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The Possible Contribution of *Chlorella vulgaris* to Uptake High Concentration Carbon Dioxide to Alberta's Climate Leadership Plan

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Abstract

In November 2015, the Government of Alberta proposed the Climate Leadership Plan (CLP). It aims to reduce carbon emissions while diversifying Alberta's economy and protecting the health and the environment. In this research, the possibility of the contribution of CLP and using *Chlorella vulgaris* (*C. vulgaris*) in different biomass concentration to sequester CO_2 in the domestic wastewater effluent as the Medium is assessed. Wastewater is an appropriate, renewable, economical medium, which contains enough nutrients. After cultivation of stock solution to 5% (v/v) CO_2 the CO_2 sequestration rate by *C. vulgaris* was measured in the presence of 20% (v/v) CO_2 in the total volume of photobioreactor headspace. *C. vulgaris* illustrated the ability of fixation of 20% CO_2 in the total gas volume of the photobioreactor headspace in optimal physical conditions in 35 hours. The CO_2 fixation rate was higher in the middle and end of the exponential growth phase compared to other growth phases.

Keywords: Wastewater treatment; *Chlorella vulgaris*; Microalgae; GHG mitigation; Water; Nutrients; CO_2

Introduction

An increase in population has led to a rise in the global demand for energy and waste treatment, and this demand has contributed to global warming. Fossil fuels are the primary source of energy all over the world, and their increasing consumption will lead to higher emissions of greenhouse gases (GHG) such as carbon dioxide (CO_2), methane (CH_4), ozone (O_3) and water vapour. GHG reflect incoming radiation from the sun and trap outgoing infrared radiation [1]. In this GHG crisis, CO_2 plays a vital role, which has raised concerns in recent years. The main industrial sources responsible for CO_2 emissions are coal-fired power plants [2]. Coal-fired power plants have historically been Alberta's inexpensive supply of electricity. Sixteen percent of Alberta GHG emissions come from the electricity sector; this amount is higher compared to other Canadian provinces. According to the latest federal regulations, all coal-fired power plants should phase-out once they become fifty years old [3]. On the other hand, Wastewater is an appropriate, renewable, economical medium, which contains enough nutrients, such as nitrogen and phosphorus, for algae to grow.

As an aquatic plant, algae lack roots, leaves, and stems and have the ability to conduct photosynthesis similar to terrestrial plants. *C. vulgaris* is a well-known green eukaryotic microalga in the *Chlorella* species [4]. Microalgae can be found everywhere in the world and grow in either fresh or saline water; they can tolerate the most severe environmental conditions [5]. As a result of the photosynthesis process and presence of carbon substrate and enough nutrients, energy is stored in the produced biomass. In recent decades, microalgae have been used to produce high-value products, such as pharmaceuticals, cosmetics, and food supplements, biodiesel as engine fuels, and biomass as livestock food [6].

November 20, 2016, Government of Alberta's Climate Leadership Plan focused on reducing carbon emissions. They implemented a $20/tonnes carbon price to all GHG emitters on January 1, 2017. Beginning in 2018 this price rose to $30/tonnes carbon and will increase by 2% every year [7]. The new regulations necessitate innovative technologies to mitigate CO_2 to meet Alberta's aggressive targets while remaining leaders in oil production. This study aims to find the range of optimum optical density that *C. vulgaris* has higher CO_2 uptake in the domestic wastewater effluent as a medium. Also, growth possibility of *C. vulgaris* in high CO_2 concentration will be assessed. Finally, assess the contribution of this research result to Alberta CLP.

