

$$f(x) = \frac{L}{1+e^{-k(x-x_0)}} \quad (2)$$

where *L* is the curve's maximum biomass concentration value, *k* is the relative steepness of the exponential phase ($time^{-1}$), x_o is the time of the sigmoidal curve's midpoint, and *x* is time.

6.2.1. Manually enter the logistic equation above. Select **Fit a curve with nonlinear regression** on the **Analysis** tab in the software. On the left of the **Parameters: Nonlinear Regression** box, choose **Create new equation** under the **New dropdown** box. Use the default explicit equation as equation type, name the new function, and define the new function as Y = L/(1+ exp(-k*(x-b))), where *b* represents x_o.

6.3. Calculate the overall biomass productivity of the microalgal batch by dividing the difference between the final and initial biomass concentrations by the difference between the final and initial times.

6.4. Calculate the maximum biomass productivity of the microalgal batch from the derivative of Equation 2 (Equation 3) at the sigmoid midpoint, when $x = x_o$.

$$\frac{\partial f(x)}{\partial x} = \frac{L\,k\,e^{-k(x-x_0)}}{(e^{-k(x-x_0)}+1)^2} \quad (3)$$

REPRESENTATIVE RESULTS:

A calibration curve for the green microalgae, *S. obliquus*, harvested in the exponential phase, was established with OD_{750} and dried biomass concentrations (**Figure 2**). The linear regression had an R^2 value of 0.9996.

An *S. obliquus* culture was started in a 250 mL Erlenmeyer flask from a culture stored on a refrigerated agar plate. The microalga was inoculated in 3N-BBM with 10 mM HEPES buffer and sparged with 2.2% CO_2 in a 2 L photobioreactor with 1.5 L working volume (0.07 vvm) (**Figure 1**).

The batch was tracked via OD_{750}; the biomass concentrations were calculated from the calibration curve, and then modeled with a logistic curve (**Figure 3**). The photobioreactor maintained the culture at pH 6.8, 100 cm^3 min^{-1} total gas flow rate, continuous 280 µmol m^{-2} s^{-1} illumination, and 27 °C. The logistic curve fit biomass concentration data from lag to exponential to stationary phase. From the logistic model, the maximum biomass concentration during the batch was 2070 ± 20 mg L^{-1}, maximum biomass productivity occurred at 4.6 day, and the rate of specific biomass productivity was 1.0 d^{-1}. The maximum biomass productivity, calculated from the derivative of the logistic curve at the time of maximum growth, was 532 ± 60 mg L^{-1} d^{-1}.

The well-mixed room model was used to calculate the accumulated concentration of NO_2, SO_2, and CO in the case of fume hood failure for 24 h. These values were compared to the exposure limits (**Table 2**). For example, in the scenario where 0.05 L min^{-1} of 400 ppm NO_2 is released during a fume hood failure period of 24 h, the well-mixed room model with inputs of calculated G = 0.0377 mg min^{-1}, Q = 0.0001 m^3 min^{-1}, V = 100 m^3, and maximum time for simulation = 1440 min predicts NO_2 accumulation to 0.54 mg m^{-3} (0.29 ppm), which is above the acceptable chronic exposure limit (American Conference of Governmental Industrial Hygienists threshold limit value [ACGIH TLV]) and below the short-term exposure limit (STEL).

A promising preliminary trial with simulated flue gas achieved a greater maximum microalgal biomass productivity rate (690 ± 70 mg L^{-1} d^{-1}) than that of 12% CO_2 and ultra-zero air (510 ± 40 mg L^{-1} d^{-1}) (**Figure 4**). Prior to the experiment, a gas monitor was calibrated with CO, NO_2, and SO_2. The simulated flue gas experiment was carried out without any risk to personnel or damage to equipment from corrosive gases.

FIGURE AND TABLE LEGENDS:

Figure 1: Bench-top photobioreactor illuminated by red and blue LED lights. The photobioreactor operates as a 2 L batch reactor with 1.5 L working volume. The photobioreactor is continuously fed with gases through the sparging ring and excess gas vents through ports in the headplate. Adapted with permission from Molitor et al.[5].

Figure 2: Calibration curve relating OD_{750} to *S. obliquus* cell dry weight. *S. obliquus* cell culture light absorption was measured at 750 nm, then cells were filtered and dried to obtain cell dry weight measurements. Reprinted with permission from Molitor et al.[5].

