ACS Sustainable Chemistry & Engineering

Research Article

Cite This: ACS Sustainable Chem. Eng. 2019, 7, 9474−9479

pubs.acs.org/journal/ascecg

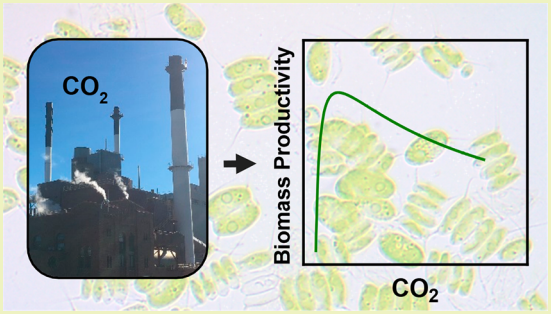
Maximum CO_2 Utilization by Nutritious Microalgae

Hannah R. Molitor,*,† Emily J. Moore,† and Jerald L. Schnoor†

†Department of Civil and Environmental Engineering, University of Iowa, 103 S. Capitol St., Iowa City, Iowa 52240, United States

Supporting Information

ABSTRACT: High-protein microalgae are a promising alternative to soy for more rapidly and sustainably produced protein-rich animal feed. However, there are still significant barriers to be overcome in growing nutritious microalgae, recovering nutrients from wastewater, and fixing CO_2 from flue gas in full-scale sustainable operations. Currently, it is generally assumed that nutritious microalgae, including *Scenedesmus obliquus*, are inhibited by CO_2 levels characteristic of industrial flue gases. Experiments in a 2 L photobioreactor with the ability to control CO_2 concentrations and pH demonstrated that the inhibition of *S. obliquus* was not important until 10% CO_2 and was not prohibitively reduced even at 35% CO_2. The rate of growth exceeded all values in the literature for *S. obliquus* at concentrations greater than 2.5% CO_2, and the amino acid content of the microalgae was equal or superior to that of soy. A substrate inhibition model indicated that CO_2 levels comparable to flue gases do not substantially inhibit *S. obliquus* growth, with careful pH control. The model indicated maximum biomass productivity of 640 ± 100 mg L^{-1} d^{-1} at 4.5% CO_2 (K_m of 0.8 ± 0.4% CO_2, K_i of 26 ± 9% CO_2, and v_{max} of 860 ± 120 mg L^{-1} d^{-1}), which exceeds previously measured biomass productivity values at inhibitory CO_2 concentrations. Protein contents of *S. obliquus* and soy were comparable.



KEYWORDS: *Carbon dioxide utilization, Nutritious microalgae, Substrate inhibition*

Downloaded by UNIV OF IOWA at 15:10:25:645 on June 03, 2019
from https://pubs.acs.org/doi/10.1021/acssuschemeng.9b00656.

INTRODUCTION

Global agriculture consumes 3% of annually produced energy and 70% of freshwater, and occupies 11% of land area.[1] Though substantial resources are devoted to agriculture, 1 billion of the earth's 7.7 billion people already lack sufficient, nutritious food on a regular basis.[2] Furthermore, the world population is predicted to increase by 2−3 billion by 2050. Due to population increases and urbanization patterns, demand for animal-derived protein is expected to double by 2050.[3] To meet the global demand for animal and human food, crop and livestock production must increase in quantity and efficiency, using finite resources. Soybean meal is the most common protein supplement in cattle feed, but conventionally grown crops are energy, water, and nutrient intensive.[4]

Inefficiencies of Soybean Meal as a Feed Supplement. Of the four billion bushels of soy grown in the United States annually, 70% becomes animal feed.[5] However, soy crops are dependent on ammonium fertilizers which are produced through the energy-intensive Haber-Bosch process, use freshwater resources, and occupy 20% of the United States cropland.[6] Not only does conventional agriculture place significant demands on natural resources but also it is inefficient. Only 16% of anthropogenically sourced nitrogen reaches the end product, plant or animal protein.[7] Instead, large quantities of nitrogen fertilizers runoff the land with precipitation water, leach into the groundwater, volatilize as ammonia, and undergo microbial denitrification. Soybeans get nitrogen from both biological fixation of inert atmospheric nitrogen and uptake of reactive nitrogen from the soil.[8] Approximately 50% of the nitrogen required for soybeans comes from the atmosphere. Mature soya plants are able to fix nitrogen from the atmosphere, but "starter nitrogen" is applied to seedlings to stimulate plant growth and eventually increase grain yield.[9] Beyond nutrient demands, conventional soybean meal requires environmentally harmful chemical inputs for growth and processing.

