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RESEARCH ARTICLE

Response of the Green Alga *Chlorococcum humicola* Developed in Chu-13 Culture Medium to Different Concentrations of CO_2

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Abstract

Microalgae response is one of the criteria that is adopted in estimating the efficacy of any species or genus in tolerance or the fixation and withdrawal of the influential concentrations of CO_2 either in its natural or supplied or combined forms with other gases. For the purpose of the application, the current study was chosen *Chlorococcum humicola* alga belonging to the green algae to follow the effect of different concentrations of CO_2 supplier were (25, 50, 75, 100%) volume/volume, of culture volume in curves, growth rates, times of multiplication, biomass and some fatty acids. The experiment and development of alga in the Chu-13 culture medium was carried out under controlled environmental conditions of temperature and density of light and pH, as they were in the order of (25 ±2, 3000 lux, 7.6) and with a lighting system of 16:8 hour light to darkness. The alga cells was incurred into mentioned concentrations for one time by injection of the algal culture by CO_2 using a device (gas-flow-meter) for three repeaters of each concentration. The results were recorded at time of Zero and daily for 18 days. The results showed that the growth curve based on the absorbency values at the wavelength 650 nm recorded from the fourth day a higher value than the standard sample of all the concentrations. The highest values of 0.71 with the concentration (50%).As for the time of multiplication, the study found that a slight differences between the standard sample and all concentrations and recorded the highest value (1.84) in the control sample for the 18th day. The dry biomass at harvest in the end of the experiment, the values were recorded (370, 360, 410, 340) mg dry weight/L at the concentrations (25, 50, 75, 100%) volume/volume respectively, while the control recorded 310 mg/dry weight/ L. The HPLC technique that has been applied to diagnose some fatty acids has also indicated the presence of acids: (Arachidic C20:4, LinoleicC18: 2(Omega6), Oleic C18:1(Omega 9), Plamitolic C16:1, Palmetic C16:0, Myristic C14:0). The highest value (403.93) % was recorded for the palmetic acid with a 100% concentration of CO_2 supplier and a lower value of (82.38) % for arachidic acid with a 25% concentration of CO_2 compared with the standard sample. The conclusion of the study that the treatment with CO_2 although it has not given growth rates and times is remarkably multiplied but on the other side it gave an increase in the biomass ranged of (19.35, 16.13, 32.25, 9.67%) for CO_2 concentrations (25, 50, 75, 100%) volume/volume respectively. As well as identified fatty acids stimulated the increase of total percentage after harvest on day 18th of the treatment with values: (32.443, 47.820, 53.443, 121.211, 144.409, 262.636) for each (Palmetic, Pamiotolic, Myristic Oleic, Linoleic, Arachidic) respectively.

Keywords: *Microalgae, CO_2, Biofixation, Capturing of CO_2, Chlorococcum humicola alga.*

Introduction

The deterioration in the quality and quantity of natural resources as a result of the growth of the population in recent years, on the one hand, and the high rate of overexploitation of these resources by the human being and the fact that serious climatic changes have taken place in different parts of the world, on the other hand. All this factors have prompted research institutions and centers science, universities and institutes even at the level of researchers and interested in the conservation of environmental resources and sustainable development to inspect renewable energies, so that they are become effective and environmentally friendly on the one hand and inexpensive on the other. Hence, start thinking about investing cells solar and wind energy produce bioethanol and bio-fuels biodiesel by thinking of investing non-economic plants or residues

