Improving Algae Photobioreactor Energy Efficiency Through Active Irradiance Control for Dynamic Carbon Dioxide Fixation

Connor B. Geiman,* Frieda B. Taub,† and Joseph L. Garbini‡
University of Washington, Seattle, WA, 98006

We have created a 40-liter *Chlorella vulgaris* algae photobioreactor testbed for carbon dioxide removal that responds to elevated levels of carbon dioxide, while reducing energy consumption during nominal operation. Energy efficiency will be an important consideration for the bioregenerative life support systems (BLSS) integrated into future space missions that will provide a crew with breathable air and other resources. A reduction in energy expenditure has the potential to reduce the overall mass, power, and volume (MPV) for a mission. However, though it is important to reduce the energy cost of BLSS components, this should not come at the expense of mission safety. The life support subsystems must retain the ability to adapt to the dynamic crewed cabin environment. In our photobioreactor, the LED lighting and circulation pump account for nearly all of the system energy consumption, at 75% and 23% respectively. In this paper we characterize energy reduction through active irradiance control. Using the Blackman relationship between photosynthetic rate and the three factors of irradiance, temperature, and environmental carbon dioxide, we designed a feedback controller to react to the sensed dynamic cabin environment by varying the photobioreactor's irradiance levels. Thus, the carbon fixation rate of the algae is matched to the cabin carbon dioxide concentration. We tested our photobioreactor in controlled and uncontrolled scenarios. Results indicated that the controlled photobioreactor responds effectively to a step increase in CO_2, while using up to 57% less energy on lighting than an uncontrolled photobioreactor over the course of the response. The development of a photobioreactor that utilizes active irradiance control to respond to carbon dioxide, as described in this paper, is an important step towards reducing BLSS MPV. Future work should focus on further optimizing LED- and pump-related energy savings as well as other BLSS subsystems through similar active controls of high-power components.

Nomenclature

BLSS	=	biological life support system
ISS	=	International Space Station
K_P	=	proportional relationship between photosynthetic rate and light intensity
K_C	=	proportional controller gain
LED	=	light-emitting diode
MPV	=	mass, power, and volume
PPFD	=	photosynthetically active photon flux density
R^2	=	coefficient of determination
% RD	=	percent of reading
CO_2	=	carbon dioxide
O_2	=	oxygen
H_2O	=	water
$C_6H_{12}O_6$	=	glucose

* Graduating Senior, Mechanical Engineering, 3900 E Stevens Way NE, Seattle, WA 98195
† Professor Emerita, School of Aquatic & Fishery Sciences, 1122 NE Boat Street, Seattle, WA 98195 Box 355020
‡ James B. Morrison Endowed Chair, Mechanical Engineering, 3900 E Stevens Way NE, Seattle, WA 98195

I. Introduction

BIOLOGICAL processes integrated into life support systems may be a compelling alternative to the pure physicochemical systems in use on the International Space Station (ISS) today. Such processes could involve air revitalizing algae, waste processing bacteria, and food-producing higher plants. Air revitalization is a promising early application of biological life support. Plants naturally consume CO_2 and release O_2, making them an excellent biological complement to human respiration, and a variety of plants have been sent to the ISS in order to assess feasibility for future space missions. Algae offer several benefits over higher plants for air revitalization including rapid growth, a liquid medium that is easy to manipulate and house, and ability be grown in a xenic environment.[1] However, algal systems exhibit higher combined mass, power, and volume (MPV) requirements than the existing ISS air revitalization system, and there is much to be learned about how MPV might be decreased.[2] There is an opportunity to take advantage of the mechanism of algal photosynthesis to reduce MPV. A 40 L algae photobioreactor was constructed for the purpose of examining algal behavior in a controlled system and reducing system energy requirements. The photobioreactor LED lighting accounted for up to 75% of the overall system power consumption. Attempting to save energy by operating an air revitalization bioreactor with minimal illumination runs the risk of rendering the system unable to respond to scenarios in which CO_2 concentration has reached an unsafe level for the crew. Based on the mechanism of photosynthesis it is possible to improve the efficiency of algal lighting by varying it using feedback control, while enabling the system to respond to elevated CO_2 scenarios.