Microalgae as a Sustainable Alternative to Soy. High-protein microalgae are a promising alternative to soy for more rapidly and sustainably produced protein-rich animal feed.[10] Two members of the family of green algae, *Desmodesmus* and *Scenedesmus*, have been researched as candidates for nutritious microbial protein because of characteristic high-protein contents and favorable amino acid profiles.[11−18] These members of the family Scenedesmaceae have demonstrated a tolerance for range of pH, temperature, nutrient variation, and shear force.[17] However, there are still significant barriers to growing nutritious salable microalgae, recovering nutrients from wastewater, and fixing CO_2 from flue gas in full-scale operations.[19] Currently, it is generally assumed that nutritious microalgae, including *Scenedesmus obliquus*, are inhibited by CO_2 levels characteristic of flue gas.[11,13,20,21]

Flue Gas as a Source of Carbon for Microalgae. Significant and prompt mitigation of greenhouse gas emissions will be required to stabilize atmospheric concentrations of

Received: February 5, 2019
Revised: April 25, 2019
Published: April 30, 2019

© 2019 American Chemical Society

DOI: 10.1021/acssuschemeng.9b00656
ACS Sustainable Chem. Eng. 2019, 7, 9474−9479

CO_2, according to the Intergovernmental Panel on Climate Change.[22] Uptake of CO_2 by photoautotrophs, including microalgae, is a means to utilize solar energy and "waste" nutrients while producing a valuable feed and removing CO_2 from the atmosphere or stack gases as a resource.[23] Waste streams of flue gas containing high levels of CO_2 can be diverted for use as microalgal substrate, especially for the species able to tolerate high concentrations of CO_2, NO_x, and SO_x.[24] CO_2 is fixed as microalgal biomass, which can be harvested for high-value compounds, biofuels, fertilizer, or animal feed.[25−27] In addition, waste heat from power plants or industrial processes may be recovered to maintain favorable culture temperatures (15 to 35 °C).[28] Revenue from these products could offset CO_2 capture and algae dewatering costs.

Microalgae have been grown across a wide range of nonideal CO_2 concentrations to which they are not adapted to attempt to produce biomass rapidly. Certain microalgae have very high CO_2 fixation rates (5−10 times faster than terrestrial plants), 70% to 80% of which goes to biomass production.[29,30] Unfortunately, the CO_2 stream itself can inhibit microalgal growth.[13,31] CO_2 is readily available in the atmosphere at 0.04%, which is only a fraction of its availability in coal-fired power plant flue gas at 12−15% CO_2.[32] Natural gas combustion, oil refining, cement manufacturing, iron and steel manufacturing, and anaerobic fermentation emission streams contain 3−4%, 8−9%, 14−33%, 20−44%, and approximately 40% CO_2, respectively.[32,33]

To date, studies of flue gas as substrate for microalgae have focused on contaminants to the CO_2 stream but generally neglected the inhibitory effect of CO_2 itself. A study of inhibition in the microalgae *Chlamydomonas reinhardtii* approximated the K_m at 0.09% CO_2.[34] Studies of *S. obliquus* where CO_2 input ranged from 0.03% to 50% indicated growth limitation at high CO_2 levels,[13] but CO_2 tolerance has yet to be determined in *S. obliquus* at high concentrations of CO_2 relevant to flue gas with promising biomass productivities.

In previous studies, decreased CO_2 tolerance was reported and was likely due to media acidification without pH correction.[13] Aqueous CO_2 forms carbonic acid in water, some of which deprotonates to bicarbonate and H^+ thus decreasing the solution pH. Media acidification occurs when CO_2 is provided in excess. Conversely, if too little CO_2 is provided, microalgae fix all the aqueous CO_2 and the solution pH increases.

Here, we show that high CO_2 concentrations typical of power plant flue gas did not greatly inhibit the growth of microalgae. In fact, at somewhat lower CO_2 concentrations, we demonstrated the highest CO_2 uptake rates in the literature. Most importantly, the microalgae product showed equal or better nutritional characteristics to the animal feed which it would replace, soy protein.

MATERIALS AND METHODS

Experimental Setup. *S. obliquus* was selected as a promising candidate after growth rate comparison with *C. vulgaris* and *D. armatus* (see Figure S1 in Supporting Information). *S. obliquus* (SAG 276-1), *N. atomus* (SAG 14.87), and *D. armatus* (SAG 276-4d) cultures (see Figures S2 and S3 in Supporting Information) were purchased from the culture collection at Göttingen University in Germany. Species identity was confirmed through DNA extraction, Sanger sequencing with primers EukA (5′-AACCTGGTTGATCCTGCCAGT-3′) and EukB (5′-TGATCCTTCTGCAGGTTCACCTAC-3′),[35] and BLAST sequence search.

A Sartorius Biostat A Plus bioreactor, fitted with two red and blue LED panels (280 μmol m^{-2} s^{-1}), served as a photobioreactor for microalgae cultivation (see Figure S4 in Supporting Information). Batch studies of *S. obliquus* were conducted in 1.5 L of 3-fold nitrogen content Bold's Basal Medium[36] at 27 °C, 10 mM HEPES buffer, and pH 6.8 under continuous illumination. Constant feedback from the bioreactor pH meter was used to control the addition of the base (1N NaOH) to maintain a pH of 6.8. The cultures were sparged continuously. A cylinder of Praxair Ultra-Zero air provided a blend of N_2 and O_2, which was mixed with gas from a second tank containing high purity CO_2. The reactor was sparged at a total rate of 0.1 L min^{-1} (0.07 vvm). CO_2 concentrations were selected to approximate the range from atmospheric levels to high CO_2 levels from industrial processes, and concentrations were confirmed with the appropriate sensors (Senseair K30 1%, Gas Sensing Solutions Ltd. cozIR 20%, or Gas Sensing Solutions Ltd. cozIR 100%) and GasLab v2.0.8.14 software. Following inoculation, the cultures' optical density at 750 nm (OD_{750}) was 0.05 ± 0.03. The reactor was sampled daily as each batch progressed from lag to stationary phase. Biomass values were calculated from a calibration curve relating OD_{750} measurements to biomass concentrations (see Figure S5 Supporting Information).

