



Mesopotamia Environmental Journal

ISSN 2410-2598

journal homepage: www.bumej.com



DOI: <http://dx.doi.org/10.31759/mej.2020.5.4.0085>

Effect of exposure to the different concentration of pure CO₂ gas on growth rates and fatty acids metabolism of green Alga *Chlorococcum humicola*

Ibrahim M.A. Al-Salman and Ragad S. M. Alani

College of Education for pure sciences, University of Baghdad, Iraq.

Corresponding Author : dr.Ibrahim.ima@gmail.com

To cite this article:

Ibrahim MA Al-Salman and Ragad S M Alani , Effect of Exposure to the Different Concentration of Pure CO₂ Gas on Growth Rates and Fatty Acids Metabolism of Green Alga *Chlorococcum humicola* , *Mesopo. environ. j.* 2020, Vol.5, No.4:72-85.

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](https://creativecommons.org/licenses/by-nc-nd/4.0/).



Abstract

The current study examined the effects of the exposure to the different concentrations of the pure CO₂ on the growth rates and fatty acids metabolism of the green algae *Chlorococcum humicola* that isolated locally. The algae cells exposure into many concentrations of CO₂ (25, 50, 75, 100%) v/v of culture media which were (BG11, Chu13, Chu10) for growing. The experiments were conducted in standard laboratory conditions such as temperature, lighting intensity, pH which were respectively (25±2°C, 3000 lux, 7.6) with lighting system 18:16 hour (light :dark). The supplementing of CO₂ every 73 hour by the device (Gas-flow-meter). The results were recorded daily for 18 days. The results have shown that present responses in the growth rates of studied algal cells by all using cultures media that included in this study for growing the algae with best result in Chu13 media. As well as, the present study found that many types of fatty acids in dry biomass of algal cells in vitro by using HPLC technique and recorded the following fatty acids: Myristic C14:0, Palmetic C16:0, Plamitolic C16:1, Arachidic C20:4, Linoleic C18: 2 (Omega:6), Oleic C18:1(Omega 9). The response value was changed between the acids depending on the type of culture media and the following fatty acid: Palmetic C16:09, Myristic C14:0, Palmitolic C16:1, Olic C18:1 and Linolic C18:2) recorded the values (440.64,

474.54, 987, 770, 1054.25, 455.64) mg/L dry weight respectively in two concentrations (100, 75%) v/v when growing the algae in the culture media Chu1. While the acid Arachidic C20:4 recorded highest value (684.35) mg/L in the culture media BG11 at 100% concentration

Keywords: *Chlorococcum humicola*, fatty acids, Growth rate, 'CO₂'

Introduction

Scientific sources indicate that the onset and evolution of microalgae cultivation began about 60 years ago. The factors influencing the growth characteristic of algae, which have been well studied are the lighting and heat factors while there are factors that have not yet been studied in detail, including pH factors and Carbon dioxide, in the case of optical saturation or abundance of light intensity, the adjusted CO₂ rate will double. [1]

As a result of all these data, in the last years of the current decade, serious studies and opinions have emerged that call for control and reduction of CO₂ emission levels without compromising the ecological balance process and the need for it as an important biological element in organic production. It is work in creating a rationing process for the production, consumption and stabilization of this gas, whatever its levels in the environment, or the production of it in technical methods and its use as purified gas from organic and inorganic compounds. Its use in plant cultivation and algae in practical form gives a production and at the same time, the excess of the percentages of the actual need is eliminated. These include the call for the use of living organisms that possess high capacity and distinguish in the photosynthetic organism's and also have the ability to process a sequential extraction and fixation of a duo or as a carbon Sequestration and Biological fixation emitted from stationary sources[2].

According to the researchers, one of these organisms targeted in this practical application to solve this environmental problem is the group of algae in general and microalgae of them in particular, because they are widespread in various saline and freshwater environments. As well as they are present in all other environmental circles wherever available Water, moisture, nutrients and the body or compound on which it is temporarily or permanently based, not to mention the large capacity of this group to capture CO₂ both from the atmosphere and from other sources of emission [3] [4] This process achieves two important goals simultaneously: the first is to get rid of excess gas and the second is to invest this gas in increasing biomass and growth rates and to develop the process of building organic compounds in these algae cells and investing them in different environmental applications.