Figure 3: *S. obliquus* growth data at 2.2% CO_2 input modeled with a logistic regression. The data points represent biomass values as calculated from optical density measurements. The data have been modeled with a logistic regression through a least squares fit; $f(x) = \frac{L}{1+e^{-k(x-x_0)}}$ where L = 1955 mg L^{-1}, k = 1.154 d^{-1}, and x_0 = 3.317 d. R^2 = 0.995.

Figure 4: Modeled *S. obliquus* growth at 12% CO_2, with and without additional simulated flue gas components. The biomass measurements from each batch of microalgae were modeled with logistic regressions.

Table 1: Composition of coal-fired power plant emissions. These quantities were averaged from the University of Iowa power plant emissions data collected at minute intervals over the span of 10 h.

Table 2: Exposure limits and descriptions for toxic gases (CO, SO_2, NO_2) in flue gas. OSHA TWA: time weighted average (usually 8 h period), CEILING: value never to be reached, STEL: short-term exposure limit (TWA over 15 min), NIOSH IDLH: danger to life and health, NIOSH REL: 15 min exposure limit, ACGIH TLV: acceptable chronic exposure limit, no ill effects.

Table 3: Composition of triple-nitrogen Bold's basal medium (3N-BBM). The quantity of nitrogen has been tripled from the original Bold's basal medium[11].

DISCUSSION:

Batch, axenic photobioreactor experiments with regulated pH, temperature, gas flow rate, and gas concentration promote meaningful results by eliminating contamination by non-target algal strains and variability in culture conditions. Accurate pure culture growth kinetics can be obtained even in the presence of corrosive gases (CO_2, SO_2, NO_2), which serve as nutrients, turning waste gases into a valuable product such as animal feed.

Prior to beginning any microalgal experiment, the chosen microalga culture should be taken from storage and readapted to liquid culture. Growing the microalgae into exponential phase improves the probability that experiments have equivalent initial conditions and that the microalgae do not stagnate in lag phase after inoculation.

Calibration curves relating optical density and biomass concentrations are especially important during studies of biomass productivity. High microalgal biomass productivity is one of the key goals of the microalgal industry and, as such, is often an indicator of research success[12]. Therefore, accurate calculations of biomass concentrations from optical density measurements must stem from species-specific, precise, and accurate calibration curve data. To avoid potential optical interferences, it is important that measurements for the calibration curve and during the experiment be made in equivalent background solutions. Additionally, the calibration curve should be made with measurements taken from microalgae in the growth phase(s) most representative of those in the experiments. Certain microalgal species can have dramatic differences in cell size during different growth phases which can alter the optical density and perceived biomass concentrations. It should be noted that biomass productivity is related to, but not equivalent to, growth rate. Specific growth rate depends on the number of cells (change in cell density over time/cell density) present, and specific biomass productivity depends on the bulk mass of cells (change in mg/L biomass over time per mg/L biomass)[13] present.

When microalgal biomass concentrations are modeled with a logistic curve, experimental results can be meaningfully compared by interpolating biomass concentrations and accurately calculating biomass productivities. However, care should be taken when interpreting these experimental results; it is inappropriate to compare overall and maximum batch biomass productivity. While maximum biomass productivity values are useful to compare batch results,

overall biomass productivity is misleading without further information on the experiment duration and microalgal growth phases. These rates change continuously during the lag, log growth, and stationary phases.

During experiments with corrosive gases which are characteristic of power plant or industrial combustion emissions, caution should be exercised for both human health and equipment longevity. Standard parts must be replaced with more robust materials, and consumables such as tubing should be inspected and replaced more frequently to resist corrosion, prevent leaks, and avoid human exposure. Extra safety measures and risk awareness are essential to safe and successful operation and sampling. The method is not appropriate for flammable gases because there is potential for gas accumulation within the headspace and the equipment is neither designed for such risks nor suitable for safe adaptation to such conditions.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure1