from large plants such as cellulose or from fermentation of organic residues. Finally moving to the algae investment especially microscopic of them after proven its superiority over all sources [1, 2, 3, 4].The researchers found that this bioproduction and the use of algae in particular requires abundant amounts of energy represented by visible light and carbon source represented by carbon dioxide CO_2. This later was the main factor in the process of organic synthesis, the increase of biomass, growth rates for algae and development their metabolism and their physiological and biochemical characteristics in achieving the highest levels of biomass and growth rates. At the same time developing and stimulating the mechanisms of the biovital compounds synthesis represented by carbohydrates, fats and total proteins furthermore fatty acids, enzymes and other organic compounds are important in practical applications for algae uses in food sources, pharmaceutical industries or dietary supplements etc[5, 6, 7]. Although scientific sources indicate that the onset and evolution of microalgae cultivation began about 60 years ago, some factors affecting algae growth have been studied well, such as lighting and heat factors, while there are factors that have not yet been studied in detail, including pH, concentrations and quality of carbon dioxide gas, optical saturation, the nature and quantity of nutrients and other factors [8, 9]. As well as the emphasis and advocacy for the use photosynthetic organisms and correspondingly also has high capacity in the process of sequestration and fixation of CO_2 gas emitted from various sources took on a great deal of interest from environmentalists, algae and biotechnology [10, 11].

Researchers believe that one of these organisms targeted in this practical application to solve this environmental problem is the group of algae in general and microalgae in particular, because they are widespread in various saline and freshwater environments and are present in all other environmental milieu wherever available water, moisture, nutrients and the body or compound on which it is temporarily or permanently based as well as the large capacity of this group to capture CO_2 both from atmosphere or from other emission sources [12, 13]. 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.

Materials and Methods

A pure culture of alga *Chlorococcum humicola* was obtained from the Advanced Algae Laboratory in the Department of Biology, college of Education for Pure Sciences (Ibn Al-Haitham), University of Baghdad, then the development of alga in the modified Chu 13 culture medium by [14]. The development was carried out under laboratory conditions of temperature 25±2, density of lighting 3000 lux, and optical system 8:16 hours light: darkness). The studied alga was exposed to four different concentrations of pure CO_2 gas compressed (25, 50, 75, 100) % of the volume of the culture (5 L development bottles) one time at the beginning of the experiment only using a German-made gas flow-meter. The samples were repeated by three duplicates per sample.

The effect of CO_2 supplier daily in the life of the alga cells was followed up for 18 days, by measuring the absorbency values at 650 nm wavelength to determine the density of the alga cells according to [15]. The calculating of cells number using transect method as stated in [16] to draw growth curves and calculate growth rate and multiplication times after exposure based on the following equation:

Growth rate (K) = t 3.322÷ Log Nt – Log N0, Multiplication times= Log2 ÷K*

The dry biomass of alga was measured at the end of the experiment and the operation (harvest), using the centrifugal method at speeds of 5000 cycles/minute for five minutes, as well as the measurement of some fatty acids of the studied alga using the technique of HPLC device as reported in [17].

Results

The growth curve shows depending on the absorbency values to expose the green alga *Ch. humicola* to the different concentrations of carbon dioxide gas when developed in the culture medium Chu13 at the beginning of the experiment a clear convergence to the

third day after exposure, while the absorbency recorded higher values of control at the fourth day to the end of the experiment (18th Day). The value recorded at this concentration was the highest compared to the rest concentrations. When relying on the number of cells for the algae culture, the results showed that their numbers recorded a clear increase from the first day higher than the control, especially in the two concentrations (50, 100%) and the rest concentrations were higher than the control to the seventh day of the experiment, then the cellular numbers became close to control and concentrations (25, 75) %. At the calculating the growth rate of alga depending on the values of absorbency, it's found that the control medium recorded higher values than the concentrations in the first and second days, but on the eighth day, there were lower growth rates than the two concentrations (25, 50%) and above (75, 100%), while higher growth rates of control were recorded in the days (4-7) with clearly, growth rates were higher in concentration than 50% of the day (7-13) and then the rates were became very close (Table 1).

