Using Simulated Flue Gas to Rapidly Grow Nutritious Microalgae with Enhanced Settleability

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ABSTRACT: Favorable microalgal nutrition from waste resources and improved harvesting methods would offset costs for a process that could be scaled up to treat pollution and produce valuable animal feed in lieu of soy protein. Co-benefits include avoidance of carbon dioxide emissions, which may provide an additional revenue stream when carbon markets begin to flourish. To sustainably achieve these goals at scale, barriers to microalgal production such as tolerance for waste streams and dramatic improvement in dewatering and settleability of the microalgae must be overcome. Presently, it is largely assumed that nutritious microalgae, including Scenedesmus obliquus, would be inhibited by SO$_2$ and NO$_x$ in flue gases and settle slowly as discrete particles. Studies conducted with a 2 L photobioreactor, sparged with simulated coal-fired power plant flue gas, demonstrated that both biomass productivity and settling rates were increased. The average maximum biomass productivity was $700 \pm 40$ mg L$^{-1}$ d$^{-1}$, which significantly exceeded that of the control culture ($510 \pm 40$ mg L$^{-1}$ d$^{-1}$). Thirty-minute trials of modeled bulk settling showed rapid coagulation, likely facilitated by extracellular polymeric substances, and compaction when the cultures were grown with simulated emissions. Control cultures, not exposed to the additional toxicants in flue gas, settled as discrete particles and did not show any settling progress within 30 min. Of the SO$_2$ sparged into the cultivation system, $(111 \pm 4\%)$ was captured as either SO$_4^{2-}$ in the medium or fixed in the S. obliquus biomass. The stress of simulated-emissions exposure decreased the S. obliquus protein contents and altered the amino acid profiles but did not decrease the fraction of methionine, a valuable amino acid in animal feed.

INTRODUCTION

Today, nearly one billion of earth’s 7.6 billion people already lack sufficient nutritious food on a regular basis, and this population is predicted to increase by 2–3 billion by the year 2050. Crop and livestock production will need to increase in quantity and efficiency using finite agricultural area and water resources to meet global demand. Microalgae are a promising supplement to conventional agriculture as rapidly and sustainably produced protein-rich biomass.

Direct reuse of waste resources to grow microalgal biomass has the potential to be more efficient than conventional agriculture and avoid direct competition for food between humans and livestock. However, for microalgae to compete as a cost-effective high-protein supplement, biomass productivity rates must increase, nutritional properties must be maintained by growth on waste resources, and harvesting efficiency must dramatically improve.

Flue Gas as a Source of Carbon for Microalgae. Flue gas waste streams containing CO$_2$ can be diverted to cultivate microalgae, especially if the species is able to tolerate NO$_x$ and SO$_2$. Tolerance is often strain-dependent and in some cases may be limited to only tolerance for SO$_2$. Kumar et al. treated coal-powered boiler flue gas, containing 6% CO$_2$ through mixotrophic cultivation of lipid-rich Chlorella vulgaris for biofuel and removed 64, 62, and 83% of CO$_2$, SO$_2$, and NO$_x$, respectively. Van Den Hende et al. achieved removal efficiencies of 48 $\pm$ 7% CO$_2$, 99 $\pm$ 1% SO$_2$, and 87 $\pm$ 5% NO$_x$ from coal-fired power plant emissions containing 12% CO$_2$ using a consortium of microalgae and bacteria. Previous studies examined the tolerance of microalgal species for NO$_x$ and SO$_2$ and achieved modest treatment of flue gas but generally ignored the potential of microalgae as animal feed and the possible stimulatory effect of sulfur on the growth of microalgae as a nutrient and on the enrichment of their amino acid profiles.

Flue Gas as a Source of Sulfur for Microalgae. Unlike animals, microalgae are able to assimilate and metabolize inorganic sulfur as sulfate. Although sulfur occurs in relatively high concentrations in oceans (28 mM SO$_4^{2-}$), microalgae adapted to freshwater can be limited by low sulfur concentrations (200 $\mu$M SO$_4^{2-}$). Given this range of environments, it is unsurprising that marine microalgae, such

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as diatoms and dinoflagellates, have characteristically low C/S ratios and freshwater microalgae, including chlorophytes, have high C/S ratios despite similar culture media. Generally, freshwater microalgal species have adapted to increase sulfate transport by an order of magnitude, to compensate for sulfur-limiting conditions.

