

The Effects of Algae on CO₂ Mitigation and Nitrate Production

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Climate change is one of the most pressing environmental issues of our time. Scientists and policymakers are seeking sustainable, affordable ways to reduce CO₂ and other greenhouse gases to mitigate climate change. One notable solution for carbon sequestration is algae. In this experiment, we hypothesized that CO₂-absorbing algae would most effectively mitigate atmospheric CO₂ under conditions of high initial algal mass and high light levels. To test this hypothesis, a total of 18 containers of varying amounts of algae were exposed to varying amounts of light: High light-high mass (HL-HM), High light-low mass (HL-LM), Low light-high mass (LL-HM), Low light-low mass (LL-LM), High light-control (HL-C), and Low light-control (LL-C). We measured CO₂ (ppm, parts per million) and nitrate (ppm) levels in each container over a 14-day period. As a result, we found that CO₂ consumption was not affected by light level, algal mass, or their interaction. However, treatments with higher algal mass exhibited greater nitrate consumption than controls. Although this study has limitations, the results suggest that algae may require genetic modification to enhance CO₂ absorption to become a viable solution for climate change mitigation.

Introduction

Climate change is one of the most pressing global issues of the 21st century. Rising global temperatures, melting ice caps, and frequent extreme weather events have completely changed ecosystems and altered the way of life for both wildlife and humans worldwide. According to the IUCN, “The 2022 Global Risk Report by the World Economic Forum argues that biodiversity loss, along with climate change, is one of the greatest global challenges of our time.”¹ According to the United Nations, human activity has impacted three-quarters of land-based environments and two-thirds of marine environments². Since the Industrial Revolution in the 1800s, human activity has driven global warming at a rate not seen for over many millennia, with CO₂ production increasing 250 times faster than after the last Ice Age³. Some of the leading human activities that drive this trend are transportation, electricity, and industry, as seen in Figure 1⁴. As a result of these trends, there has been an increase in greenhouse gases in our atmosphere, particularly CO₂. With the effects of climate change intensifying, researchers are looking towards clean and sustainable solutions to reduce CO₂ levels. Many current solutions for carbon reduction focus on artificial or engineered approaches, such as carbon capture and storage (CCS), renewable energy systems, and direct air capture technologies⁵. Even though these approaches can mitigate climate change, they are often costly, energy-intensive, and difficult to deploy on a global scale. However, this paves the way for nature-based solutions (NbS). Some NbS’s include restoring

forests, protecting peatlands, and using photosynthetic organisms. These offer low-cost, renewable alternatives that harness natural processes to capture carbon¹.

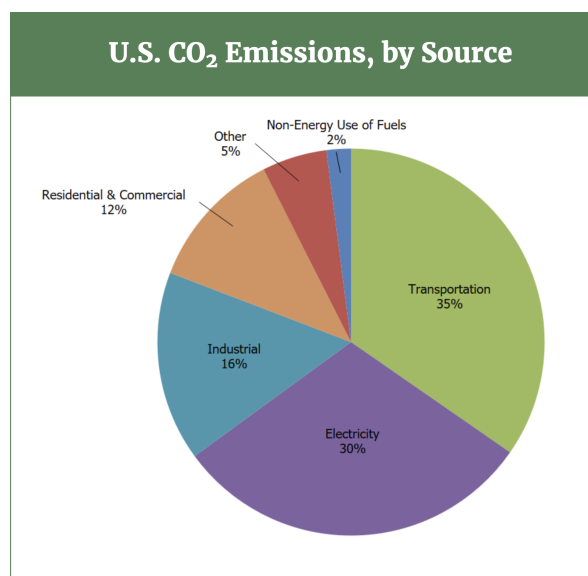


Figure. 1 U.S. CO₂ Emissions, by Source. Most CO₂ emissions come from human activities, with the largest sources being transportation, electricity, and industrial production.