Table 1: Growth rate of *Ch. humicula* under the influence of different concentrations of CO_2 depending on the absorbency values

Growth rate (K)	Cont. Chu13	25%	50%	75%	100%
1	0.67	0.39	0.35	0.33	0.58
2	0.76	0.74	0.71	0.59	0.66
3	0.55	0.57	0.60	0.50	0.55
4	0.41	0.48	0.53	0.44	0.47
5	0.34	0.42	0.47	0.40	0.45
6	0.33	0.38	0.43	0.36	0.38
7	0.33	0.34	0.36	0.33	0.34
8	0.30	0.31	0.33	0.31	0.30
9	0.29	0.29	0.32	0.29	0.30
10	0.26	0.27	0.29	0.27	0.28
11	0.24	0.25	0.27	0.25	0.26
12	0.22	0.23	0.25	0.23	0.24
13	0.21	0.22	0.23	0.21	0.23
14	0.20	0.20	0.22	0.21	0.22
15	0.19	0.19	0.21	0.20	0.21
16	0.18	0.18	0.20	0.19	0.20
17	0.17	0.17	0.19	0.18	0.19
18	0.16	0.16	0.18	0.17	0.18
Mean	0.32	0.32	0.34	0.30	0.34

While calculating the time of multiplication based on the values of absorbency, the differences were slight and intangible among all the concentrations in the experiment and control. The highest value for the time of multiplication at the control on the eighteenth day and reached (1.84) day whereas the lowest time of multiplication at the control also reached (0.4) day (Table 2).

Table 2: Time of multiplication of *Ch. humicula* under the influence of different concentrations of CO_2 depending on the absorbency values

Time of multiplication (G.)	Cont. Chu13	25%	50%	75%	100%
1	0.45	0.76	0.85	0.91	0.51
2	0.40	0.41	0.42	0.51	0.46
3	0.55	0.53	0.50	0.60	0.55
4	0.73	0.63	0.57	0.68	0.64
5	0.89	0.72	0.63	0.75	0.67
6	0.92	0.79	0.70	0.83	0.79
7	0.92	0.89	0.83	0.91	0.88
8	0.99	0.97	0.91	0.97	1.01
9	1.05	1.04	0.95	1.03	1.00
10	1.15	1.13	1.04	1.13	1.08
11	1.25	1.21	1.12	1.23	1.16
12	1.35	1.29	1.21	1.32	1.23
13	1.44	1.36	1.29	1.42	1.29
14	1.50	1.48	1.35	1.45	1.36
15	1.56	1.60	1.41	1.52	1.42
16	1.66	1.69	1.49	1.60	1.51
17	1.75	1.73	1.56	1.68	1.60
18	1.84	1.86	1.63	1.76	1.68
Mean	1.13	1.12	1.03	1.13	1.05

The relying on the number of cells to calculate the growth rate, the results show that the highest growth rate of the record at the concentration (100%) in all days of experience while the

concentration was at 25% convergent with control and recorded at 75% lower rates compared to the control Table (3).

Table 3: Growth rate of alga *Ch. humicola* under exposed to CO_2 gas depending on the number of cells

Growth rate (K)No.	Cont. Chu13	25%	50%	75%	100%
1	0.11	0.21	1.30	0.02	2.75
2	0.06	0.19	0.98	0.00	1.82
3	0.06	0.37	1.00	0.29	1.15
4	0.06	0.42	0.91	0.35	0.81
5	0.17	0.42	0.81	0.36	0.60
6	0.15	0.26	0.60	0.22	0.45
7	0.13	0.08	0.47	0.09	0.33
8	0.11	0.08	0.41	0.08	0.29
9	0.10	0.07	0.37	0.07	0.31
10	0.09	0.04	0.33	0.06	0.29
11	0.07	0.04	0.30	0.05	0.26
12	0.05	0.03	0.26	0.04	0.23
13	0.02	0.03	0.21	0.01	0.19
14	0.01	0.02	0.20	0.01	0.14
15	0.01	0.03	0.18	0.02	0.13
16	0.01	0.03	0.17	0.03	0.13
17	0.01	0.03	0.16	0.03	0.12
18	0.01	0.03	0.15	0.03	0.11
Mean	0.07	0.13	0.49	0.10	0.56

While calculating the multiplication time depending on the number of cells, the lowest values for the multiplication time at the concentration 100% compared to the control, but the highest multiplication times recorded in the concentration 75% (Table 4).