Material and Methods

Microalgae and medium

Chlorella vulgaris was obtained from the Canadian Phycological Culture Center (CPCC) located at the University of Waterloo, Ontario. The medium used in this experiment was Bold's Basal Medium (BBM) which consist of KH_2PO_4 0.12 g l^{-1}, $CaCl_2 \cdot H_2O$ 0.025 g l^{-1}, $MgSO_4 \cdot 7 H_2O$ 0.075 g l^{-1}, $NaNO_3$ 0.25 g l^{-1}, K_2HPO_4 0.075 g l^{-1}, NaCl 0.025 g l^{-1}, $Na_2EDTA \cdot 2 H_2O$ 0.01 g l^{-1}, KOH 6.2 g l^{-1}, $FeSO_4 \cdot 7 H_2O$ 0.005 g l^{-1}, H_2SO_4 1 ml l^{-1}, trace metal solution 1 ml, H_3BO_3 0.0081 g l^{-1} and F/2 vitamin solution 1 ml. Moreover, since the goal is to use wastewater to grow algae, the BBM was not sterilized afterwards for scaling up to higher volumes nor for use in the follow-up experiments.

Experimental setup

C. vulgaris was cultivated in BBM in three 500 ml Erlenmeyer flasks to have enough stock culture. A 2- litres vacuum flask was chosen as a photobioreactor. The volume of the vacuum flask was checked with a graduated cylinder. 1.5 litres of the flask was occupied by microalgae culture. Using a graduated cylinder, the headspace volume was measured which was 484 mL. According to Nielsen [8], an appropriate CO_2 supply for the saturation of microalgae growth is approximately 5% of the unicellular green algae *Chlorella*. If there is an excess amount of CO_2, the pH can drop, and the growth of microalgae can be inhibited. Figure 1 illustrates the schematic of the apparatus setup. Also, a closed system is chosen based on its advantages compared to an open system such as reduction of water evaporation and lowering the growth of competitive algal weeds and predators and pathogens that may kill the desired microalgae [9].

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In a closed system, the gas in the headspace of the photobioreactor is continuously recirculated throughout the *C. vulgaris* culture in a loop, passing through the gas sensors. Consequently, gas concentrations changes, due to photosynthesis and respiration of *C. vulgaris*, so it can be easily monitored in real time. Moreover, using such a configuration is financially beneficial since it avoids the necessity of having more sensors for the gas inlet and outlet simultaneously. To protect the sensors by water trap from the moisture the desiccant beads were bought from Sigma-Aldrich Canada. Also, UV Flux 25% oxygen sensor and COZIR-WR high-performance CO_2 infrared gas analyzer (IRGA) and the air pump were purchased from CO_2 Meter Inc. In this experiment, 20% of the headspace was occupied by CO_2. Since we calculated the volume of headspace, we were able to feed the proper amount of CO_2 to the microalgae culture while not inhibiting the growth. Considering the ideal gas law and CO_2 molecular weight and knowing that the concentration of CO_2 in the air which is 400 mg/L so 0.387 liter of air has 0.155 gram of CO_2 therefore, the total CO_2 mass in the headspace is 0.35 gram. Knowing that for 0.35 g of CO_2 is equal to 20% in the CO_2 sensor detector; the initial mass of CO_2 in four experiments can be easily measured. Applying the same method above the mass of oxygen in the headspace was found to be 0.58 g for 15.58% of oxygen detected by the O_2 sensor. In- addition, based on the growth of microalgae, which is similar to other living microorganisms, they have different growth phases, as shown in Figure 2.

In this experiment, the photobioreactor was filled with *Chlorella vulgaris*, which was in various growth phases, to monitor the ability of microalgae to fix CO_2 in different phases four OD680 were chosen to test the CO_2 mitigation ability of *C. vulgaris* (Table 1).

The exponential growth phase of microalgae has the most rapid growth possible under the conditions presented in this batch system. Four optical densities were chosen from the exponential phase to conduct this experiment. For the first test, an OD was selected from the beginning of the exponential growth phase; the second test used an average OD from the entire exponential phase. The third test used an OD that was chosen from just before the end of the exponential growth phase, and finally, the fourth OD was selected from the end of the exponential growth phase and the beginning of the stationary phase.