Material and Methods:

Preparation of the culture medium: Three culture medium were prepared in the form of stock solutions and were sterilized by the combine of the harvester at 121 °c for 15 minutes under pressure of 1.5 atmosphere. Except for the phosphate-containing solutions in the synthesis, they were sterilized through filter paper with a size of (0.45) micrometers, to ensure that it was not deposited by heat on the walls of the tubes when sterilized by the autoclave [5] All the solutions prepared left to cool at the degree of 25 °c then preserved in a degree 4 °c for the use of the media are Chu13 *, BG11 and Chu10 * as noted in [6], [7] and [8].

The preparation of the Algae culture and the design of experiments: The Algae culture for *C.humicola* in the three medium and the skilling up method (i.e. increase in the size of the culture every

3-5 days of cultivation until reaching the target size required for each experiment). The algae were exposed the green algae at the beginning the only one-time experience for different concentrations of carbon dioxide gas when it is developed in the middle Chu13 * by following the steps below:

5 liter plastic containers with UV rays were sterilized for a quarter-hour wavelength of 270-250nm. The necessary size was prepared from the required algae culture not due to the experience, as the medium CHU13 were prepared from stock solutions and the size needed for the experiment. Use the German Supply system-generated CO₂ gas processing device from Sera company shown in the Panel (1).

The gas flow meter device has been used by the (SKC) English company to determine the amount of gas processed for the culture during a given time and measured in litres/minute units.

The prepared cultures were equipped with carbon dioxide gas in the concentrations (100, 75, 50, 25% of the final size of the culture and considered the CHU13 media not equipped with gas as a treatment for control



Panel (1): Two processors and a CO₂ gas processing gauge for pure cultures used in experiments.

The growth rate (k) is calculated based on the following equations: Growth rate (k) = $\frac{\log N_t - \log N_0}{t} \times 3.322$ as N_t = The optical density in time t or the number of cells in time t., N_0 = optical density or number of cells at the beginning of the experiment according to Huang *et al.* (2002):

pH measurement: The pH value of the control and samples as well as the stock solutions and culture medium are measured by pH meter model (ATC) of (Hanna) company daily for control and treatment throughout the duration of the experiment.

Identified of cell count: The cross-sectional method (Transect) was followed according to [9] The number of cells was determined daily for control and treatment and the results were expressed in units (cell $\times 10^6$ /ml) and calculated based on the following rates:

The size per sector (ml) = sector length $\times$ width $\times$ depth of slide, number of sectors in (1 ml) of sample = $1000 \div$ sample size per sector, the number of cells in (1 ml) of the sample = the average number of cells in one sector $\times$ the number of sectors in 1 ml of the sample.

-Harvest of algae culture: The algae culture was harvested by the centrifuge (Japanese Hittachi company) with a speed of 3000 rpm for 5 minutes. The deposit was placed in a

laboratory dish and the samples were left to dry at 45 °C at oven for 48 hours or until dry. Then weighed in the delicate balance of four mattresses and the samples were preserved in sterile plastic bags sealed in the darkness at a temperature of 25°C to determine the amount of the living mass of the treatments, and preserved for use [10] [11].

Measurement of fatty acids in algae: -

1. The weight (100 mg) of dry moss used in the experiment for the purpose of extracting fatty acids. The algae was added to (1 ml) of the extraction solution (1:1 methanol: chlorine foam) for a period of (15 minutes) and requested a device (Vortex).
2. The samples were placed in the centrifuge system at speed (3000) rpm for 5 minutes. After the remove of the steel deposit took (1 ml) of the extracted fat and added (4 ml) of solution (water: isopropanol by 3:2) and used (1 ml) of solution (KOH) with concentration (5 N) after pulling (50 micro and Liter) of the Add (1 ml) of solution (water: ISO propanol by 3:2) (1 ml) of KOH with a concentration (5 N).
3. The samples were transported to a water bath at a temperature (80°C) for a full hour with the continuous shake by Shaker water bath. Then the samples were left to cool at room temperature. A (500 micro-liter) of each sample has been added (25 micro-liters) of formic acid (neutralize) samples.

-Use Millipore filter paper with openings (0.2 mm) for the purpose of some times the samples in a device column (HPLC) under standard separation conditions. The column specification is particle size (um3) and dimensions (50mm × 4.6).

Mobil phase: A Cetone nitrite: tetra hydro furan (THF): 0.1%. Phosphoric acid in the (50.4:21.6: 28 v/v).