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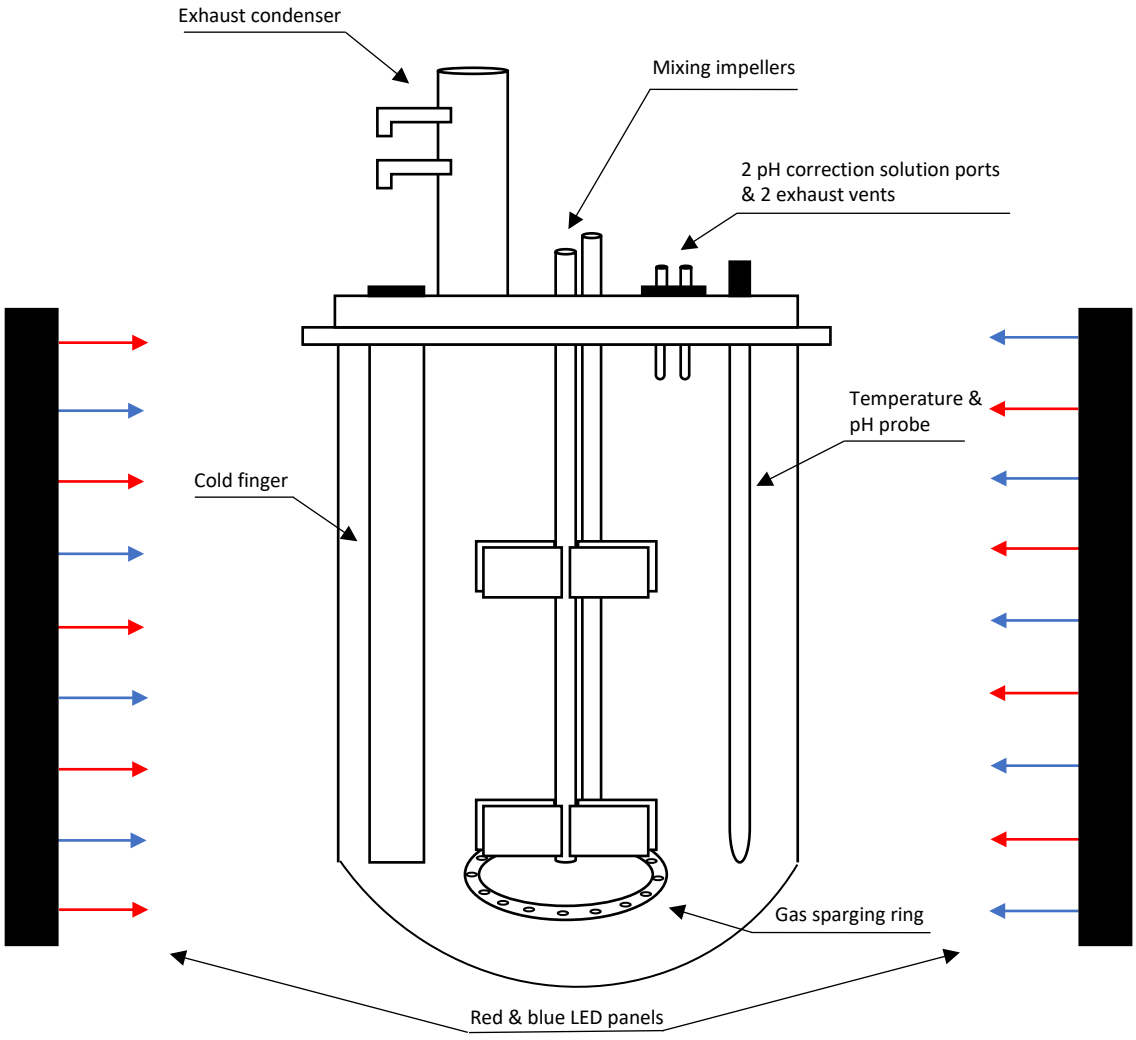

