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# Use of *Chlorella vulgaris* for $CO_2$ mitigation in a photobioreactor

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**Carbon dioxide ($CO_2$) is a colorless gas that exists at a concentration of approximately 330 ppm in the atmosphere and is released in great quantities when fossil fuels are burned. The current flux of carbon out of fossil fuels is about 600 times greater than that into fossil fuels. With increased concerns about global warming and greenhouse gas emissions, there have been several approaches proposed for managing the levels of $CO_2$ emitted into the atmosphere. One of the most understudied methods for $CO_2$ mitigation is the use of biological processes in engineered systems such as photobioreactors. This research project describes the effectiveness of *Chlorella vulgaris*, used in a photobioreactor with a very short gas residence time, in sequestering $CO_2$ from an elevated $CO_2$ airstream. We evaluated a flow-through photobioreactor's operational parameters, as well as the growth characteristics of the *C. vulgaris* inoculum when exposed to an airstream with over 1850 ppm $CO_2$. When using dry weight, chlorophyll, and direct microscopic measurements, it was apparent that the photobioreactor's algal inoculum responded well to the elevated $CO_2$ levels and there was no build-up of $CO_2$ or carbonic acid in the photobioreactor. The photobioreactor, with a gas residence time of approximately 2 s, was able to remove up to 74% of the $CO_2$ in the airstream to ambient levels. This corresponded to a 63.9-g/$m^3$/h bulk removal for the experimental photobioreactor. Consequently, this photobioreactor shows that biological processes may have some promise for treating point source emissions of $CO_2$ and deserve further study.**
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## Introduction

Currently, the flux of carbon dioxide ($CO_2$) out of fossil fuels is about 600 times greater than that into fossil fuels [6]. In 1997, 7.4 billion tons of $CO_2$ was released into the atmosphere from anthropogenic sources and it has been estimated that by the year 2100, this number will increase to 26 billion tons [6]. There are four major atmospheric reservoirs for $CO_2$: vegetation and soil, fossil fuels, oceans, and ocean sediments [8].

There are several approaches to managing the levels of $CO_2$ emitted into the atmosphere. The first is to increase the efficiency of energy conversion. A second approach is to use energy sources that are lower in carbon or are carbon-free. One of the most understudied approaches is carbon sequestration [6]. Carbon sequestration technologies can be used to manage emissions from both point and nonpoint sources and can be used in conjunction with other carbon management methods.

There have been several proposed types of carbon sequestration technology including ocean sequestration such as deep ocean injection or increasing the amount of $CO_2$ dissolved in the ocean. In order to evaluate this type of sequestration, a recent study looked at the effects of adding iron to a phytoplankton bloom in the southern Atlantic Ocean [1,16]. Results of this study indicated that there was an increase in the amount of phytoplankton biomass and photosynthesis rate in the surface waters. However, the iron was not able to penetrate deep into the ocean and there was no significant increase in the amount of $CO_2$ that was exported downward from the atmosphere [4]. Another proposed form of sequestration is to sequester the $CO_2$ into terrestrial ecosystems. Again, the rates and effects of the increase in the flux of $CO_2$ into the terrestrial ecosystem are unknown [8]. Sequestration of $CO_2$ into more permanent geological formations has also been suggested but not tested. Carbon sequestration can also be accomplished through chemical approaches. Some problems with these approaches are that they must be safe for the environment, stable for long-term storage, and cost-competitive to other sequestration options.

One highly understudied method of carbon sequestration technology is the use of biological processes in a direct $CO_2$-to-biomass conversion from point source emissions of $CO_2$. Algae are a much better option than most plants and trees because they are better able to handle extreme environments, are more efficient at using $CO_2$ for photosynthesis, have a higher proliferation rate, and can be more readily incorporated into engineered systems [10]. However, it has become clear that biological carbon sequestration technologies have been poorly studied and are in their infancy of development [6].