UV ultraviolet radiation was detected at 10 wavelength UV with a flow rate equal to (1.5 ml) min and temperature (40°C). The final concentrations of fatty acids are calculated according to the equation:

(cons.sug = $A_2/A_1 \times \text{Cons of Standard} \times D.F$).: Cons. S = lipid concentration in the sample (micro gram. 1-), A_1 = confined distance under the fatty acid curve, A_2 = the distance trapped under the acid curve in the sample, Consecration of standard = standard acid concentration, d. F = investigation coefficient [12] [13] The results were analyzed by using the statistical analysis system-SAS (2012) program in analyzing the data and comparing the moral differences between the averages with the test of the lesser significant difference Least significant difference – LSD and [14] Multiple range test).

Results and Discussion.

Table (1) shows the growth rates of the laboratory in the Chu13 media after exposure to CO₂ gas in the concentrations used in the experiment and rely on the number of cells recorded rates (0.52, 2.13, 3.27, 0.54) for the concentrations (100, 75, 50, 25)% volume/volume respectively. While control (0.39) was recorded on the first day, on the third day, growth rates were lower than control in the two concentrations (100, 25%). the concentration (75, 50%) scored higher than the third day. However, in the sixth and ninth days all the concentrations were recorded, except for (25%) higher levels of control and since the 12th day of the experiment the rates have become higher for all concentrations compared to control until the end of the experiment.

Table (1): The growth rate of the algae *Ch. humicola* when it is exposed to CO₂ gas every 72 hours depending on the number of cells in the Chu13 media during the duration of the study.

Time of treatment/Days	Concentrations				
	CONT	25%	50%	75%	100%
1	0.39	0.52	2.13	3.27	0.54
2	0.50	0.13	0.92	0.89	0.27
3	0.54	0.07	0.69	0.57	0.47
4	0.43	0.05	0.49	0.38	0.43
5	0.36	0.09	0.35	0.26	0.52
6	0.31	0.10	0.26	0.17	0.52
7	0.34	0.01	0.26	0.26	0.44
8	0.35	0.08	0.25	0.29	0.38
9	0.33	0.12	0.25	0.30	0.35
10	0.29	0.30	0.31	0.34	0.31
11	0.23	0.35	0.33	0.35	0.28
12	0.19	0.37	0.34	0.35	0.27
13	0.19	0.33	0.30	0.32	0.26
14	0.19	0.30	0.27	0.29	0.25
15	0.18	0.27	0.24	0.27	0.24
16	0.18	0.24	0.21	0.25	0.24
17	0.18	0.22	0.18	0.22	0.23
18	0.18	0.20	0.17	0.21	0.21

When developing algae in the media Chu-10 table (2) the study found that the results of growth rates depending on the number of cells after exposure to gas every 72 hours recorded higher values of control on the first day, especially in the 100, 25% (1.83, 2.03) respectively. On the third day amounted to (0.67, 0.71) for two concentrations 100, 25% respectively, while the control recorded 0.57 on the sixth day, rates were lower, for all concentrations and for moral differences. Whereas the differences were slight on the ninth day of the treatment, from the twelfth day to the end of the experiment recorded higher rates of control in the treatments (100, 75, 50, 25)% (100, 75, 50, 25%).

Table (2): The growth rate of the algae *Ch. humicola* when exposed to CO₂ gas every 72 hours depending on the number of cells in the CHU10 media during the duration of the study.

Time of treatment/Days	Concentrations				
	Cont Chu10	25%	50%	75%	100%
1	1.09	2.03	1.55	1.59	1.83
2	0.72	1.03	0.84	0.84	0.96
3	0.57	0.69	0.60	0.59	0.67
4	0.49	0.52	0.46	0.45	0.51
5	0.43	0.42	0.38	0.37	0.41
6	0.39	0.35	0.33	0.32	0.35
7	0.30	0.30	0.26	0.26	0.30
8	0.23	0.27	0.21	0.22	0.27
9	0.18	0.24	0.17	0.18	0.24
10	0.12	0.21	0.13	0.15	0.21
11	0.07	0.20	0.10	0.13	0.19

12	0.02	0.18	0.07	0.11	0.18
13	0.02	0.17	0.06	0.10	0.17
14	0.02	0.15	0.06	0.09	0.15
15	0.02	0.14	0.05	0.08	0.14
16	0.01	0.14	0.05	0.08	0.14
17	0.03	0.13	0.04	0.08	0.13
18	0.04	0.12	0.06	0.08	0.12

Table (3) shows the growth rate (K) of the laboratory milking when exposure to CO₂ gas every 72 hours after its development in the media BG11, depending on the number of cells, if the concentrations (100, 50)% rates (1.68, 1.85) respectively for the first day. While control (0.90) while the growth rates decreased and for all the concentrations of control on the third day, the sixth day recorded a rise in the growth rate at the concentrations (75)% (0.51) and recorded control and concentrations 25% (0.31). Whereas other concentrations decreased from control, the ninth day recorded a rise in concentrations 25 and 75% (0.38, 0.36) respectively while the control recorded the growth rate (0.24). As this rise was shown on the twelfth day to the end of the experiment.

