



## EFFECT OF THE AQUEOUS CARBON SOURCE ON GROWTH RATE OF THE MICROALGAE *COELASTRELLA* SP. MH923012

Zainab H. Razooki<sup>1</sup>, Ibrahim J. Abed<sup>1</sup> and Mahmood K.H. Al-Mashhadani\*<sup>2</sup>

<sup>1</sup>. Department of Biology, College of Science, University of Baghdad, Baghdad. Iraq.

<sup>2</sup> Department of Chemical Engineering, College of Engineering, University of Baghdad, Baghdad-Al-Jadiria, Iraq

\*Corresponding author: Mahmood K.H. Al-Mashhadani; e-mail: m.hummadi@coeng.uobaghdad.edu.iq.

### Abstract

The present study addressed the effect of the carbon source on the growth of Microalgae *Coelastrella* sp. MH923012, which newly registered in Iraq. In this research, which has a novelty via using this type of Microalgae strain as sample test, five different volumetric quantities (5, 10, 15, 20, and 25 ml) of the carbonic solution have been adopted in all experiments. Preliminary results showed that the addition of carbonic solution had less effect on the pH culture media compared to distilled water. The presence of buffer component in the BG11 media played a vital role in determining the consumption of hydrogen ions resulting from reversible reactions. However, the carbonic source remained abundant in the culture flasks that affected the response of Microalgae growth. Therefore, the experimental data showed that the addition of this solution (regardless of the dosing times), clearly improves the growth of *Coelastrella*. Nevertheless, increasing dosing times increases the rate of growth. For example, the addition of 5 ml of aqueous carbonic solution to the *Coelastrella* flasks caused increases the optical density by (1.18 for unadjusted pH flasks and 1.42 for adjusted pH flasks) times greater than that of the control flask. The other thing to consider is the continued growth of *Coelastrella* sp. in all the flasks (except control flask) despite of passing 22 days on the beginning of cultivation. This is the period in which the control flasks reached the stationary phase and then reached to death phase. This is clear evidence that the carbon source is the main limiting factor of the microalga *Coelastrella* sp.

**Keywords:** Microalgae, Carbon dioxide, *Coelastrella* sp., Le Chatelier's principle

### Introduction

Biofuel, with its various sources, is one of the most promising forms of energy to ensure that global energy demand is met, while achieving new economic benefits (Nogueira, 2011; Chu *et al.*, 2007). In addition, the biofuel has important role in addressing the climate change, which was due to anthropogenic carbon dioxide emissions that reached critical and alarming limits (Rutherford *et al.*, 2018). The scientific solutions and logic of application for the biofuels may achieve this goal. There are several methods for biofuel production (Kubba *et al.*, 2016). Indeed, it has been hoped that biofuel could be produced from cellulosic feedstock that do not directly compete with food crops. However, cellulosic fuels have failed to become viable at a significant scale, in spite of main supports and high expectations when renewable fuel delegation were established over a decade ago (Lynd, 2017).

In contrast, the algae have the ability to grow faster, production of valuable materials (such as carbohydrates), and fixation the carbon dioxide (Chisti, 2007) or extracts production (Karm, 2016). Depending on the type of Microalgae strains, the Microalgae can produce different lipids, hydrocarbons and other complex oil content, which is suitable for the production of biodiesel. The known total lipid content of Microalgae differs from 5 to 77% and can produce 10-30 times higher amount of biodiesel than any other sources (feedstock crops, as example) (Gouveia & Oliveira, 2009, Yeh *et al.*, 2010). However, there are several technological problems still related to the production and cultivation process using this source. In fact, absence of technological substructures and managed practices of the algae based fuels is still a weakness (Noraini *et al.*, 2014; Ziolkowska & Simon, 2014). Thus, the cost factor and the lack of production are clearly present as one of the important

obstacles to the development of this source (Slade & Bauen, 2013, Bharathiraja *et al.*, 2015, Ziolkowska & Simon, 2014).