Although work has been done to quantify sulfur limitation of microalgal growth, little has been done to quantify the effects of high sulfate concentrations. Modeled sodium sulfate effects on *Chlamydomonas moewusii* predicted growth inhibition at 25 mM, but the inhibitory effects were attributed to the increase in culture ionic strength rather than the sulfate ion concentrations specifically. An additional cause of growth inhibition by SO$_4^{2-}$ is the increase in medium acidity, which may be overcome by cultivating acidophilic microalgae or by adding a base, decreasing the flue gas flow rate, or using very high inoculum culture densities. Although confounding inhibition factors and minimum necessary sulfur concentrations have been identified for certain species, this is the first study to demonstrate biomass productivity increases, nutritional changes, and stoichiometric differences attributed to high sulfate concentrations.

**Nutritional Qualities of Microalgae as Cattle Feed.** Several species of microalgae have high protein contents, but the value of the protein is determined by the amino acid profile and how it compares to that of commercial feed. The first limiting amino acid in cereal crops, lysine, is supplied in adequate quantities by legumes. However, legumes such as soy that are high in protein and lysine are deficient in methionine. In fact, methionine is the first limiting amino acid in soy-based animal feed and is often required as a feed supplement in cattle operations. Certain microalgal species are able to supply greater fractions of the sulfur amino acid, methionine, relative to soy, in addition to providing comparable crude protein. Plants and algae assimilate and reduce sulfate before it is used to synthesize cysteine and subsequently methionine. Animals are unable to synthesize methionine from cysteine but can convert methionine to cysteine, thus requiring specific quantities of methionine. The next limiting amino acids are then histidine or threonine for dairy or beef cattle, respectively.

**Harvesting as an Economic Obstacle.** Although microalgae are advantageous in their nutritional qualities, potential to remediate pollution, and use of solar energy, harvesting remains an overwhelming challenge for scale-up and commercialization. Microalgae have similar density as water and are cultured in very dilute solutions, which makes separation difficult. Popular separation techniques that rely upon uneconomical chemical or energy inputs include chemical flocculation, centrifugation, filtration, flotation, and electrophoresis. Although gravity sedimentation avoids high energy usage or compromising the product with flocculants, it is often prohibitively slow. All told, microalgal harvesting is often reported as 20−30% (and as much as 57%) of production costs. Alternatives to popular separation techniques are attractive to producers looking to cultivate microalgae in an economical and sustainable manner.

Easily settleable, self-flocculating microalgae would be beneficial for economical cultivation. Extracellular polymeric substances (EPS) are biopolymers excreted by microalgae for aggregation, adhesion, and protection that change microalgal surface charge, flocculation, and settling. EPS can protect cells from toxic substances, dewatering, or other sources of stress while forming multicellular aggregates that can be rapidly settleable. The characteristics of EPS depend on the species, culture conditions, physiology, and cell age. Under stress from unfavorable culture conditions, some microalgae will produce EPS as a protectant. The rate of floc formation can depend on the composition of EPS; among other components, sulfate is strongly associated with floc generation.

Here, we show that higher rates of biomass productivity are achieved by *Scenedesmus obliquus* grown with simulated flue gas than those shown previously in the literature. Also, the potentially inhibitory compounds NO$_x$ and SO$_x$ did not prove to be inhibitory; on the contrary, they stimulated the growth rate of *S. obliquus*. In fact, the biomass productivity of *S. obliquus* grown with simulated flue gas outpaced that of *S. obliquus* grown with CO$_2$-supplemented air. Under the exposure to simulated power plant emissions, microalgal protein content decreased relative to control cultures and the amino acid profile was somewhat altered. However, methionine contents were maintained. Most advantageously, cultivation with simulated flue gas dramatically increased the culture settleability through coagulation and bulk settling.

**RESULTS & DISCUSSION**

**Microalgal Growth and Nutrient Utilization.** To investigate the effect of potentially inhibitory components of power plant emissions on microalgal biomass productivity, *S. obliquus* was grown with simulated coal-fired power plant emissions and air (with equal CO$_2$ concentrations in each) for comparison. Microalgae grown with simulated emissions reached the stationary phase on Day 6, rather than Day 9 as in the control, and high biomass productivities were achieved (Figure 1). Trial 3 ended one day early, when the cylinder supplying SO$_2$ was depleted. The average overall biomass productivity for the three simulated flue gas trials was $323 \pm 2$ mg L$^{-1}$ d$^{-1}$, whereas the control overall biomass productivity was $223 \pm 13$ mg L$^{-1}$ d$^{-1}$.