Table (3): The rate of growth of the milking *Ch. humicola* when exposed to CO₂ gas every 72 hours depending on the number of cells in the BG11 media during the duration of the study.

Time of treatment/Days	Concentrations				
	Cont. BG11	25%	50%	75%	100%
1	0.90	0.74	1.85	0.42	1.68
2	0.52	0.00	0.58	0.23	0.50
3	0.36	0.19	0.08	0.30	0.44
4	0.34	0.18	0.05	0.50	0.33
5	0.32	0.28	0.09	0.53	0.15
6	0.31	0.31	0.14	0.51	0.12
7	0.28	0.35	0.18	0.45	0.00
8	0.26	0.35	0.20	0.41	0.03
9	0.24	0.36	0.21	0.38	0.03
10	0.22	0.32	0.19	0.34	0.03
11	0.21	0.30	0.18	0.32	0.02
12	0.20	0.27	0.16	0.29	0.02
13	0.19	0.25	0.15	0.28	0.02
14	0.18	0.24	0.14	0.26	0.02
15	0.17	0.22	0.13	0.25	0.02

16	0.16	0.21	0.13	0.23	0.02
17	0.16	0.20	0.12	0.22	0.02
18	0.15	0.19	0.11	0.21	0.01

From the tables (4, 5, 6) in the order showing the results of the detection of fatty acids after the exposure of the laboratory algae to gas every 72 hours, table 3 shows the chemical content of these acids for the green milking when it is developed in the media of Chu13. The results were found that the acid myristic record values (87.2, 322.9, 236.57, 194.4) mg/ G dry weight of the track (100, 75, 50, 25%) respectively, while control (90.86) mg/g dry weight was recorded as either acid palmitic C16:0 (242.3) m/g dry weight in control while the value of the values (319.2, 364.4, 378.30, 390.8) mg/g dry weight in the trigrams used in the experiment respectively. While the acid record palmitolic C16:1 recorded control (481.9) while rose in concentration and recorded (627.2, 820.8, 624.34, 728.3) mg/g dry weight. The acid control (OMEGA9) Oleic C18:1 registered (187.6) mg/g dry weight while the concentrations (239.7, 428.9, 331.65, 339.96) registered mg/g dry weight respectively. As C18 acid control record: 1 linolic (OMEGA6) control (288.9) mg/g dry weight. Whereas the treatments were recorded every 72 hours (264.9, 350.2, 364.75, 192.24) mg/g dry weight. The arachidic C20 acid recorded: 4 (290.4) mg/g weight compared to non-treatment from the Chu13 media between the countries (149.32 269.6, 341.3, 295.90) mg/g dry weight in the concentrations (100, 75, 50, 25)% respectively.

Table (4): Chemical content of fatty acids for green milking *Ch. humicola* when exposed to different concentrations of CO₂ gas every 72 hours and its growing in the Chu13 media.

Compounds	Concentration (%)					LS D
	Control	25 %	50 %	75 %	100 %	
Myristic	90.86 ± 5.19 d	194.4 ± 5.77 c	236.57 ± 11.5 b	322.9 ± 17.3 a	87.2 ± 4.62 d	31. 98 *
Palmetic C16:0	242.3 ± 11.55 c	390.8 ± 17.3 a	378.30 ± 17.3 a	364.4 ± 12.3 a	319.2 ± 17.3 b	51. 45 *
Palmitolic C16:1	481.9 ± 23.09 c	728.3 ± 40.4 ab	624.34 ± 34.6 b	820.8 ± 46.2 a	627.2 ± 34.6 b	11 5.4 *
Oleic 18:1 Omega 9	187.6 ± 5.77 d	339.96 ± 17.3 b	331.65 ± 16.5 b	428.9 ± 23.1 a	239.7 ± 1.5 c	50. 23 *
Linoleic C18:1 Omega 6	288.9 ± 11.51 b	192.24 ± 5.77 c	364.75 ± 17.3 a	350.2 ± 16.3 a	264.9 ± 11.5 b	42. 25 *
Arachidic C20:4	290.4 ± 11.54 b	149.32 ± 5.77 c	295.90 ± 11.5 b	341.3 ± 17.3 a	269.6 ± 16.5 b	38. 16 *