Recently, several studies have evaluated specific species of algae for their potential to reduce $CO_2$ levels from industrial waste gas [5,11–20]. Not only did the algae used in these experiments have to be tolerant of high levels of $CO_2$, but they would also have to be tolerant of sulfur dioxides, nitrogen oxides, and volatile organic compounds (VOCs) present in the waste gas. One study found a decrease in the compounds present in these gases, as well as an increase in algal biomass [17]. Other studies found that the algae they used did not adapt well to environmental conditions present in outside ponds [9]. While other studies found that *Chlorella* species, *Phaeodactylum* species, and *Monoraphidium minutum* were tolerant of the flue gas that was passed through the system, actual removal of chemicals was difficult to determine [5,11,13]. However, researchers still felt that these systems would be feasible but required additional research [12]. To our knowledge, none of

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these studies evaluated the relationship between algal growth and direct removal potential of $CO_2$ from an airstream. Nor did any of these studies establish a rate of $CO_2$ removal from the various air streams. In many studies, the potential for $CO_2$ removal by the algae was assumed due to the indirect measurement of biomass without an investigation of direct $CO_2$ removal.

This research project describes the effectiveness of *Chlorella vulgaris*, used in a photobioreactor with a very short gas residence time, in sequestering $CO_2$ from an elevated $CO_2$ airstream. *Chlorella* was chosen because it tolerates high levels of $CO_2$, as well as other compounds such as sulfur dioxides, nitrogen oxides, and VOCs. We evaluated a flow-through photobioreactor's operational parameters, as well as the growth characteristics of the *C. vulgaris* inoculum, when exposed to an airstream with over 1850 ppm $CO_2$. Finally, we were able to determine the effectiveness of the photobioreactor in bulk $CO_2$ from the airstream relative to various parameters of algal growth and establish a rate of $CO_2$ removal for this system.

## Materials and methods

### Stock cultures and chemicals

Cultures of *C. vulgaris* were purchased from Carolina Biological Supply (Chicago, IL). The stock culture was placed in a mineral medium containing $NH_4Cl$ 110 mg/l, $K_2HPO_4$ 25 mg/l, $MgSO_4$ 50 mg/l, $CaCl_2$ 13 mg/l, Fe(II)EDTA 75 mg/l, and G9 trace metal solution 1 ml/l. The G9 trace metal solution contained $H_3BO_3$ 3.25 g/l, $MnSO_4{\cdot}H_2O$ 1.5 g/l, $ZnSO_4{\cdot}7H_2O$ 0.3 g/l, $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$ 0.08 g/l, $CuSO_4{\cdot}5H_2O$ 0.05 g/l, $Co(NO_3)_2{\cdot}6H_2O$ 0.15 g/l, and KI 0.01 g/l. These cultures were placed on a bench at room temperature under continuous, cool white, fluorescent lighting for 1 week (the same light used for the photobioreactor). Filtered and sterile room air was continuously pumped into the stock culture to provide a constant source of $CO_2$. The culture was swirled daily to mix the medium as well as the culture. Subcultures were made weekly. All chemicals and media were purchased from Fisher Scientific (Chicago, IL).

### Photobioreactor design and set-up

Figure 1 illustrates the set-up of the photobioreactor. The photobioreactor was constructed of borosilicate glass by Ace Glass (Appleton, WI). It measured 51 mm (OD) and 90 cm in length with 2.0-mm-thick sidewall glass. Preliminary studies showed that this size reactor allowed for excellent light penetration and algal growth. The photobioreactor had four no. 7 Ace threads to allow tubing to be attached. Each side thread was approximately 12 in. from the ends (Figure 1). Once operational, the system was completely closed in order to prevent contamination.

Airflow into the photobioreactor was provided *via* filtered hydrocarbon-free building air and a 99%+ pure $CO_2$ cylinder (AGA Gas, Oshkosh, WI) through Teflon tubing. The building airflow was adjusted using a stainless steel micrometering valve (Badger Valve and Fitting, Neenah, WI). $CO_2$ airflow was adjusted using two micrometering valves (Figure 1). The $CO_2$/air mixture was adjusted to achieve the desired concentration of $CO_2$ in the airstream.