This paper describes the photosynthetic relationship between light intensity and algal CO_2 uptake rate, and how that phenomenon can be used in bioreactor control. The experimental setup and details of the bioreactor apparatus are described. Several experiments were performed in order to verify that the bioreactor behaved as expected based on photosynthetic theory, followed by several experiments demonstrating the efficiency and response of the bioreactor under a constant light level compared to its response under a controlled light level.

II. Materials and Methods

A. Defining a safe level of ambient CO_2

Crew of early ISS missions regularly reported headaches, lethargy, and a variety of other symptoms attributed to excess CO_2, and a conversation with an early ISS crewmember indicated that CO_2-related symptoms were a recurring event.[3] Although serious health effects do not occur until CO_2 reaches extreme levels above NASA recommendations,[4] recent studies have reported that CO_2 levels at or above 1000 ppm are likely to cause cognitive declines.[5] As astronauts and tourists from a greater array of backgrounds begin spending time in space it will become increasingly important to maintain a comfortable, optimal level of carbon dioxide. For the experiments described in this paper, consistent with the results of Satish et al. (2012), the "safe" CO_2 concentration threshold for controller design was defined at 1000 ppm.

B. Theory

1. Photosynthetic theory

Many have proposed and experimented with algae as a key organism for biological life support systems.[1] Algae produce a variety of useful resources for humans, including breathable oxygen and potentially nutrition.[2] Since most algae are effectively a respiratory opposite to humans they have been investigated extensively as a candidate for air revitalization in a biological life support system (BLSS), as they require only light, carbon dioxide, and a growth medium containing nutrients including nitrogen and phosphorus in order to thrive. Algae remove CO_2 from their ambient environment through photosynthesis. The equation for oxygenic photosynthesis is as follows:[6]

$$6CO_2 + 12H_2O + Light\ Energy \longrightarrow C_6H_{12}O_6 + 6O_2 + 6H_2O \quad (1)$$

A photosynthetic organism is considered to be light limited when *Light Energy* is the variable in Equation (1) which limits the photosynthetic reaction speed. Experiments by Blackman revealed that, while an organism is light-limited, the rate of photosynthesis corresponds proportionally to the light intensity to which it is exposed.[§] This is

[§] Discovering the Secrets of Photosynthesis, https://www.biology-pages.info/P/Photosynthesis_history.html, (accessed March 8, 2021)

shown as a linear region in Figure 1. As photosynthetic rate increases, so does the rate of photosynthetic CO_2 fixation. Thus, brightness can be dynamically selected to allow an algal culture to maintain an appropriate level of ambient CO_2. This approach avoids expending any more energy on lighting than necessary for effective carbon dioxide removal.

2. Control theory

If all variables other than light level are held constant in an algal system, a simple feedback controller can be used to provide actuation to respond to elevated CO_2. A high-level diagram of such a control system is shown in Figure 2. *T(s)* represents a controller responsible for actuating the light level, *P(s)* represents a model of the bioreactor's behavior in response to varying light levels, and *C(s)* is the CO_2 concentration within the bioreactor ambient environment. *R(s)* and *δ(s)* represent, respectively, the desired reference concentration of ambient CO_2 and a disturbance in ambient CO_2 concentration.

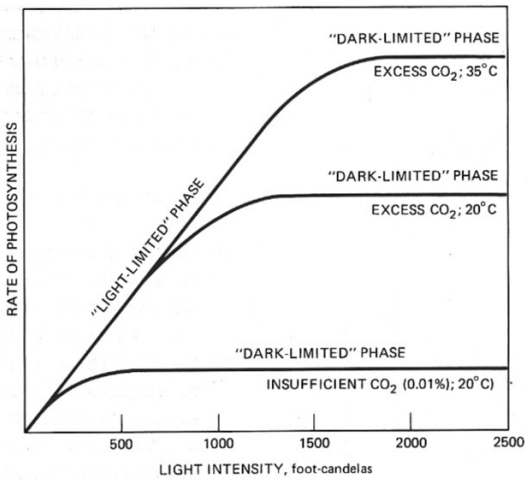

Figure 1. Relationship between photosynthesis rate and light intensity at various operating conditions.[§]

