Comparison of Different Media Formulations and the Optimal Growing Conditions on Growth, Morphology and Chlorophyll Content of Green Alga, *Chlorella vulgaris*.

Ghada W. Abou-El-Souod[1], Lamiaa H. S. Hassan[1] and Ebtsam M. Morsy[2]

[1]Botany Department, Faculty of Science, Menoufia University, Egypt
[2]Microbiology Department, Soils, Water and Environment Research Institute. Agriculture Research Center, Egypt
Lam_san@rocketmail.com

Abstract: *Chlorella vulgaris* is a green, spherical, single celled fresh water microalga belongs to the phylum Chlorophyta. The aim of this work is to evaluate the effect of five different defined inorganic medium such as BG-11 Medium, Bold's basal medium, modified Chu-10 medium, Zarrouk's Medium Modified, and Kuhl's medium, on the growth, morphology and pigment content of *Chlorella vulgaris*. The best growth was obtained in modified Chu-10 medium as compared to other medium. The growth of *Chlorella vulgaris* in terms of optical density had increased by (2.48) at 670 nm, cell count (3.44), the amount of (chlorophyll-a 2.92) and (chlorophyll-b 6.62) times of the initial record respectively, after a period of five weeks. Growth media formulations were varied to optimize the growth media composition for maximized algal biomass production. The effects of culture conditions at Nitrogen source, pH values, lighting source and Aeration on growth, morphology and the contents of chlorophyll-a, chlorophyll-b, of *Chlorella vulgaris* were determined. The best Nitrogen source was ammonium nitrate (NH_4NO_3) had increased by (1.56 OD, (1.47 CC), (2.08 chlorophyll-a) and (chlorophyll-b 6.54) times of the initial record respectively, after a period of five weeks. The best pH was recorded at pH 10 (7.38 OD), (8.85 CC), (10.52 chlorophyll-a) and (chlorophyll-b 6.7). The influence of aeration on biomass production in *Chlorella vulgaris* the aerated culture increased by (doubled growth rate by OD), (1.89 CC), chlorophyll a and b content by (3.24 and 1.31) times over that of the non-aerated culture. Light source also effects on the growth rate the sunlight culture increased by (1.72 OD) and (1.76 CC) chlorophyll a and b content by (1.21 and 1.21) times over than of the non-aerated culture.

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1. Introduction

Algae are photosynthetic organisms; however, they are masked by photosynthetic pigments that give them a distinguishing color that is used to identify key divisions **(Menetrez, 2012)**. Based on their morphology and size, algae are typically divided into two major categories macroalgae and microalgae. Macroalgae, commonly known as seaweed, are usually found in coastal areas in both intertidal and sub tidal habitats and are composed of multiple cells, which organize to structures resembling roots, stems, and leaves of higher plants; some species have gas-filled structures to provide buoyancy (**Chen *et al*., 2009**). They are classified into three broad groups based on their pigmentation: (i) brown seaweed (Phaeophyceae), which includes the large kelps; (ii) green seaweed (Chlorophyceae) such as sea lettuce; and (iii) red seaweed (Rhodophyceae), the most diverse group of all (**Singh *et al*., 2011**). In contrast, microalgae are a large group of microscopic photosynthetic organisms, many of which are unicellular (**Chen *et al*., 2009**). Microalgae, capable of performing photosynthesis, are important for life on earth; they produce approximately half of the atmospheric oxygen and use simultaneously the carbon dioxide to grow photo-autotrophically. The three most important classes of microalgae in terms of abundance are the diatoms (Bacillariophyceae), the green algae (Chlorophyceae), and the golden algae (Chrysophyceae) (**Carlsson *et al.*, 2007**).