These values were greater than previous literature rates for *S. obliquus* biomass productivity with simulated emissions. The
The average maximum biomass productivities of the simulated flue gas and control experiments were 700 ± 40 and 510 ± 40 mg L⁻¹ d⁻¹, respectively. Thus, equally high rates of maximum biomass productivity were achieved by *S. obliquus* grown with simulated flue gas (at 12% CO₂) as the highest literature values (at an optimal CO₂ input of 4.1%).

From the average maximum biomass productivity and measurements of mass percent carbon, the maximum rate of CO₂ utilization was calculated to be 1300 ± 80 mg L⁻¹ d⁻¹, which corresponds to (5.79 ± 0.01)% CO₂ utilization during the exponential growth phase. The calculated overall average rate of CO₂ utilization was 600 ± 4 mg L⁻¹ d⁻¹. Assuming that carbonate species concentrations in the culture medium (pH 6.8) were in chemical equilibrium with the sparged CO₂, less than 0.1% CO₂ (0.02 mol total bicarbonate) went into solution, (5.79 ± 0.01)% CO₂ was captured by the microalgae, and approximately, 94% CO₂ escaped the reactor. The percent captured by the microalgae could be improved by decreasing the gas flow rate, recycling the off-gas, or using reactors in series.

During each of the three batch trials, sparged SO₂ was oxidized in the photobioreactor and accumulated as SO₄²⁻ in the culture medium (Figure 2). Because the original medium was sulfur-free, all accumulated SO₄²⁻ was attributed to the simulated flue gas. Sulfate ions were accumulated in the medium at a rate of (202 ± 23) mg d⁻¹ SO₄²⁻. From the total produced biomass and measurements of mass percent sulfur, the average total sulfur capture was estimated to be (111 ± 4)%.

Nitrate was removed at an average rate of (80 ± 23) mg d⁻¹ NO₃⁻ (Figure 3). Phosphate was removed at an average rate of (19 ± 7) mg d⁻¹ PO₄³⁻ (Figure 4). A lesser quantity of phosphorus was fixed in microalgae grown with simulated emissions than the control microalgae, (54 ± 6)% and (77 ± 8)%, respectively. At the end of the batch trials, control and simulated emission-sparged media had (61 ± 6)% and (28 ± 9)% of the initial phosphorus, respectively. The microalgal growth was nitrogen-limited; in each batch, NO₃⁻ was exhausted before PO₄³⁻.

Experimental conditions and CO₂, NO₃⁻, and PO₄³⁻ concentrations were comparable between control and simulated-emissions experiments, while the oxidation of SO₂ to SO₄²⁻ provided dramatically more available sulfur to the microalgal cultures sparged with simulated emissions, which stimulated *S. obliquus* biomass productivity.
Stoichiometric Comparison of Microalgae Grown with Control and Simulated Flue Gases. The mass percentages of carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur were quantified in order to compare the stoichiometry of *S. obliquus* biomass, grown either with simulated flue gas or under control conditions, in a pH-stat system. From the mass percentage values (Table 1), the stoichiometric equations for microalgal growth and composition, under the experimental and control conditions, were developed (eqs 1 and 2).

Control

\[
\text{H}_3\text{PO}_4 + 92\text{CO}_2 + 0.3\text{SO}_4^{2-} + 15\text{NO}_3^- + 16.6\text{H}^+ \\
+ 73.2\text{H}_2\text{O} \rightarrow \text{C}_{92}\text{H}_{163}\text{O}_{68}\text{N}_{13}\text{P}_{0.5} + 129.7\text{O}_2
\] (1)

With simulated flue gas

\[
\text{H}_3\text{PO}_4 + 130\text{CO}_2 + 0.5\text{SO}_4^{2-} + 14\text{NO}_3^- + 16\text{H}^+ \\
+ 102.5\text{H}_2\text{O} \\
\rightarrow \text{C}_{130}\text{H}_{223}\text{O}_{59}\text{N}_{14}\text{P}_{0.5} + 175.7\text{O}_2
\] (2)