For all that, serious attempts to overcome these obstacles, or some of them, are still continuing. For instance, use of cheap and available alternatives as requirements in cultivation process (Huber *et al.*, 2006, Abbasi & Abbasi, 2010, Sambusiti *et al.*, 2015). The use of carbon dioxide produced from electrical stations is one such of those attempts (Rezvani *et al.*, 2016, Duarte *et al.*, 2017). This gas is main requirement in the photosynthesis of the Microalgae cells for glucose production. A range of studies have demonstrated that the presence of CO<sub>2</sub> gas (with control ratios) in cultivation processes increases the growth rate of algae (Derakhshandeh & Un, 2019, Al-Mashhadani & Khudhair, 2017). In fact, the use of carbon dioxide in sparing system in the bioreactor may cause an increase in the carbonic source for the bioprocess. Thus, the growth of Microalgae as a biomass increases, as a result of contributing in increasing the Gibbs free energy in the negative direction according to Le Chatelier's principle (Al-Mashhadani *et al.*, 2011). In other hand, the bubbling system of the bioreactor leads to remove of oxygen, which produced (as a by-product) from the metabolic processes. This removal is carried out through the mass transfer phenomena of the gas between the culture solution and gas bubbles. According to principle of mass transfer phenomena (Bird *et al.*, 2007), this process depending on the un-equilibrium state of oxygen concentrations between the two phases, which will cause the rejuvenation of the Microalgae environment and increases their susceptibility to breeding more. Therefore, the use of air in bioreactors, although containing low carbon dioxide, has the primary task of reducing the oxygen concentration in the culture solution to be about 7-8 mg/L as was confirmed by (Ibrahim & Abdulmajeed, 2018). Thus, both processes

(dissolution or stripping process) improve the growth of Microalgae. For the above reason, the current research sought to separate the two processes above, by focusing on the effect of the carbonic source on the growth of algae as far away as possible from the removal of the oxygen produced from the process of photosynthesis. All experiments work in this study, were performed using a solution saturated with carbon source with a constant concentration. In addition, the effect of this gas in the current work was tested using type of Microalgae has been recently diagnosed from an Iraqi environment and has been registered globally under accession No. MH 923012.

## Material and Methods

### Culture Media

Preparation of the BG-11 media for the cultivation purpose in this work was conducted according to the guidance and advices provided by the company (BioReady TM media, China).

### Isolation and purification of Microalgae

Microalgae *Coelastrella* sp.MH 923012, which used in the present study, was isolated from the Tigris River and registered in Gen Bank database (accession No. MH 923012) (Abed *et al.*, 2018).

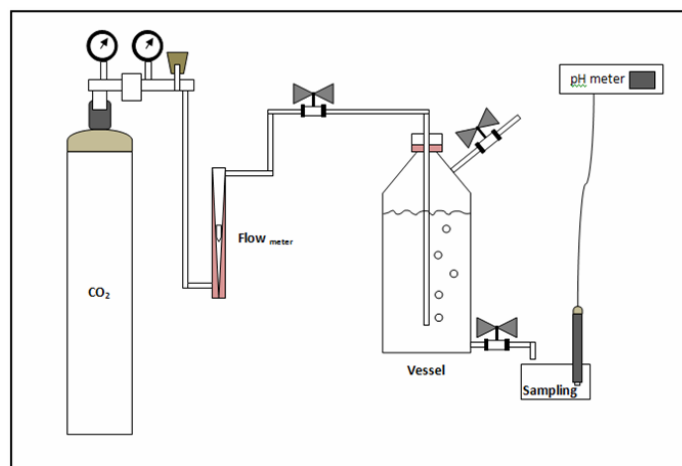
### Culturing the Microalgae species

The isolated *Coelastrella* sp.MH 923012 Microalgae were cultivated in the prepared BG-11 media. The process was carried out via inoculation of that media with 2 ml of Microalgae samples. Then, they were incubated in illuminated incubator (Conviro Canada origin) under light intensity  $168 \mu\text{E m}^{-2}\text{s}^{-1}$  and controlled temperature ( $24 \pm 2$  °C). When the species reached the stationary phase of the growing, the harvesting was applied.