Table 4: Multiplication time of *Ch. humicola* under exposed to CO_2 gas depending on the number of cells

Time of multiplication (G)	Cont. Chu13	25%	50%	75%	100%
1	2.66	1.45	0.23	16.64	0.11
2	5.13	1.55	0.31	132.48	0.17
3	4.88	0.82	0.30	1.04	0.26
4	5.06	0.72	0.33	0.86	0.37
5	1.76	0.71	0.37	0.84	0.50
6	2.02	1.17	0.50	1.35	0.67
7	2.40	3.57	0.64	3.23	0.91
8	2.77	3.89	0.73	3.91	1.04
9	3.09	4.59	0.82	4.60	0.97
10	3.46	7.74	0.91	5.36	1.05
11	4.42	8.52	1.00	6.20	1.17
12	5.76	10.54	1.18	7.01	1.28
13	13.56	11.83	1.42	35.73	1.59
14	37.31	15.58	1.53	22.96	2.10
15	45.22	10.16	1.65	12.77	2.27
16	52.89	9.47	1.77	11.86	2.40
17	43.63	8.82	1.89	10.53	2.53
18	47.06	11.90	2.02	9.97	2.65
Mean	15.73	6.28	0.98	15.96	1.22

The results of the final harvest process for estimating the dry biomass of alga culture showed that the four treatment cultures with CO_2 concentrations have different effect on biomass than the other, and the highest value of 410 gm dry weight/L at the concentration of 50%; while the concentrations (100, 75, 25)% gave the values (370, 360, 340) gm dry weight/L respectively; whereas control recorded 310 gm dry weight/L. Table (6) shows the chemical composition of some of the fatty acids detected after the exposure of alga cells to the concentrations used in the experiment of CO_2 gas, through the application of the HLPC technology. The study found that it gave varying values and recorded (154.6, 90.11) in concentration (25, 100%) as the highest and the lowest values respectively of the myristiacid acid, while plametic recorded the highest value at concentration 100% that reached 302.1. Whereas the lowest value with a concentration 50% that reached 197.81. Palmitolic recorded the highest value with concentration 100% and lowest value in the control medium, reached 273.2. Oleic (Omega-9) recorded the highest value with a concentration 25%, reached 389 and the lowest concentration in the control sample, reached 148.65. While linoleic (Omega 6) has recorded with the concentration 100% higher value (298.02) and lower value with control,

reached 121.9. Finally, the highest value for the acid arachidic with a concentration 100%, reached 249.8 and the lowest value in control (68.89).

Table 6: Fatty acids of alga *Ch. humicola* under exposed to different concentrations of CO_2 when developed in the modified culture medium Chu 13. (Mg/l)

Fatty Acids	Cont. Chu13	25%	50%	75%	100%	Total% increase after 18 days
C14:0 Myristic Acid	100.76	90.11	112.00	134.03	154.61	53.443
Palmetic AcidC16:0	227.64	232.00	197.81	279.77	302.10	32.079
Palmitolic Acid C16:1	273.26	387.00	380.47	378.11	403.93	47.820
Oleic C18:1(Omegaa 9)	148.65	389.00	327.97	307.86	337.75	121.211
Linoleic AcidC18:2 (Omega 6)	121.91	164.88	287.16	285.68	298.02	144.409
Arachidic Acid C20:4	68.89	82.38	236.15	237.84	249.82	262.636

Below is the proportional distribution of each type of fatty acid in the four concentrations of CO_2 that treated in the culture medium of the alga *Ch. humicola.*

Discussion

The results of the current study shown in Tables (1, 2, 3, 4), found that the one-time treatment and different concentrations of CO_2 have given different responses to the cells of alga *Ch.humicola*, the effect of the growth curves, its rates and times of multiplication. As well as noted in the first four days the case of the initial stimulation or reaction of alga cells to the increase the levels of CO_2 supplier for the culture medium, then a state of equilibrium and gradual rise, which shows the rise of the values of absorbency, or equilibrium and stability then maintain the height of some values, especially in the case of high concentrations and convergence the other with the standard sample in the few concentrations that represents the growth curve as well it is dependent on the calculation of cells number, or primary stimulation then adjustment in the number and convergence with the standard sample of different concentrations, which represents the rate of growth dependent on the number of cells or when calculating the multiplication time that shown in table (2).