Comparison of *S. obliquus* growth on NO_3^- and NH_4^+, prior to the CO_2 inhibition experiments, yielded no significant difference in growth rates (see Figure S6 in Supporting Information). Nitrate and phosphate depletion in select *S. obliquus* culture batches was measured over time using HACH kits TNT836 and TNT846, respectively.

Kinetic Calculations. *S. obliquus* was grown at several CO_2 concentrations, and a substrate inhibition curve was modeled according to Monod kinetics to determine the appropriate CO_2 input to maximize biomass productivity and the impacts of those CO_2 levels on growth rates. At each CO_2 level, microalgae biomass was tracked via OD_{750} and then fit with a logistic regression (eq 1 and Figure S7 in Supporting Information) in GraphPad Prism Version 7.03

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

where L is the curve's maximum value (mg L^{-1}), k is the relative steepness of the exponential phase (d^{-1}), and x_0 is the time of the sigmoidal growth curve's midpoint (d). The maximum biomass productivity at each CO_2 input was calculated from the derivative of eq 1 at the sigmoid midpoint, where $x = x_0$. The standard errors of the logistic regression parameters were used to calculate the standard error of the calculated maximum biomass productivity values.

Substrate inhibition was also modeled in GraphPad Prism, according to the substrate inhibition model (eq 2)

$$v = \frac{v_{max} \times x}{K_m + x\left(1 + \frac{x}{K_i}\right)} \tag{2}$$

where v_{max} is the maximum growth velocity, K_m is the Michaelis−Menten constant, and K_i is the inhibitor constant.

Nutritional Analysis. Total protein contents of dried *S. obliquus*, *N. atomus*, *D. armatus*, and soybean flour were quantified via total nitrogen analysis by Dairy One Forage Laboratories (Ithaca, NY). Total microalgal protein was estimated from total nitrogen, with a conversion factor of 5.05 ± 0.03 to exclude nonprotein nitrogen.[37]

Dried microalgae and soy samples were submitted to Bio-Synthesis Inc. (Lewisville, Texas, U.S.A.) for amino acid profile analysis. Samples were hydrolyzed in 6 N HCl for 24 h at 110 °C, dried, resuspended, and added to a reaction buffer before injection onto an HPLC column.

RESULTS AND DISCUSSION

Substrate Inhibition. The greatest measured maximum and overall biomass productivities were measured here to be 700 ± 110 and 510 ± 20 mg L^{-1} d^{-1}, respectively, at 4.1% CO_2 (Figures 1 and 2). The maximum overall biomass productivity

DOI: 10.1021/acssuschemeng.9b00656
ACS Sustainable Chem. Eng. 2019, 7, 9474−9479

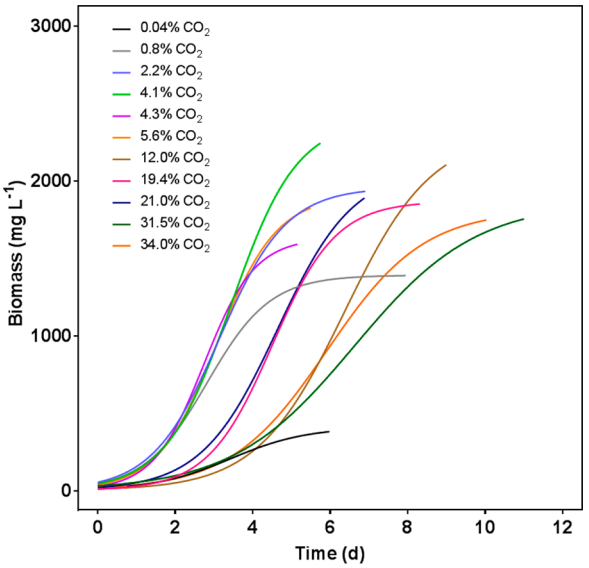


Figure 1. Modeled *S. obliquus* growth across a range of CO_2 inputs: 0.04% to 34% CO_2. Raw data are included in Supporting Information Figure S8.