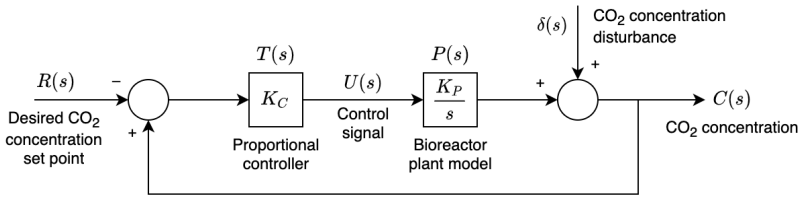

Figure 2. High-level representation of feedback control loop for a biological air revitalization system subject to a step increase in CO_2.

The plant, *P(s)*, includes the proportional relationship between the electronic signal provided by the controller to the LEDs and the photosynthetic rate, K_P, described by Blackman. K_P is always less than zero, thus the positive feedback loop. K_P is placed in series with an integrator, $\frac{1}{s}$, to convert from carbon dioxide rate to concentration. There are a variety of options for the controller, *T(s)*, but in order to demonstrate the benefits of feedback control succinctly the focus of this paper is on proportional control, with other controllers left for future analyses. Therefore, the controller is a constant gain, K_C. In order to meet the safe CO_2 level of 1000 ppm with room to spare, the reference value is set at 800 ppm. The relevant variables are described in Table 1. With the control system defined, the principles described above can be tested on a laboratory scale bioreactor.

Table 1. Variables shown in control loop, their associated value, and a description of each.

Symbol	Equation	Description
T(s)	K_C	Proportional bioreactor controller
P(s)	K_P/s	Model of bioreactor behavior; note that $K_P < 0$
C(s)	-	Ambient CO_2 concentration within bioreactor
R(s)	800 ppm	Desired (reference) CO_2 concentration
δ(s)	-	Carbon dioxide concentration step disturbance

C. Experimental setup

1. *Bioreactor*

In order to observe whether closed loop feedback control can be used to regulate algae photosynthetic rate and improve the efficiency of an algae photobioreactor, a bioreactor testbed was built, and several experiments were designed and run on the testbed. The bubble column algae photobioreactor built for this purpose is shown in Figure 3. The bioreactor was designed to be materially closed, although experimental success was not contingent on a perfectly sealed system. The bioreactor was constructed from panels of transparent acrylic and held 40 L of algae in media in contact with a 36 L gas volume. In a flight-like system, the bioreactor liquid volume will be exposed to air from the cabin environment, rather than a closed gas volume. Control of the concentration of gases exposed to the algal culture using the gas volume within the bioreactor acts as a model of the ambient spacecraft environment. *Chlorella vulgaris* algae were selected due to their optimal qualities for experimentation and potential for space use.[7] The algae were grown in a modified Bold's Basal Medium, whose components are listed in Table 3 in the Appendix, at a temperature of 21.4ºC ± 0.8ºC and a pressure of 101 kPa. A CO_2 supply was connected to the bioreactor in order to raise the CO_2 concentration inside the bioreactor on demand. One 60 cm × 60 cm LED grow light panel was positioned on each face of the bioreactor.[**] The brightness (PPFD) of the light panels was adjustable from 0 to 80 µmol m^{-2} s^{-1}, which prior literature indicates is within the light limited range of *C. vulgaris*.[8] For the remainder of the paper, this light intensity range will be referred to in terms of percent brightness, from 0% to 100% light level.

Figure 3. 40-liter *Chlorella vulgaris* bioreactor testbed with CO_2 dosing cylinder.