Overall, *S. obliquus* elemental composition was significantly altered by the simulated flue gas (Table 1). The N/C mass ratios for the control microalgae and the microalgae exposed to simulated flue gas were 0.18:1 and 0.13:1, respectively. Under the simulated-emissions condition, microalgae had lower N/C ratios, which supports the decreased N-rich protein content. However, the high N/C values suggested that the biomass would be slow to compost as fertilizer. The P/C mass ratios for the control microalgae and the microalgae exposed to simulated flue gas were 0.028:1 and 0.020:1, respectively, which is corroborated by the differences in PO₄³⁻ uptake. The microalgal cultures grown with simulated emissions are less N- and P-rich than the control cultures. Microalgae grown under stressful conditions are often less N-rich, and fast-growing producers are less-nutritious. The energy content, indicated by the H/C ratio, was comparable between microalgae grown with control gases and simulated emissions, 0.15:1 and 0.14:1, respectively. For the microalgal biomass grown under control and experimental conditions, the average oxidation states of carbon were −0.31 and −0.52, respectively. These values indicated that the whole biomass had relatively low energy content and if unprocessed would be better suited for animal feed than as fuel/feedstock. If the biomass was to be combusted for energy, the biomass grown under control conditions would produce 1.76 g CO₂/g biomass and the biomass grown with simulated emissions would produce 1.92 g CO₂/g biomass (per stoichiometric mole of biomass, under the control and experimental conditions, combusion would produce 92 and 130 mol CO₂, respectively).

*S. obliquus* Protein Nutritional Qualities. Microalgae are a proven viable food source for domesticated livestock. Ruminants, through their specialized digestive systems, better obtain the nutritional value from algae than other animals. In soy- and corn-based cattle feed, methionine and lysine, respectively, are the first limiting amino acids and methionine is frequently required as feed supplements.

Certain microalgae have high protein contents when grown under ideal conditions. To ascertain the nutritional value of microalgae grown with simulated flue gas, the protein content of *S. obliquus* grown with simulated flue gas, *S. obliquus* grown with CO₂-supplemented air (control), and whole soybean was compared (Figure 5). Although the *S. obliquus* control culture protein content exceeded that of soy, that of *S. obliquus* grown with simulated emissions was 30.8 ± 0.8%, which is lower than that of soy at 40.3 ± 0.8%. This can be explained by the stress created on the microalgae by the toxic gas mixture, causing the protein synthesis to decrease and the amino acid profile to be altered. The decrease in protein content was consistent with the decrease in mass percent nitrogen under the simulated-emissions condition.

To quantify the changes in microalgal protein quality under the stress of simulated flue gas, the amino acid profiles of *S. obliquus* grown with simulated flue gas, *S. obliquus* grown with CO₂-supplemented air (control), and soy were compared (Figure 6). Advantageously, the percent protein that was methionine under both *S. obliquus* culture conditions was equivalent, each more than double that of soy. The lysine content of *S. obliquus* grown with simulated emissions was equal to that of soy and better than that of the control. Overall, the *S. obliquus* grown with simulated emissions was sufficiently rich in lysine and methionine. The next limiting amino acids,
after methionine and lysine, are histidine or threonine for dairy or beef cattle, respectively. *S. obliquus* grown with simulated emissions had poorer histidine content than control *S. obliquus* and soy. However, both *S. obliquus* grown with simulated emissions and control *S. obliquus* resulted in (53 ± 6) and (51 ± 8)% greater threonine values than soy, respectively.

**Settleability.** Harvesting is a serious challenge to the microalgal industry. The size and density of microalgae make discreet cells, or even small clusters, very difficult to filter or settle from the culture medium without additional energy or chemical inputs. Fortunately, the settleability of *S. obliquus* grown with simulated flue gas emissions was dramatically superior to that grown with the control gases.

During the batch simulated flue gas experiments, microalgal settling was enhanced and it is hypothesized that production of EPS caused foaming, coagulation, and enhanced settling. Stress-induced production of EPS has previously been shown to cause beneficial coagulation, in this case, stress is attributed to acidic or toxic flue gas components. EPS facilitated the formation of flocs with good attachment, but no flocs formed under control conditions (Figure 7). Foaming began on Day 2 and an antifoaming agent was required by Day 3 of each microalgal batch, as foam would otherwise fill the headspace of the photobioreactor and displace significant quantities of biomass from the liquid culture. Foaming did not occur in control cultures. After bacterial contamination and extensive cell death were ruled out as potential causes, it was hypothesized that foaming and coagulation were due to EPS production.