Figure2

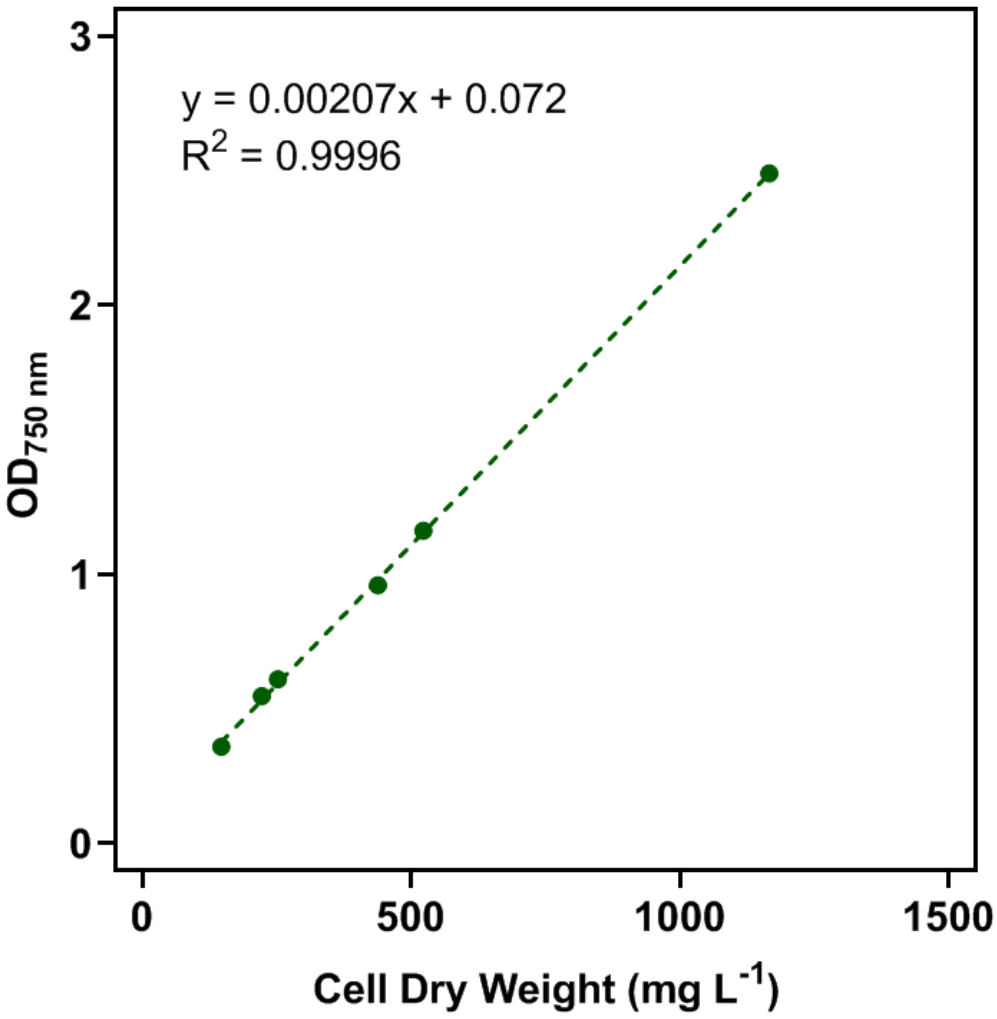

Figure3

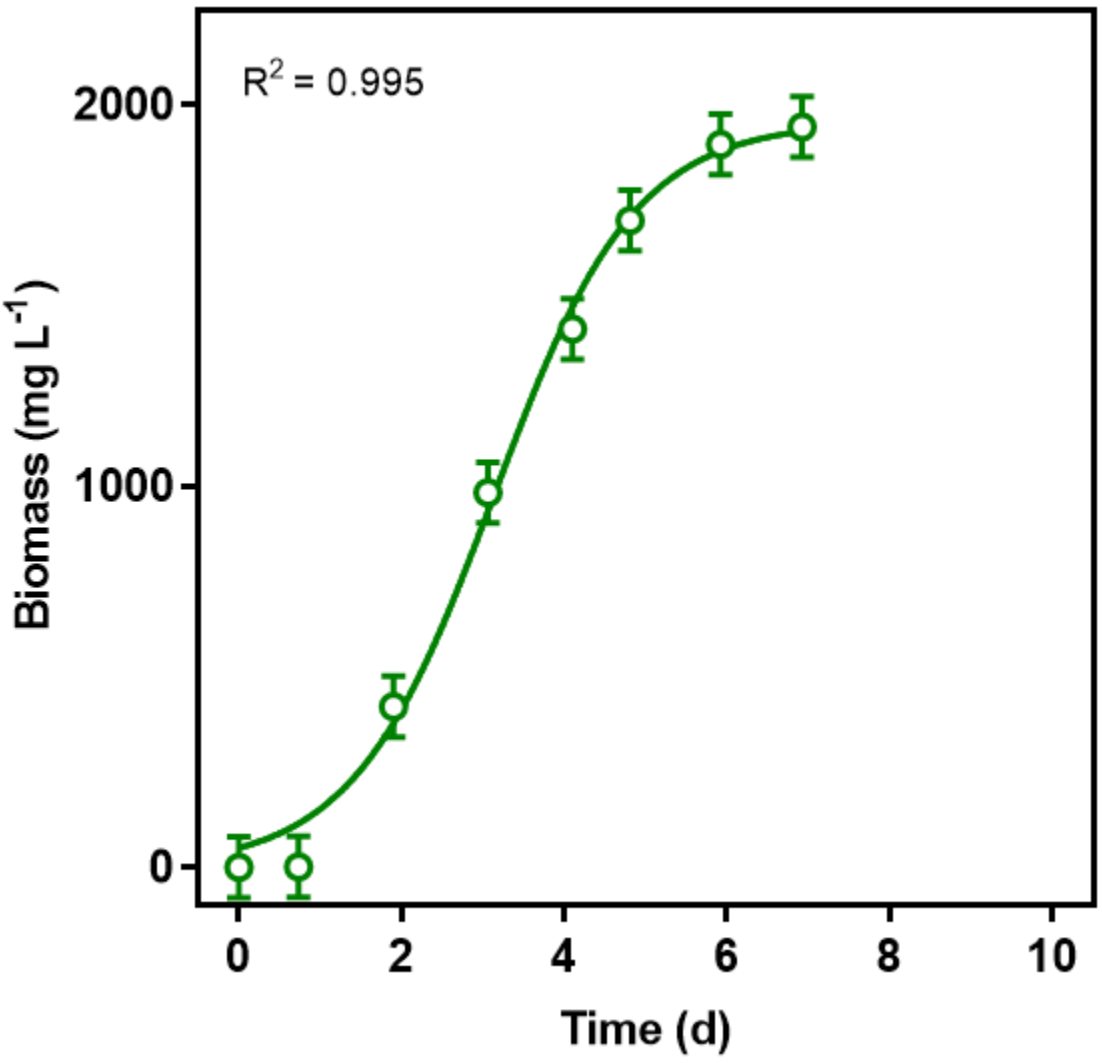

Figure4

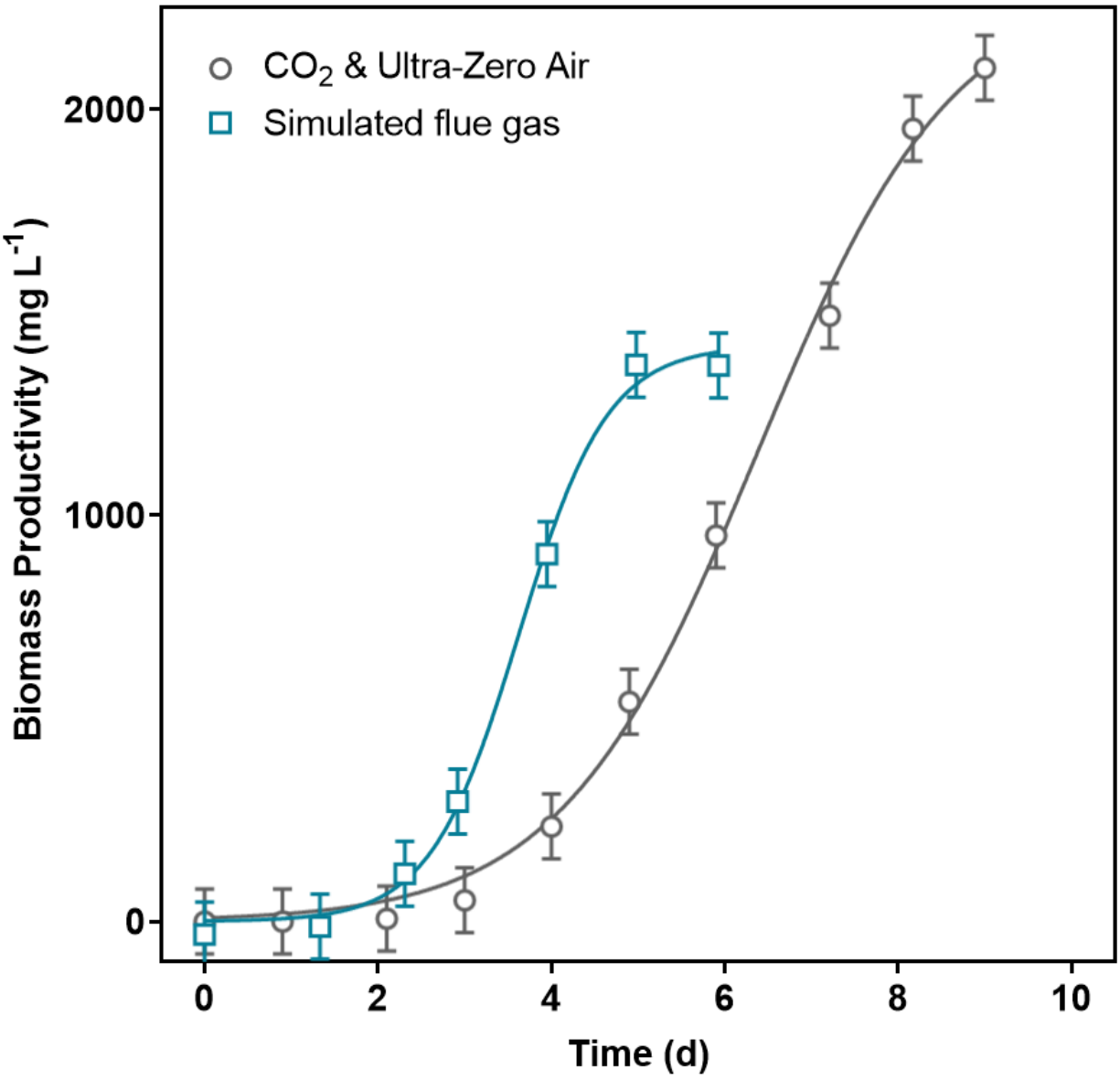

Table1

Component	Percent
H_2O	12.6%
CO_2	11.6%
O_2	5.8%
CO	0.048%
SO_2	0.045%
NO_2	0.022%
N_2	69.9%

Table 2

Toxic gas	TWA	CEILING	STEL	NIOSH IDLH	NIOSH REL	ACGIH TLV
CO	35 ppm	200 ppm	-	1,200 ppm	35 ppm	25 ppm
SO_2	2 ppm	100 ppm	5 ppm	100 ppm	2 ppm	2 ppm
NO_2	3 ppm	5 ppm	1 ppm	13 ppm	1 ppm	0.2 ppm

CDC Description

Colorless, odorless

Colorless gas with a characteristic, irritating, pungent odor

Yellowish-brown liquid or reddish-brown gas (above 70 °F) with a pungent, acrid odor

Table 3

Compound	mM
$NaNO_3$	8.82×10^{0}
$MgSO_4 \cdot 7H_2O$	3.04×10^{-1}
NaCl	4.28×10^{-1}
K_2HPO_4	4.31×10^{-1}