As shown in Figure 4, the CO_2-enriched air contained in the bioreactor traveled in a closed loop through the system. A diaphragm pump continuously circulated the gas through the bioreactor, bubbling it through the algal culture in order to facilitate gas transfer. The bioreactor gas loop consisted of 1⁄4" vinyl tubing connected such that the gas would pass from the bioreactor tank into a sensor chamber containing a carbon dioxide sensor, then through a mixing chamber to the gas pump inlet. The gas pump outlet was connected directly to the air stones via airline tubing. After passing through the air stones and percolating through the algae tank the gas returned to the sensor chamber. This configuration improves the mixing of the CO_2 before it reaches the sensor chamber and promotes higher gas pressure for the air stones compared to other elements of the gas loop, as they are the most resistive element. A small acrylic cap was installed at the top of the bioreactor to vent the slight excess pressure that occurred when adding CO_2. If lower bioreactor carbon dioxide levels were desired while experiments were not taking place, the gas loop was opened by disconnecting one tube, allowing ambient air to enter the bioreactor loop.

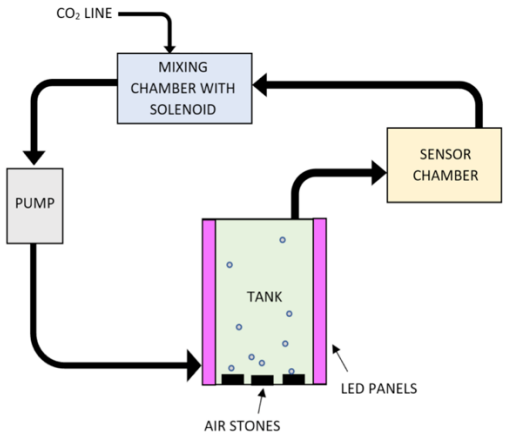

Figure 4. Diagram of closed-loop bioreactor system arrangement.

2. *Electronics*

The carbon dioxide levels within the bioreactor were measured with a SenseAir S8 carbon dioxide sensor, which is capable of detecting carbon dioxide concentrations between 400 ppm and 50,000 ppm with ± 10% RD

[**] Each panel contained 660 red (620-630 nm) and 240 blue (460-470 nm) individual LEDs, evenly distributed.

accuracy and 10 ppm resolution. An Arduino Nano was used to record data and control the system. The two sets of LED panels were connected via a brightness controller to the Arduino, which provided a pulse-width modulated signal to the brightness controller in order to actuate the light panel brightness. The carbon dioxide release solenoid was also connected to the Arduino and actuated when an impulse in carbon dioxide was needed for an experiment.

D. Experimental procedure

1. Verification of linearity

Because a linear relationship between light intensity and photosynthetic rate (K_P) was assumed in the feedback control system, it was important to ensure that the bioreactor was operating with the linear region of Figure 1. Photosynthesis experiments were conducted at several light levels from low (10%, 8 µmol m^{-2} s^{-1}) to maximum (100%, 80 µmol m^{-2} s^{-1}). For each experiment, the light level was set and CO_2 was rapidly added to the bioreactor, causing a spike to approximately 2000 ppm, a level which would be uncomfortable for a crewmember. The CO_2 was allowed to circulate for several minutes to mix uniformly through the system. Photosynthetic rate was expected to enter steady state as the CO_2 mixed through the system.[9] The bioreactor entered a steady state CO_2 consumption period just below 2000 ppm, which appears as the beginning of a linear region on a semilog plot. The plot of CO_2 concentration versus time for each of the four experiments is shown in Figure 5. In each of the four experiments, the system reached a steady state carbon dioxide decrease within 15 minutes of carbon dioxide being added to the system. The curves remained linear on a semilog scale for the duration of each experiment.

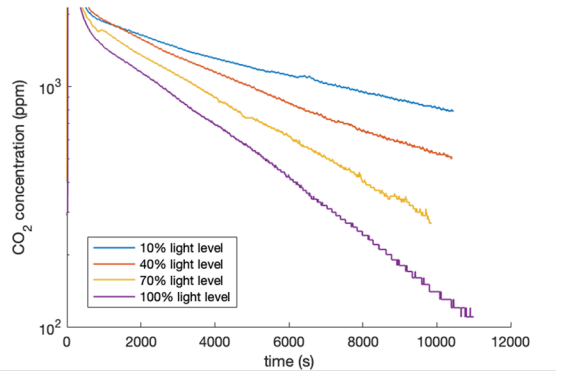

Figure 5. Carbon dioxide *concentration* versus time during photosynthesis at four different light levels.