Chlorella vulgaris is a eukaryotic green coccoid species of single celled fresh water microalgae belonging to Chlorellaceae family. The cells are without flagella and have thin cell wall. It can grow prolifically doubling its biomass weight in few hours of sunlight. So, *Chlorella vulgaris* is known as one of the fastest growing microalgae. **(Christi, 2007 and Hu *et al.*, 2008)**. *Chlorella* contains green photosynthetic pigments known as chlorophyll (a) and (b) in its chloroplasts. Through photosynthesis, it multiplies rapidly, requiring only carbon dioxide, water, sunlight, and a small amount of minerals to reproduce. *Chlorella* has much higher utilization rate (10-20%) of light energy for photosynthesis when

compared to common plants **(Zhang *et al.,* 2000)**. According to **(Belasco, 1997)** and **(Zelitch, 1971)**, it contains 45% protein (w/w, dry basis), 20% fat, 20% carbohydrates, 5% fiber and 10% minerals and vitamins. *Chlorella* is the richest source of chlorophyll which is widely used as a health food and feed supplement, as well as in the pharmaceutical and cosmetics industry **(Sharma *et al.,* 2011)**. It has been produced commercially in several countries for its use as neutraceutical food and medicinal purpose due to its valuable contents particularly pigments, proteins carotenoids, lipids, immune stimulator compounds, polysaccharides, vitamins, antioxidants and minerals **(Sharma *et al.,* 2011)**. Other health promoting effects of *Chlorella* are the lowering of cholesterol, preventive action against atherosclerosis or antitumor action. Extracts of *Chlorella vulgaris* are considered to have antimicrobial effects **(Gors *et al.*, 2010)**. Large scale production of *Chlorella* biomass depends on many factors, the most important of which are nutrient availability, temperature, aeration and light. These factors influence the growth of *Chlorella* and the composition of the biomass produced by causing changes in metabolism. Number of media compositions for the cultivation of microalgae has been proposed that were based on the analysis of the natural habitats where the algae grows **(Vonshak, 1986)**. The growth of *Chlorella* at various concentrations of macronutrients and micronutrients has been studied **(Eyster *et al.,* 1958)** and some media formulations have been made which were based on detailed study of nutrient requirements of algae **(Provasoli and Printner, 1953)**. The elements required for the growth of green algae are N, P, K, Mg, Ca, S, Fe, Cu, Mn, and Zn **(Oh-Hama and Miyachi, 1988)** and these elements are added in the form of salts, a list of which is found in Kaplan and co-workers published work **(Kaplan *et al.,* 1986)**. Variation in the elemental composition of *Chlorella* under different conditions and different stages of growth has been reported (**Oh-Hama and Miyachi, 1988**; **Harrison *et al.,* 1990)**.

This research is aimed to study the effect of different media compositions and then evaluate the influence of different culture conditions on the growth of microalgae *Chlorella vulgaris* to optimize the best culture condition.

2. Material and Methods

Organisms

The experimental organism *Chlorella vulgaris* was obtained from Phycology laboratory, Faculty of Science, Menouifa University, Egypt. Chlorella *vulgaris* was earlier identified according to (**Prescott, 1962)**.

The effect of different media on *Chlorella vulgaris* growth

In order to find out the best culture medium, cultures were subjected to five different media of different chemical compositions and pH:-

1. BG-11 Medium **(Ilavarasi *et al*., 2011).**
2. Bold's Basal (B.B.) Medium **(Bischoff, and Bold, 1963).**
3. Modified Chu-10 Medium **(Stein, 1973).**
4. Zarrouk's Medium Modified **(Zarrouk, 1966).**
5. Kuhl's Medium **(Kuhl and Lorenzen, 1964).**

Five conical flasks containing 250 ml of each medium and 50 ml freshly growing *Chlorella vulgaris* were followed through optical density (OD) by using colorimeter at (670 nm), cell count (CC) was performed using haemocytometer according to **(Gerloff *et al.*, 1950)** and pigment estimation by using UV visible spectrophotometer, The chlorophyll (a) and chlorophyll (b) content of the samples were estimated by **(MacKinney, 1941)**. All medium in the flask were sterilized in autoclave at 121°C for 20 min. before inoculation. Cultures were shaken gently, three times a day to avoid clumping and accelerate the growth process. Experiment for each medium was carried out in three replicates Optical density (using spectrophotometer), cell count (using hemocytometer) and chlorophyll contents were done every 7 days for 5 weeks.