Microalgal biomass settled rapidly if reactor mixing ceased, even temporarily. Experiments conducted to quantify the settling rates (Figure 8) revealed that bulk settling occurred at a nonlinear rate, characterized by first coagulation and then compaction, which was modeled after eq 4. According to the model, the region of coagulation and rapid settling occurred across the upper 30.2 ± 0.5 cm, the region of compaction was at 1.8 ± 0.4 cm, the rate of rapid settling was 1.6 ± 0.2 min⁻¹, the rate of slowed settling was 0.10 ± 0.05 min⁻¹, and the minimum compacted bulk height was predicted to be 1.2 ± 0.2 cm (Figure 9).

The average fraction of biomass that settled in bulk, rather than discrete particles, was 58 ± 17%. The control, *S. obliquus* grown with CO₂-supplemented air, only settles slowly as discrete particles and did not settle significantly within the 30 min trials.

**CONCLUSIONS**

The biomass productivity rates of *S. obliquus* grown with simulated power plant flue gas surpassed those of research conducted with CO₂-supplemented air at equal CO₂ concentrations, despite the additional stresses of nitrogen dioxide, sulfur dioxide, and carbon monoxide. Indeed, the biomass productivity of *S. obliquus* was significantly increased even as SO₄²⁻ concentrations reached 1300 mg L⁻¹. This work exceeds the highest biomass productivity rates, for *S. obliquus* grown with simulated emissions, in the literature.

Experiments with simulated emissions resulted in dramatically improved culture separability, a major obstacle to economical microalgal production. Often, microalgae settle slowly as discrete particles. In this study, coagulated microalgal biomass in each trial was rapidly settled and compacted.

![Figure 6. Percent amino acid comparison among whole soybean, *S. obliquus* grown in 3N-BBM and CO₂ blended with ultra-zero air, and *S. obliquus* grown in sulfur-free 3N-BBM and simulated coal-fired power plant flue gas. Error was 5% of the measured values.](https://pubs.acs.org/doi/abs/10.1021/acsomega.0c03492)

![Figure 7. Comparison of (a) *S. obliquus* grown with simulated flue gas, which coagulated, with (b) *S. obliquus* grown under control conditions, which did not coagulate.](https://pubs.acs.org/doi/abs/10.1021/acsomega.0c03492)

![Figure 8. Comparison of (a) *S. obliquus* grown with simulated flue gas, which settled rapidly in a backlit graduated cylinder, with (b) *S. obliquus* grown under control conditions, which settled slowly.](https://pubs.acs.org/doi/abs/10.1021/acsomega.0c03492)
S. obliquus had greater fractions of methionine, the limiting amino acid in cattle feed, relative to soy, although the crude protein value of S. obliquus grown with simulated emissions was significantly lower. These results will move forward microalgal CO2 and SO2 capture from industrial and municipal sources and production of sustainable nutritious animal feed.

Our work will help overcome efficiency barriers in producing nutritious salable microalgae and fixing pollutants from flue gas (CO2, SO2, and NOx) in full-scale operations. Thus, use of energy-intensive fertilizer and freshwater resources will decrease, wastewater treatment costs will be offset, and greenhouse gas emissions will be reduced to produce an economical protein source.

**MATERIALS AND METHODS**

**Experimental Setup.** S. obliquus (SAG 276-1) was purchased from the culture collection at Göttingen University in Germany. Species identity was confirmed through DNA extraction, Sanger sequencing with the primers EukA (5′-AACCTGGTTGATCCTGCCAGT-3′) and EukB (5′-TGATCCTTCTGCAGG-TTCACCTAC-3′), and BLAST sequence search.

A 2 L Sartorius Biostat A bioreactor (Sartorius Stedim Biotech GmbH, Göttingen, Germany), fitted with two red and blue LED panels (280 μmol·m−2·s−1; Roleadro, San Francisco, CA, USA), served as a photobioreactor and pH-stat system for microalgal cultivation (see Figure S1 in the Supporting Information). Batch studies of S. obliquus were conducted in 1.5 L of 3-fold nitrogen content, sulfur-free Bold’s Basal Medium59 (S-free 3N-BBM) at 27 °C, 10 mM HEPES buffer, and pH 6.8 under continuous illumination and at a mixing rate of 200 rpm. Constant feedback from the bioreactor pH meter controlled the addition of the base (1 N NaOH) to maintain pH 6.8.