Microalgae growth conditions

The stock culture was cultivated in the pH of 7 and ambient temperature (20- 22°C) the light intensity was 2200 Lux provided by ABI cool light LED light. To provide carbon substrate and proper mixing, 5% of CO_2 in the gas mixture (Air-CO_2) was diffused into culture flask, utilizing stone diffusers. *C. vulgaris* growth was determined by measuring the OD using HACH (DR/4000 U Spectrophotometer).

Results and Discussion

It is important to know that the efficiency of CO_2 sequestration in a closed system depends on microalgal species, CO_2 concentration, photobioreactor design and physical growth conditions [10]. Also, Figure 3 shows the depletion of CO_2 in the algae photobioreactor. Interestingly, the CO_2 concentration approaches zero for all four experiments. Since the system is airtight and has a fixed solubility of CO_2 in water, the removed CO_2 only can be sequestered by algae cells in the photobioreactor. To prove the photosynthesis process, the potential oxygen production was measured at the same time as CO_2 depleted.

Figure 4 shows the increase of oxygen in the headspace of the photobioreactor. As previously discussed, four algae cultures with varying initial OD-680 were added to the photobioreactor. Figure 4 illustrates that an OD-680 of 0.650, which is at the beginning of the exponential growth phase, had a higher oxygen production rate compared to other tested optical densities. Table 2 presents the results of applying the same method as described before to determine the producer of biomass concentration. Also, the concentration of biomass has converted to the mass of biomass per litre of the bioreactor.

Table 2 illustrates the rate of CO_2 consumption per produced biomass in a period of 35 hours. Razzak et al. and Hu [10,11] reported that most microalgae strains need to uptake 1.7 kg of CO_2 to produce 1 kg of dry weight biomass. However, the reported rate belongs to open pond raceway with constant nutrient feeding. Clearly, in such a closed system such as our experiment, this rate would be way lower because of lack of nutrient and carbon substrate over the time. In the table above, the result shows that in OD of 0.493, both algae production and CO_2 uptake are high values compared to other values. Moreover, in OD of 1.030, although the algae production is the lowest, the CO_2 uptake is the highest value compared to the other OD's. This is because of the presence of high concentration of biomass which existed in the photobioreactor from the beginning of the experiment. Moreover, Vaičiulyte et al. [12] reported that they could achieve the highest biomass productivity of *C. vulgaris* at 0.3 g L^{-1} d^{-1} with a constant 2% CO_2 flow into the culture. This result is because of the presence of an adequate amount of nutrients and carbon substrate. However, the above experiment conducted over

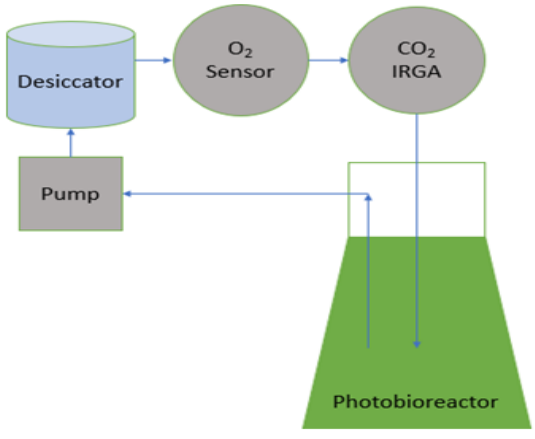

Figure 1: Experiment setup-schematic.

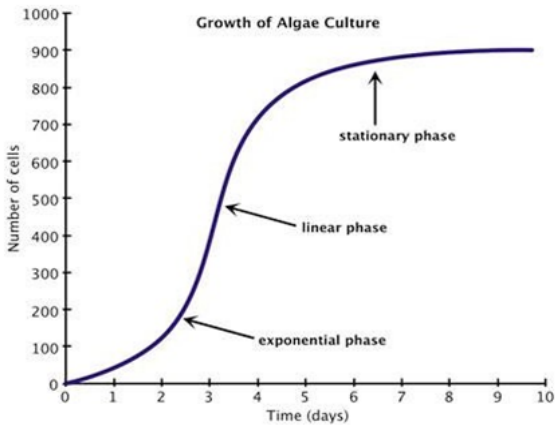

Figure 2: Schematic representation of algae growth rate in batch culture.