Using data from the four experiments, K_P can be determined. The rate of CO_2 consumption was determined for each curve at four different CO_2 concentrations. Because CO_2 concentration has an effect on the rate of photosynthesis, a decrease in CO_2 removal rate is observed as each experiment progresses. This decline in photosynthetic rate occurs despite the light level being held constant for the duration of each experiment. If a CO_2 concentration is specified, a linear relationship can be calculated between light intensity and photosynthetic rate. CO_2 fixation rate can be used as a proxy for photosynthetic rate. Figure 6 shows a plot of CO_2 fixation rate versus light intensity at four different CO_2 concentrations. A negative CO_2 fixation rate indicates that CO_2 is being removed from the air. Each line is approximately linear with a slope of K_P. By choosing to calculate K_P at a low CO_2 concentration (750 or 1000 ppm), the relationship between photosynthetic rate and light level will be conservatively estimated and the system will respond somewhat faster in reality than in simulations involving K_P.

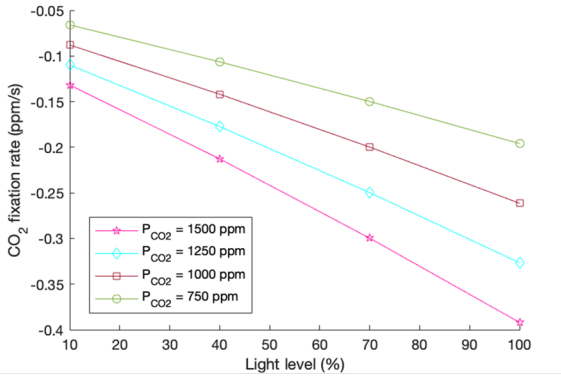

Figure 6. Carbon dioxide fixation *rate* at varying light intensities for four different concentrations of carbon dioxide.

2. Impulse disturbance experiments

An experimental scenario was designed to examine the theoretical and actual performance of the controller for response time and energy consumption. Similar to the linearity verification experiments described above, each experiment began with the addition of CO_2 to the system, causing a rapid spike to approximately 2000 ppm. Data collection began once the carbon dioxide level descended to 2000 ppm. CO_2 was allowed to be consumed through algal photosynthesis until passing the safe threshold of 1000 ppm. Three experiments were run, all with similarly sized carbon dioxide impulses. One was run with the light panels set to 20% light level (Experiment 1), one with the panels set to 100% (Experiment 2), and one with the LED panels under proportional feedback control (Experiment 3). The proportional feedback controller gain, K_C was selected so that the light level of the LED panels would range from 0%

at the reference value (800 ppm) to 100% at 500 ppm above the reference value (1300 ppm). Above 1300 ppm the lights remain at full brightness for maximal photosynthetic rate. Two days after supplementing the algal culture with 600 mL of modified Bold's Basal Medium concentrate, all experiments were done within a 24-hour period at 21.4ºC ± 0.8ºC. These steps were taken in order to ensure the algal culture was not nutrient limited and to minimize the changes in variables other than carbon dioxide concentration and photosynthetic rate. Because the impulse disturbance experiments were performed on a different algae culture with different population dynamics than that of the linearity verification experiments, K_P was recalculated from Experiments 1 and 2 in order to accurately predict Experiment 3.