A custom Praxair cylinder provided a blend of 1000 ppm NO2, 1000 ppm CO2, 12% O2, and balance N2. The reactor was sparged continuously at a total rate of 0.1 L min−1 (0.07 vvm).40 Gas concentrations were selected to simulate those from coal combustion emissions from the University of Iowa power plant (Table 2).

**Table 2. University of Iowa Power Plant Boiler 10 Coal Combustion Emissions Composition**

| component | mole percent (%) |
|-----------|-----------------|
| H2O       | 12.6            |
| CO2       | 11.6            |
| O2        | 5.8             |
| CO        | 0.048           |
| SO2       | 0.045           |
| NO2       | 0.022           |
| N2        | 69.9            |

Because of the toxic nature of the combustion emission gases, experiments were conducted in a walk-in fume hood that housed the entire experimental setup.41 The experiments were continually monitored with MultiRAE toxic-gas sensors (RAE Systems, Inc., San Jose, CA, USA).

For each batch experiment, the bioreactor was inoculated to an optical density at 750 nm (OD750) of 0.015 ± 0.005 and sampled daily as each batch progressed from lag to stationary phase. Biomass values were calculated from a calibration curve relating OD750 measurements to cell dry weight concentrations (see Figure S2 in the Supporting Information).

Microalgal concentrations were fit with a logistic regression (eq 3) in GraphPad Prism Version 8.1.2

\[
f(x) = \frac{L}{1 + e^{-k(x-x_o)}}
\]

where \( L \) is the curve’s maximum value (mg L−1), \( k \) is the relative steepness of the exponential phase (d−1), and \( x_o \) is the time of the sigmoidal growth curve’s midpoint (d). The maximum biomass productivity was calculated from the derivative of eq 3 at the sigmoid midpoint, where \( x = x_o \).

Foaming, which may cause inaccurate biomass concentration measurements, was prevented with 1–2 mL daily additions (never exceeding 6 mL per trial) of sterile 1% antifoam SE-15 solution (Sigma-Aldrich, Inc., Saint Louis, MO, USA) beginning on Day 3 of each microalgal batch.42

**Inoculum Preparation.** S. obliquus inoculum was prepared from pure cultures stored on refrigerated 3N-BBM agar plates. Colonies from the stored plates were used to inoculate 100 mL of sterile 3N-BBM in 500 mL Erlenmeyer flasks capped with foam plugs. Cultures were grown in the flasks for approximately 6 d (the approximate midpoint of the exponential growth phase after transfer from the refrigerated stock), at 25 °C and 16:8 h light cycle, before use in bioreactor experiments.

**Control Conditions.** Control experiments used Praxair high-purity CO2 and ultra-zero air to attain 12% CO2. The control cultures had the same temperature, pH, gas flow rate, stirring rate, optical density upon inoculation, and HEPES buffer concentration as the experimental cultures. The culture medium (3N-BBM) was identical with the exception that sulfate (34 mg L−1) was included in the medium for the control experiments, whereas simulated flue gas provided sulfate during the experimental batches. The initial ionic strengths of the media were approximately equal.

Figure 9. Modeled bulk settling, through coagulation and compaction, of microalgae grown with simulated power plant emissions. The model error is represented with a 95% confidence interval. Settling of control microalgae was not observed within the 30 min period.
Nutrient Quantification in Flue Gas and the Culture Medium. The concentration of CO₂ sparged into the reactor was confirmed using GasLab software and a coziR wide-range 0–20% CO₂ sensor (CM-0123, Gas Sensing Solutions Ltd., Glasgow, UK).

Sulfate concentrations accumulated, from the sparged simulated flue gases, during the 50 h bioreactor experiment were quantified using SulfaVer4 Method 8051 (HACH, Loveland, CO, USA) with a HACH DR6000 UV–Vis spectrophotometer (see Figures S3 & S4 in the Supporting Information).

During the cultured simulated flue gas trials, sulfate, phosphate, and nitrate concentrations were measured in daily samples of the culture medium (0.2 μm filtered) using an ion chromatograph (Thermo Fisher ICS-2100) equipped with a Dionex IonPac AS18 column. Combined Seven Anion Standard II (Dionex, Sunnyvale, CA) was used to calibrate the instrument.