KH_2PO_4	1.29×10^{0}
$CaCl_2 \cdot 2H_2O$	1.70×10^{-1}
$ZnSO_4 \cdot 7H_2O$	3.07×10^{-2}
$MnCl_2 \cdot 4H_2O$	7.28×10^{-3}
MoO_3	4.93×10^{-3}
$CuSO_4 \cdot 5H_2O$	6.29×10^{-3}
$Co(NO_3)_2 \cdot 6H_2O$	1.68×10^{-3}
H_3BO_3	1.85×10^{-1}
EDTA	1.71×10^{-1}
KOH	5.52×10^{-1}
$FeSO_4 \cdot 7H_2O$	1.79×10^{-2}
H_2SO_4 (concentrated)	1×10^{-3} µL

Name of Material/Equipment	Company	Catalog Number
Biostat A bioreactor	Sartorius Stedim	
Bump test NO_2 gas	Grainger	GAS34L-112-5
Bump test O_2, CO, LEL gas	Grainger	GAS44ES-301A
Bump test SO_2 gas	Grainger	GAS34L-175-5
Corrosion resistant tubing for NO_2 gas	Swagelok	SS-XT4TA4TA4-6
Corrosion resistant tubing for SO_2 gas	QC Supply	120325
cozIR 100% CO_2 meter	Gas Sensing Solutions Ltd.	CM-0121 at CO2meter.com
cozIR 20% CO_2 meter	Gas Sensing Solutions Ltd.	CM-0123 at CO2meter.com
Durapore Membrane Filter, 0.45 µm	Millipore Sigma	HVLP04700
Gas cylinder regulators	Praxair	PRS 40221331-660
Gas cylinders	Praxair	Ulta-zero air, high purity CO2, or custom gas composition
Gas monitoring and leak detection system	RAE Systems by Honeywell	MAB3000235E020
GasLab software	GasLab	v2.0.8.14
Hose barb	Grainger	Item # 3DTN3
K30 1% CO2 meter	Senseair	CM-0024 at CO2meter.com
LED grow panels	Roleadro	HY-MD-D169-S
Memosens dissolved oxygen probe	Endress+ Hauser	COS22D-19M6/0
Memosens pH probe	Endress+ Hauser	CPS71D-7TB41
Oven, Isotemp 500 Series	Fisher Scientific	13246516GAQ
Prism GraphPad software	GraphPad Software	Version 7.03 or 8.0.1
Stem to hose barb fitting	Swagelok	SS-4-HC-A-6MTA