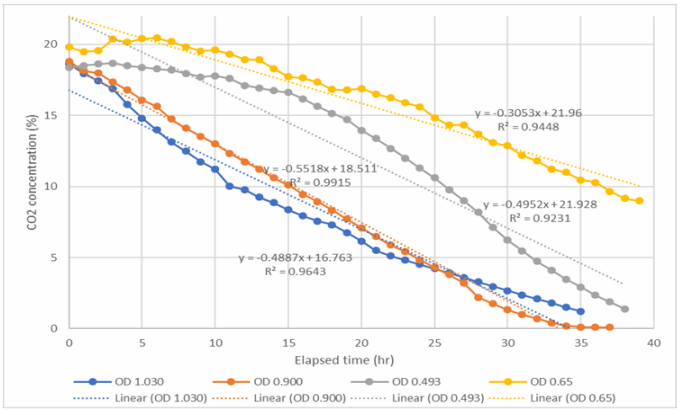

Figure 3: CO_2 (%) sequestration with 20% CO_2 in the headspace.

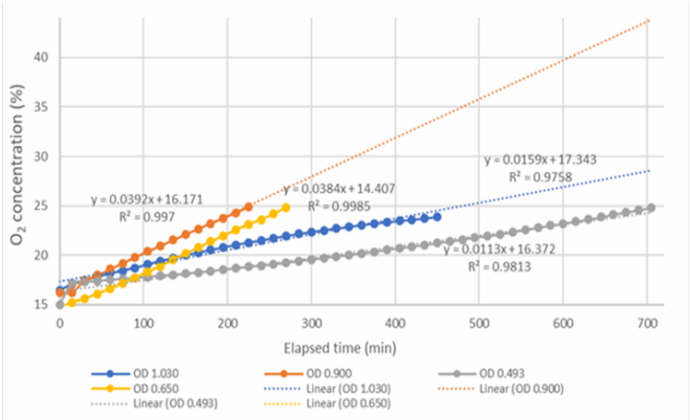

Figure 4: CO_2 production by *C. vulgaris* fixing 20% CO_2 in the headspace.

C	Chosen OD 680
1	0.49
2	0.65
3	0.9

Table 1: Select optical densities in 680 wavelength.

OD initial (680nm)	Initial CO_2 mass (g)	CO_2 mass after 35 hrs (g)	Consumed CO_2 mass (g) after 35 hrs
0.493	0.263	0.041	0.222
0.65	0.231	0.122	0.109
0.9	0.234	0.005	0.229
1.03	0.276	0.021	0.255

Table 2: The initial and final mass of *C. vulgaris* for every test.

two days without adding any nutrients or carbon substrate. Based on the above experiments, the highest productivity of biomass in 24 hours is 0.039 g L^{-1} d^{-1} for OD of 0.4. Also, it is highly recommended to measure the biomass increase or growth rate to assess the potential of a microalgae culture system for direct CO_2 removal [10]. Table 3 shows the increase in biomass concentration after 35 hours the experiment started which is proof of CO_2 removal.

Table 4 summarizes the existing literature data on the amount of provided CO_2 and produced biomass for *C. vulgaris* while providing a basis for comparison, differing experimental conditions between this work and the presented literature should be considered Table 5. Specifically, the bioreactors presented in the literature are roughly one third the size of our bioreactor as well as using different mediums and 15 days duration. One of the reasons for low biomass productivity is because the photobioreactor was air-tight. To maintain the system air-tight we could not top nutrient and definitely, after a certain period, the nutrient became limiting for algae growth. Also, Bhola et al. [13] stated that in the similar experiment 15% of CO_2 in the photobioreactor led to a three-time lower yield of biomass compared to the presence of 4% CO_2. This is because of the toxic effect of CO_2 by converting CO_2 to carbonic acid. Produced carbonic acid decreases pH and making an acidic environment for algae [14].