Effects of Nitrogen source on Growth and Biochemical Profile of *Chlorella Vulgaris:* According to **(Rai *et al.,* 2013)**

Suitable nitrogen source for optimum algal growth was determined by subjecting the algal culture to medium (no. 3) containing different sources of nitrogen such as $Ca(NO_3)_2$, $NaNO_3$, NH_4Cl, NH_4NO_3 and urea $[CO.(NH_2)_2]$. Culture flasks with sterile medium were inoculated and incubated in the environmental chamber at 25^{o}C, with fluorescent illumination. Optical density (670 nm), cell counts were done and chlorophyll contents every 7 days for 5 weeks.

Effects of pH optimization on Growth and Biochemical Profile of *Chlorella Vulgaris*. According to **(Rai *et al.,* 2013).**

The effect of pH on growth of the algae was studied using modified Chu-10 media in the pH range of 5, 6, 7, 8, 9, 10, 11 and 12 in 250 ml conical flasks. All the flasks were inoculated at 15% (v/v) inoculum and incubated at 25± 1ºC temperature. Optical density (using spectrophotometer), cell count (using hemocytometer) and chlorophyll contents were done every 7 days for 5 weeks.

Effects of lighting source on Growth and Biochemical Profile of *Chlorella vulgaris:* According to **(Anaga and Abu, 1996).**

Light sources were sunlight, and artificial illumination provided from white fluorescent lamps. A growth chamber was constructed and illuminated by two 4ft-white fluorescent lamps, which were mounted in the chamber at a height of about 30 cm from the bench top. Six 100 ml-conical flasks each containing 50 ml of the growth medium (no. 3) and inoculated with 10 ml of the culture were used. One set (three replicates) was incubated in natural light (sunlight) and the second set (three replicates) was incubated in the growth chamber with the fluorescent lamps. Optical density (using spectrophotometer), cell count (using hemocytometer) and chlorophyll contents were done every 7 days for 5 weeks.

Effects of Aeration on Growth and Biochemical Profile of *Chlorella vulgaris:* According to **(Anaga and Abu, 1996).**

Conical flasks 100 ml containing 50 ml of the growth medium (no. 3) were set up. Each flask was inoculated with 10 ml of the culture. One set of three flasks was aerated using an aquarium pump which pumped air at 150 bubbles per min through a drip set (plastic tubing) fitted with a regulator. Optical density (using spectrophotometer), cell count (using hemocytometer) and chlorophyll contents were done every 7 days for 5 weeks.

3. Results and Discussion

Growth estimation through Optical density (OD), cell count (CC) and Chlorophyll content of *Chlorella vulgaris* in different culture conditions shows different growth pattern, in spite of all culture conditions started with similar initial inoculums. Among all five culture conditions, clearly indicated that the best growth of *Chlorella vulgaris* was obtained in modified Chu-10 medium as compared to that in other media.

The present results agree with those of who showed that **(Sharma *et al.*, 2011)**. Optical density (OD) and cell count (CC) had increased by 2.48 and 3.44 times of the initial record respectively, after a period of five weeks, Bold's basal medium was next to modified Chu-10 in promoting the growth of *Chlorella vulgaris*. Growth was increased about 1.81 times in OD and 2.31 times in CC. In BG-11 medium OD and CC were increased 1.78 and 2.03 times, of the initial record. In Khul's medium OD was increased 1.71 times and CC was increased 1.95 times the initial record. Zarrouk's medium proved to be insufficient in supporting the growth of *Chlorella vulgaris* as has been observed through OD and CC (Table 1 and Table 2), which increased up to 1.63 and 1.44 times the initial record, respectively. The growth curve did not show lag phase and it demonstrates that there was a quick adaptation of *Chlorella vulgaris* to the media.