Table (5) shows the chemical content of some fatty acids of *Ch. humicola*, when it is developed in the (CHU10) media if the acid registers C14:0 myristic (181.56) mg/g dry weight in non-treated control either the concentration (100, 75, 50, 25)% recorded (400.7, 474.5, 212.8, 213.4) mg/g dry weight respectively. Acid control (palmetic C16:0) was recorded as a value (336.58) mg/g dry weight while the concentrations (548.4, 770.9, 422.9, 436.89) mg/g dry weight were recorded, as well as the acid palmitolic C16:1 control was recorded (635.15) mg/g dry weight, while the Triakis (1054, 1048, 920.3, 837.62) m Mine/g dry weight. As the Oleic C18 acid record: 1 (omega9) amounts amounted to (455.6, 266.5334.4, 401.17) mg/g dry weight while non-treated (control) recorded (190.98) mg/L. C18 acid: 2 linoleic (OMEGA6) (440.6, 327.4, 351, 357.17) mg/g dry weight of the used in the experiment while the control recorded (222.44) mg/g dry weight, whereas the acid record Arachidic C20:0 (441.3, 347.9, 306.52, 156.99) mg/g dry weight in the concentrations (100, 75, 50, 25)% control of the Chu 10 non-gas treatment (253.75) mg/g dry weight.

Table (5): Chemical content of fatty acids for *Ch. humicola* when exposed to different concentrations of CO₂ gas every 72 hours and its development in the Chu10 media.

Compounds	Concentration (%)	LSD				
	Control	25 %	50 %	75 %	100 %	
Myristic	181.56 ± 5.77 c	213.4 ± 11.5 c	213.8 ± 13.0 c	474.5 ± 23.1 a	400.7 ± 33.1 b	52.8 *
Palmetic C16:0	336.58 ± 17.32 d	436.89 ± 33.1 c	422.9 ± 23.1 cd	770.9 ± 40.4 a	548.4 ± 28.9 b	87.2 *
Palmitolic C16:1	635.15 ± 34.64 c	837.62 ± 46.2 b	920.3 ± 51.9 ab	1048 ± 57.7 a	1054 ± 57.7 a	158.8 *
Oleic 18:1 Omega 9	190.98 ± 5.77 d	401.17 ± 23.1 a	334.4 ± 17.3 b	266.5 ± 11.5 c	455.6 ± 23.1 a	55.2 *
Linoleic C18:1 Omega 6	222.44 ± 11.54 c	375.17 ± 17.3 b	351.0 ± 17.3 b	327.4 ± 17.3 b	440.6 ± 13.1 a	55.8 *
Arachidic C20:4	253.75 ± 11.54 c	156.99 ± 5.77 d	306.52 ± 17.3 b	347.9 ± 17.3 b	441.3 ± 21.2 a	50.8 *

The values of fatty acids of green milking used in the experiment at the time of treatment every 72 hours with the CO₂ used and the growing medium BG11 of the Myristic acid recorded C14:0 (171.14) mg/g dry weight in non-gas-treated (control), while (324.7, 365.5, 302.9, 224.2) mg/g dry weight of the concentrations (%) (100, 75, 50, 25, respectively). Whereas the acid record palmitic C16:0 (361.51) mg/g dry weight in control and recorded in the four concentrations (457.3, 333.8, 507.8, 317.9) mg/g dry weight respectively. As well as control of palmitic acid (616.91) mg/g dry weight, while in used concentrations recorded values (998.3, 880.9, 703.4, 736.9) mg/g dry weight. For C18:1 Oleic in control (200.58) for concentrations recorded (245.7, 399.5, 310.2, 274.8) mg/g dry weight respectively. In acid linoleic control (188.55) and for treatment in the concentrations (561.0, 378.6, 339.5, 320.53) mg/g dry weight, the acid C20:4 Arachidic recorded Control it (198.32) mg/g dry weight while registered (684.4, 304.6, 305.6, 314.67) mg/g dry weight for treatments in the concentrations (100, 75, 50, 25%) respectively (Table 6).

Table (6): Chemical content of fatty acids for green milking *Ch. humicola* when exposed to different concentrations of CO₂ gas every 72 hours and its development in the BG11 media.

