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# Evaluating the impact of CO<sub>2</sub> concentration on algal growth and bio-fixation, using a fabricated experimental set up

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## Abstract

The increasing level of CO<sub>2</sub> emissions during the industrialization has turned the world into a major hazard and studies are required to focus on practical method for environmental protection. This study aims at increasing the carbon sequestration capability through biological method using microalgae, *chlorella sp.* . In this research, the effect of 3 different CO<sub>2</sub> concentrartions (5, 10 and 15%) and aeration condition were investigated on algal growth. The experiment were all performed under the optimum growth conditions based on previous researches. The maxium biomass concentration was achieved through pumping 15% CO<sub>2</sub> at 2.7 g/L. Growth factors including specific growth rate ( $\mu$ ) and overall biomass productivity ( $P_{\text{overall}}$ ) were also observed. Another experiment was a shorter cultivation process which was performed at 10% CO<sub>2</sub>. In this experiment, sampling were done every couple hours to find out the CO<sub>2</sub> fraction in the batch photobioreactor. In the finall stage, after 56 hours cultivation the *chlorella vulgaris* could stabilize 67.7% of the whole CO<sub>2</sub> injected.

**Keywords:** microalgae, CO<sub>2</sub> biofixation, CO<sub>2</sub> concentration, algal growth



## 1. Introduction

Over-population and uncontrolled vitality utilization has driven to the consumption of global energy assets saves. Furthermore, excessive energy consumption such as heat, electricity and transportation fuel has been recognized as one of the chief culprits of global warming and environmental pollution [1,2]. The impact of warming threaten both marine and terrestrial environment [3]. Global warming can put the marine food networks at risk by changing the foundation and function of main producers [4, 5].

Carbon dioxide is known to be a considerable contributor to global warming and climate change [6,7]. This is due to the fact that carbon dioxide as one the main greenhouse gases (GHG) can take in sun rays and trap it within the atmosphere. Besides, burning of fossil fuels has released large amounts of carbon dioxide (CO<sub>2</sub>) into the atmosphere, which could affect worldwide climate change in a very short period of time[8]. A power plant, transportation, industrial plants, and cement production emit about 5 Gt carbon dioxide on a yearly basis [9]. The data of Global Carbon Project (GCP, 2022) shows that the global CO<sub>2</sub> emission from industrial fossil fuels in 2021 exceeds the earth's annual natural carbon capture capability[10]. Generally, there are two major approaches for CO<sub>2</sub> emissions reduction. First, the decreased fossil fuels utilization combined with an expansion in use of renewable energy sources; second, carbon stabilization and storage through various biological, physical, or chemical methods[11,12]. Microalgae species are unicellular or simple multicellular photosynthetic microorganisms that can grow much faster than higher plants and are able to sequester CO<sub>2</sub> emissions from different sources involving CO<sub>2</sub> in the atmosphere, industrial exhaust gases, and soluble carbonate salt [13]. They are found in aquatic environments. They play a significant role in marine environment, forming the foundation of food web as basic producers [14, 28]. Estimates mention that there are still more than 20000 species which haven't been studied yet [15,32]. Marine microalgae are potential feed stock due to their valuable metabolites like pigments, vitamins, proteins, carbohydrates and lipids with valued fatty acids [16, 29]. Currently, in many countries, the cultivation of this microorganism has gained a great popularity for the production of nutrients needed by humans, and it is produced as a product for diet foods. Cultivation of microalgae does not compete with arable and resources required by traditional crops as it can develop on inappropriate lands for agriculture therefore, issues related to food security are avoided. Moreover, its production is much more proficient than land crops. Its high protein substance shows extraordinary potential to cope with the nutritional needs of the growing population. Separated from being rich in protein, the presence of bioactive components in microalgae gives it a health benefit[17]. Among microalgae, *Chlorella Sp.* is one of the most studied because it is rich in nutritional value and owns several biocompounds. It is that grows with a pH of approximately 10 in water bodies [18, 31]. During the past few decades, microalgae and cyanobacteria have found a considerable attention by researchers, toward developing biofuels, such as biodiesel, biohydrogen and biogas utilizing. In the field of bio-pharmaceuticals, natural products in algae extract are used in cosmetic and medicinal cases[19, 30].