Table (1): Effect of different media on growth (OD) of *Chlorella vulgaris*

Media / Weeks	BG-11	BBM	Chu-10	Zarrouk	Kuhl
Initial week	0.300±0.002	0.300±0.002	0.300±0.002	0.300±0.002	0.300±0.002
1st week	0.342±0.021	0.319±0.003	0.406±0.013	0.371±0.014	0.326±0.007
2nd week	0.393±0.013	0.380±0.026	0.487±0.009	0.405±0.01	0.347±0.01
3rd week	0.441±0.001	0.419±0.029	0.565±0.009	0.419±0.002	0.380±0.016
4th week	0.477±0.020	0.507±0.049	0.641±0.013	0.458±0.007	0.461±0.033
5th week	0.533±0.034	0.544±0.023	0.744±0.024	0.490±0.016	0.512±0.035
LSD*	0.061	0.364	0.000	0.047	0.117

Each value is Mean ± SD (n=3), 0ptical density (OD); * The mean difference is significant at the 0.05 level.

Table (2): Effect of different media on growth (CC) of *Chlorella vulgaris* (10^4 cells/ml)

Media / Weeks	BG-11	BBM	Chu-10	Zarrouk	Kuhl
Initial week	114.35±0.0	114.35±0.0	114.35±0.0	114.35±0.0	114.35±0.0
1st week	126.67±4.88	134.17±3.97	134.92±7.36	119.5±1.5	112.17±7.61
2nd week	157.5±7.3	145.25±4.88	182.92±7.67	126.42±5.07	158.25±3.64
3rd week	162.42±13.78	218.08±7.21	233.58±3.58	150.58±8.35	170.08±2.31
4th week	193.33±6.44	234.47±16.94	297.58±11.83	157.5±10.21	212.75±2.40
5th week	231.83±10.67	263.58±9.55	393.75±22.58	164.58±10.20	223.00±11.25
LSD*	0.007	0.038	0.000	0.496	0.061

Each value is Mean ± SD (n=3), Cell count (CC); * The mean difference is significant at the 0.05 level.

The pigment content of the algae also correlates with the growth of *Chlorella vulgaris*. Maximum Chlorophyll -a and Chlorophyll -b content were found in cultures of modified Chu-10 medium i.e. 2.95% and 6.86%, respectively, after a period of five weeks, followed by 2.26% and 4.11% in Bold's basal

medium, 1.66% and 2.28% in BG-11 medium, 2.07% and 3.14% in Zarrouk's medium and minimum Chlorophyll -a and Chlorophyll -b content were found in Kuhl's medium i.e. 2.22% and 2.44%, respectively (Figure 1). **(Sharma *et al.*, 2011)**.