III. Results and Discussion

A. Bioreactor system response

1. Constant-light-level response experiments

Of the two experiments run at constant levels, Experiment 1 resulted in low energy consumption over the course of the experiment at the cost of slower response time, whereas Experiment 2 resulted in a quick system response but large energy consumption over the course of the experiment. During Experiment 1, run with the lights at 20% brightness, the system took 2.88 hr to reach the 1000 ppm carbon dioxide target and the lighting required a constant 13.4 W of power. Over the first 3 hours of the experiment 144.72 kJ of energy were consumed by the light panels. During Experiment 2, which was run at 100% brightness, the system took 0.94 hr to reach the target level of carbon dioxide and the lighting required a constant 62.4 W of power. Over a 3-hour time period 673.92 kJ of energy would have been consumed, had the experiment been left to run. The plots of CO_2 concentration for Experiments 1 and 2 are shown in Figure 7. Data shown in that figure was collected as the bioreactor entered the steady state region and crossed 2000 ppm. Noise in the data was present due to the effect of high levels of humidity in the bioreactor on the sensor. Lines of best fit were calculated for Experiments 1 and 2, which showed R^2 values of 0.941 and 0.983, respectively.

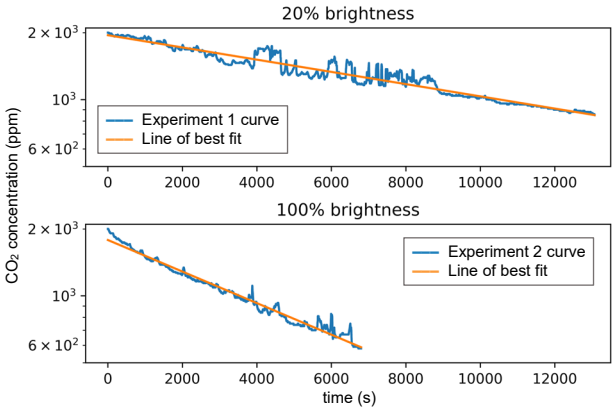

Figure 7. Experiments 1 (top) and 2 (bottom), demonstrating constant low and high light level bioreactor CO_2 concentration responses to a CO_2 spike to 2000 ppm, each plotted with a line-of-best fit.

2. Controlled-light-level response simulation and experiment

Using the method described in the experimental procedure, the plant gain K_P, relating light level to photosynthetic rate, was calculated from Experiments 1 and 2. K_P was -1.18×10^{-3} $\frac{\text{ppm/s}}{\text{\% light level}}$.

The system response to a CO_2 impulse to 2000 ppm was simulated using the feedback control model. The impulse response experiment, Experiment 3, was then run on the bioreactor. The simulated and experimental results are shown in Figure 8. As noted in the experimental section, K_P was calculated at a

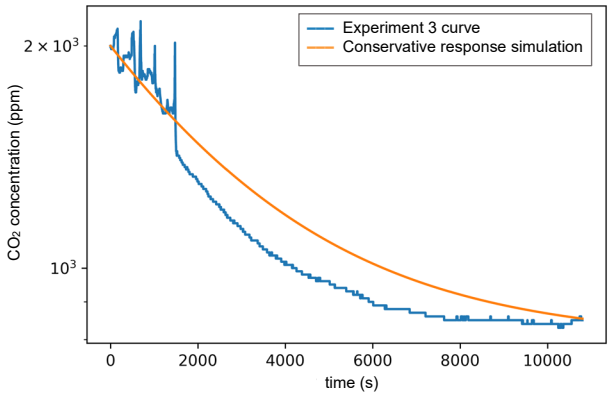

Figure 8. Conservative simulation and Experiment 3 controlled response to a spike in CO_2 concentration to 2000 ppm.

low CO_2 concentration, 1000 ppm. Calculating K_P at a low concentration results in a more conservative system response projection than reality, so the simulated curve in Figure 8 has a shallower trajectory. During Experiment 3, the system took 1.17 hr to reach the target level of carbon dioxide, and the lighting consumed 286.74 kJ of energy over the 3-hour experiment. The controlled bioreactor of Experiment 3 took 59% less time to reach a safe CO_2 level than the dim bioreactor of Experiment 1 and consumed 57% less energy through lighting than the bright bioreactor of Experiment 2 over the course of 3 hours. These results are summarized in Table 2.