Microalgal Nutritional Analysis. The protein contents of dried S. obliquus samples were quantified via total nitrogen analysis by Dairy One Forage Labs (Ithaca, NY, USA). The fraction of microalgal protein was estimated from total nitrogen measurements, with a genus-specific conversion factor of 5.05 ± 0.03 to exclude nonprotein nitrogen.33

Dried microalgal samples were submitted to Bio-Synthesis Inc. (Lewisville, Texas, USA) for amino acid profile analysis using high-performance liquid chromatography.

Microalgal Elemental Composition. The mass percentages of carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur were determined at the Iowa State Materials Analysis & Research Laboratory (see Figures S5 through S10 in the Supporting Information). Carbon, oxygen, hydrogen, sulfur, and nitrogen were quantified through elemental analysis. Sulfur and phosphorus were quantified through energy-dispersive X-ray spectroscopy (FEI Quanta-250 SEM/EDS equipped with an Oxford Instruments X-Max 80 detector).

Bulk Settling Experiments. Static column settling tests, modeled after sludge volume index tests in the wastewater field, were conducted with a 1000 mL graduated cylinder. Microalgal culture, grown with simulated power plant emissions and harvested on Day 6, was well mixed (200–300 rpm) prior to pouring the culture into the graduated cylinder to a height of 33.3 cm. The bulk height of the biomass was observed for a period of 30 min. The same procedure was applied to a microalgal control culture grown with 12% CO₂ and ultra-zero air.

Bulk settling was modeled with two-phase exponential decay

\[ f(x) = (\text{Span}_1)e^{-K_{1x}} + (\text{Span}_2)e^{-K_{2x}} + \text{plateau} \quad (4) \]

where Span₁ is the region dominated by coagulation and rapid settling, Span₂ is the region dominated by compaction, K₁ is the rate of rapid settling, K₂ is the rate of compaction, and the plateau is the minimum compacted bulk height.

The fractions of biomass in the settled bulk and the suspended remainder were quantified by measuring the volume and OD₇₅₀ of each portion. The absence of bacterial contamination as a cause of flocculation was confirmed by phase-contrast microscopy at 100X magnification and using agar streak plates. The lack of cell death (and subsequent release of cell debris) was confirmed through microscopy at 100X to search for cell debris and those lacking the green pigment. Prior to microscopy and plating, flocculated samples were rapidly mixed to break up the flocs.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c03492.

Image of the experimental setup, biomass versus OD₇₅₀ calibration curve, sulfate concentration versus OD₇₅₀ calibration curve, 75 h sulfate accumulation, and supplementary elemental analysis and SEM–EDS data (PDF).

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Author Contributions

H.R.M. performed the experiments in the laboratory. The idea for the research was from both H.R.M. and J.L.S. equally, and the writing of the manuscript was performed by H.R.M. and edited by J.L.S. Both authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

1. Robinson, T. P.; Pozzi, F. Mapping supply and demand for animal-source foods to 2030. Animal Production and Health Working Paper; Rome, 2011; p 3.
2. Searchinger, T.; Waite, R.; Hanson, C.; Ranganathan, J. Creating a Sustainable Food Future: A Menu of Solutions to Feed Nearly 10 Billion People by 2050; World Resources Institute, 2018; pp 6–9.
(17) Tabe, L.; Higgins, T. J. V. Engineering plant protein composition for improved nutrition. Trends Plant Sci. 1998, 3, 282–286.

(23) Gerardo, M. L.; Oatley-Radcliffe, D. L.; Lovitt, R. W. Minimizing the Energy Requirement of Dewatering Scenedesmus sp. by Microfiltration: Performance, Costs, and Feasibility. Environ. Sci. Technol. 2014, 48, 845–853.

(24) Uddman, N.; Qi, Y.; Danquah, M. K.; Forde, G. M.; Hoadley, A. Dewatering of microalgal cultures: A major bottleneck to algae-based fuels. J. Renew. Sustain. Energy 2010, 2, 012701.

(25) Fasaei, F.; Bitter, J. H.; Slegers, P. M.; van Boxtel, A. J. B. Techno-economic evaluation of microalgae harvesting and dewatering systems. Algal Res. 2018, 31, 347–362.