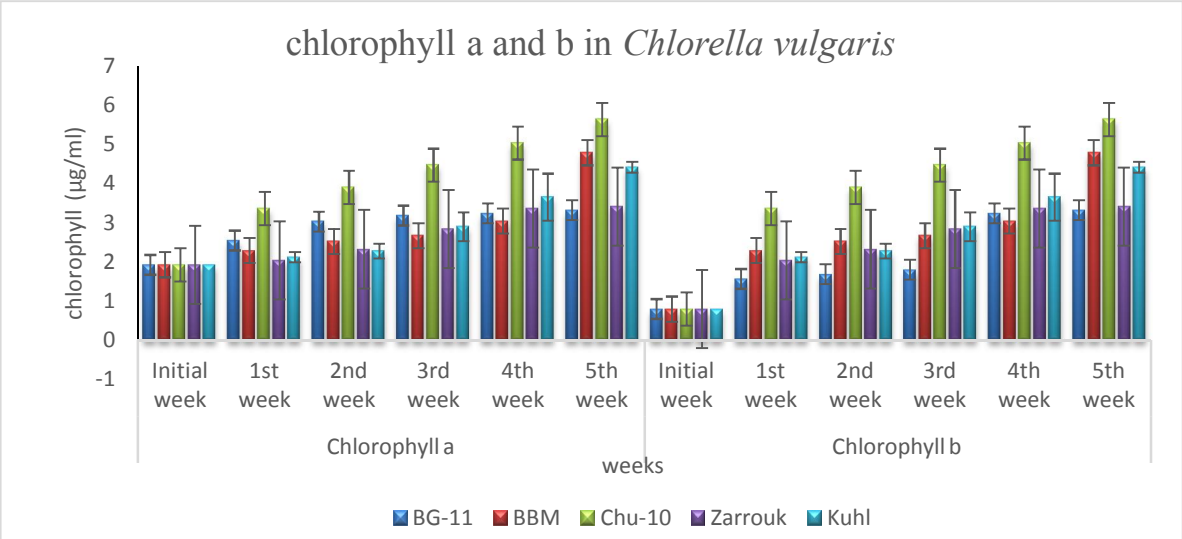

Fig. 1: Effect of different media on growth (Chlorophyll (a) and Chlorophyll (b)) of *Chlorella vulgaris* (µg/ml)

Effect of different factors on Chu-10 medium on growth of *Chlorella vulgaris*

1. Nitrogen source

Nitrogen being important constituent of the cell protein was needed for algal growth, either in combined or in molecular form. Estimation of growth through Optical density (OD), cell count (CC) and Chlorophyll content of *Chlorella vulgaris* in different culture conditions on Chu-10 medium shows different growth pattern, in spite of all culture conditions started with similar initial inoculums. Among all five different nitrogen sources, clearly indicated that the best growth of *Chlorella vulgaris* was obtained in ammonium nitrate (NH_4NO_3). **(Madkour *et al.*, 2012).**

The results in Tables (3 and 4) also are in agreement with **(Tepe *et al.*, 2006).** Optical density (OD), cell count (CC) of ammonium nitrate (NH_4NO_3) had increased by 1.56 times and 1.40 times of the initial record respectively, after a period of five weeks. Calcium nitrate (Ca $(NO_3)_2$) was next to ammonium nitrate (NH_4NO_3) solution in promoting the growth of *Chlorella vulgaris*. Growth was increased about 1.52 times in terms of OD and 1.40 times in terms of CC. In sodium nitrate ($NaNO_3$) OD and CC were increased 1.48 and 1.39 times, of the initial record. In ammonium chloride (NH_4Cl) OD was increased 1.46 times and CC was increased 1.37 times the initial record. urea ($CO(NO_2)_2$) proved to be insufficient in supporting the growth of *Chlorella vulgaris* as has been observed through OD as well as CC (Table 3 and Table 4), which increased up to 1.33 and 1.36 times the initial record, respectively. The growth curve did not show lag phase and it demonstrates that there was a quick adaptation of *Chlorella vulgaris* to the media.

Table (3): Effect of different Nitrogen source on Chu-10 medium on growth (OD) of *Chlorella vulgaris* at 670 nm

N source / Weeks	$Ca(NO_3)_2$	$NaNO_3$	NH_4Cl	NH_4NO_3	Urea
Initial week	0.632±0.029	0.632±0.029	0.632±0.029	0.632±0.029	0.632±0.029
1st week	0.702±0.017	0.645±0.007	0.714±0.018	0.725±0.019	0.673±.044
2nd week	0.798±0.025	0.731±0.023	0.731±0.005	0.790±0.043	0.726±0.040
3rd week	0.863±0.024	0.867±0.023	0.781±0.036	0.843±0.092	0.786±0.036
4th week	0.887±0.018	0.891±0.005	0.86±0.034	0.928±0.048	0.842±0.058
5th week	0.958±0.04	0.940±0.028	0.925±0.11	0.983±0.037	0.881±0.047
LSD*	0.699	0.048	0.075	0.199	0.284

Each value is Mean± SD (n=3), 0ptical density (OD); * The mean difference is significant at the 0.05 level.