The bottom of the photobioreactor (~1 cm) was filled with sterile Celite R-635 beads (1 mm in diameter), which assisted the airstream to be dispersed into smaller air bubbles for passage through the system. During periods of nutrient addition, liquid in the photobioreactor exited *via* the bottom connector, which was connected to the biomass settling flask. Four full-spectrum fluorescent lamps provided light for the system. The lamps were located 15 cm from the photobioreactor on either side (Figure 1) and the system light intensity was maintained at a level between $2.4\times10^{19}$ and $3.0\times10^{19}$ photons/s/m$^2$.

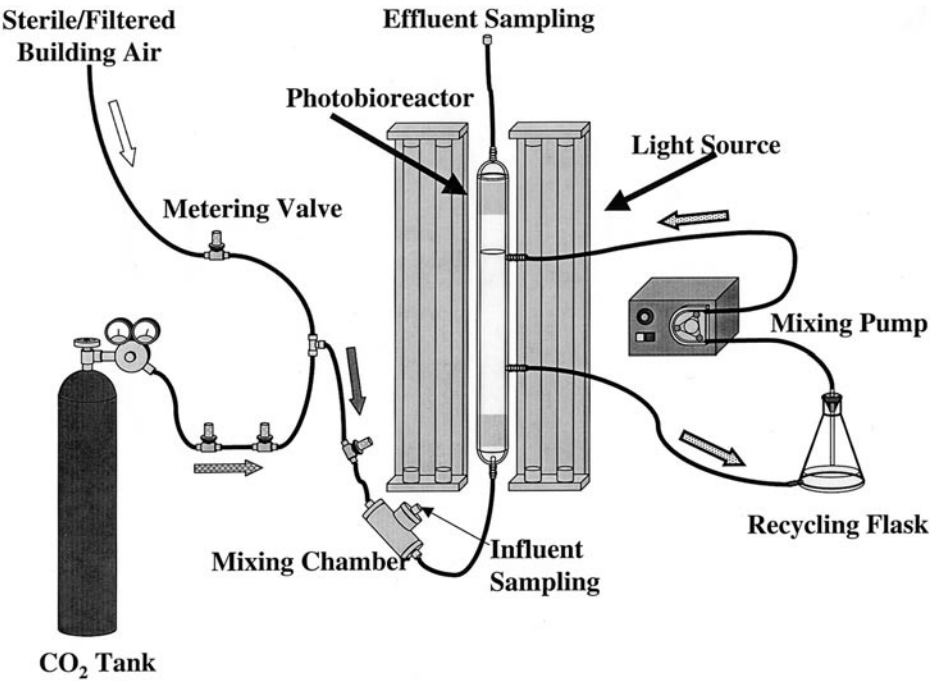


**Figure 1** Diagram of the experimental photobioreactor.

When VOCs were added to the system, a syringe pump (KD Scientific, Boston, MA) was used to inject a mixture of benzene, toluene, acetone, methanol, and naphthalene (1:1:1:1:1, vol/vol) into the system's mixing chamber to achieve the desired VOC concentration.

### *Photobioreactor algae inoculum*

The initial inoculum for the photobioreactor was prepared by aseptically placing 100 ml of algal stock culture into two Nalgene 50-ml centrifuge tubes. The solution was centrifuged at $4000 \times g$, after which the supernatant was discarded (Beckman GP Centrifuge; Beckman Instruments, Palo Alto, CA). Pelleted algal cells in each centrifuge tube were washed with 50 ml of double-strength mineral medium and centrifuged again for 10 min at the same speed. The supernatant was discarded and the cells in each centrifuge tube were resuspended in 50 ml of double-strength mineral medium.

Before inoculating the system, the photobioreactor was filled with 1500 ml of double-strength mineral medium. After filling the photobioreactor, both 50-ml centrifuge tubes were emptied into the recycling flask (Figure 1). The mixing pump (Simon Varistaltic Pump; Manostat, Barrington, IL) was turned on for 60 min to allow complete mixing of the algae into the system (Figure 1). After 60 min, the pump was turned off and the contents of the recycling flask were emptied into a separate Erlenmeyer flask to allow for the various analyses to be conducted.