### Aqueous carbon stock preparation

The carbon dioxide saturated solution has been prepared for the current study. The preparation approach was carried out by injecting carbon dioxide at 500 ml / min in a tank containing distilled water as shown in Figure (1). During this process, the pH (HANNA HI, 9811-5) was monitored to ensure that the gas saturation process was completed. After that all the ports of the reservoir were closed to prevent any

gas leaks into the atmosphere. Different volumetric quantities of aqueous carbon solution were taken in the preparation stock based on our previous experiments on distilled water and BG-11 media.



**Fig. 1:** Schematic diagram of aqueous carbon stock preparation

### Set-Up of the experiential work

The experiments included three seed groups. The first group (G1) represents the control (without addition of aqueous carbonic solution). While, the second group (G2) included a flask for each carbonic solution with two replicates to confirm the accuracy of the results and for statistical purposes (error bar). Each flask contains 500 ml of BG-11 media, while 2 ml of isolated Microalgae, as inoculation was added into each flask. Finally the third group (G3) is quite similar to the second group (G2), but, the only difference was that the second group (G2) operates without controlling the pH, while the third group (G3) operates under the control of the pH to reach the value of 7 as can be seen in Table (1).

All the additions were made at the same time. After 24 hours the pH was measured before and after addition process with optical density measurement for each day by spectrophotometer (Shimadzu, Japan) to observe the level of algal growth until reach to stationary phase. Finally, the specific growth rate and doubling time was calculated according to (AL-Mashhadani & Khudhair, 2017).

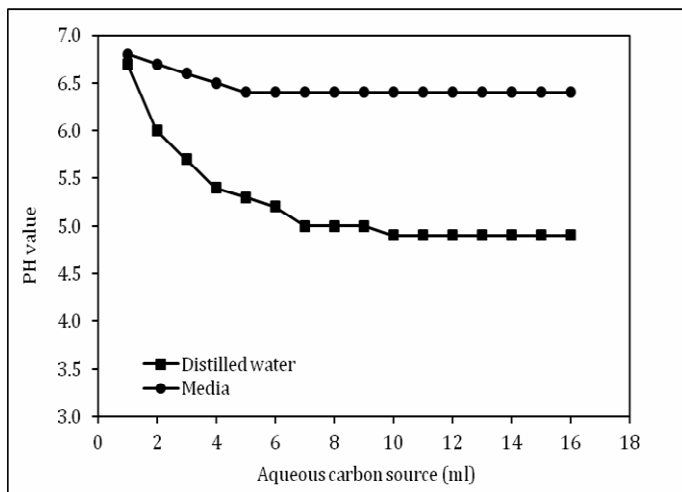
**Table 1:** Groups of aqueous carbon stock preparation

Groups	Groups contents				
G1	(a) BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)				
	CO <sub>2</sub> volumes				
	5 ml	10 ml	15 ml	20 ml	25 ml
G2	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)
G3	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)	BG-11 media (500 ml) + <i>Coelastrella</i> sp. algae (2ml)

## Results and discussions

### Determine of CO<sub>2</sub> volume

The equilibrium state of the carbonic acid with aqueous carbon dioxide in the culture solution was determined in the current study. The objective of this step was to investigate the minimum and maximum volume of saturated solution. These determinants were adopted in subsequent experiments in this work. Figure (2) shows the variance between the measured pH values versus time where the aqueous carbon stock was added to the distilled water and BG11 media. In general, aqueous carbon dioxide reacts with H<sub>2</sub>O to form weak acid (H<sub>2</sub>CO<sub>3</sub>). The latter turns into bicarbonate (HCO<sub>3</sub><sup>-</sup>) and carbonate (CO<sub>3</sub><sup>-</sup>) in reverse reactions by which two protons are donated. Increase the solubility of the carbon dioxide represents a driving force to increase hydrogen ions until the equilibrium state occurs. Therefore, in this figure it can be seen that the effect was clear on both flasks. However; the pH value was dropping faster in the distilled water compared with flask media. In fact, the presence of the buffer compound (Na<sub>2</sub>CO<sub>3</sub>) in BG11 media played an important role in determining that decline by resisting any change in hydrogen ions that present in the solution. It can be seen that the immediate treatment of the hydrogen ions produced from these reactions is sufficient to limit that decline. However, the buffer substance in this media targets the hydrogen ions without compromising the amount of carbon present in the solution. According to this comparison, the appropriate volumetric quantities were (5, 10, 15, 20, 25 ml) of the aqueous solution and adopted for the current study.

