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CO_2 bio-fixation by *Chlamydomonas reinhardtii* using different periodic CO_2 dosing strategies

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Highlights: The *CO_2 sequestration potential of the green microalga Chlamydomonas reinhardtii was investigated with different CO_2 dosing strategies. A gas mixture containing 30% CO_2 and N_2 was used in these experiments in order to simulate the treatment of flue gases from various industries containing high concentrations of CO_2. Alongside the CO_2 sequestration, the results suggest that the microalgal biomass was rich in carbohydrates and lipids, and thus suitable to be used for biofuel production.*

Keywords: Periodic CO_2 dosing, CO_2 sequestration, biochemical profiles, chlorophyll, *Chlamydomonas reinhardtii.*

1. Introduction

The atmospheric carbon dioxide (CO_2) concentrations reached 411.31 parts per million (ppm) in May 2018, i.e. 2.54% higher than the 400.83 ppm registered in 2015. This recent unusual increase in CO_2 emissions is due to overuse of fossil fuels and the strong 2015-16 El Niño event, which has ultimately reduced the natural capacity of forests and oceans to absorb CO_2 from the atmosphere (CO_2 earth 2018).

The natural photosynthetic capacity of microalgae has grabbed world's attention due to their stronger CO_2 biofixation capacity and the potential to couple this CO_2 sequestration to the production of wide range of products (biofuels and value added compounds). Microalgae can also use wastewater as source of nutrients, contributing to an environmental benefit along with biofuel production. It was found that the green microalga *Chlamydomonas reinhardtii* can capture the CO_2 at a high rate, because of its well developed photosynthetic apparatus and active ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme. Most of the studies on CO_2 fixation by *C. reinhardtii* were based on the production of biomass and biofuel like bio-H2, bio-ethanol. While very little information exists about the growth of *C. reinhardtii* under elevated concentrations of CO_2 (Fan et. al. 2017).

In this research, *C. reinhardtii* was cultivated under various CO_2 dosing strategies with the objective to increase the biomass growth rate as well as biochemical compounds production. In our previous study, the highest CO_2 sequestration rates were observed at a CO_2 concentration of 30%, which was used as a fixed CO_2 concentration in this study. The production rate of sugars, proteins, lipids and pigments with different CO_2 feeding strategies was also investigated. A detailed understanding of the effect of dosing strategies is essential to apply the CO_2 sequestration process by microalgae for the treatment of industrial flue gases containing high concentrations of CO_2.

2. Material and Methods

Microalgae culture

The microalgae culture of *C. reinhardtii* used in this study was obtained from the algae collection center of the University of Naples Federico II. The high salt medium (HSM) used for the cultivation of *C. reinhardtii* consisted of ($mg \cdot L^{-1}$): NH_4Cl (500), $MgSO_4 \cdot 7H_2O$ (20), $CaCl_2 \cdot 2H_2O$ (10), K_2HPO_4 (1440), KH_2PO_4 (720), EDTA disodium salt (50), $ZnSO_4 \cdot 7H_2O$ (22), H_3BO_3 (11), $MnCl_2 \cdot 4H_2O$ (5.01), $CoCl_2 \cdot 6H_2O$ (1.61), $CuSO_4 \cdot 5H_2O$ (1.57), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (1.1), $FeSO_4 \cdot 7H_2O$ (4.99), KOH (15).

CO_2 sequestration experiments

Batch experiments for CO_2 sequestration were carried out in 500 mL glass flasks containing 300 mL of HSM and inoculated with microalgae in exponential growth phase. The bottles were kept on rotary shakers at 160 rpm,

while a 30% CO_2 – 70% N_2 gas mixture was supplied using a DAS GIP MX 4/4 gas mixer. Continuous light was supplied by Philips TLD Eco 51W/840 fluorescent lamps at an intensity of 130 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and measured with Digital Lux metre (Dr. Meter, China). Temperature was kept between 22 and 25 °C. Prior to starting the biosequestration experiments, the initial pH of the medium was adjusted to 7.