### *Algal cell counting, dry weight, and chlorophyll concentration*

A direct microscopic count was performed on the algal suspension that was removed from the recycling flask. This procedure was conducted when the photobioreactor was inoculated and each time nutrients were added to the system. Direct counts were conducted using a Brightline Hemacytometer and an Olympus CH-2 Light Microscope (Leeds Precision Instruments, Minneapolis, MN).

Algal dry weight was calculated [2] when the photobioreactor was inoculated and every time nutrients were added.

Chlorophyll concentration was calculated from the algal suspension removed from the photobioreactor. The method for determining total chlorophyll concentration was similar to the one used by Graan and Ort [7]. Chlorophyll concentration was measured when the photobioreactor was inoculated and every time nutrients were added. Three milliliters of 80% acetone was added to a glass cuvette in the fume hood and placed in a spectrophotometer, which had been calibrated using 100% acetone. The spectrophotometer was "blanked" using 3 ml of 80% acetone. Five microliters of the algal solution was added to the acetone and the absorbance was recorded. Absorption was measured at two wavelengths, 647 and 664 nm. The values obtained at each wavelength were used to calculate the final micromolar ($\mu$m) concentration of chlorophyll. The equation for determining chlorophyll concentration was [7]: Total $(C_a + C_b) = 7.93A_{664} + 19.53A_{647}$.

### *pH and light measurements*

The pH meter was calibrated daily using pH 4, 7, and 10 solutions. It was measured when the system was inoculated and every time nutrients were added. Light intensity was measured adjacent to the bioreactor at liquid level using a Li-Cor Model LI-189 photometer (Quantum Sensor, Lincoln, NE).

### *Airstream analysis and $CO_2$ in aqueous phase*

Influent and effluent $CO_2$ measurements were taken four to five times per week. Effluent measurements were made prior to influent measurements and were taken for approximately 120 min. Air measurements were recorded every 10 s, for a total of 720 data points at each sampling event. A sampling pump (Conspec P3000 Sampling Pump; Conspec, Charleroi, PA) was used to draw a slipstream of the elevated $CO_2$ airstream. The sample airstream was analyzed for $CO_2$ concentration using an Engelhard Telaire 7001 Carbon Dioxide Monitor (Engelhard Technologies, Goleta, CA), and the monitor was connected to an Engelhard Recordaire Model 1058 Data Logger, which was connected to a personal computer (Figure 1). $CO_2$ measurements were recorded using VG-16 Graphing Software (Engelhard, Version 4.02). The monitor was factory-calibrated using a 900-ppm $CO_2$ standard.

Hydrocarbons were monitored *via* a JUM 32-200 (JUM, Lone Star, TX) portable total hydrocarbon analyzer using the EPA Method 25A protocol for air analysis.

Free $CO_2$ in the aqueous solution was tested in the recycling flask; a La Motte Carbon Dioxide Test Kit was used (model PCO-DR; La Motte, Chestertown, MA). The limit of detection was 1 ppm $CO_2$.

### *Addition of nutrients*

Double-strength mineral medium was added to the system three times a week to ensure that the system did not become nutrient-limiting. The recycling flask was first emptied and the contents were discarded. Next, 210 ml of fresh mineral medium was added to the recycling flask, and the varistaltic pump was turned on for 60 min to allow complete mixing of the nutrients into the system. After 60 min, the pump was turned off and the contents of the recycling flask were emptied into a separate Erlenmeyer flask. The recycling flask was refilled with mineral medium to weigh down the flask. The same tests that initially were done on the algal solution were done on the algal solution that was removed after nutrient recycling (pH, free $CO_2$, total chlorophyll concentration, direct algal cell count, and algal dry weight).

## Results and discussion

Except for the runs including total hydrocarbon data, the photobioreactor was operated three separate times with triplicate measurements of each biological parameter taken. Thus, the data presented represent the average of three independent runs of the photobioreactor and nine independent measurements of each biological parameter. The runs, including total hydrocarbon data (VOCs), consisted of two independent runs of the photobioreactor. Each $CO_2$ or VOC data point represents the average of 720 data points collected over 120 min during each independent run.