According to the IUCN, “The latest IPCC report demonstrated that nature-based solutions such as reducing the destruction of forests and other ecosystems, restoring them, and improving the management of working lands, such as farms

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— are among the top five most effective strategies for mitigating carbon emissions by 2030”⁶. Among these natural solutions, algae have emerged as quite the promising option⁷. Algae are simple, plant-like organisms found in both freshwater and marine environments. They also have an extensive and broad phylogenetic range, allowing them to grow in varied environments, allowing them to grow 10-50 times quicker than terrestrial plants⁸. As a result, they can fix CO₂ 10-50 times faster⁹. Algae naturally absorb CO₂ from the atmosphere and release O₂ via photosynthesis. Under optimal conditions, they could be completely carbon-negative. However, it is unknown whether this is true at small scales or under varying conditions. Unlike many terrestrial plants, algae grow rapidly, do not require fertile soil, and can thrive in diverse environments using only sunlight and water¹⁰. In addition, they can be used to clean wastewater by removing nitrogen compounds, ammonia, nitrate, and nitrite¹¹. Because of these traits, algae present itself as an accessible and sustainable approach to carbon removal¹². Cultivating algae also requires minimal infrastructure. This could enable implementation in wastewater systems or bioreactors, which makes it a cheaper and more scalable option than many technological alternatives¹³. However, large-scale implementation has not yet been achieved, primarily because research is still in its early, laboratory-based stages.

Altogether, this begs the question: what are the effects of CO₂-absorbing algae on climate change mitigation? To better understand algae as a natural solution to climate change, we investigated how different algal biomass and light levels affect CO₂ absorption in a closed-container system, aiming to find the most optimal conditions for maximizing algal CO₂ uptake. We hypothesized that CO₂-absorbing algae at high algal densities and high light levels could mitigate atmospheric CO₂ production. By understanding the environmental impacts on algae, we can determine whether algae could serve as a low-cost, conventional strategy for reducing global CO₂ levels.

To investigate the potential of algae, we conducted this experiment to identify the optimal conditions for maximizing CO₂ uptake. We measured CO₂, algal mass growth, and nitrate levels as indications of photosynthetic activity and gas absorption, using initial algal mass and light level in a 12-hour cycle as the primary independent variables. The next sections describe the experimental design and analytical methods we used for our study.

Methods

Experimental Design

We decided to use a factorial design to investigate how different environmental factors, such as light intensity and ini-

tial algal biomass, affect CO₂ uptake by freshwater algae (*Scenedesmus* spp.). We used 18 clear, food-grade plastic containers, with three replicates per treatment combination. *Scenedesmus* spp. Algae cultures and Alga-Gro® Freshwater Medium were obtained from Carolina Biological Supply (Items #152510 and #153752, respectively). Each container was equipped with the following: 0.8L of growth medium, a label with a unique identifier, and a vented lid to allow gas exchange. We then placed each container in a Ziploc bag to trap the gases produced and kept them in the same room to maintain a uniform ambient temperature.

The two independent variables were: (1) light intensity—high (HL; defined as the highest light level, light level 5 of the GooingTop LED Grow Light) or low (LL; defined as the lowest light level, light level 1 of the GooingTop LED Grow Light)—and (2) starting algal mass—high (HAM; two vials of *Scenedesmus* spp. Algae cultures, each 0.03 L), low (LAM; one vial of *Scenedesmus* spp. Algae cultures, each 0.03 L), or zero (control). Of the six combinations of independent variables, there were three containers for each to ensure the accuracy and validity of the results. Three containers of each combination of independent variables were chosen because it was similar to the amount used in “Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuel production”¹⁴. Light treatments were established using GooingTop LED Grow Light lamps set on a “12-hour light”: “12-hour dark” cycle using an outlet timer. HL containers were placed under a GooingTop LED Grow Light, set at level 5 brightness (60,000 lux), while LL containers were placed under a GooingTop LED Grow Light set at level 1 brightness (13,000 lux). These grow lamps were considered sufficient based on a previous study of *Scenedesmus obliquus*, a close relative to *Scenedesmus* ssp., which reported an optimal light intensity of about 13,000 lux¹⁵. One GooingTop LED Grow Light at each brightness level was placed over each group to ensure uniform light exposure within each treatment. Control containers received only medium, while experimental containers were inoculated with either high or low algal mass. The exact volumes of the algal cultures were recorded at inoculation. The six treatment combinations were: High light-high mass (HL-HM), High light-low mass (HL-LM), Low light-high mass (LL-HM), Low light-low mass (LL-LM), High light-control (HL-C), and Low light-control (LL-C).