Table (4): Effect of different Nitrogen sources on Chu-10 medium on growth cell count (cc) of *Chlorella vulgaris* (10^4cells/ml)

N source / Weeks	$Ca(NO_3)_2$	$NaNO_3$	NH_4Cl	NH_4NO_3	Urea
Initial week	448.27±31.45	448.27±31.45	448.27±31.45	448.27±31.45	448.27±31.45
1st week	482.33±22.30	469.67±0.007	497.00±0.018	524.67±0.019	483.00±.044
2nd week	502.25±17.89	567.83±0.023	517.17±0.005	537.42±0.043	516.50±0.040
3rd week	525.92±17.81	597.25±0.023	598.25±0.036	597.67±0.092	581.00±0.036
4th week	571.08±1.77	598.67±0.005	608.00±0.034	617.42±0.048	597.67±0.058
5th week	628.42±8.58	620.92±0.028	614.50±0.11	656.92±0.037	608.92±0.047
LSD*	0.000	0.066	0.542	0.003	0.247

Each value is Mean ± SD (n=3), Cell count (CC); * The mean difference is significant at the 0.05 level.

Maximum Chlorophyll -a and Chlorophyll -b content were found in ammonium nitrate (NH_4NO_3) i.e. 2.08 times and 6.55 times, respectively, after a period of five weeks, followed by 1.96 times and 6.47 times in Calcium nitrate ($Ca(NO_3)_2$), 1.92 times and 5.19 times in sodium nitrate ($NaNO_3$), 1.8 times and 4.9 times in ammonium chloride (NH_4Cl) and minimum Chlorophyll -a and Chlorophyll -b content were found in urea ($CO(NO_2)_2$) i.e. 1.48 times and 4.84 times, respectively (Figure 2).

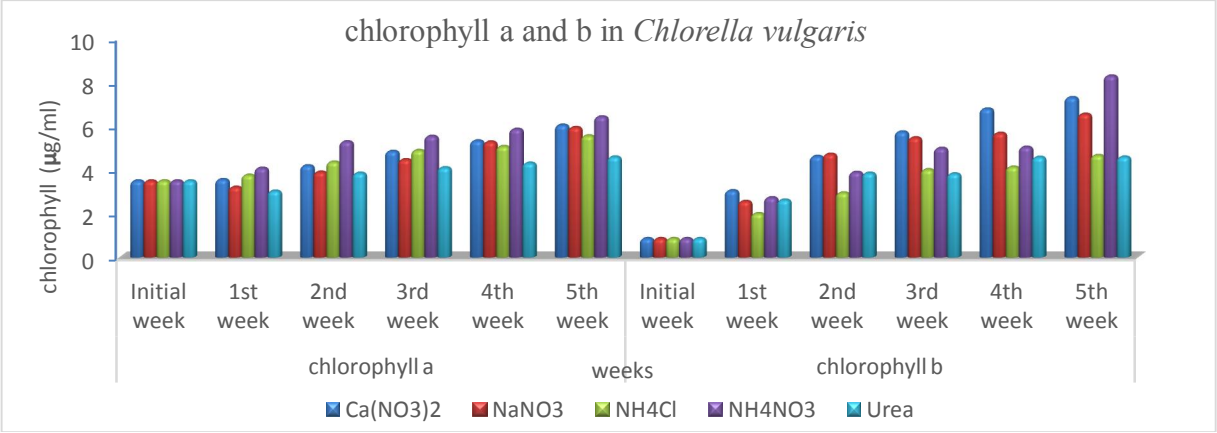

Fig. 2: Effect of different Nitrogen sources on Chus-10 medium on growth Chlorophyll (a) and (b) content of *Chlorella vulgaris* (µg/ml)

2. pH values

Optical density (OD), cell count (CC) and chlorophyll content had increased by 7.38 times and 8.85 times of the initial record respectively at pH (10), after a period of five weeks. At pH (11) was the next growth of *Chlorella vulgaris*. Growth was increased about 4.64 times in terms of OD and 6.14 times in terms of CC. Then followed by pH (7), the growth increased about 4.40 and 5.9 times OD and CC respectively .At pH (9), the growth was increased about 4.29 times in terms of OD and 5.85 times in terms of CC. The growth increased in pH (6) OD and CC were increased 3.33 and 5.77 times, of the initial record. pH (5) is followed the growth at pH (6) had increased by 1.43 times and 2.41 times of the initial record respectively. The growth at pH (8) has the lowest increases 1.07 times in terms of OD and 1.7 times in terms of CC. At pH (12) the *Chlorella vulgaris* inoculum had decreased 0.51 OD and 0.019 CC from the initial record respectively.