The controller successfully improves the energy efficiency of the bioreactor by keeping the system in a low-power state when CO_2 is at a desirable level, while maintaining a rapid response to elevated levels of CO_2. Furthermore, because lighting accounts for most of the energy cost of the bioreactor, the total system energy consumption over 3 hours was reduced to just over half of the original high-light level energy consumption, from 894.24 kJ to 507.06 kJ.

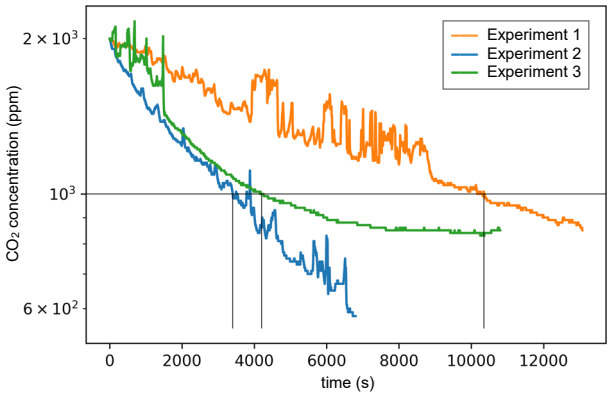

Figure 9. Responses produced during Experiments 1 (low light), 2 (high light), and 3 (controlled light).

Table 2. Response speeds to CO_2 impulse and energy use during three photobioreactor response experiments.

Experiment	Time to desirable CO_2 level	Lighting energy use over 3 hours
Experiment 1 (20%)	2.88 hr	144.72 kJ
Experiment 2 (100%)	0.94 hr	673.92 kJ
Experiment 3 (Controlled)	1.17 hr	286.74 kJ

B. Sources of error

The CO_2 sensor suffered from error likely induced by humidity during Experiments 1 through 3. Linear curve fits to Experiments 1 and 2 resulted in high R^2 values, indicating that the sensor data remained within an appropriate range.

The system was sealed but not pressure tested, so a small amount of air transfer between the bioreactor and the lab environment did occur during experimentation. However, the bioreactor demonstrated markedly and consistently different responses at each light level, indicating that photosynthesis was the primary mechanism of carbon dioxide decrease. In this experimental design, emphasis was placed on demonstrating that algae respond to light levels in a predictable way, allowing the system to be controlled to save energy. In future works, if the priority is a precise analysis of the response, efforts should be made to quantify the leakage rate and use more precise sensors and construction methods.

As temperature is a variable that affects photosynthetic rate, heat from the light panels is another potential source of error. *C. vulgaris* photosynthesize more rapidly at increasing temperatures. In order to confirm that the LED temperature would not have a significant impact on the response of the algae, the lights were turned to high for 3 hours. The culture temperature changed from 21.2ºC to 22.2ºC over the course of 3 hours, which is not expected to cause a meaningful increase in the photosynthetic rate of the culture.[10]

C. The algae population as a controllable system

By managing relevant variables, including CO_2 concentration, light intensity, temperature, nutrient levels, and culture density, it is possible to treat this biological system as if it behaves like a more conventional, electromechanical control system. As biological systems and the microorganisms that comprise them become better understood, biological systems will increase in their controllability. Historically, mechanical and chemical systems have been chosen due to their efficiency, predictability, and reliability. Physicochemical interactions are well understood, whereas biological ecosystem interactions can be more complex and hard to predict which can lead to unexpected system failures, such as those which occurred during the Biosphere 2 experiments.[11] Algae in particular have been

known to exhibit unpredictable behavior and undesirable characteristics, such as biofilm buildup.[12] In this study, an unexpected population death never occurred in the *C. vulgaris* cultures used to seed the bioreactor, even when stored at room temperature with no added nutrients for six months. Biofilm buildup did occur, particularly on the air stones, which might have resulted in increased flow resistance and therefore increased pump energy requirements over the life of the system.

Algae in nature receive varying levels of sunlight throughout the day, and it has been shown that *C. vulgaris* can thrive under 24-hour lighting.[13] Although the effects of increasing and decreasing light levels on an experimental algal system are not fully understood, algae are normally subject to variable light levels in nature, indicating that they have developed to tolerate it. Experiments have examined the effect of a non-natural intervention, strobing lights, on a *C. vulgaris* culture and observed an increase in biomass production.[14] Ultimately, care should be taken when attempting to control a biological system in a life support situation as there may be unknown variables that could impact the stability or behavior of the culture.