The dependent variables measured were carbon dioxide (CO₂), algal mass growth, and nitrate (NO₃⁻) concentrations over time, which indicated photosynthetic activity and CO₂ absorption. CO₂ was measured daily using a Vernier CO₂ Gas Sensor (Item #CO2-BTA). The sensor was placed in the Ziploc bag of each container until the reading stabilized. We then recorded the CO₂ concentration in ppm on our data sheet. CO₂ measurements were taken daily at 20:00 HRS, whilst Ni-

trate concentration was measured every other day using the API® 5-in-1 Test Strips at the same time. We chose these nitrate measurement instruments for their cost-effectiveness within a limited budget. For every measurement, the strip was dipped into the container, avoiding algae clumps, and was held at a consistent angle and lighting. Then a colorimetric test was performed as per manufacturers instructions and the nitrate concentration was recorded. In addition, we measured room and water temperatures (°C) with a standard thermometer during each CO₂ measurement, and we conducted daily visual observations, including water color (e.g., clear, light green, dense green), turbidity, algal clumping, and other noteworthy changes.

Data Collection

The data was collected over the course of a 14-day period. On inoculation day, all containers were labeled, the growth medium batch was prepared, the equipment was rinsed, and the light timer was set. The initial baseline measurements of CO₂, temperature, and nitrate concentration were recorded prior to algal inoculation. Post-inoculation, a second set of measurements was taken to establish post-inoculation baseline values. We recorded the date, time, and volume of algae added for both measurements.

During Days 1–14, measurements were taken at the same time each day (20:00 HRS). During each measurement, the following was collected: CO₂ concentration (ppm), temperature (°C), nitrate concentration (ppm; every other day), and the previously described qualitative observations. All of the measurements were recorded with the date, time, container ID, treatment group, and replicate number. To minimize contamination, the containers were handled with clean hands; the bags and lids were kept sealed between measurements; and instruments were kept clean between uses. The control containers served as contamination checks. Any significant changes in their parameters would indicate the presence of external factors affecting the results.

Data Analysis

After the experiment, two time-series graphs were created for both CO₂ and nitrate concentrations over time for each treatment group. We then calculated the mean and standard deviation for each treatment group on each day in order to assess trends and variability. To determine whether light intensity and/or starting algae mass had any effect on the algae, a two-way ANOVA test was performed¹⁶. Additionally, we calculated rates of change for CO₂ (ppm/day) and nitrate (ppm/day) to quantify how quickly each treatment group absorbed CO₂ and consumed nutrients.

Any decreases in nitrate concentration over time provided

indication of algal growth, photosynthetic activity, and nitrate consumption from the medium¹⁷.

Results

CO₂ Consumption Patterns

Over the 14-day experimental period (4-17th December 2025), CO₂ concentrations had decreased across all treatment groups (Figure 2). After stabilizing from the inoculation day values (276.57 to 1251.22 ppm) (761.13 ± 318.68 , mean $\pm$ sd), the initial CO₂ readings on Day 1 ranged from 93.46 to 488.28 ppm (253.76 ± 107.76), whilst on Day 8, CO₂ levels had lessened to a smaller range of 185.01-307.08 ppm across all treatment groups. Lastly, the final CO₂ readings on Day 14 ranged from 154.5 to 246.05 ppm.

The CO₂ consumption rates varied among treatment groups (Figure 2). The low-light control group (LL-C) displayed the highest rate of CO₂ consumption (-9.40 ppm/day), whereas the high-light low-mass (HL-LM) (-1.45 ppm/day each; sd = 4.54 ppm/day) and low-light low-mass (LL-LM) (-1.45 ppm/day each; sd = 7.37 ppm/day) treatments showed the lowest rates.

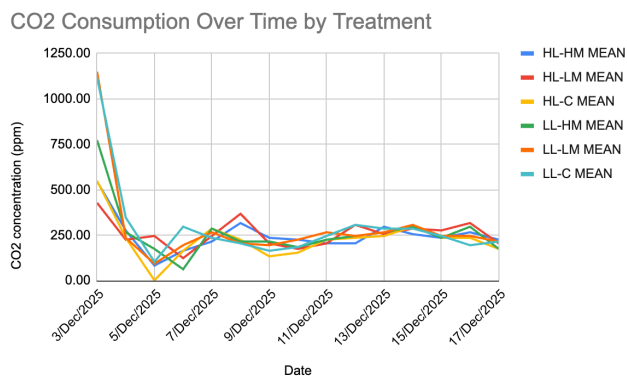


Figure. 2 CO₂ Consumption Over Time by Treatment. Across all treatments, the mean CO₂ concentrations decreased, with values hovering 250 ppm after inoculation.