In this study, an experimental set up is designed and constructed to perform a series of tests. These tests mainly focus on evaluating the impact of different CO<sub>2</sub> concentration on *chlorella* sp. growth in a batch cultivation system. The growth factors were investigated and results were reported through the curves. The culture pH was also observed during the microalgae growth and CO<sub>2</sub> assimilation process. Another important factor was the CO<sub>2</sub> bio-fixation rate which was calculated through a determined equation after obtaining the data from GC (gas chromatography) device. The amount of CO<sub>2</sub> fraction in each sample were analysed and determined with GC precisely. It was firstly targeted to recognize the effect of CO<sub>2</sub> concentration as the inorganic carbon source for photosynthesis, on algal growth then to find the CO<sub>2</sub> sequestration capability of the microalgae *Chlorella vulgaris* in a short run cultivation.

## 1. Materials and methods

### 1.1 Microalgae strain

The microalgae strain used in this research, *chlorella vulgaris* was obtained from Tarbiat Modares University: Natural Resources and Marine Sciences.

### 1.2 Microalgae cultivation medium

*chlorella vulgaris* was maintained in Bold's basal medium (BBM). The medium contained 0.25 g/L of NaNO<sub>3</sub>, 0.075 g/L of K<sub>2</sub>HPO<sub>4</sub>, 0.175 g/L of KH<sub>2</sub>PO<sub>4</sub>, 0.075 g/L of MgSO<sub>4</sub>, 0.025 g/L of CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.025 g/L of NaCl, 0.005 g/L of FeSO<sub>4</sub>.7H<sub>2</sub>O, 0.05 g/L of Na<sub>2</sub> EDTA, 0.031 g/L KOH, 0.0015 g/L of ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.0003 g/L of CuSO<sub>4</sub>.5H<sub>2</sub>O, 0.0003 g/L of MnCl<sub>2</sub>.4H<sub>2</sub>O, 0.0001 g/L of CoCl<sub>2</sub>.6H<sub>2</sub>O, 0.00025 g/L of MnO<sub>3</sub>, 0.008 g/L of H<sub>3</sub>BO<sub>3</sub>. The pH was adjusted to 6.4-6.8 with 1M NaOH solution prior to autoclaving at 120°C for 15 min.

The cultures were maintained in an Erlenmeyer flask (250 mL) containing 120 mL medium with the initial cell density of 0.15 g/L. After 5 days (exponential phase), the seed culture was transferred to 1 L of Erlenmeyer flasks containing 500 mL BBM medium with 0.15 g/L inoculum and cultured for 10 days under the same condition. Cultures were aerated with sterile air at a gas flow rate of 0.3 vvm (volume gas per volume medium per min). After 10 days cells were harvested for further experiments.

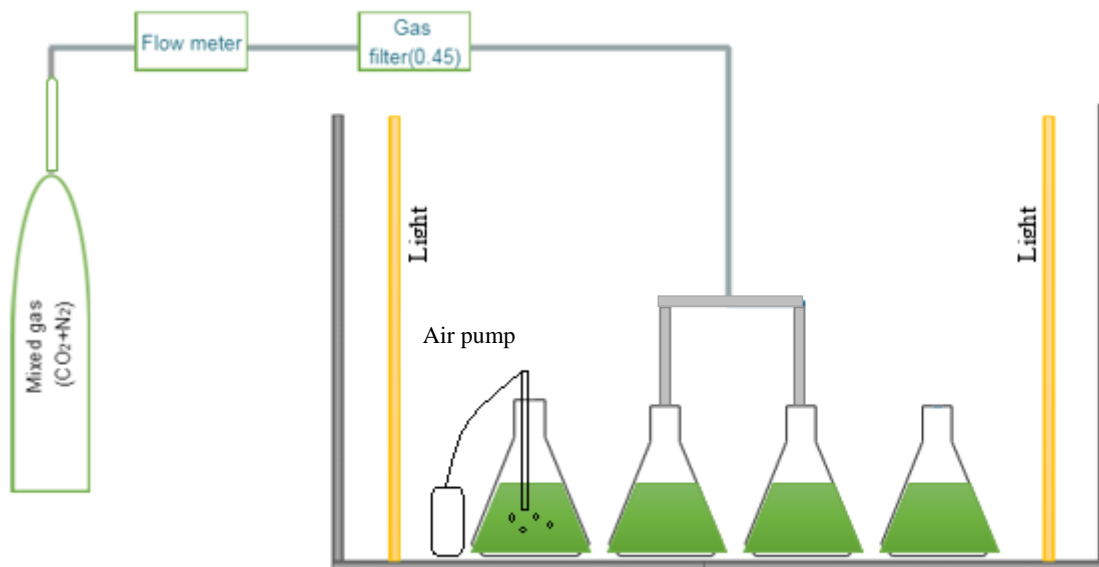
### 1.3 Experimental procedure and design

The schematic diagram of the assembled experimental set up is shown in the figure 1. In this study, the microalgae cultivations were performed in the batch bioreactors (500 mL Erlenmeyer flasks) which is filled up to 300 mL working liquid volum.