Therefore, these mechanisms can be explained by the reaction and inverse reaction between the cells of the alga and the change of the CO_2 supplier concentrations as a condition that begins with the cell adaption of this increase and dealt with several physiological aspects represented the increase of the number or change in color or volume and even the form of cells to deal with an external influence surplus in the growth medium outside the adaptation contexts of the components of the medium gradually whatever the nature of additive (salt, vitamin, heavy ingredient, pesticide or different plant nutrients). These conclusions are consistent with 11, 18, 19] who have registered a stimulating process in the early days and have changed the volume and shape of the tested organisms cells (algae and fungi) under the influence of various factors. Sometimes the response was became at the level of internal construction of compounds and the alteration or quantitative and qualitative development of pigments or fats, proteins, carbohydrates or amino acids, fatty acids and other biological compounds. Therefore, some increases occur in some of them or the disappearance of others or change the original composition, thus the general activity of the metabolism and the photosynthesis were affected especially in algae. This results agreed with [2, 4, 20, 21] who have indicated that the type of treatment, the nature of the medium, the quantity and quality of the added factor and the method and time of addition all factors that affect the nature of cell response. These cells change the volume, shape and nature of pigments 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. Some researchers also argue that the species or genus of the alga used was variable even in the case of the development of several algae in the same culture medium, since [22].

Tested the efficacy of different types of algae in fixating CO_3 and CO_2 for sewage water as a medium of development, and they found that the algae *Chlorella valgaris* was able to stabilize 624 CO_3/L/day and 26.0 gm/m^2/h of gas and in the medium of 15 v/v% CO_2, while algae *Synechocystis aquatiliscan* stabilized about 1500 gm/m^2/h of CO_2 [23]. Have carried out an experiment to learn the effect of different concentrations of CO_2 on the group of green freshwater algae (*Chlamydomonas*, *Chlorella* and *Scenedasmus*) were developed on the culture medium of modified Bristol medium which added CO_2 to the concentrations (3-168%), with the three algae having varying growth rates, the highest rate

of concentration of 30, 100% of CO_2, as well as the highest activated level of the photosynthesis in *Chlorella* and *Scenedasmus* [20]. Discussed the process of electing the algal genus and species the most suitable for the extraction and fixation of CO_2 supplier from the environmental milieu (CO_2 sequestration) and according to their study on *Chlorella*, *Spirulina* and *Dunaliella* algae under the concentrations of 0.04-100% v/v for the production of carotene in different temperatures of 25-100 °C, they found a difference in the susceptibility of the tested algal genus to tolerate and interact with the gas as well as the suppling method and the system used in the suppling (bottles, tank, or photovoltaic reactors) as well as the nature and composition of the culture medium used for algae development. This opinion is supported by [24] who found that the algae *Ch. humicola* recorded the highest biomass and amount of fat when cultured in the Blood basal medium compared to the sodium alginate medium. The diversity of fatty acids also changed, with 70% of the saturated acids recorded and 12.2% of the multi-unsaturated.

Following the results in Table (6), there is also a clear variation in the values and distribution of fatty acids detected in HPLC-mediated alga samples: (Arachidic C20:4, Linoleicc18:2 (OMEGA6), Oleic C18:1 (omega-9), plamitolic C16:1, palmetic C16:0, Myristic C14:0) toward the four concentrations (25, 50, 75, 100%) of CO_2 supplier gas. Although all of these acids are in response to the increase in CO_2 supplier levels for the medium of development starting from the 25% concentration treatment with the exception of Myristic C14 acid: 0 which gave a value of (90.11) less than the standard sample (100.76). This response varies depending on the fatty acid type and the amounts of concentration despite the components of the medium are similar to all the treatments.