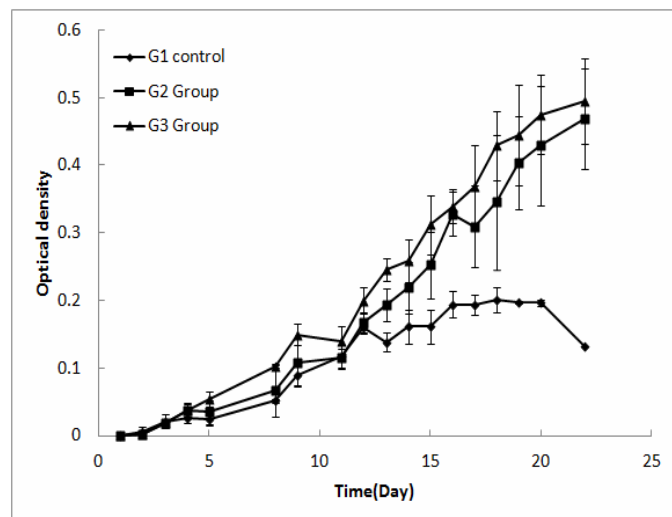


**Fig. 2:** The comparison between distilled water and BG11 media after addition of acidic solution

### Growth rate estimation

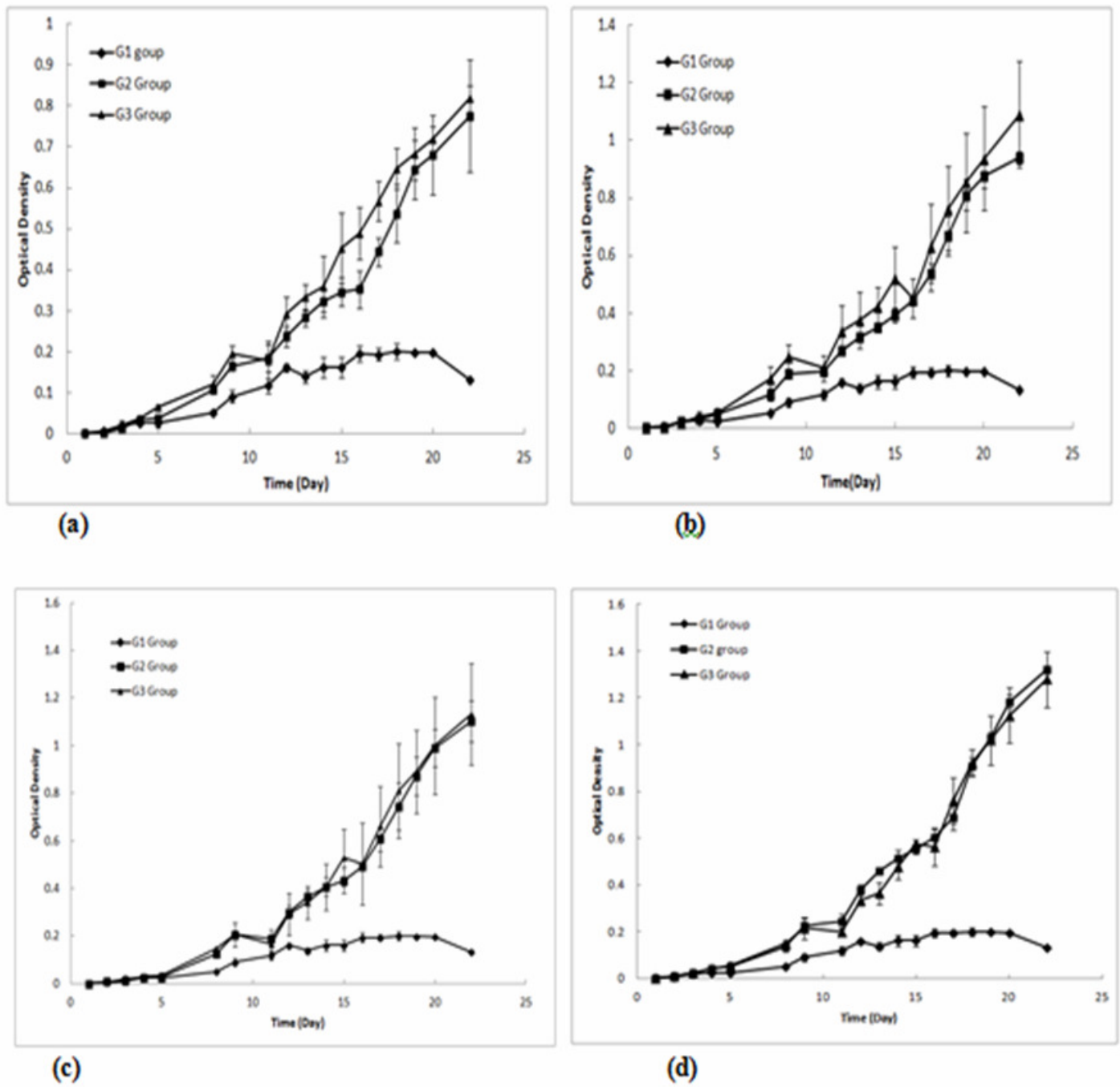
*Coelastrella* sp. MH 923012 has been grown in BG-11 media. The experiment based on three groups (G1, G2, and G3), while optical density was measured spectrophotometrically every day to observe the growth rate of Microalgae until reach to stationary phase of the control