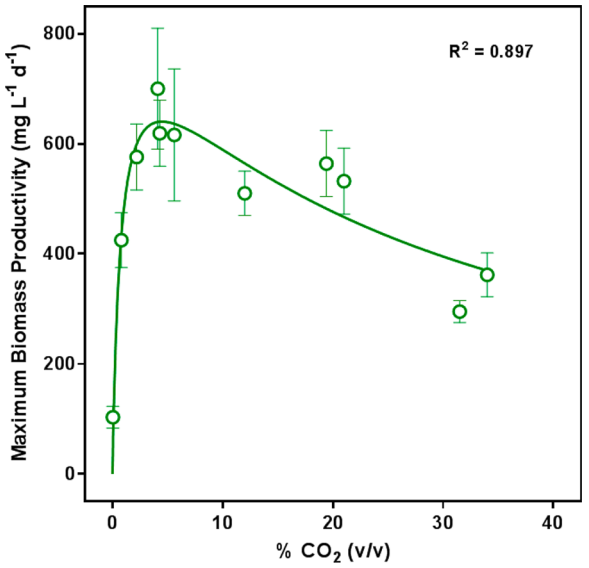


Figure 2. *S. obliquus* maximum biomass productivity over a range of CO_2 inputs. Data were fitted with a substrate inhibition curve. Error bars represent the standard error of the modeled maximum growth rate.

literature value for *S. obliquus* was 626.6 mg L^{-1} d^{-1} (error and maximum biomass productivity not reported) at 420 μmol m^{-2} s^{-1} and 2.5% CO_2 in a nitrogen starvation study for lipids production.[38] The same *S. obliquus* strain produced biomass at overall rates of 292.5 mg L^{-1} d^{-1} under 60 μmol m^{-2} s^{-1} and 10% CO_2,[39] and 460−490 mg L^{-1} d^{-1} under 2.5% and 180 μmol m^{-2} s^{-1}.[40]

S. obliquus growth was predicted to be optimized at 4.5% CO_2 and inhibited above 26 ± 9% CO_2, as indicated by the substrate inhibition model, where v_{max} is the maximum growth velocity (860 ± 120 mg L^{-1} d^{-1}), K_m is the Michaelis−Menten constant (0.8 ± 0.4% CO_2), and K_i is the inhibitor constant (26 ± 9% CO_2).

This work's substrate inhibition model indicated a K_m value approximately 10-fold greater than that of a previous study of *C. reinhardtii*, which indicated that this study's biomass productivity peaked at much greater CO_2 levels. Previous studies maximized microalgal productivity when pH was relatively steady at neutral values but did not have the feedback control to properly maintain pH at an exact value; in other cases, pH was managed by periodically adding of ammonium salts, varying the CO_2 concentration, or adding higher concentrations of buffer and letting the solution gradually acidify.[31,41−45] In small batch studies (less than 100 mL) where the pH was initially 6.8, this study found that biomass productivity was much less than that of *S. obliquus* in the pH-stat system. The characteristic high biomass productivity of *S. obliquus* is enabled under constant pH conditions.

At constant pH, HCO_3^- and dissolved CO_2 concentrations increase proportionally with headspace CO_2 concentrations. Ribulose-1,5-bisphosphate carboxylate (RuBisCO), a key enzyme in photoautotrophs, catalyzes the fixation of CO_2 and HCO_3^-.[46] Biomass productivity was used to estimate the rates of CO_2 consumption based on the typical carbon content of *S. obliquus* cells.[13] Estimated maximum and overall CO_2 consumption rates were 1,280 ± 200 and 930 ± 40 mg L^{-1} d^{-1}, respectively, at 4.1% CO_2. High *S. obliquus* biomass productivity and high CO_2 fixation rates can be achieved at levels typical of natural gas and coal-fired power plant flue gas (see Table S9 in Supporting Information), with careful pH control.

In each measured batch, nitrate was depleted from the culture medium as the culture reached stationary phase (see Figure S10 in Supporting Information). Phosphate concentrations in the culture medium generally declined at a decreasing rate and were not depleted (See Figure S11 in Supporting Information).

Operation Scale-Up. Though this is bench-scale research and the scale-up of economical microalgal production systems is difficult, these results are promising. Biomass productivity, scale, cultivation method, and nutrient costs determine the economic viability of microalgal products.[25] The potential for higher biomass productivities at much higher CO_2 concentrations is advantageous to resource-efficient production. In this study, there were significant losses of CO_2 from the system as an optimal mass flow rate of CO_2 was not investigated. However, these results elucidate equilibrium CO_2 concentrations required for maximum microalgal growth and CO_2 utilization efficiency. When sparged CO_2 mass flow rates exceed uptake rates by microalgae growth at scale, then bioreactors may be utilized in series for increased capture and/or CO_2 flow rates may be decreased.

Bioreactor pH control, at scale, is difficult due to the nonlinearity of the reaction and time delays. Depending on nutritional uptake by the microalgae in the photobioreactor, pH may either increase or decrease. In our experience, no more than 10 mL of 1 N NaOH, per batch, was needed to hold the pH constant against high CO_2 concentrations. At a larger scale, several investigators have successfully achieved pH control using proportional control, or proportional-integral (PI) control with or without feedback or cascade controllers.[47,48]

Microalgae as cattle feed is cost prohibitive relative to soybean meal because it requires separation from a dilute solution and is susceptible to contamination.[49] However, optimized microalgal biomass productivity on waste substrates provides an opportunity to meet animal feed demands and

DOI: 10.1021/acssuschemeng.9b00656
ACS Sustainable Chem. Eng. 2019, 7, 9474−9479

reduce global energy, freshwater, and land use.[50] Under these conditions, the usage of energy-intensive fertilizer and freshwater resources would decrease, wastewater treatment costs would be offset, and greenhouse gas emissions would be reduced to produce an economical protein source.