Tubing, dilute acid/base transfer	Allied Electronics and Automation	6678441
Tubing, gas transfer	Allied Electronics and Automation	6678444

Comments/Description

2-liter bioreactor for microbial fermentation; designed to be autoclaved; pH, temperature, gas flow rate control

Calibration gas for MultiRAE gas detector

Calibration gas for MultiRAE gas detector

Calibration gas for MultiRAE gas detector

PTFE Core Hose Smooth Bore X Series—Fiber Braid and 304 SS Braid Reinforcement

Reinforced Braided Natural EVA Tubing - 1/4" ID

CO2 meter for concentrations up to 100%

CO2 meter for concentrations up to 20%

Hydrophilic, plain white, 47 mm diameter, 0.45 µm pore size, PVFD membrane filters

Single-stage stainless steel regulator configured for 0-15 psi outlet assembly diaphragm valve with ¼”MNPT threads, Stainless steel to resist corrosion from NOx and SOx

Dependent on study objectives

Pumped model that detects O2, SO2, NO2, CO, and LEL

Software for CO2 meter measurements and data logging

Used to adapt regulators to tubing, Stainless steel to resist corrosion from NOx and SOx

CO2 meter for concentrations less than 1%

Red & blue LED light panels

Autoclavable (with precautions) dissolved oxygen probe for bioreactor

Autoclavable (with precautions) pH probe for bioreactor

Small oven for drying

Graphing software for data organization, data analysis, and publication-quality graphs

Stainless Steel Hose Connector, 6 mm Tube Adapter, 1/4 in. Hose ID

Silicone TP Process Tubing; 1.6mm Bore Size; 3000mm Long; Food Grade

Silicone TP Process Tubing; 3.2mm Bore Size; 3000mm Long; Food Grade

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Author(s):	Hannah Molitor, Deborah Williard, Jerald Schnoor

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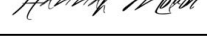
Signature:	
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3. *Figure 4: Please delete the unnecessary white space between characters.*
Figure 4 has been replaced.

4. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. Please be as specific as you can with respect to your experiment providing all necessary details. Please provide all volumes and concentrations used throughout. See examples below.

---

5. 1.1: Please specify the species of microalgae used.
This has been amended in Step 2.1 to say " Prepare *the Scenedesmus obliquus* inoculum, or other microalgal inoculum species selected for the photobioreactor…"

6. 1.1.1: Please specify the composition of microalgal growth medium.
Table 3 has been added to detail the medium composition, and the medium has been cited.

7. 1.1.2: Please specify at what conditions the microalgae are cultured (culture medium, temperature, light/dark hours, shaking speed, etc.).
These details have been added in Step 2.1.3.

8. 1.1.3: How long does it take for the microalgae to reach its exponential growth phase? Please be specific whether the cells are rinsed or concentrated in this protocol.
This has been corrected: "Allow the microalgae to reach its exponential growth phase (2-4 days) before transferring cells to the photobioreactor. Depending on the goal of the experiment, the cells may be rinsed of culture medium (this study) and/or concentrated with multiple centrifugation steps prior to inoculation of the bioreactor." See 2.1.4.

9. Section 2: Referencing Figure 2 (schematic of the photobioreactor) would be helpful here.
Figure 1 and referenced in Section 3 (formerly Section 2).

10. 2.1.2: Please list an approximate volume of solutions to prepare.
The solutions are now listed as 100 mL. See 3.1.1.

11. 2.2.4: What buffer is used here? Please provide its composition.

10 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) buffer has been specified. See 3.2.4.

12. 2.6: Please specify the volume of culture medium added.
1.5 L of culture medium has been specified. The 2-L volume of the bioreactor has been specified in the last paragraph of the introduction. See 3.6.