flask. At the beginning of experiment all groups shows gradient increase in growth. However, after ten days, the G2 and G3 shows sharply growth compared with G1 as shown in Figure (3). While it shows that the stationary phase reaches at 15th day in G1 group and for seven days. Then the decline phase in that control group was occurred. On the contrary it was in the other two groups, since it can be seen that there are continuation of the growth rate in both groups.

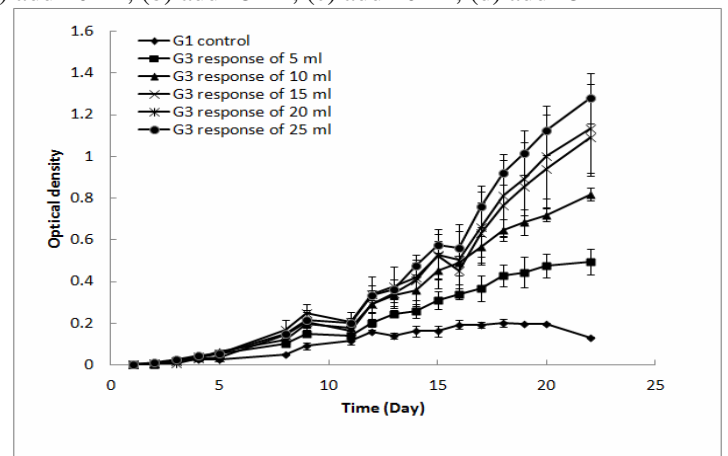
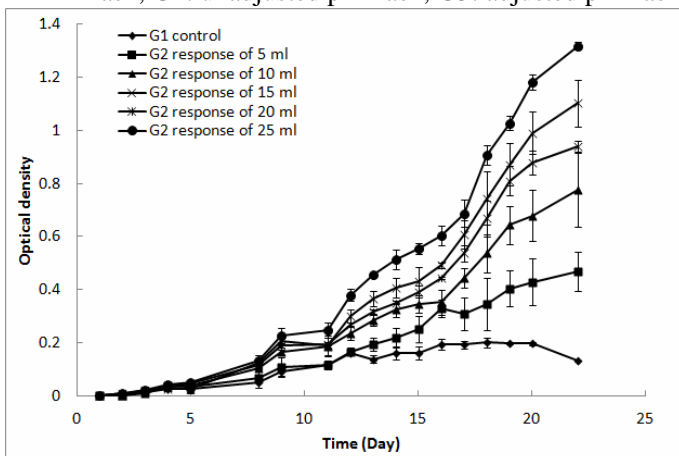


**Fig. 3:** Optical Density of *Coelastrella* sp. MH 923012 microgreen algae when 5 ml of carbonic solution was added; G1: control flask; G2: unadjusted pH flask; G3: adjusted pH flask.