Targeted Wastewater Characteristics. Treatment of high nitrate wastewaters, without carbon source supplementation, is generally considered challenging for microbes.[51] Fortunately, there are thousands of sources of domestic secondary effluent, some of which are colocated with power plants. Additionally, industrial and agricultural wastewater streams with low organic carbon and high nitrogen concentrations (such as explosives factory wastewater, fertilizer plant wastewater, agricultural runoff, and irrigation return waters)[52] are advantageous options for microalgae substrates. However, contamination by heavy metals and pathogens from certain wastewaters would be hurdles in the production of microalgae for animal feed.

Growing microalgae on low organics waste streams or tertiary wastewater will limit microbial competitors because the majority of carbon sources will have already been removed using prior treatment steps. Microalgae are also given a competitive edge over potential contaminating species when media conditions such as pH or salinity are tailored to their growth and hinder other microbes.[53] Microalgae may also dominate in nutrient-rich systems; in eutrophic lakes, autotrophic microbes outcompete their heterotrophic counterparts.[54] This condition necessitates that the wastewater substrate have low turbidity to allow solar energy to reach microalgae, which already require shallow depths to avoid self-shading.

Nutritional Qualities. Microalgae is an alternative protein source with greater resource-to-protein conversion efficiency than soy. However, nutritious microalgae currently costs 10-fold the price of soy, which is $0.10/lb to $0.20/lb.[53] High microalgal biomass productivity using inexpensive and sustainably sourced substrates would significantly offset the overall cost of production. *S. obliquus*, *D. armatus*, and *N. atomus* each have high growth rates and protein contents comparable to soy (Figure 3).

In addition to resource efficiency, certain microalgae may be able to supply greater fractions of the sulfur amino acids, cysteine and methionine, in comparison to soy.[19] Methionine is the first limiting amino acid in cattle feed supplemented by soybean meal and is often required as a feed supplement at extra expense to the farmer.[4] The next limiting amino acids are lysine, then histidine or threonine depending on whether the cattle are designated for milk or beef production.[55,56] Ruminants, in general, more efficiently digest and extract the nutritional value from algae-supplemented feed than other animals.[10] To determine the nutritional value of microalgal protein, the amino acid profiles of *S. obliquus*, *D. armatus*, *N. atomus*, and soybean flour were compared (Figure 4). The selected microalgal species were twice as rich in methionine, relative to soy.

This work demonstrates the highest *S. obliquus* biomass productivity rates at CO_2 concentrations that imitate power plant or industrial emissions. Photobioreactor experiments and a modeled substrate inhibition curve predicted maximum biomass productivity at 4.5% CO_2, but *S. obliquus* was not much inhibited even at 35%. The control of system pH appears to be a key parameter in achieving high biomass productivity under the acidifying conditions of high CO_2 concentrations.

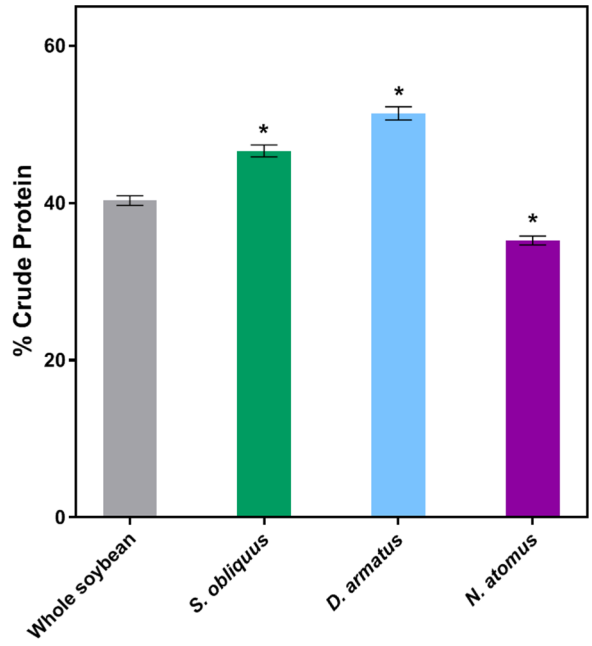


Figure 3. Percent crude protein comparison among whole soybean, *S. obliquus*, *D. armatus*, and *N. atomus*. Error bars represent the standard deviation. *Indicates statistical difference from the soy control at $p < 0.01$, $\alpha = 0.05$.

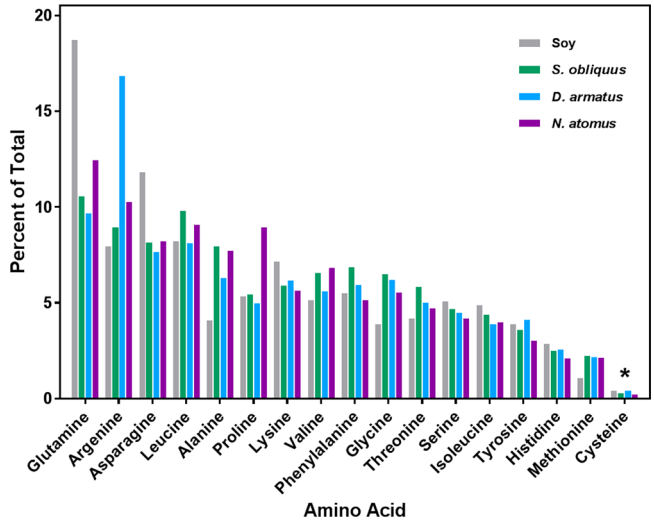


Figure 4. Percent amino acid comparison among soy, *S. obliquus*, *D. armatus*, and *N. atomus*. Percent error was 5% of the measured values. *Indicates low recovery of cysteine.