Nitrate Consumption Patterns

Across the experimental period, nitrate concentrations generally decreased (Figure 3). Initial nitrate readings on Day 1 ranged from 20-80 ppm (50 ± 19.70), with the high-light (HL) container mainly at 80 ppm. By Day 8, nitrate levels stabilized at 40 ppm across all containers. At the end of the experimental period, the nitrate concentrations ranged from 20-80ppm (44.44 ± 17.56).

Nitrate consumption rates varied across all treatment groups. The high-light treatments with algae (HL-HM and HL-LM) were the most photosynthetically active, with rates of -2.0 ppm/day (0.00 ppm/day) and -3.0 ppm/day (1.65 ppm/day), respectively, while the control containers (HL-C and LL-C) showed net increases of $+2.0$ ppm/day (1.65 ppm/day) and $+1.0$ ppm/day (1.65 ppm/day), respectively. This indicates that high lighting had a significant effect on nitrate uptake and played a greater role than initial algal mass.

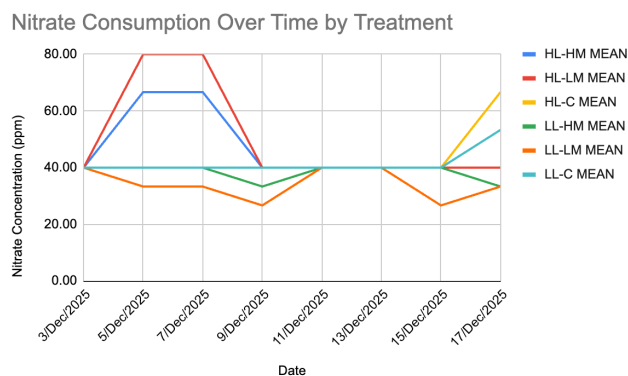


Figure. 3 Nitrate Consumption Over Time by Treatment. High-light treatments produced greater, earlier nitrate fluctuations, whilst low-light treatments remained stable near a baseline of 40 ppm.

Algal Growth Observations

Our qualitative data revealed an increase in algal growth throughout the experiment. Observations on Day 1 showed minimal algal clumping, which was barely noticeable in high-mass containers. However, no clumping was observed in low-mass or control containers. By Day 8, a distinct 1 cm-thick layer had formed at the bottom of all containers containing algae. Dense, dark-green algal clumps were present by Day 12 across all treatment groups with algae. The most prominent growth was seen in the HL-HM, LL-HM, and LL-LM containers on the last day, which had the darkest algal clumps.

Statistical Analyses

We used a two-way ANOVA test to assess the effects of light level and algae mass on CO_2 and nitrate consumption rates. Based on our test results, it was concluded that CO_2 consumption, light level ($*F_{1,12} = 0.561$, $*p = 0.468$), algal mass ($*F_{2,12} = 0.815$, $*p = 0.466$), and their interaction ($*F_{2,12} = 0.171$, $*p = 0.845$) had no significant effects on CO_2 uptake or nitrate consumption.

As for nitrate consumption, we found that algae mass did show a significant effect ($*F_{2,12} = 10.231$, $*p = 0.00255$),

whilst light level ($*F_{1,12} = 3.769$, $*p = 0.07605$) and the light level:algal mass interaction ($*F_{2,12} = 3.769$, $*p = 0.05367$) showed little to no effect. Therefore, the treatment groups with higher algal mass showed greater nitrate consumption than the controls, indicating photosynthetically active algae.

Discussion

This study investigated the effects of light intensity and algal biomass on CO_2 and nitrate consumption over a 14-day period. We hypothesized that a high-light, high-algal-mass environment would increase CO_2 consumption. However, the statistical analyses showed no significant differences between treatments. Suggesting that our system was more sensitive to nutrient uptake than to CO_2 uptake. Through our experiment and results, we concluded that the algae were photosynthetically active. Therefore, we can attribute these unexpected results for CO_2 consumption to supplier-specific algal conditions, methodological challenges, and inconsistencies in baseline conditions.