Compounds	Concentration (%)					LSD
	Control	25 %	50 %	75 %	100 %	
Myristic	171.14 ± 5.77 d	224.2 ± 11.5 c	302.9 ± 17.3 b	365.5 ± 17.3 a	324.7 ± 17.3 ab	46.0 *
Palmitic C16:0	361.51 ± 17.32 b	317.9 ± 12.3b	507.8 ± 28.8 a	333.8 ± 17.3 b	457.3 ± 23.1 a	67.1 *
Palmitic C16:1	616.91 ± 14.64 b	736.9 ± 40.4 b	703.4 ± 26.4 b	880.9 ± 46.2 a	998.3 ± 51.9 a	128.9 *
Oleic 18:1 Omega 9	200.58 ± 11.54 d	274.8 ± 17.6 bc	310.2 ± 13.1 b	399.5 ± 17.3 a	254.7 ± 11.5 c	48.4 *
Linoleic C18:1 Omega 6	188.55 ± 5.77 c	320.53 ± 14.3 b	339.5 ± 15.3 b	378.6 ± 16.2 b	561.0 ± 28.9 a	58.6 *
Arachidic C20:4	198.32 ± 5.77 c	314.67 ± 16.3 b	305.6 ± 17.3 b	304.6 ± 17.3 b	684.4 ± 34.6 a	65.1 *

From the results of measuring the fatty acids of algae after exposure every 72 hours to CO₂ gas and its development in the Chu13 and BG11 medium and Chu10 we find that the highest values recorded for acids palmitic C16:0 Myristic C14:0 and C16:1 palmitic and linoleic C18:1 oleic C18:1 in the Chu-10 medium in the two concentrations (100 – 75)% respectively and amounted to (440.6, 455.6, 1054.0, 770.9, 474.5) mg/g dry weight respectively. While arachidic (OMEGA9) scored the highest value in the BG11 media when 100% concentration in gas it was amounted to (684.4) mg/g dry weight, while the lowest amount of fatty acids after treatment with gas was recorded every 72 hours at the Chu13 media in the non-treated control samples and amounted to (187.6, 481.9, 242.3, 90.86) mg/g dry weight of acids myristic, palmitic, palmitic and oleic respectively. Whereas the minimum amount of arachidic was reduced when the concentration was

25% for the same medium Chul3 while it was less valuable for the acid linolic when control was not treated to the center BG11 and amounted to (188.55) mg/g dry wt. dry weight (tables 4, 5, 6). By following the growth rate response of the laboratory algal cells to the different concentrations of CO₂ as in the tables (3, 2, 1) the results found that they have indicated different values. The results can be explained what happened in the first four days that is a state of stimulation or initial reaction of moss cells to increase the levels of CO₂ processed for the growing medium, followed by a state of gradual adaptation of the presence of the additive. Then the onset of a state of equilibrium and gradual rise of growth, and thus the growth curve reaches the state of stability. The results also find very similar similarities in some of the results when relying on the calculation of the number of cells, where the initial stimulation. Then adjustment in the number and convergence with the standard sample of different concentrations, especially in values representing the growth rate dependent on the number of cells or when calculating the time of multiplication. Therefore, these mechanisms can be explained by the reaction and inverse reaction between the cells of the algae and the change of the equipped CO₂ concentrations as a condition that begins with the condition of the cell acclimatization of this increase and dealt with several aspects of the increase of the number or change in color or size and even the shape of the cells to deal with a new external influence in the medium of growing outside the adaptation contexts of the components of the center gradually, whatever the nature of the additive (gas, salt, vitamin, heavy metals, pesticide or different plant nutrients), These findings are in line with researchers' studies [15], [16] [17] [18] [19]. which recorded a stimulating process in the early days and a change in the size and shape of the cells of the tested organisms (algae and fungi) under the influence of various factors of catalysts or growth inhibitors.

When the results are followed in tables (6, 5, 4) showing the results of changes in fatty acid values at the time of treatment every 72 hours. The results also found a clear variation in the values and distribution of fatty acids detected in algae samples by HPLC technique: (Arachidic C20:4, Linoleic C18:2) Omega-6), Oleic C18:1 (Omega-9), Plamitolic C16:1, Palmetic C16:0, Myristic C14:0. The direction of the four concentrations (100, 75, 50, 25%) of the processed CO₂ gas. Although all of these acids are in response to the increase in CO₂ levels prepared for the culture media, starting from a 25% concentration treatment, this response varies depending on the type of fatty acid, the concentration quantity and the composition of the culture medium.