Additionally, *S. obliquus* was more nutritious than soy, a conventional protein supplement in cattle feed. Protein contents were similar, but the microalgae had greater fractions of methionine, the limiting amino acid in cattle feed. These results will move forward efforts to upcycle waste to high-protein microalgal biomass for cattle feed as a large-scale, economical alternative to soybean meal.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.9b00656.

DOI: 10.1021/acssuschemeng.9b00656
ACS Sustainable Chem. Eng. 2019, 7, 9474−9479

Microalgal species comparison, culture medium nutrient depletion, nitrogen source comparison, photobioreactor schematic, and microalgae micrographs (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: hannah-molitor@uiowa.edu.

ORCID

Hannah R. Molitor: 0000-0003-4855-5358

Author Contributions

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

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship under Grant No. 1546595. Any opinion, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the National Science Foundation. The authors would also like to acknowledge Deborah Williard, Ellen Black, and Emily Greene for instrumentation and sampling assistance.

REFERENCES

(1) Bruinsma, J.; Fischer, G.; Nachtergaele, F.; Faurès, J.-M.; Hoogeveen, J.; Poulisse, J.; Tran, D.; Griffee, P.; Nguyen, N.; Bishop, C.; Clarke, L. *World agriculture: towards 2015/2030*; Food and Agriculture Organization of the United Nations: 2003; pp 127−138.

(2) FAO; IFAD; UNICEF; WFP; WHO *The State of Food Security and Nutrition in the World 2017*; FAO: Rome, 2017; p 2.

(3) Bodirsky, B. L.; Popp, A.; Lotze-Campen, H.; Dietrich, J. P.; Rolinski, S.; Weindl, I.; Schmitz, C.; Muller, C.; Bonsch, M.; Humpenoder, F.; Biewald, A.; Stevanovic, M. Reactive nitrogen requirements to feed the world in 2050 and potential to mitigate nitrogen pollution. *Nat. Commun.* **2014**, *5*, 1−7.

(4) Hard, D. L. In Protein sources for the animal feed industry, *FAO Expert Consultation and Workshop on Protein Sources for the Animal Feed Industry, Bangkok, Thailand*; Food and Agriculture Organization of the United Nations: Bangkok, Thailand, 2002; pp 125−139.

(5) USDA *USDA Coexistence Fact Sheets: Soybeans.*; USDA Office of Communications: Washington, DC, 2015; pp 1−3.

(6) USDA *Census of Agriculture.*; USDA NASS: 2012; Vol. *1*, p 7.

(7) Matassa, S.; Batstone, D. J.; Hulsen, T.; Schnoor, J.; Verstraete, W. Can Direct Conversion of Used Nitrogen to New Feed and Protein Help Feed the World? *Environ. Sci. Technol.* **2015**, *49* (9), 5247−5254.

(8) Gutiérrez-Boem, F. H.; Scheiner, J. D.; Rimski-Korsakov, H.; Lavado, R. Late season nitrogen fertilization of soybeans: effects on leaf senescence, yield and environment. *Nutr. Cycling Agroecosyst.* **2004**, *68* (2), 109−115.

(9) Gai, Z.; Zhang, J.; Li, C. Effects of starter nitrogen fertilizer on soybean root activity, leaf photosynthesis and grain yield. *PLoS One* **2017**, *12* (4), 1−15.

(10) Bleakley, S.; Hayes, M. Algal Proteins: Extraction, Application, and Challenges Concerning Production. *Foods* **2017**, *6* (5), 1−34.

(11) Ho, S. H.; Chen, W. M.; Chang, J. S. Scenedesmus obliquus CNW-N as a potential candidate for CO(2) mitigation and biodiesel production. *Bioresour. Technol.* **2010**, *101* (22), 8725−8730.

(12) Gardner-Dale, D. A.; Bradley, I. M.; Guest, J. S. Influence of solids residence time and carbon storage on nitrogen and phosphorus recovery by microalgae across diel cycles. *Water Res.* **2017**, *121*, 231−239.

(13) Tang, D.; Han, W.; Li, P.; Miao, X.; Zhong, J. CO2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO2 levels. *Bioresour. Technol.* **2011**, *102* (3), 3071−3076.

(14) De Morais, M. G.; Costa, J. A. V. Biofixation of carbon dioxide by Spirulina sp. and Scenedesmus obliquus cultivated in a three-stage serial tubular photobioreactor. *J. Biotechnol.* **2007**, *129* (3), 439−445.

(15) Kaewkannetra, P.; Enmak, P.; Chiu, T. The effect of CO2 and salinity on the cultivation of Scenedesmus obliquus for biodiesel production. *Biotechnol. Bioprocess Eng.* **2012**, *17* (3), 591−597.