Figure (3) also shows the response of the growth rate of *Coelasterella* sp. MH 923012 during the addition of 5ml carbonic solution. All groups shows gradient increment in growth, however and at 22 day, the optical density of G2 and G3 recorded (0.429) and (0.475) respectively, compared with G1 group that recorded (0.196) before entering the decline phase. Other additives also gave a response at a growth rate similar to that of Figure 3. However, increasing the amount of carbonic solution to green micro-algae flasks has led to a significant improvement in growth rate as can be seen from Figure (4). In addition, the adjusting the pH value to 7 has a significant effect when adding a small amount of carbonic solution (5 and 10 ml), while the matching was almost the predominant in the responses when dosing increases (i.e. 15, 20, and 25 ml). For example, the optical density of G2 reached to (0.679, 0.877, 0.990 and 1.182) and G3 (0.718, 0.938, 0.999 and 1.124) at 22 days of experimental working days, when 10, 15, 20, and 25 ml were added respectively. Figure (5) shows clear comparison of the effect of all dosing the aqueous carbon solution in growth rate of G2. It can be observed that the increase of growth association with dosing increment. While Figure (6) shows the comparison of growth rates of G3 flasks under the effect of all aqueous carbon solution dosing and also dramatically increase of growth rate for each one.



**Fig. 4:** Optical Density of *Coelastrella* sp. MH 923012 microgreen algae when the carbonic solution was added; G1: control flask; G2: unadjusted pH flask; G3: adjusted pH flask; (a) add 10 ml; (b) add 15 ml; (c) add 20 ml; (d) add 25 ml



**Fig. 5:** Growth rate estimation of G<sub>2</sub> during addition of five CO<sub>2</sub> concentrations compared with G<sub>1</sub>

**Fig. 6:** Growth rate estimation of G<sub>3</sub> during addition of five CO<sub>2</sub> concentrations compared with G<sub>1</sub>

Table 2 shows that efficiency of the growth rate of *Coelastrella* sp. MH 923012 increases with increasing the supplied amount of carbon source solution into the BG11 flasks. Thus, with simple calculations conducted on sample

of previous studies, Table 3 was extracted. This evolution shows how much Microalgae growth is improved in the current study when compared with previous studies if the exception of the type of algal used was adopted.

**Table 2:** The growth efficiency (as X factor) of G2 and G3 compare with G1

Volume carbonic solution (ml)	Efficiency of G2/G1 (X factor)	Efficiency of G3/G1 (X factor)
5	1.182	1.42
10	2.452	2.65
15	3.462	3.77
20	4.04	4.08
25	5.01	4.71

**Table 3:** The growth efficiency (as X factor) in several previous studies

Type of Microalgae	CO <sub>2</sub>	X factor	References
<i>Nannochloropsis</i> sp.	10%	1.0631	(Saifuddin <i>et al.</i> , 2015)
<i>Nannochloropsis</i> sp.	15%	1.321	(Saifuddin <i>et al.</i> , 2015)
<i>Nannochloropsis oculata</i>	2%	0.621	(Chiu <i>et al.</i> , 2009)
<i>Nannochloropsis oculata</i>	15%	0.257	(Chiu <i>et al.</i> , 2009)
<i>Chlorella</i> PY-ZU1	15%	1.249	(Cheng <i>et al.</i> , 2013)
<i>Chlorella vulgaris</i>	8.50%	4.372	(Rendon <i>et al.</i> , 2013)

The specific growth rate and doubling time of the culture flasks in all groups; G1 control flasks; G2 culture flasks (unadjusted pH), and G3 culture flasks (adjusted pH) were also calculated in the present study. The results showed clearly that the specific growth rate of Microalgae *Coelastrella* sp.MH923012 increases with increasing the amount of carbonic solution dosing, while the doubling time decreases. For example, in 5 ml dosing, the specific growth rate were (0.20971 and 0.21511 day<sup>-1</sup>) and doubling time were (3.3052 and 3.222 day) in G2 and G3 respectively, while in the control flasks (G1), the specific growth rate was (0.15506 day<sup>-1</sup>) and doubling time was (4.4701 day).