The flasks are all spaced into an enclosure. This box contains lights (LED Pwm 220v) around the reactors, which is supplied by the optimum level of energy. The light intensity was measured by the lux meter device (Mastech, MS 6610). The temperature was regulated and then recorded on a daily basis using a temperature controller (R<sub>1</sub> & BNT, STC-100). Based on studies light was adjusted on 4000Lux and the temperature was regulated on 28 degree celsius



[21]. The *Chlorella vulgaris* was initially precultured to avoid the lag phase and after that placed into the photobioreactors for cultivation. On the outside of the enclosure, a mixed gas cylinder including CO<sub>2</sub> and N<sub>2</sub> was installed. Based on previous studies, this gas flows through a line to feed microalgae cells inside the reactors and was adjusted at a flow rate of 200 mL.min<sup>-1</sup> (~ 0.06 VVM) using the flow meter[22]. Beside this, a gas filter (0.45 μm) was attached to avoid contamination. Cultivation with continuous aeration and a non-aerated condition batch were carried out simultaneously, with the optimized condition of light and temperature.



**Figure 1: Schematic diagram of the experimental set up**

#### **1.4 Biomass concentration, specific growth rate and productivity**

The biomass concentration of the microalgae cells were determined by washing and drying the cellular suspensions. In this process, a centrifuge (Hermle Z 233 M-2, Germany) and oven (Binder 230V/10A, Germany) have been utilized. Through centrifuging the microalgae suspension and discarding the supernatant and washing it with distilled water for 3 times, a concentrated suspension has been achieved. It got dried in the oven at 70 °C for 24 h. The standard curve of cell dry weight-light absorbance was plotted through sampling 100 cc of the same cells and dilution making in 10 falcon tubes. The optical density in different concentration range was measured at the wavelength of 680 (OD<sub>680</sub>) in a UV spectrophotometer (Jenway



6320D, United Kingdom). The correlation between OD<sub>680</sub> and biomass concentration (C) (g.L<sup>-1</sup>) was obtained according to the Eq (1) through drawing standard curve:

$$C \text{ (g.L}^{-1}\text{)} = 2.9583 \text{ OD}_{680} - 0.1024, \text{ (R}^2\text{=0.99)} \quad \text{Eq (1)}$$

The specific growth rate,  $\mu_g$  (d<sup>-1</sup>) and biomass productivity,  $P_{\text{overall}}$  (mg.L<sup>-1</sup>.day<sup>-1</sup>), were determined from dry biomass weight data[23].

$$\mu_{g(d^{-1})} = \text{Ln}(X_2/X_1) / (t_2-t_1), \quad \text{Eq (2)}$$

$$P_{\text{overall}} \text{ (mg.L}^{-1}\text{.day}^{-1}\text{)} = (X_f - X_0) / (t_f-t_0), \quad \text{Eq (3)}$$

where  $X_1$  and  $X_2$  represent cell dry weight at the time  $t_1$  and  $t_2$  of the exponential growth phase, respectively.  $X_f$  and  $X_0$  are likewise the cell dry weight at the time  $t_f$  and  $t_0$ .

## 1.5 CO<sub>2</sub> biofixation rate

Gas samples were collected from the photobioreactors(Erlenmeyer flasks) since the beginning of culturing on a daily basis. For determination of CO<sub>2</sub> concentration, gas chromatography was used. Gas analysis was carried out in a GC (Agilent 7220A, USA) equipped with a thermal conductivity detector (TCD) and a packed column (Carboxene 1000, Supelco, USA). The injector and detector temperatures were 150 and 220 °C, respectively. Temperature programming was assigned for oven temperature starting from 40 to 150 °C at a heating rate of 20 °C min<sup>-1</sup>. The CO<sub>2</sub> removal percentage (%) and CO<sub>2</sub> fixation rate (g. L<sup>-1</sup>.h<sup>-1</sup>) for the experiment conducted in the airlift photobioreactor were defined by Eqs. (3) and (4) [20].