It's found that under acidic conditions, the magnesium atom of chlorophyll molecule (Mg^{+2}) is lost and the color changes to the characteristic pheophytin olive green color, while under alkaline conditions the methyl and phenyl esters are removed, producing chlorophyll in which is a bright green color. The pH of modified Chu-10 medium was maintained from 7.2 to 7.4, thus this medium favors the growth of algae. The pigment content of *Chlorella ellipsoidea* was highest at pH 4.0, 6.0 and 7.5 **(Khalil *et al.*, 2009)** and some Scientists maintained unialgal and axenic cultures of *Chlorella vulgaris* in modified Chu-10 medium in their research work but they had not mentioned the impact of this medium on growth and morphology of *Chlorella vulgaris* **(Prasad *et al.*, 2006 and Mallick and Rai, 1993)**. The green alga *Botryococcus protuberans* has shown enhanced growth rate in the modified Chu-10 medium **(Rai *et al.*, 2007)**.

Table (5): Effect of different pH values on Chu-10 medium on growth (OD) of *Chlorella vulgaris*

pH values / Weeks	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11	pH 12
Initial week	0.084±0.0	0.084±0.0	0.084±0.0	0.084±0.0	0.084±0.0	0.084±0.0	0.084±0.0	0.084±0.0
1st week	0.16±0.003	0.15±0.003	0.16±0.002	0.15±0.002	0.16±.002	0.18±0.002	0.20±0.002	0.13±0.001
2nd week	0.18±0.002	0.19±0.004	0.19±0.004	0.17±0.003	0.20±0.004	0.25±0.003	0.27±0.004	0.024±0.002
3rd week	0.19±0.004	0.25±0.003	0.32±0.002	0.18±0.004	0.23±0.032	0.37±0.001	0.35±0.003	0.034±0.002
4th week	0.16±0.002	0.27±0.003	0.33±0.003	0.16±0.002	0.29±0.003	0.50±0.002	0.36±0.002	0.039±0.003
5th week	0.12±0.002	0.28±0.004	0.37±0.002	0.09±0.003	0.36±0.002	0.62±0.003	0.39±0.004	0.043±0.004
LSD*	0.000	0.000	0.000	0.000	0.003	0.000	0.000	0.222

Each value is Mean ±SD (n=3), 0ptical density (OD); * The mean difference is significant at the 0.05 level.

Table (6): Effect of different pH values on Chu-10 medium on growth cell count (CC) of *Chlorella vulgaris* (10^4cells/ml)

pH values / Weeks	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11	pH 12
Initial week	212±0.0	212±0.0	212±0.0	212±0.0	212±0.0	212±0.0	212±0.0	212±0.0
1st week	348±4.58	263±7.69	361±5.2	262±2.96	269±.3.53	332±2.91	329±3.28	68±2.31
2nd week	376±3.28	363±5.81	448±4.98	297±5.49	288±4.91	585±14.57	468±9.87	20±0.67
3rd week	410±4.04	509±2.19	579±1.45	362±5.84	624±1.76	1109±2.03	784±2.2	12±0.88
4th week	575±3.28	810±8.5	812±4.93	436±5.04	1049±7.94	1829±6.69	1143±2.6	5±0.58
5th week	510±4.51	1224±5.24	1321±2.03	360±7.51	1241±1.15	1877±7.21	1302±2.6	4±0.58
LSD*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.531

Each value is Mean ± SD (n=3), Cell count (CC); * The mean difference is significant at the 0.05 level.

Maximum Chlorophyll -a and Chlorophyll -b content were found in (pH 10) i.e. 10.52 and 6.7 times, respectively, after a period of five weeks, followed by 8.61 times and 4.19 times in (pH 11), 8.24 and 4.08 times in (pH 9), 6.5 and 2.66 times in (pH 7), 3.71 and 2.58 times in (pH 6), 2.21 and 0.92 times in (pH 5), 1.34 and 0.91 times in (pH 8) and minimum Chlorophyll -a and Chlorophyll -b content were found in (pH 12) i.e. 0.67 and 0.57 times, respectively (Figure 3).