13. 2.9.3.4: Please specify the desired total flowrate and CO2 concentration.
The total flowrate of 0.01 L/min and CO2 concentration of 12% have been added. See 3.11.4.

14. 3.1.1: Please specify species of interest.
This step has been amended to read "inoculate with the species of interest (this study: *S. obliquus*)." See 5.1.1.

15. 3.1.2: Please specify the volume of the contents filtered. Please specify the type of filter.
The volume has been specified as a minimum of 100 mL. The filter has been detailed as a MillaporeSigma Durapore 47 mm 0.45 um filter membrane in the Materials List.

16. 3.1.3: How to dry the biomass and filters? At what temperature?
These specifications have been moved from Steps 5.1.2.- 5.1.3.

17. After you have made all the recommended changes to your protocol section (listed above), please highlight in yellow up to 2.75 pages (no less than 1 page) of protocol text (including headers and spacing) to be featured in the video. Bear in mind the goal of the protocol and highlight the critical steps to be filmed. Our scriptwriters will derive the video script directly from the highlighted text.

18. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. The highlighted text must include at least one action that is written in the imperative voice per step. Notes cannot usually be filmed and should be excluded from the highlighting.

19. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

Reviewers' comments:

Reviewer #1:
Manuscript Summary: Clear
Major Concerns: No
Minor Concerns: No

Reviewer #2:
Manuscript Summary: This is a good work with great contribution to the research community.
Major Concerns: None
Minor Concerns: None

Reviewer #3:
Manuscript Summary:
This manuscript has done a good job describing the construction and operating of a bench-scale

photobioreactor. The system turned the corrosive gases into nutrients. The author has stated clearly in the introduction the motivation of presenting this protocol, which is to minimize the risk of human and environmental exposure to corrosive and toxic contaminants during research and scale-up processes. Overall, the manuscript is well written.

Minor Concerns:
Introduction: It is preferred that the author shows an example of flue gas concentration in realistic industrial situation.
Thank you for this suggestion. Lines 72-73 have been changed to specify coal-fired power plant flue gas and include Table 1, which details the flue gas composition.

1.1.3: Please explain how you determine the exponential growth phase.
Step 2.1.4 has been amended to specify that the microalgae optical density should be tracked as in Section 5 & 6, and that exponential phase will be reached 2-4 days after inoculation.

Section 2: how do you attach the tubes and probes? Do you use glue to seal? Please provide the gas flow rate in addition to vvm. Please also provide the gas concentration and gas type as an example.
Tubes are attached with hose barbs and the probe is connected with a twist to lock mechanism (now both mentioned in Section 3). Also, probes are part of the Sartorius biostat assembly and should be attached according to manufacturer instructions. No glue is used to seal the connections.
The gas flow rate is now included in Step 3.11.4. Additionally, Step 3.10 has been updated to specify "simulated coal-fired power plant emissions" as in Table 1.
Instructions for calculating vvm are included in the note below 3.10.2; vvm will differ depending on chosen gas flow rates and medium working volumes.

2.9.3.2: Are the venting tubes in figure 2? the Exhaust condenser?
The exhaust condenser was labeled in the figure and the venting tubes have been added.

4.1: What's the calibration curve linear regression?
The calibration curve linear regression mentioned in Step 6.1 would have been determined in Section 5. Step 6.1 has now been updated to specify this. The example calibration curve regression equation is included in the caption of Figure 2.

4.2: Please define x as well.
Thank you. *x* has been defined as time in Step 6.2.

Section 6: Is part of this section included in the hazardous chemical management program within the university? I suggest the author to refer to the university regulations or make a reference.
Per the University of Iowa's Chemical Hygiene Plan (https://ehs.research.uiowa.edu/sites/ehs.research.uiowa.edu/files/ChemicalHygienePlan19.pdf), we worked with the University Fire Safety Coordinator and University EHS Industrial Hygiene Officer to develop this method for safely working with the toxic gases.

6.2: How do you calculate the laboratory air turnover rate?
Calculating parameters of air exchange and possible toxic gas concentration accumulations are now included in Section 1.

Figure 4 legend: "Microalgal growth under the simulated flue gas condition stopped because the CO2

supply was depleted." Should this be the flue gas supply? In the figure, CO2 was supplied until Day 9, while flue gas was supplied only till Day 4.

This amendment is correct. However, for better comparison, the Figure 4 has been replaced with a graph in which both microalgae batches successfully reach stationary phase and the sentence has been removed.

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