Moreover, the results also confirmed that algal growth is determined by the amount of carbon source available in the culture medium. The depletion of carbonate present in the sodium bicarbonate compound reduced growth and reached stable phase within a period of no more than 15 days and 7 days to reach the stage of death. While the other groups (G2 and G3), the growth rate continues to grow confirming that the carbon source is the main limiting factor of the Microalgae *Coelastrella* sp.MH923012.

### Conclusions

The present study showed the ability of *Coelastrella* sp.MH 923012 Microalgae to adapt and survive under different aqueous carbonic solution. The research concluded that the addition of this solution into the culture medium contributes to the abundance of carbon source necessary for the growth of Microalgae, despite the depletion of hydrogen ions by the buffer compound that found in that media. This has been clearly demonstrated by the apparent improvement in Microalgae growth, whatever the dosing times. However, an increase in this quantity has a positive effect on the growth rate. In addition, the present study suggests that the adjusting the pH may not be necessary for the process, as the Microalgae adaptation of the pH decrease is evident by the responses, especially when adding the 15, 20, and 25 ml of carbonic solution. This contributes to improving the economic perspective of that process. The study also

confirmed the importance of carbonic source on the growth of Microalgae if the standard media (such as BG11) is used in the cultivation process.

### Acknowledgements

The authors thank the Iraqi Ministry of Higher Education and the University of Baghdad (College of Science and College of Engineering) for the support for this research.

### References

- Abbasi, T. and Abbasi, S. (2010). Biomass energy and the environmental impacts associated with its production and utilization. *Renewable and sustainable energy reviews*, 14: 919-937.
- Abed, I.J.; Abdulhasan, G.A. and Najem, A.M. (2018). Genotype versus phenotype to determine the definitive identification of the genera *Chlorella beijerinck*, 1890 (chlorellaceae) and *Coelastrella chodat*, 1922 (scendesmaceae). *Bulletin of the Iraq Natural History Museum* (P-ISSN: 1017-8678, E-ISSN: 2311-9799), 15: 101-111.
- Al-Mashhadani, M.K.; Bandulasena, H.H. and Zimmerman, W.B. (2011). CO<sub>2</sub> mass transfer induced through an airlift loop by a microbubble cloud generated by fluidic oscillation. *Industrial & Engineering Chemistry Research*, 51: 1864-1877.
- AL-Mashhadani, M.K.H. and Khudhair, E.M. (2017). Cultivation of *Chlorella vulgaris* Using Airlift Photobioreactor Sparged with 5% CO<sub>2</sub>-Air as a Biofixing Process. *Journal of Engineering*, 23: 22-41.
- Bharathiraja, B.; Chakravarthy, M.; Kumar, R.R.; Yogendran, D.; Yuvaraj, D.; Jayamuthunagai, J.; Kumar, R.P. and Palani, S. (2015). Aquatic biomass (algae) as a future feed stock for bio-refineries: A review on cultivation, processing and products. *Renewable and Sustainable Energy Reviews*, 47: 634-653.
- Bird, R.B.; Stewart, W.E. and Lightfoot, E.N. (2007). *Transport phenomena*. John Wiley & Sons.