These conclusions are consistent with the researchers [20] [21] and [22] who have been told that the microalgae response represent one of the criteria that is adopted in estimating the efficacy of any species or genus in endurance or fixation and pulling effective concentrations of any additive or reduced from the culture medium. This view is further supported by researchers [23] [24] who found sex algae *Chlorococcum* spp. that vary in life, growth rates, and time of multiplication when the components of the median are altered, including CO₂ gas values, whether natural, processed or combined with gases. As well as this effect in the hematological physiological properties of cells, for example, that algae *C. humicola* has registered the highest biomass and the amount of fat when cultivated in the of blood base medium compared to the center of sodium alginate and the diversity of fatty acids also changed, as 70% of the of saturated acids and 12.2% of the polyunsaturated.

These conclusions are also in line with the findings of the current study with researchers [25] [26], [27], [28], [29]. that the response extends to the level of internal construction of compounds

within the algal cells work to modulating or develop quantitative and qualitative amounts of pigments, fats, proteins, carbohydrates, amino acids, fatty acids and other biological compounds. Some of which increase or disappear or change in their original composition. As a result of the effect of public activity for the light and the process of photosynthesis especially in algae, through several studies they have done to know the impact of the type of treatment and the nature of the medium and the quantity and quality of the added factor and the method and time of addition, which found from the results of experiments that all factors affect the nature of the response of cells and that these cells change the size. The form and nature of tinctures as well as the chemical content through studies on different types of microalgae including *Chlorococcum* sp., *Chlorella* sp., *Scenedesmus* sp., *Anabena* sp., *Danella* sp, *Spirulina* sp. and others.

While some researchers argue that the genus or species of algae used is the variable even in the case of the development of several algae in the middle of the development of unified cultured medium. [30] tested the efficacy of different types of algae in installing CO₃ and CO₂ from water (sewage) as a medium of development for the team, they found that *Chlorella vulgaris* algae could install what values of 624 CO₃/L/day and 26.0 gm/m²/h of CO₂ gas and in the media of 15 v/v% while algae *Synechocystis aquatilis* can stabilize about 1500 gm/m²/h of CO₂.

The researchers [31], according to an experiment to follow the influence of different concentrations of CO₂ on several green algae from freshwater algae (*Chlorella*, *Scenedesmus quadricauda*, *S. dimorphus*) were developed in the center of the (Bristol medium) The axis that added CO₂ in the concentrations (168-3%). The results of the experiment indicated that the three algae gave varying growth rates, and that the highest rates were in the concentrations of 100, 30% CO₂ and the highest level of activity of photosynthesis in algae cells (*Scenedesmus*, *Chlorella*).

References:

[1]- Abdel-rahman, M.H.M; ALI, R.M and SAID, H.A (2005). Alleviation of NaCl-induced Effects on *Chlorella vulgaris* and *Chlorococcum humicola* by Riboflavin Application. international journal of agriculture & biology. 07-i58-62 .

[2]- Al - Akaily. T.M and Alsalman, I.M.A (2016). Stimulation of growth rate and doubling times for green alga *Scenedesmus dimorpha* by using different culture medium. J of Dyala Unever- Iraq

[3]- Al-Aarajy, M. (1996). Studies on the mass culture of some microalgae as food for fish larvae. Ph. D. thesis, Univ. Basrah, Pp: 107 .

[4]- Al-Hattab, M and Ghaly, A (2015). Production of Biodiesel from Marine and Freshwater Microalgae: A Review. Advances in Res, .3(2): 107-155 .

[5]- Alsalman, I.M ; Svetlova, E.N and Plehkanov, S.E (1989). Sulfate stress on culture of *Scenedesmus quadricauda*. J of Biol- Sci, Moscow state University, no,7 pp 70-74. Moscow-USSR .

[6]- Alsalman, I.M, A and Al- Akaily, T.M.I (2018). Stimulation of total protein in three locally isolated green algae. 5th Inter Sci , conf of Gent and Environ . 27-28 March. Baghdad- Iraq .