(16) Xia, L.; Yang, H. J.; He, Q. N.; Hu, C. X. Physiological responses of freshwater oleaginous microalgae Desmodesmus sp NMX451 under nitrogen deficiency and alkaline pH-induced lipid accumulation. *J. Appl. Phycol.* **2015**, *27* (2), 649−659.

(17) Li, Q. F.; Powers, W.; Rozeboom, D.; Liu, Y.; Liao, W. An integrated Water Curtain-Microalgal Culture system (WCMC) to mitigate air emissions and recover nutrients from animal feeding operations. *Algal Res.* **2016**, *18*, 166−174.

(18) Ji, F.; Liu, Y.; Hao, R.; Li, G.; Zhou, Y. G.; Dong, R. J. Biomass production and nutrients removal by a new microalgae strain Desmodesmus sp in anaerobic digestion wastewater. *Bioresour. Technol.* **2014**, *161*, 200−207.

(19) Becker, E. W. Micro-algae as a source of protein. *Biotechnol. Adv.* **2007**, *25* (2), 207−210.

(20) Hanagata, N.; Takeuchi, T.; Fukuju, Y.; Barnes, D. J.; Karube, I. Tolerance of microalgae to high CO2 and high temperature. *Phytochemistry* **1992**, *31* (10), 3345−3348.

(21) De Morais, M. G.; Costa, J. A. Carbon dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnol. Lett.* **2007**, *29* (9), 1349−1352.

(22) IPCC Climate Change 2014: Mitigation of Climate Change. *Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change; 9781107058217*; Cambridge University Press: Cambridge, 2014; pp 111−150.

(23) Munguía-López, A. d. C.; Rico-Ramírez, V.; Ponce-Ortega, J. M. Analysis of Carbon Policies in the Optimal Integration of Power Plants Involving Chemical Looping Combustion with Algal Cultivation Systems. *ACS Sustainable Chem. Eng.* **2018**, *6* (4), 5248−5264.

(24) Cheah, W. Y.; Pau Loke, S.; Chang, J.-S.; Ling, T.; Juan, J. C. Biosequestration of atmospheric CO2 and flue gas-containing CO2 by microalgae. *Bioresour. Technol.* **2015**, *184*, 190−201.

(25) Vuppaladadiyam, A. K.; Prinsen, P.; Raheem, A.; Luque, R.; Zhao, M. Sustainability Analysis of Microalgae Production Systems: A Review on Resource with Unexploited High-Value Reserves. *Environ. Sci. Technol.* **2018**, *52*, 14031−14049.

(26) Gutiérrez-Arriaga, C. G.; Serna-González, M.; Ponce-Ortega, J. M.; El-Halwagi, M. M. Sustainable Integration of Algal Biodiesel Production with Steam Electric Power Plants for Greenhouse Gas Mitigation. *ACS Sustainable Chem. Eng.* **2014**, *2* (6), 1388−1403.

(27) Gong, J.; You, F. Value-Added Chemicals from Microalgae: Greener, More Economical, or Both? *ACS Sustainable Chem. Eng.* **2015**, *3* (1), 82−96.

(28) Gassan, H.; Eugenia, M. M.; Sebastián, S. Influence of temperature on growth of Scenedesmus obliquus in diluted olive mill wastewater as culture medium. *Engineering in Life Sciences* **2010**, *10* (3), 257−264.

(29) Ho, S.-H.; Chen, C.-Y.; Lee, D.-J.; Chang, J.-S. Perspectives on microalgal CO2-emission mitigation systems — A review. *Biotechnol. Adv.* **2011**, *29* (2), 189−198.

(30) Sydney, E. B.; Sturm, W.; De Carvalho, J. C.; Thomaz-Soccol, V.; Larroche, C.; Pandey, A.; Soccol, C. R. Potential carbon dioxide fixation by industrially important microalgae. *Bioresour. Technol.* **2010**, *101* (15), 5892−5896.