Table 6 shows the ratio of produced O_2 mass and consumed CO_2 mass in the period of four hours. The reason for four hours measurement is because the O_2 sensor cannot detect the O_2 concentration above 25% and the O_2 concentration in all four experiments reached the highest point the sensor could read in four hours. Finding corresponding CO_2 uptake in four hours we measured the ratio of produced O_2 and consumed CO_2. Based on the photosynthesis equation stoichiometry, for every CO_2 molecular mass (44 g) which is uptaken by *C. vulgaris* one O_2 molecular mass (32 g) should be produced (ratio=0.73). In this experiment, the ratio in Table 6 is lower than 0.73 for all four sets of experiments. Comparing the exponential growth phase, the beginning,

OD initial (680 nm)	OD Final (680 nm)	Algae initial concentration (g)	Algae final concentration (g)	Produced algae (g)	Consumed CO_2 (g)	CO_2 consumed / Produced algae (g CO_2 / g biomass)	CO_2 consumed / Produced algae/hour (g CO_2 / g biomass/ hr)
0.493	0.977	0.056	0.113	0.057	0.222	3.9	0.11
0.65	1.072	0.074	0.124	0.05	0.109	2.18	0.06
0.9	1.032	0.104	0.12	0.016	0.229	14.31	0.42
1.03	1.115	0.119	0.13	0.01	0.255	25.5	0.72

Table 3: Consumption of CO_2 and biomass production over 35 hours.

Amount of CO_2 provided (%)	Produced biomass (g L^{-1} day^{-1})	References
0.03	0.226	Bhola et al. [13]
4	1.222	Bhola et al. [13]
6	0.21	Chinnasamy et al. [14]

Table 4: Biomass produced by microalgae species at a CO2 concentration in literature.

Initial OD (680 nm)	Amount of CO_2 provided (%)	Produced biomass (g L^{-1} day^{-1})
0.493	20	0.039
0.65	20	0.034
0.9	20	0.011
1.03	20	0.007

Table 5: Provided CO_2 and produced biomass based on the different initial concentration.

OD initial (680 nm)	Produced O_2 (g)	Consumed CO_2 (g)	Ratio of Produced O_2 (g)/Consumed CO_2 (g)
0.493	0.15	0.067	0.45
0.65	0.335	0.028	0.08
0.9	0.327	0.047	0.14
1.03	0.186	0.109	0.6

Table 6: The O_2 production of *C. vulgaris* in the presence of 20% CO_2 after 4 hours.

for its high rate of biomass production and end of the exponential growth phase, for its high accumulate biomass concentration have a greater ratio compared to the middle of the exponential growth phase [15,16].

Conclusion

The closed loop is an easy and direct measurement of CO_2 sequestration with minimal sample preparation and without any pre-measurement. Moreover, an additional benefit of this method is simultaneous oxygen production measurements. As shown in Figures 2 and 3, *C. vulgaris* has a higher CO_2 sequestration rate and biomass production rate during the beginning of the exponential phase compared to other parts of the exponential phase and stationary phase. During the exponential growth phase, the rate of increase of cells in the culture is correlated to the number of cells present at any time. As a result, if more algae cells are present, then more CO_2 will be fixed. Overall, the efficiency of CO_2 fixation in a closed cultivation system depends on the microalgae species, CO_2 concentration, photobioreactor design, and operating conditions. Klinthong states that *C. vulgaris* has a maximum CO_2 removal efficiency of 55.3% at 0.15% CO_2 in a membrane photobioreactor while this experiment shows almost 100% of CO_2 removed at 20% of total volume in 36 hours.

The outdoor cultivation is harder to control but usually leads to higher yield and lower cost. On the other hand, indoor cultivation is easier to control but more expensive. According to Alberta's long cold season outdoor algae cultivation is not possible because of inappropriate growth conditions. Thus this method would not have a great contribution to CLP to reduce GHG emission.

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