- [7]- Berges, L; Defaria, B; Oderbrect, C and Abreu, P (2007). Potintial adsorption of CO₂ by microalgae cultivated in aquaculture. Attantica, Rio Grande. 296: 35-46
- [8]- Currasi,V.;Mangione,M.R.;Sanfratello,V.(2010).Quotation of fatty acid from vegetable oil by HPLC with UV detection chromatographic science ,pp:48
- [9]- Eppley, R. W. ; Harrison, W. G.; Ghisholm, S. W. and Stewart, E. (1977). Particulate organic matter in surface waters off southern California and its relationship to phytoplankton. J. Mar. Res., (34): 671-696
- [10]-Flakowski ,P.G and Raven, J.A (1997). Aquatic photosynthesis. Electronic resource. Book: <http://trove.Nla.gov.au/version/220768508:375>
- [11]- Gilmour, D.J and Zimmerman, W.B (2012). Can algal biofuels play a major role in meeting future energy needs? Biofuels 3: 511-513
- [12]- Hopkinson, B; Dalin, Y. Xu; Patrick J; McGinn,1 and Morel, F(2010).The effect of CO₂ on the photosynthetic physiology of phytoplankton in the Gulf
- [13]- Huang , X.H.; Li, C.L.;Liu, C.W. and Zeng , D,Q.(2002). Studies on borgei . J . Zhanjiang Ocean Univstily 22(3):8-12
- [14]- Jawad, A.M. (1982). Interaction between cyanobacteria and other microorganisms. Ph.D. thesis. Liverpool University. England. 5(9):1139-1142
- [15]- Jones,J.;Manning,S.;Montorya,M.;Keller,K.and Poenie,M.(2012).Extraction of algae lipids and their analysis by HPLC and mass spectrometry. J.Am.Oil Chem.Soc.,(89):1371-1381
- [16]- Kassim,T.I.; AL-saadiah and Salman,N.A.(1999).Production of some phytoplankton and zooplankton and their use as live food for fish larva. Iraqi.J.agric proc.of 2ndsa.confer.4(5):188-201
- [17]- Kuei, L .Y; Jo-Shu C and Chen, W (2010). Effect of light supply and carbon source on cell growth and cellular composition of a newly isolated microalga *Chlorella vulgaris* ESP-31. EngLifeSci;10(3):201–8
- [18]- Martinez , M.R.; chakroff , R.P. and pantastico , J.B.(1975). Note on direct phy to plankton Hacmocy to meter.phil. Agric . 57:1-12. of Alaska. Oceanogr., 55(5):2011–2024.Phycol2017;29(2):949-982
- [19]- Minillo, A; Cardamoni, and Fonseca, N (2013). Growth performance of microalgae exposed to CO₂. Clean Ener Technol. 1 (2): 110-14
- [20]- Olaizola, M (2003). Microalgal removal of CO₂ from flue gases: changes in medium pH and flue gas composition do not appear to affect the photochemical yield of microalgal cultures. Biotechnology and Bioprocess Engineering 8: 360-367
- [21]- Ono, E and Cuello, J.L (2003). Selection of optimal microalgae species for CO₂ sequestration

- [22]- Rippka, R. & Herdman, M. (1992). Pasteur Culture Collection of Cyanobacterial Catalogue and taxonomic handbook ,vol . 1: Catalogue of Strains, Inst. Pasteur, Paris ,PP: 103
- [23]- Santhoshkumar, K.1; Prasanthkumar, S.1and Ray, J. G (2016). Chlorococcum humicola (Nageli) Rabenhorst as a Renewable Source of Bioproducts and Biofuel. Plant Studies. 5(1): 49-57
- [24]- SAS. (2012). Statistical Analysis System Users Guide. Statistical.Version 9.1 th ed. SAS. Inst. Inc. Cary. N.C. USA
- [25]- Shaker, B.K; Alsalman, I.M and ALatabi, M.S (2018). Bioremediation of pesticide Glyphosate (Ground –UP SL) and remove Cd, Cr elements from polluted aquatic water medium by using Fungi (Asprgilus niger , Tricoderma harzanium). Biochem and Cel. Archi(Accepted 13 May).
- [26]Yamaguchi,K.;Nakano,H.;Murakami,M.;Kansu,S.;Nakayama,O.;Kanda,M.;Nakmura,A.and Iwamoto,H.(1987).Lipid composition of green algae Botryococcus branrii,agric.biol.chem.51(2):493-498
- [27]- Yang, Y and Gao,K (2003). Effect of CO2 concentrations on the freshwater microalgae (Chlamydomonas, Scenedesmus and Chlorella). Appli Phycol. 55:1-11
- [28]- Yang,Y (2013). Effect of temperature , light intensity , carbon dioxide and culture medium nutrient on growth and lipid production of Ettlia oleoabundans algae. Ph.D thesis, Worcester polytec, insti, Chaina.151pp
- [29]-Ying, K; James G,D and Zimmerman W.B (2018) Effects of CO2 and pH on Growth of the Microalga Dunaliella salina. J Microbiol, Biochem Technol 6:167-173. doi: 10.4172/1948-5948.1000138