In our previous study, the effect of different concentrations of CO_2 in the feed gas on the growth of *C. reinhardtii* was investigated by sparing the gas just once per day for 10 minutes. According to Ying et al. (2015), pediodic dosing of CO_2 helps to achieve a higher gas/liquid mass transfer rate than continuous dosing and increase the biofixation capacity of microalgae. In the present study, besides a single sparging for 10 minutes each day at a flowrate of 1 L/h, CO_2 was sparged with the same flowrate for 10 minutes two and three times a day (double and triple sparging, respectively) and twice a day for 10 minutes each by doubling the volume of fed gas using a flow rate of 2 L/h (double volume – double sparging)

Analytical Method

Measurement of cell growth

Absorbance was measured by spectrophotometric method using an UV/Visible spectrophotometer (Agilant G1103A 8453) at 680 nm weavelength.

Biochemical analysis

Samples for biochemical analyses were collected each day to measure the concentrations of sugars, proteins and lipids. Samples were pretreated using ultrasonication at 60 °C for 10 min in order to, to break the cell wall so that the biochemical compounds could be more accurately measured. The sugars were estimated using phenol sulfuric acid assay (Dubois et al., 1956), proteins using Lowry's assay (Lowry et al., 1951), and lipids using sulfo phosopho vanillin assay (Min et al., 2014).

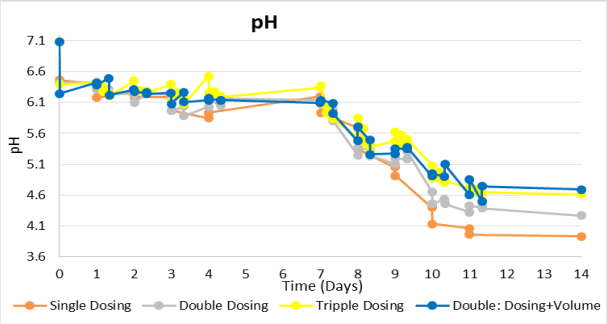
Measurement of total pigments

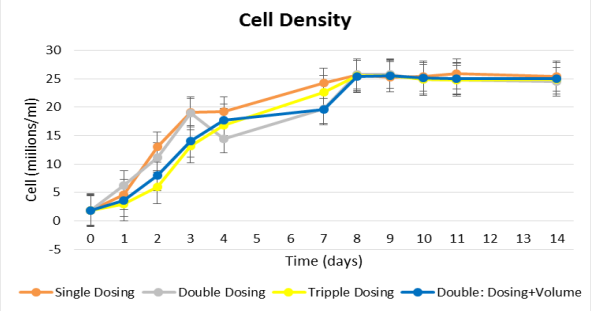
A spectrophotometric method was used to measure the total pigments (chlorophyll and carotenoids) produced by the microalgae. 1 mL sample was centrifuged at 8000 rpm for 5 min and the supernantant was discared. The algal pellet was dissolved in 1 mL pre heated dimethyl sulfoxide at 60°C using vortex shaker, then the solution was centrifuged at 8000 rpm for 5 min. The supernantant was transferred to another tube and used to measure the absorbance at different weavelengths (480, 630, 649, 665 and 710 nm). The measured absorbance was used to calculate the amount of chlorophyll a and b as well as the carotenoids by using the equations developed by Wellburn (Wellburn 1994).

3. Results and Discussion

The first significant result shown in Figure 1 is the gradual decrease of pH from … ± … to approximately 4.5 (triple dosing and double dosing + double volume), 4.2 (double dosing) and 3.8 (single dosing) over the period of sequestration. This decrease of pH can be attributed tothe CO_2 solubilization and conversion into carbonic acid as well as ammonium (NH_4^+) uptake by microalgae. The cell density increased significantly until the pH dropped to 5.5 (day 8), since a further decrease of pH did not correspond to an extra cell density increase (Figure 1).

The Biochemical analyses (Figure 1) were performed to study the fate of the carbon captured by the microalgae inside the cells. The total sugar concentration obtained with the single and double dosing strategy was comparatively higher compared to that achieved with the other two strategies. While the protein and lipid concentration was higher with the double dosing strategy, the total pigment concentration was substantially the same under all conditions.