- Cheng, J.; Huang, Y.; Feng, J.; Sun, J.; Zhou, J. and Cen, K. (2013). Improving CO<sub>2</sub> fixation efficiency by optimizing *Chlorella* PY-ZU1 culture conditions in sequential bioreactors. *Bioresour Technol*, 144: 321-327.
- Chisti, Y. (2007). Biodiesel from Microalgae. *Biotechnology advances*, 25: 294-306.
- Chiu, S.-Y.; Kao, C.-Y.; Tsai, M.-T.; Ong, S.-C.; Chen, C.-H. and Lin, C.-S. (2009). Lipid accumulation and CO<sub>2</sub> utilization of *Nannochloropsis oculata* in response to CO<sub>2</sub> aeration. *Bioresource technology*, 100: 833-838.
- Chu, S.; Goldemberg, J.; Arungu-Olende, S.; El-Ashry, M.; Davis, G. and Nakicenovic, N. (2007). Lighting the way: Toward a sustainable energy future. InterAcademy Council.
- Derakhshandeh, M. and Un, U.T. (2019). Optimization of Microalgae *Scenedesmus* SP. growth rate using a central composite design statistical approach. *Biomass and Bioenergy*, 122: 211-220.
- Duarte, J.H.; de Morais, E.G.; Radmann, E.M. and Costa, J.A.V. (2017). Biological CO<sub>2</sub> mitigation from coal power plant by *Chlorella fusca* and *Spirulina* sp. *Bioresource technology*, 234: 472-475.
- Gouveia, L. and Oliveira, A.C. (2009). Microalgae as a raw material for biofuels production. *Journal of industrial microbiology & biotechnology*, 36: 269-274.
- Huber, G.W.; Iborra, S. and Corma, A. (2006). Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. *Chemical reviews*, 106: 4044-4098.
- Ibrahim, A.R. and Abdulmajeed, B.A. (2018). Biological Co-existence of the Microalgae–Bacteria System in Dairy Wastewater using photo-bioreactor. *Iraqi Journal of Chemical and Petroleum Engineering*, 19: 1-9.
- Karm, I., 2016: the effect of some fresh water algae extracts in the inhibition of the growth of some microorganism that cause food spoilage. *The Iraqi Journal of Agricultural Science*, 47: 1118-1123.
- Kubba, A.; Al Zubaedi, I.S.; Salih, E.A. and Al Saadi, A. (2016). Evaluation of the cellulolytic fungi isolated from agricultural wastes producing of bioethanol. *The Iraqi Journal of Agricultural Science*, 47: 1111-1117.
- Lynd, L.R. (2017). The grand challenge of cellulosic biofuels. *Nature biotechnology*, 35: 912.
- Nogueira, L.A. (2011). Does biodiesel make sense? *Energy*, 36: 3659-3666.
- Noraini, M.; Ong, H.C.; Badrul, M.J. and Chong, W. (2014). A review on potential enzymatic reaction for biofuel production from algae. *Renewable and sustainable energy reviews*, 39: 24-34.
- Rendon, S.M.; Roldan, G.J.C. and Voroney, R.P. (2013). Effect of carbon dioxide concentration on the growth response of *Chlorella vulgaris* under four different LED illumination. *International Journal of Biotechnology for Wellness Industries*, 2: 125-131.
- Rezvani, S.; Moheimani, N.R. and Bahri, P.A. (2016). Techno-economic assessment of CO<sub>2</sub> bio-fixation using Microalgae in connection with three different state-of-the-art power plants. *Computers & Chemical Engineering*, 84: 290-301.
- Rutherford, J.S.; Wiegman, A.R.; Day, J.W. and Lane, R.R. (2018). Energy and Climate–Global Trends and Their Implications for Delta Restoration. *Mississippi Delta Restoration*. Springer.
- Saifuddin, N.; Aisswarya, K.; Juan, Y. and Priatharsini, P. (2015). Sequestration of high carbon dioxide concentration for induction of lipids in Microalgae for biodiesel production. *Journal of Applied Sciences*, 15: 1045.
- Sambusiti, C.; Bellucci, M.; Zabaniotou, A.; Beneduce, L. and Monlau, F. (2015). Algae as promising feedstocks for fermentative biohydrogen production according to a biorefinery approach: a comprehensive review. *Renewable and Sustainable Energy Reviews*, 44: 20-36.
- Slade, R. and Bauen, A. (2013). Micro-algae cultivation for biofuels: cost, energy balance, environmental impacts and future prospects. *Biomass and bioenergy*, 53: 29-38.
- Yeh, K.L.; Chang, J.S. and Chen, W.M. (2010). Effect of light supply and carbon source on cell growth and cellular composition of a newly isolated Microalgae *Chlorella vulgaris* ESP-31. *Engineering in Life Sciences*, 10: 201-208.
- Ziolkowska, J.R. and Simon, L. (2014). Recent developments and prospects for algae-based fuels in the US. *Renewable and Sustainable Energy Reviews*, 29: 847-853.