$$\text{CO}_2 \text{ removal (\%)} = (\text{CO}_{2\text{inlet}} - \text{CO}_{2\text{outlet}}) / \text{CO}_{2\text{inlet}} * 100 \quad \text{Eq(4)}$$

$$\text{CO}_2 \text{ consumption rate} = 1.88 \times P_{\text{overall}} \quad \text{Eq(5)}$$

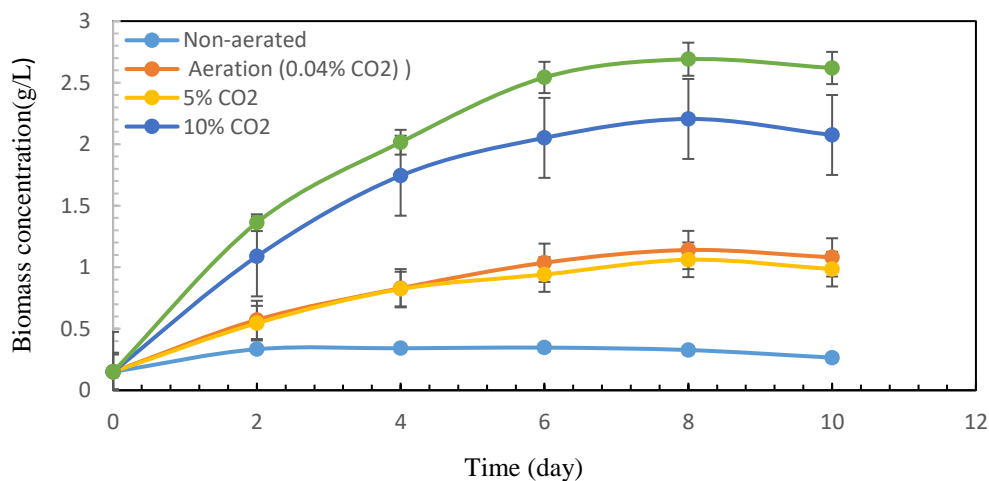
CO<sub>2inlet</sub> (%) and CO<sub>2outlet</sub> (%) are the percentage of CO<sub>2</sub> concentration in the inlet and outlet gas, respectively and  $P_{\text{overall}}$  is the biomass productivity (g.L<sup>-1</sup>.d<sup>-1</sup>) The pH and dissolved oxygen (DO) was measured daily by pH meter and dissolved oxygen meter (WA-2017SD LUTRON, Japan), respectively.

## 2. Result and Discussion



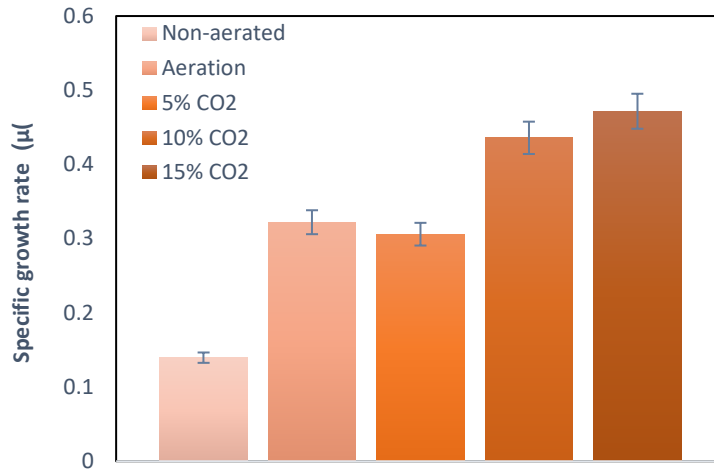
## 2.1 Effect of CO<sub>2</sub> concentration on microalgal growth