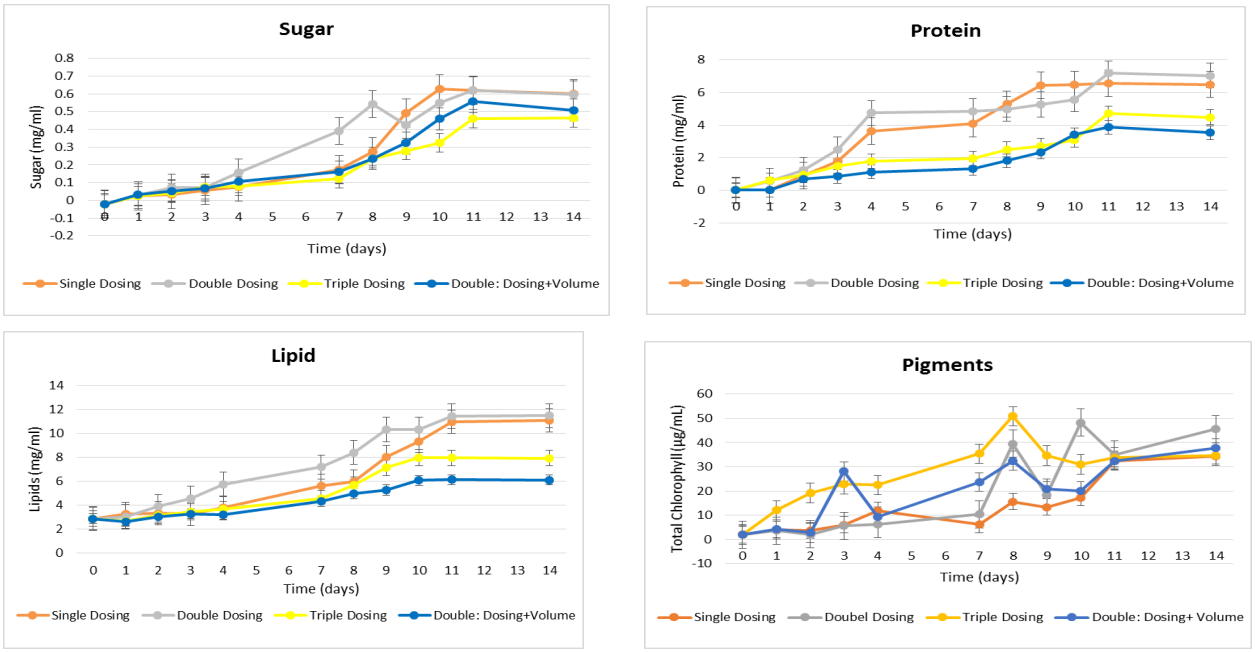


Figure 1: Different parameters investigated during the biosequestration of CO_2 by *C. reinhardtii.* 1. pH of the medium, 2. Cell density, 3. Sugar concentration, 4. Protein concentration, 5. Lipid concentration, 6. Pigment concentration.

4. Conclusion

The CO_2 sequestration experiments with *C. reinhardtii* showed that the double dosing strategy resulted in a significantly higher concentration of biochemical compounds compared to the other strategies tested. In particular, the production of sugars, proteins and lipids at the end of the experiments was 0.6, 6.8 and 11.5 mg/mL, respectively. Cell density at the end of the experiments was approximately the same (25 millions/mL) with all dosing strategies. In order to better understand the rate and pathways of CO_2 biofixation by *C. reinhartdii*, the effect of different gas flow rates on the growth of microalgae, cell dry weight and total organic carbon should be analysed. However, the results of this study show that the flue gases from industries containing 30% CO_2 can be efficiently used for enhancing the growth of *C. reinhardtii* and the production of biomolecules of commercial interest.

References

CO_2.earth (2018). Are we stabilizing yet? https://www.co2.earth/

Kumar A., Ergas S., Yuan X., Sahu A., Zhang Q., Dewulf J., Malcata X. F., Lagenhove H., (2010). Enhanced CO_2 fixation and biofuel production via microalgae: recent developments and future directions. *Trends in Biotechnology* 28, 371–380

Fan Q., Yan X., Yi Y., Xiaosheng L., Li Z., Hui Z., Haiying W., (2017). Enhancing growth of *Chlamydomonas reinhardtii* and nutrient removal in diluted primary piggery wastewater by elevated CO_2 supply. *Water Sci Technol.* 75(10):2281-2290

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem*., 28 (3), pp 350–356

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*. 193 (1): 265–75

Min S. Park, Ji-Won Yang, Wasif Farooq, Myonghoon Moon, Anupama Srivastava, Min S Park, Ji-Won Hyang, (2014) Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method. Bioresource Technology 155 330–333

Wellburn, A.R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology* 144; 307-313