Prior to (and after) the photobioreactor being (was) operated with algae present, it was emptied and operated for several days without algae to test for any abiotic removal of $CO_2$. There was no abiotic removal of $CO_2$. During these tests, the average influent $CO_2$ was 1774.9 ppm ($\pm$124.4 ppm) and the average effluent was 1863.9 ppm ($\pm$45.4 ppm), illustrating that $CO_2$ was not being removed *via* an abiotic mechanism. The pH of the liquid reactor dropped to 6.5 without algae present. The influent variations in $CO_2$ concentration were due to changes in air pressure as a result of the building air compression system.

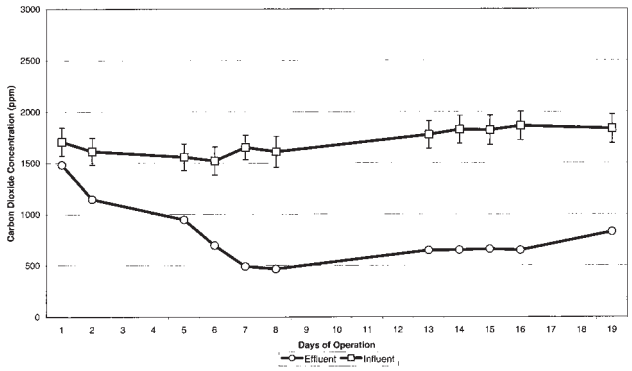


**Figure 2** Photobioreactor influent *versus* effluent $CO_2$ concentration. Each data point is the average of nine sample events with 720 samples collected at each event. Error bars are ±SD.

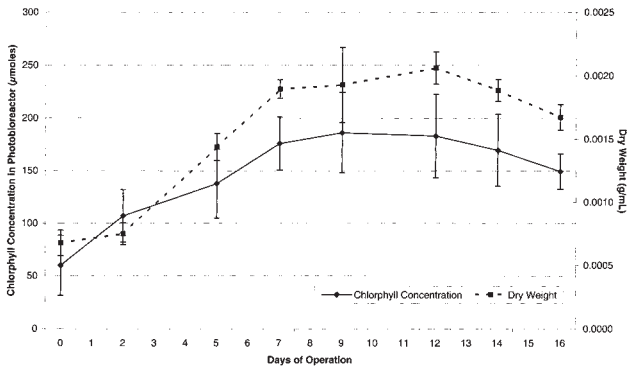


**Figure 4** Chlorophyll concentration and algal dry weight measurements within the photobioreactor. Each data point is the average of nine samples. Error bars are ±SD.

There was a high degree of homology between the various measurements obtained during all trials of the photobioreactor. At all times, the light intensity was maintained between $2.37\times10^{19}$ and $3.04\times10^{19}$ photons/s/m$^2$. Dissolved $CO_2$ within the photobioreactor was not detected during any of the runs. Knowing this, it is thought that biological activity was not the rate-limiting step in this system and $CO_2$ removal followed first-order kinetics. Interestingly, the pH of the photobioreactor was also maintained at approximately 9 (±0.5) during the entire study, indicating that the $CO_2$ was not readily converted to carbonic acid in the photobioreactor when algae were present. Since the reaction of $CO_2$ to carbonic acid reaction is based on $CO_2$ equilibrium in the aqueous phase, it is thought that very little $CO_2$ was allowed to solubilize into the liquid phase. The data support the supposition that the biological activity was able to assimilate all free $CO_2$ in the aqueous phase since there was no free $CO_2$ detected in the aqueous phase. However, chemical and physical properties of the $CO_2$ and the airstream may have limited bioavailability of $CO_2$ in the photobioreactor. It is plausible that if a more efficient air-to-water distribution system can be developed for future studies, a better removal rate may be achieved by making more $CO_2$ bioavailable.