When we received the algae, the amounts of algal mass in each vial were noticeably unequal. This led to minute inconsistencies across the treatment groups. The manufacturer only states that each vial contains 0.03 L of the substance, including algae and growth medium. However, some vials could have been older and have grown over time compared to newer-packaged vials. This explanation seems plausible, as some vials had noticeably larger clumps of algae and a darker hue, indicating that there was more algae growth than in the other vials.

Unlike CO_2 , nitrate consumption is a more reliable indicator of algal activity because it is less susceptible to physical loss and does not mix with the environment as easily¹⁸. We also chose nitrate for its environmental relevance, since combining nitrate and phosphorus in excess amounts can “accelerate eutrophication, causing dramatic increases in aquatic plant growth and changes in the types of plants and animals that live in the stream”¹⁹. In this experiment, algal mass had a noticeable effect on nitrate consumption as the treatment groups containing algae consistently removed nitrate from the medium, whilst the control groups gained nitrate. It should also be noted that there may have been inconsistencies in the nitrate data collection, as the quantitative data are based on qualitative color observations, subjecting it to observer bias. This pattern seems reasonable, since algae require nitrogen to synthesize proteins, chlorophyll, and other compounds¹⁴. Consequently, containers with stronger nitrate uptake developed thicker algal layers. This makes sense, as eutrophication, the presence of excess nitrate in bodies of water, creates enormous algae blooms which cause an imbalance in oxygen levels in the water, suffocating other wildlife²⁰. However, due to the nitrate-

limited environment, this poses the question whether the maximum CO₂ fixation had been reached due to the lack of nutrients within the container²¹. A lack of nitrogen can inhibit key cellular pathways, making it unable to use nitrate-based pathways to produce energy, consequently lowering CO₂ fixation²². According to Jin et al. (2006), maintaining a 15-20 ppm of nitrate allowed for an increase in exponential growth by 3 days, resulting in a high CO₂ fixation²³. Therefore, it is plausible that the addition of nitrate to each treatment group could have increased overall CO₂ uptake rates.

The results raise the question of whether light mattered at all. A possible explanation is that both light levels were just sufficient for growth. Other factors that may have limited growth include nutrient levels in the initial algal culture and container conditions. In addition, temperature may have contributed to the limitations of our results. After further investigation, temperature can be a major factor, as we kept the treatment groups at a consistent room temperature. This could have caused a slower CO₂ fixation along with preventing the synthesis of the D1 protein, which slows down PSII repair¹⁴. An increase in temperature could have resulted in a higher CO₂ uptake and nitrate assimilation. Overall, nitrate consumption indicates that the algae were metabolically active, although we were unable to obtain the corresponding predicted CO₂ values. Based on our results, algae may appear to be a very limited solution for climate mitigation, since this experiment detected only small differences in CO₂ consumption. This may help explain why many researchers now focus on improving algal photosynthetic efficiency rather than only altering algal environmental conditions⁸. Our inability to detect strong environmental effects on CO₂ consumption suggests that more precise measurements and careful experimental design are crucial for evaluating the true potential of algae towards climate change mitigation. However, our study outcomes are not completely null, as the strong nitrate consumption indicates the algae are metabolically active. Meaning that the main challenge in detecting CO₂ consumption in this experiment may lie in instrument accuracy and in maintaining optimal conditions for CO₂ measurement, such as in an enclosed system.

We found several limitations in our study, including sensor defects, a non-airtight system, a shortened experimental period, and a small scale. The CO₂ sensor used was an older model that produced anomalous readings and occasionally caused repeated measurements. We originally calibrated the sensor using Vernier's online instructions. It seemed to have functioned properly during calibration. However, this may have led to a loss of functionality over time. This seems plausible, as inoculation conditions varied across the experimental groups. Our design also implemented a semi-open system using Ziploc bags (zip-lock bags), which could have allowed gases to escape and mix with ambient air. As a result,

this likely reduced our measurement accuracy and masked algal CO₂ uptake. We used standard plastic zip-lock bags due to budget constraints and the lack of a specialized controlled-air enclosure. This could have been another significant factor in our analogous readings. However, the loss of CO₂ was less of a concern because CO₂ is heavier than oxygen, so most of the CO₂ remaining in the bag would settle at the bottom and would not have been as easy to lose. However, we still took cautionary actions such as not disturbing the gas inside the bag before opening it to take measurements, quickly opening and sealing the bag during measurements, and ensuring the bags were not compressed, which could release gas. Another factor that limited our study could have been that the 14-day duration was too short for the algae to acclimate and reach peak CO₂ consumption and growth rate. There was also the risk of contamination from the containers, which we had not tested for any harmful substances to the algae. Finally, the small scale introduced many challenges. Such as limited gas exchange, measurement sensitivity, and time frames, which may differ from conditions in larger laboratories or natural systems. This limits our ability to predict how algae would behave in large-scale production.