(26) Xiao, R.; Zheng, Y. Overview of microalgal extracellular polymeric substances (EPS) and their applications. Biotechnol. Adv. 2016, 34, 1225–1244.

(27) Mishra, A.; Jha, B. Isolation and characterization of extracellular polymeric substances from micro-algae Dunaliella salina under salt stress. Bioresour. Technol. 2009, 100, 3382–3386.

(28) Ma, S.; Li, D.; Yu, Y.; Li, D.; Yadav, R. S.; Feng, Y. Application of a microalgae, Scenedesmus obliquus PF3, for the biological removal of nitric oxide (NO) and carbon dioxide. Environ. Pollut. 2019, 252, 344–351.

(29) Molitor, H. R.; Moore, E. J.; Schnoor, J. L. Maximum CO2 Utilization by Nutritious Microalgae. ACS Sustainable Chem. Eng. 2019, 7, 9474–9479.

(30) Loelde, I.; Kuang, Y.; Elser, J. J. Stoichiometry in Producer-Grazer Systems: Linking Energy Flow with Element Cycling. Bull. Math. Biol. 2000, 62, 1137–1162.

(31) Madeira, M. S.; Cardoso, C.; Lopes, P. A.; Coelho, D.; Afonso, C.; Bandarra, N. M.; Prates, J. A. M. Microalgae as feed ingredients for livestock production and meat quality: A review. Livest. Sci. 2017, 205, 111–121.

(32) Bleakley, S.; Hayes, M. Algal Proteins: Extraction, Application, and Challenges Concerning Production. Foods 2017, 6, 33.

(33) González-Fernández, C.; Ballesteros, M. Microalgae auto-flocculation: an alternative to high-energy consuming harvesting methods. J. Appl. Phycol. 2013, 25, 991–999.

(34) Shipin, O. V.; Meiring, P. G. J.; Phawansra, R.; Kluever, H. Integrating ponds and activated sludge process in the PETRO concept. Water Res. 1999, 33, 1767–1774.

(35) El-Sheekh, M. M.; Khalir, H. M.; El-Shenody, R. Algal production of extra and intra-cellular polysaccharides as an adaptive response to the toxin crude extract of Microcystis aeruginosa. Iran. J. Environ. Health Sci. Eng. 2012, 9, 10.

(36) Angelicin, M.; Senthilkumar, N.; Karapag, R.; Kumar, G. A.; Ashokkumar, B.; Varalakshmi, P. Enhanced Extracellular Polysaccharide Production and Self-Sustainable Electricity Generation for PAMFCs byScenedesmus sp. Biotechnol. Adv. 2014, 32, 362–376.

(37) Fleming, H.-C.; Wingender, J. The biofilm matrix. Nat. Rev. Microbiol. 2010, 8, 623–633.

(38) Bradley, I. M.; Pinto, A. J.; Guest, J. S. Design and Evaluation of Illumina MiSeq-Compatible, 18S rRNA Gene-Specific Primers for Improved Characterization of Mixed Phototrophic Communities. Appl. Environ. Microbiol. 2016, 82, 5878–5891.

(39) Bischoff, H. W.; Bold, H. C. Some Soil Algae from Enchanted Rock and Related Algal Species; University of Texas: Austin, Tex., 1963; p 1–95.

(40) Huang, Q.; Jiang, F.; Wang, L.; Yang, C. Design of Photobioreactors for Mass Cultivation of Photosynthetic Organisms. Engineering 2017, 3, 318–329.

(41) Molitor, H. R.; Willard, D. E.; Schnoor, J. L. Microalgae Cultivation and Biomass Quantification in a Bench-Scale Photobioreactor with Corrosive Flue Gases. J. Visualized Exp. 2019, 154, No. e60566.

(42) Breuer, G.; de Jaeger, L.; Artus, V. P. G.; Martens, D. E.; Springer, J.; Draisma, R. B.; Eggink, G.; Wijffels, R. H.; Lamers, P. P. Superior triacylglycerol (TAG) accumulation in starless mutants of Scenedesmus obliquus: (I) evaluation of TAG yield and productivity in controlled photobioreactors. Biotechnol. Biofuels 2014, 7, 70.
(43) Templeton, D. W.; Laurens, L. M. L. Nitrogen-to-protein conversion factors revisited for applications of microalgal biomass conversion to food, feed and fuel. *Algal Res.* **2015**, *11*, 359–367.