The average influent $CO_2$ concentration over the three runs with no VOCs present was maintained between 1600 and 1800 ppm (Figure 2) or approximately five times the ambient levels. The fluctuation in influent $CO_2$ was due to the building air supply and its changing pressure with respect to the central air compressor. The average $CO_2$ concentration in the effluent over the three runs decreased to 489.5 ppm (±12.0 ppm), increased to 722.8 ppm (±11.6 ppm), and stabilized (Figure 2). $CO_2$ measurements of the building air were found to be 485.2 ppm (±7.0 ppm). All runs were remarkably consistent, showing a consistent pattern among the influent and effluent $CO_2$ measurements. Visual observation of the photobioreactor appeared to confirm this homology of operation. During each run, there was the same progressive change in visual appearance (reactor turning more green with increased algal growth). During this same period of operation, the average $CO_2$ influent load was 22.5 g/h (±1.3 g/h), whereas the average effluent load was 9.0 g/h (±1.5 g/h). This represented an overall reduction in $CO_2$ load of 56%. If the effluent $CO_2$ concentration is evaluated as it reached its lowest point (days 7–19) and compared to the influent $CO_2$ concentration, a reduction of 74% is observed. The $CO_2$ removal range was between 4.4 and 14.2 g/h and the average $CO_2$ removal was 12.5 g/h (±2.5 g/h) (Figure 3).

Total chlorophyll concentration at start-up was 59.97 $\mu$mol (±28.5 $\mu$mol) and reached its peak on day 9 at 186.2 $\mu$mol (±91.5 $\mu$mol) (Figure 4). Chlorophyll measurements showed a degree of variability. Algal dry weight began at $6.77\times10^{-4}$ g/ml ($\pm1.01\times10^{-4}$ g/ml) and reached a peak on day 12 at 2.06×

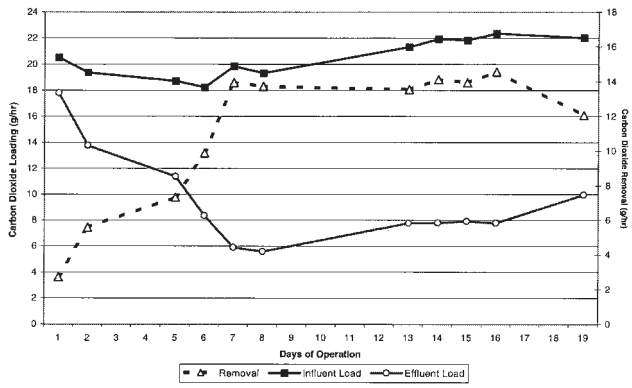


**Figure 3** Influent *versus* effluent loading of $CO_2$ during operation of the photobioreactor. $CO_2$ percent removal is also shown. Each data point is the average of nine sampling events with 720 samples collected at each event. Standard deviation error bars are not visible.

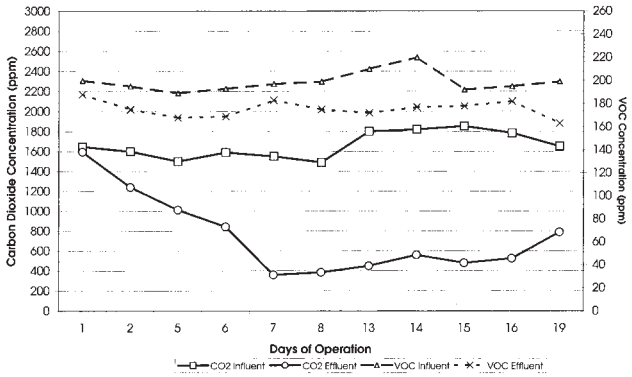


**Figure 5** Photobioreactor influent *versus* effluent $CO_2$ concentrations with VOCs present. Each data point is the average of six sampling events with 720 data points collected at each event. Error bars are ±SD.