Whereas a proportional controller is sufficient for demonstrative experiments on a short timescale under controlled conditions, it is not likely to be sufficient for long term experiments with fluctuating system dynamics. Manual control of relevant variables could be reduced by using a more complex controller. The system dynamics will change over the lifetime of the system, and others have proposed control methods that could account for these changing dynamics.[15-17] A more complex controller may be better able to model system performance. Furthermore, if predictive indicators of system performance are identified, vehicle operators may be able to better allocate energy usage. Once again, as more research is done to assess the behavior of microbiological systems, controllers will be able to better predict and modify system behavior for increased efficiency, stability, and reliability.

D. Future work

The bioreactor was designed as a tall, slim device in order to maximize the surface area of algae exposed to the light panels. Due to the height of the water column in the bioreactor, early experimentation resulted in excessive hydrostatic pressure at the bottom which caused a crack that emptied the bioreactor. Also, bowing caused the lid to fit loosely, making the system more difficult to seal. In the future, an alternative design or more robust building materials should be selected in order to minimize the chances of catastrophic mechanical failure.

Several modifications could be made to the apparatus in order to approach a flight-like system. The bioreactor described in this paper relies on gravity to percolate the bubbles from the air stones up through the medium. Bioreactor designs capable of operating in microgravity have been developed, such as the membrane photobioreactor sent to the ISS in 2019.[18] In addition, the bioreactor described in this paper was designed for simplicity, not for maximal CO_2 consumption. 2000 ppm is an undesirable level of CO_2 and an inconvenience, but true emergencies would involve much higher concentrations of CO_2. Bioreactors in future, related studies should be designed to consume CO_2 more rapidly, in order to respond effectively to extremely high, life-threatening CO_2 levels.

Ultimately, further steps should be taken to reduce the MPV of bioreactor systems. This includes improved culture density and effective system construction. Additional efforts to develop effective control methods for space BLSS should be undertaken and could involve responding to CO_2 rate increases, responding to changing system dynamics, and identifying predictive indicators of system performance. Future work should focus on responding to complex and evolving system characteristics through control of high-energy system components.

IV. Conclusion

Using feedback control, the energy consumed by an air revitalization algae photobioreactor was reduced, while still allowing the system to respond to elevated levels of carbon dioxide. The relationship between the illumination intensity of an algal culture and its corresponding carbon dioxide fixation rate is linear, if the algae are within a light-limited region of growth. Using this observation, a proportional feedback control system was developed and tested on a 40 L photobioreactor. Experimental results confirmed that light level and carbon dioxide concentration were the primary drivers of carbon dioxide removal rate from the bioreactor, allowing the system behavior to be modeled to use proportional feedback control for energy reduction. The proportional feedback controller resulted in a 57% reduction in lighting energy use as compared to a constant-light level experiment. These experiments also revealed the limitations of simple closed-loop control in biological systems. For increased flightworthiness, further effort should be made to develop a bioreactor control system that can better predict and respond to evolving system dynamics and multiple variables simultaneously. The energy efficiency gains from improved bioreactor control will contribute to overall reductions in BLSS MPV, making biological systems a more compelling option for future space missions.

Appendix

Table 3. Modified Bold's Basal Medium composition.

Concentrate composition	Amount added to initial algal culture
2.94×10^{-1} M $NaNO_3$	10 mL/L
0.17×10^{-1} M $CaCl_2{\bullet}2H_2O$	10 mL/L
0.30×10^{-1} M $MgSO_4{\bullet}7H_2O$	10 mL/L
0.43×10^{-1} M K_2HPO_4	10 mL/L
1.29×10^{-1} M KH_2PO_4	10 mL/L
0.43×10^{-1} M $NaCl$	10 mL/L
T82 trace metals[19]	2 mL/L
Total	62 mL/L

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