The highest response was to the palmetic C16:0 with concentration 100% and Oleic C18:1 at the concentration of 25% the weaker response was to the arachidic acid C20:4 (82.38) with a concentration 25%. Therefore, these acids are arranged based on the degree of response and increase in percentage, they are found at the end of the experiment (32.443, 47.820, 53.443, 121.211, 144.409, 262.636) % for the diagnosed IBC (palmetic, pamiotolic, myristic, oleic, linoleic, arachidic) respectively. As for dry biomass the current result found that indicate an increase in all values after the day (18) of the treatment compared with the standard sample ranged between (19.35, 16.13, 32.25, 9.67)% of the concentrations (50, 25 100, 75)% from CO_2 respectively. This due to the role of gas CO_2 to increase the percentage of carbon in the walls of the algal cells as well as to develop the efficient level of photosynthesis and increase the bio-productivity and organic matter in general. This conclusion is consistent with [25, 26, 27] who have been concluded through several studies aimed at comparing plants cultivated with normal levels of CO_2 and among those cultivated at elevated levels by direct suppling or in conditions where there was abnormal increase of this gas concentration due to different factors. The second group of plants achieved a much higher level of photosynthesis and organic production than the first group, moreover, with increased carbon dioxide enrichment; these plants respond to this effect, increase biomass and improve the efficiency of water use, and rise of tolerant ability for low light level.

These conclusions are also consistent with the increase in growth rates and the amount of fatty acids in the current study and other studies [24, 28, 29, 30, 31, 32] of the correlation between the increase of biomass and the amount of fat and fatty acids of different forms when controlling the levels of lighting, nutrients, pH, the quality of the culture medium, the concentration of supplier CO_2 and its quality, through experiments on various these applications. Researchers also see a close relationship between the increase of biomass and the amount of fats and fatty acids produced by algae [32].Indicates that increased CO_2 increases the biomass and production capacity of fuel and fixes about 60% of the fat within the algal cells [33].

Reported that the synthesis of fatty acids in microalgae and the role of CO_2 in their activity begins exclusively in the reservoir of carbon pool fixed in pigments. This synthesis requires the consumption of a large amount of ATP molecules and NADPH. The type and amount of light directing on cells play an important role in the interactions of photosynthesis and increase the efficiency of cells in the formation of total fat due to the high effectiveness of cellular metabolism and

the physiological efficacy of the algae. Also [34] found that the use of CO_2 and exposure of microalgae cultures to different proportions from it, affects the productivity of fats, and leads to a large extent in the change of values, especially Triacylglycerides (TAGs) and the polar fat composition such as glycolipid and sterols [35]. And [36] reported that the increase of CO_2 leads to an increase in biomass and the fixation of fat by the type of algae as well as their study on the types of green alga genus *Scenedasmus* sp. to follow their ability to stabilize CO_2 as well as the production of fats under different environmental conditions and different levels of CO_2 start from (0.03-50) % v/v. The results have indicated that the species *Sce. Obliquus-STU-3* and *Sce. pyrenoidsa* when increasing CO_2 concentrations record a larger biomass, the best optimal increase of alga was obtained when treating alga *Sce. obliquus* by 23 gm/L at concentration 15% of carbon dioxide [36]. Found that *Chlamydomonas* sp., *Chlorella* sp., *Coleastrum* sp., *Chlorococcum* sp. and *Ankistrodesmus* sp. had accumulated fat amount ranging from (46.6-55%). Additionally, the numbers of fatty acids diagnosed were differed, when exposed to temperatures and CO_2 gas in similar conditions [37].Reported that alga *Chlorococcum* sp. cells gave a good biomass with an increase of total fat by about 27.7% when increasing the flow air, the amount of light and phosphorus element available in culture medium.

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