Microalgae as photosynthetic microorganisms have different resistance against different concentrations of carbon dioxide. Most species do not have high resistance in this regard and some are also resistant to this gas in a higher range. In this experiment, the tolerance of this type of microalgae against different concentrations of carbon dioxide is investigated. Studies have reported the production of *Chlorella* biomass using CO<sub>2</sub> as a carbon source [24,25]. Considering the above explanations, the experiments were carried out at CO<sub>2</sub> concentrations of 5%, 10% and 15%. Two other culture conditions including continuous aeration with flow rate of 0.3 vvm and non-aerated were also performed simultaneously, in order to find the impact of CO<sub>2</sub> amount on the specified microalgae growth. As it can be observed in the fig. 1, all five batch conditions had ascending curves as the time passed by and found to be 0.34, 1.14, 1.06, 2.2 and 2.7 g/L at non-aerated, continuous aeration, 5%, 10% and 15% CO<sub>2</sub>, respectively after 10 days of cultivation. These results properly indicated that, increasing the intake CO<sub>2</sub> percentage resulted in an improved growth level and showed a high CO<sub>2</sub> sequestration capability in this microalgae. The other growth factor that can be referred to is specific growth rate ( $\mu$ ) factor which has been calculated through the Eq. 2, and is shown in the Fig. 2. As can be seen, the highest level of specific growth rate has been achieved by injection of 15% CO<sub>2</sub> through the culture medium reaching 0.471 d<sup>-1</sup>. However, the least amount of  $\mu$  belongs to the non-aerated condition at the number 0.139 d<sup>-1</sup>. What is significant in this graph, is increased growth condition with continuous aeration in comparison with 5% CO<sub>2</sub> injection, which could be because of the fact that aeration has been performed through the liquid medium and has become able to create a mixing condition as well, so the growth level will consequently improve. Figure shows the impact of these 5 culture conditions in the same way, and shows the overall biomass productivity at the number of 0.398 mg.L<sup>-1</sup>.day<sup>-1</sup> which is directly associated with the level of CO<sub>2</sub> biofixation rate through Eq. 5.

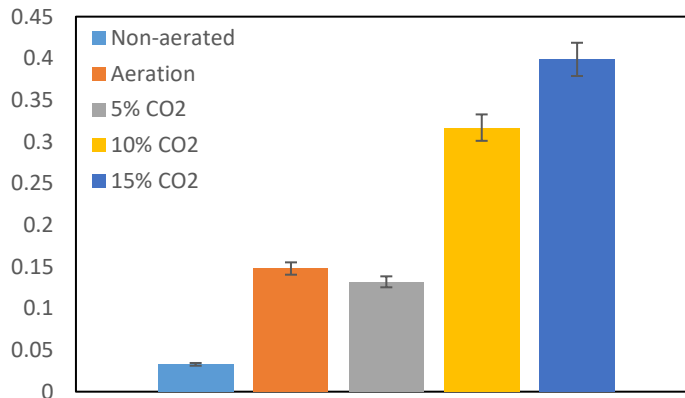




**Figure 1 :The effect of CO<sub>2</sub> concentrations at 5%,10%, 15%, aeration and non-aerated condition on algal growth**



**Figure3: The achieved specif growth rates at different CO<sub>2</sub> concentrations of 5%,10%, 15%, aeration and non-aerated condition**



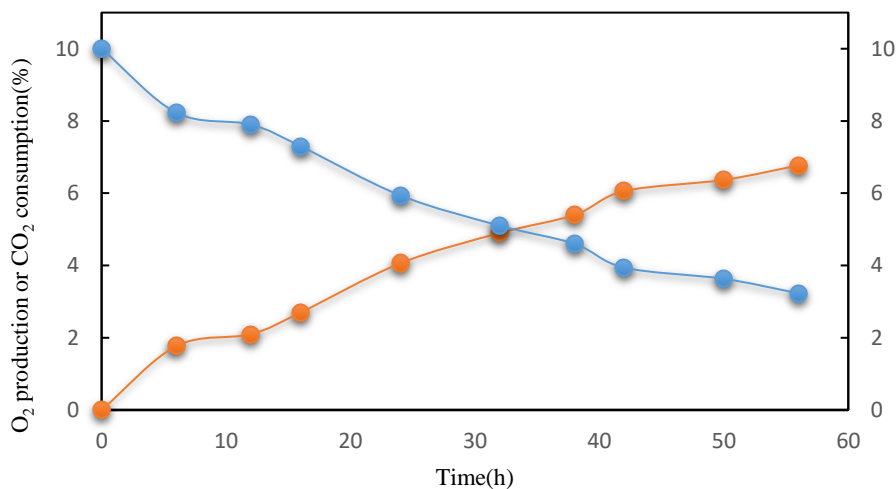
**Figure 4: The Overall biomass productivity at different CO<sub>2</sub> concentrations of 5%,10%, 15%, aeration and non-aerated condition**