DOI: 10.1021/acssuschemeng.9b00656
ACS Sustainable Chem. Eng. 2019, 7, 9474−9479

(31) Silva, H. J.; Pirt, S. J. Carbon Dioxide Inhibition of Photosynthetic Growth of Chlorella. *Microbiology* **1984**, *130* (11), 2833−2838.
(32) Songolzadeh, M.; Soleimani, M.; Takht Ravanchi, M.; Songolzadeh, R. Carbon dioxide separation from flue gases: a technological review emphasizing reduction in greenhouse gas emissions. *Sci. World J.* **2014**, *2014*, 1−34.
(33) Doušková, I.; Kaštánek, F.; Maléterová, Y.; Kaštánek, P.; Doucha, J.; Zachleder, V. Utilization of distillery stillage for energy generation and concurrent production of valuable microalgal biomass in the sequence: Biogas-cogeneration-microalgae-products. *Energy Convers. Manage.* **2010**, *51* (3), 606−611.
(34) Genkov, T.; Spreitzer, R. J. Highly conserved small subunit residues influence rubisco large subunit catalysis. *J. Biol. Chem.* **2009**, *284* (44), 30105−30112.
(35) 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* (19), 5878−5891.
(36) Bischoff, H. W.; Bold, H. C. *Some soil algae from Enchanted Rock and related algal species*; University of Texas: Austin, TX, 1963; p 1−95.
(37) 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.
(38) Ho, S.-H.; Chen, C.-Y.; Chang, J.-S. Effect of light intensity and nitrogen starvation on CO2 fixation and lipid/carbohydrate production of an indigenous microalga Scenedesmus obliquus CNW-N. *Bioresour. Technol.* **2012**, *113*, 244−252.
(39) Ho, S. H.; Chen, W. M.; Chang, J. S. Scenedesmus obliquus CNW-N as a potential candidate for CO(2) mitigation and biodiesel production. *Bioresour. Technol.* **2010**, *101* (22), 8725−8730.
(40) Ho, S.-H.; Lu, W.-B.; Chang, J.-S. Photobioreactor strategies for improving the CO2 fixation efficiency of indigenous Scenedesmus obliquus CNW-N: Statistical optimization of CO2 feeding, illumination, and operation mode. *Bioresour. Technol.* **2012**, *105*, 106−113.
(41) Hodaifa, G.; Martínez, M. E.; Sánchez, S. Influence of pH on the culture of Scenedesmus obliquus in olive-mill wastewater. *Biotechnol. Bioprocess Eng.* **2009**, *14* (6), 854−860.
(42) Goldman, J. C.; Azov, Y.; Riley, C. B.; Dennett, M. R. The effect of pH in intensive microalgal cultures. I. Biomass regulation. *J. Exp. Mar. Biol. Ecol.* **1982**, *57* (1), 1−13.
(43) Danilov, R. A.; Ekelund, N. G. A. Effects of pH on the growth rate, motility and photosynthesis inEuglena gracilis. *Folia Microbiol.* **2001**, *46* (6), 549−554.
(44) Taher, H.; Al-Zuhair, S.; Al-Marzouqi, A.; Haik, Y.; Farid, M. Growth of microalgae using CO2 enriched air for biodiesel production in supercritical CO2. *Renewable Energy* **2015**, *82*, 61−70.
(45) Scherholz, M. L.; Curtis, W. R. Achieving pH control in microalgal cultures through fed-batch addition of stoichiometrically-balanced growth media. *BMC Biotechnol.* **2013**, *13*, 39−39.
(46) Amoroso, G.; Sültemeyer, D.; Thyssen, C.; Fock, H. P. Uptake of HCO(3)(−) and CO(2) in Cells and Chloroplasts from the Microalgae Chlamydomonas reinhardtii and Dunaliella tertiolecta. *Plant Physiol.* **1998**, *116* (1), 193−201.
(47) Kang, J.; Wang, M.; Xiao, Z. Modeling and Control of pH in Pulp and Paper Wastewater Treatment Process. *J. Water Resour. Prot.* **2009**, *1* (2), 122−127.
(48) Fernández, I.; Peña, J.; Guzman, J. L.; Berenguel, M.; Acién, F. G. Modelling and Control Issues of pH in Tubular Photobioreactors. *IFAC Proceedings Volumes* **2010**, *43* (6), 186−191.
(49) Menetrez, M. Y. An Overview of Algae Biofuel Production and Potential Environmental Impact. *Environ. Sci. Technol.* **2012**, *46* (13), 7073−7085.
(50) Menger-Krug, E.; Niederste-Hollenberg, J.; Hillenbrand, T.; Hiessl, H. Integration of Microalgae Systems at Municipal Wastewater Treatment Plants: Implications for Energy and Emission Balances. *Environ. Sci. Technol.* **2012**, *46* (21), 11505−11514.
(51) Pinar, G.; Duque, E.; Haidour, A.; Oliva, J.; Sanchez-Barbero, L.; Calvo, V.; Ramos, J. L. Removal of high concentrations of nitrate from industrial wastewaters by bacteria. *Biotechnol. Bioeng.* **1998**, *63* (5), 2071−2073.
(52) Ji, F.; Yin, H.; Zhang, H.; Zhang, Y.; Lai, B. Treatment of military primary explosives wastewater containing lead styphnate (LS) and lead azide (LA) by mFe0-PS-O3 process. *J. Cleaner Prod.* **2018**, *188*, 860−870.
(53) Lundquist, T. J.; Woertz, I. C.; Quinn, N. W. T.; Benemann, J. R. *A Realistic Technology and Engineering Assessment of Algae Biofuel Production*; Energy Biosciences Institute: 2010, 1−178.
(54) McMahon, K. D.; Read, E. K. Microbial Contributions to Phosphorus Cycling in Eutrophic Lakes and Wastewater. *Annu. Rev. Microbiol.* **2013**, *67* (1), 199−219.
(55) Schwab, C. G. Amino acids and their appications in formulateing diets for cattle. *Anim. Feed Sci. Technol.* **1996**, *59*, 19−32.
(56) Schwab, C. G.; Broderick, G. A. A 100-Year Review: Protein and amino acid nutrition in dairy cows. *J. Dairy Sci.* **2017**, *100* (12), 10094−10112.

DOI: 10.1021/acssuschemeng.9b00656
ACS Sustainable Chem. Eng. 2019, 7, 9474−9479