For future work, we recommend using sealed, enclosed systems and more sensitive instruments to ensure accurate gas measurements and extend the experimental duration to allow for maximum algal growth. Future experiments should also explore the sensitivity required for instruments for this scale of experiment. Additional measurements, such as O₂, pH, and algal biomass (in weight), would provide further insight into algal growth and gas exchange. Since light level and algal mass had no significant effect on CO₂ consumption in this study, future experiments should test a wider range of light intensities, light colors, and algal masses.

Published research suggests that genetic modification may be necessary to boost CO₂ absorption efficiency within algae. In addition, there can be further improvement in microalgal culture systems to better suit algal growth requirements²⁴. However, these techniques are still emerging, and their application remains limited, as they depend on specialized and regulatory support in specific countries⁸. The modified algae could be scaled for real-world application to combat climate change; however, this requires further insight into nutrient constraints and ecological trade-offs for it to be a large-scale strategy²⁵.

Consequently, this conclusion exceeds what this study can suggest. Although this experiment encountered challenges that masked clear CO₂ results, the nitrate consumption by algae indicates biological activity and nutrient uptake. We concluded that, via improved experimental design and measurement techniques, algae's role in CO₂ uptake can be accurately assessed. Once improved, we will be able to determine if environmental optimization alone is sufficient or whether genetic

enhancement is required for climate-mitigation applications.

Conclusion

In conclusion, contrary to our hypothesis, neither light level nor algal mass significantly affected CO₂ consumption rates ($p > 0.05$), whilst algal mass significantly influenced nitrate consumption ($p < 0.05$), with higher biomass associated with greater nitrate uptake. CO₂ consumption rates varied among treatments during the 14-day period. The low-light control group (LL-C) exhibited the highest rate of decline in CO₂ consumption (-9.40 ppm/day), whereas the high-light low-mass (HL-LM) (-1.45 ppm/day each; $sd = 4.54$ ppm/day) and low-light low-mass (LL-LM) (-1.45 ppm/day each; $sd = 7.37$ ppm/day) treatments showed the lowest rates. Meanwhile, nitrate consumption rates varied across all treatment groups. The high-light treatments with algae (HL-HM and HL-LM) were the most photosynthetically active, with rates of -2.0 ppm/day (0.00 ppm/day) and -3.0 ppm/day (1.65 ppm/day), respectively, while the control containers (HL-C and LL-C) showed net increases of +2.0 ppm/day (1.65 ppm/day) and +1.0 ppm/day (1.65 ppm/day), respectively.

After analysis, we concluded that there were several limitations that could have affected our study's accuracy and precision. These limitations include the use of an older Vernier CO₂ gas sensor model, an experimental design that uses a semi-open system with Ziploc bags, an insufficient growing period, possible chemical contamination, and a small experimental scale. Even though they limit and complicate our interpretations, these limitations do not invalidate the entire study. As a matter of fact, they highlight the technical challenges of measuring CO₂ on a small-scale and semi-open system and provide insight into the best approach for future experiments.

By implementing sealed systems and more sensitive devices, it will ensure more accurate gas measurements for future research. In addition, extending the experimental duration could allow for maximum algal growth rate, allowing full analysis of the maximum potential of algae. To provide further insight, additional measurements, such as O₂, pH, and algal biomass, could be taken. This study suggests that environmental factors may play a limited role in CO₂ uptake and that the algae may require genetic modification to enhance photosynthetic activity. However, these techniques are still emerging, and their application remains limited because they require specialized expertise and regulatory support in specific countries.

Although the limitations of this study prevented us from drawing conclusions about algal CO₂ uptake, the apparent nitrate consumption indicates that the algae were photosynthetically active. To accurately evaluate algae's ability to lessen atmospheric CO₂, we recommend that future studies use more airtight experimental setups and more precise measurement

tools. Through such refinements, the potential of algae can be studied to its full extent, determining whether it requires genetic modification or can naturally achieve meaningful real-world climate mitigation.

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