### 2.1.1 CO<sub>2</sub> consumption or O<sub>2</sub> production

Industrial exhaust gases including stack gases have 10-20% CO<sub>2</sub> within and is a rich source for microalgae cultivation[26]. On the other hand, microalgae which is able to tolerate a high level of CO<sub>2</sub> is valuable microorganism to cultivate. In this experiment, a mixed CO<sub>2</sub> and N<sub>2</sub> cylinder with a determined CO<sub>2</sub> percentage has been utilized. Sampling were carried out from batch photobioreactors every couple hours. These samples were injected into GC device to make the CO<sub>2</sub> fraction in the gas atmosphere above medium clear and find the amount it can assimilate within the 56 hours cultivation. Another factor that was reported during the GC experiment was



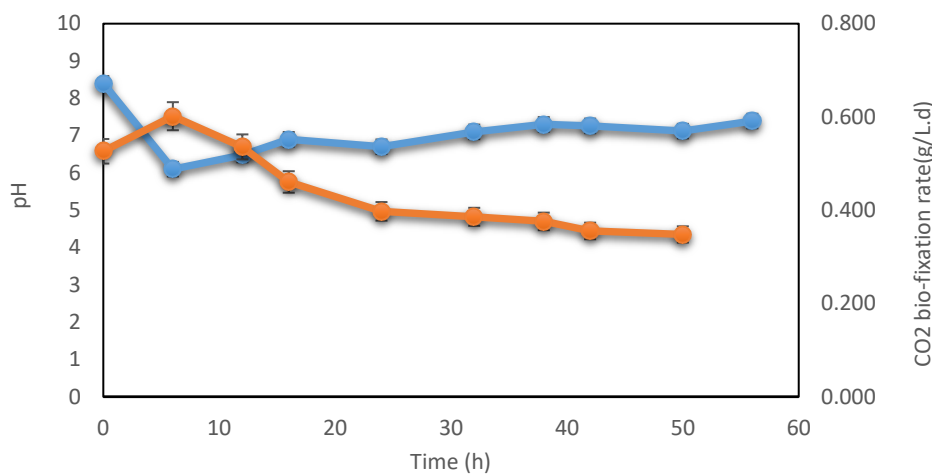
the amount of O<sub>2</sub> production. Given the fact that, N<sub>2</sub> is a neutral gas, the CO<sub>2</sub> mitigation could equals with increasing O<sub>2</sub>. Figure 5, shows a high percentage of CO<sub>2</sub> removal during the time. Considering the Eq. 4, 67.7% CO<sub>2</sub> removal has been obtained. Consequently, 6.77% O<sub>2</sub> has been produced during this time.



**Figure 5 :the percentage of O2 production or CO2 consumption (%)**

## 2.2 CO<sub>2</sub> biofixation rate and pH variations

In the present study, the CO<sub>2</sub> bio-fixation rate and pH alteration were investigated after sparging the initial of 10% CO<sub>2</sub> gas fraction during the 56 hours. As it is indicated in the fig. 5, in the first 12 hours the maximum level of CO<sub>2</sub> bio-fixation rate at 0.602 g/L.d is obtained. Which is higher than many past and recent researches. *C. vulgaris ARC1* could fix 38.4 mg CO<sub>2</sub>/L.d at 6% CO<sub>2</sub>. Another study reported 251.64 mg/L/day CO<sub>2</sub> fixation rate by *C. vulgaris* [27]. In comparison *chlorella vulgaris* proved to have a satisfactory capability for CO<sub>2</sub> absorption. This number did not change widely till the end of cultivation but decreased smoothly with the time and reached at 0.348 g/L.d. Another factor observed was pH variations in the culture which was not adjusted during the cultivation. By increasing CO<sub>2</sub> concentration, the overall pH decreased from 8.4 to 7.4. This is due to the fact that, carbon dioxide dissolves into solution and converts to carbonic acid which leads to a low pH.



**Figure 6: The amount of CO<sub>2</sub> bio-fixation rate achieved at 10% CO<sub>2</sub> and pH variations during 56 hours.**

### 3. Conclusion

In this study, the impact of different CO<sub>2</sub> concentrations including 5,10, and 15% and continuous aeration condition were investigated on the microalgae growth. The maximum biomass concentration was achieved by 15% CO<sub>2</sub> at 2.7 g/L microalgae cells. The other experiment was performed to achieve the CO<sub>2</sub> bio-fixation capability through utilizing the fabricated cultivation set up. The CO<sub>2</sub> bio-fixation rate was promising in comparison with previous studies and reached a number of 0.602 g/L.

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