$10^{-3}$ g/ml ($\pm 1.28\times10^{-4}$ g/ml) (Figure 4). Finally, direct algal cell count began at $1.54\times10^{7}$ cells/ml ($\pm 1.29\times10^{6}$ cells/ml) and reached a peak on day 9 at $1.53\times10^{8}$ cells/ml ($\pm 1.71\times10^{7}$ cells/ml). The pattern for all biological parameters was very similar. However, the dry weight and direct cell count possessed much less variability than the chlorophyll determinations. By using all three measurements, we were able to obtain a more accurate measure of activity than chlorophyll concentration alone. In fact, variability in chlorophyll concentration is likely due to variability of levels within individual cells and not as a result of changes in overall biomass. If overall biomass were variable, we would see high degrees of variability in both direct cell count and dry weight. Conversely, we observed more similar data between direct cell count and dry weight than with chlorophyll and any other parameter.

The photobioreactor, when operated with VOCs present, also maintained an average $CO_2$ concentration of between 1500 and 1800 ppm. In addition, a VOC concentration of 2330 ppm ($\pm$80.1 ppm) was also introduced to the airstream. The $CO_2$ removal of these runs was virtually identical to the results discussed above (Figure 5). In addition, 11% of the VOCs was also removed. While we do not know whether this removal was attributable to the *Chlorella* or to other abiotic factors, such as photooxidation, the VOCs did not appear to impede the *Chlorella* in removing $CO_2$ from the airstream. We had anticipated some toxicity to the algal culture as a result of the VOC addition. However, based upon $CO_2$ removal data, this did not occur. It should be noted that this was only a small portion of this study and the direct effects on the physiology of the algal cells should be further evaluated.

## Conclusions

The $CO_2$ removal during this laboratory photobioreactor study suggests that there may be some applicability of these types of systems to deal with point source emissions of $CO_2$. While this is only one study, it demonstrates that photobioreactors can operate under air retention times ($\sim$2 s) similar to other air treatment systems such as biofilters. By being able to operate under these conditions, it may make these systems applicable to some industrial waste streams. Likely the limiting factor in full-scale photobioreactors will be their geometric configuration and achieving adequate light penetration in the system.

Removal of $CO_2$ from this photobioreactor was likely not limited by the biological processes, since one would expect to see either a build-up of free $CO_2$ in the water or a drop in the pH of the system due to the formation of carbonic acid. The data suggest that once the algae reached a certain density, the photobioreactor could not support any larger population of algae, or it had reached its carrying capacity.

This system has successfully shown that a biologically based reactor can be used to sequester $CO_2$ from an airstream containing increased concentrations of $CO_2$. The overall average elimination of $CO_2$ from the photobioreactor was 63.9 g/m$^3$/h $CO_2$ ($\pm$14.1 g/m$^3$/h). When the maximum elimination of the system is compared to actual biomass present, the removal of $CO_2$ per unit of biomass is $5.63\times10^{-7}$ g $CO_2$ removed/g algal culture. The experimental photobioreactor discussed in this paper had an aqueous volume of only 2000 cm$^3$. If you had a treatment system with a volume of 1000 m$^3$, which would be in-line with the sizes of other biologically based industrial treatment systems, you could remove 63.9 kg of $CO_2$ per hour of operation (assuming similar removal rates). That is over 1533 kg of $CO_2$ mitigated per day of operation. For photobioreactors that are significantly larger, the bulk removal would be proportionally larger.

One question that arises when discussing full-scale biological carbon sequestration technologies is what to do with the biomass generated. The options for this appear to be diverse and intriguing. A recent study determined that using *Chlorella* species as filler when making polyvinyl chloride (PVC), which would be called PVC-*Chlorella*, could add tensile strength to PVC due to the physical properties of the algae [20]. Another researcher has suggested that the *Chlorella* obtained from such systems could be used as a food supplement [3].

This was the first study, which we are aware of, that used *C. vulgaris* to quantitatively remove $CO_2$ from an elevated $CO_2$ airstream in a laboratory photobioreactor. *C. vulgaris* was found to be a very effective organism in sequestering $CO_2$ and did not appear to be affected by a complex mixture of VOCs present in the airstream. Given the removal of $CO_2$ from the system ($\sim$64 g/m$^3$/h), it may be prudent to further evaluate the use of biological photobioreactors and *C. vulgaris* for treatment of point source $CO_2$ emissions.

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