3. Aeration

Chlorella vulgaris produced higher biomass when the growth medium was bubbled with air (aerated). This results are in agreement with (**Kemka *et al.*, 2007)** who reported that The *Spirulina* species produced higher biomass when the growth medium was bubbled with air (aerated) than when the medium was not bubbled with air (non-aerated).At the end of the 35 days incubation period, OD and CC had increased of the biomass concentration in the aerated culture increased by 2 times and 1.89 times over than of the non-aerated culture respectively Table (7 and 8).The growth was measured also by chlorophyll a and b estimation Figures (4). That show increase in the chlorophyll a and b content by 3.24 times and 1.31 times over than of the non-aerated culture respectively.

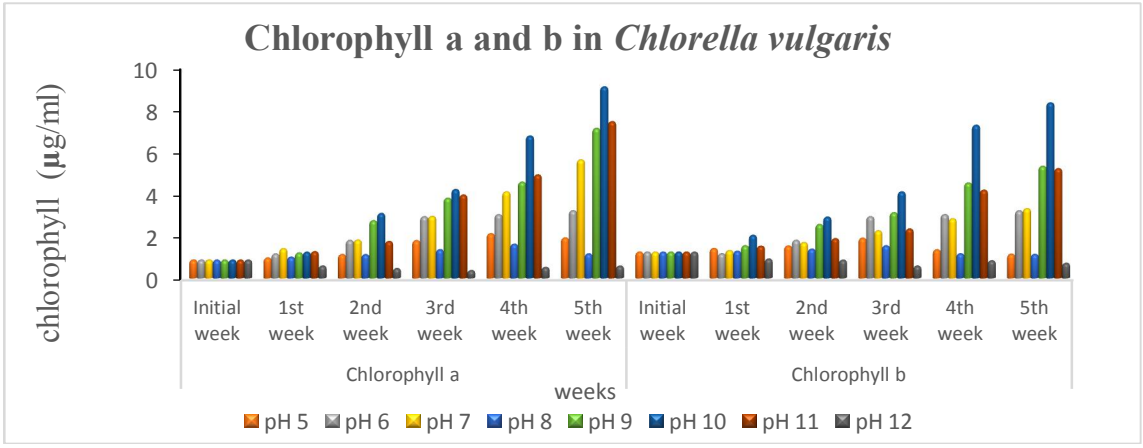

Fig. 3: Effect of different pH values on Chu-10 medium on growth Chlorophyll (a) and (b) content of *Chlorella vulgaris* (µg/ml).

Table (7): Effect of Aeration on Chu-10 medium on growth (OD) of *Chlorella vulgaris* (n.m.)

Media / Weeks	Static	Aeration
Initial week	0.634 ± 0.0	0.634 ± 0.0
1st week	0.702 ± 0.01	0.813 ± 0.037
2nd week	0.798 ± 0.014	1.341 ± 0.25
3rd week	0.814 ± 0.014	1.565 ± 0.22
4th week	0.823 ± 0.011	1.617 ± 0.02
5th week	0.848 ± 0.023	1.696 ± 0.10
LSD	0.221	0.702

Each value is Mean ± SD (n= 3), 0ptical density (OD); * The mean difference is significant at the 0.05 level.

Table (8): Effect of Aeration on Chu-10 medium on growth (Cell Count) of *Chlorella vulgaris* (10^4cells/ml)

Media / Weeks	Static	Aeration
Initial week	429±0.00	429±0.00
1st week	482.33±12.88	550.33±4.81
2nd week	502.25±10.33	700.5±1.18
3rd week	525.92±10.28	820.67±15.45
4th week	571.08±1.02	1044.17±16.54
5th week	628.42±4.95	1187.42±7.20
LSD*	0.000	0.000

Each value is Mean ± SD (n=3), Cell count (CC); * The mean difference is significant at the 0.05 level.

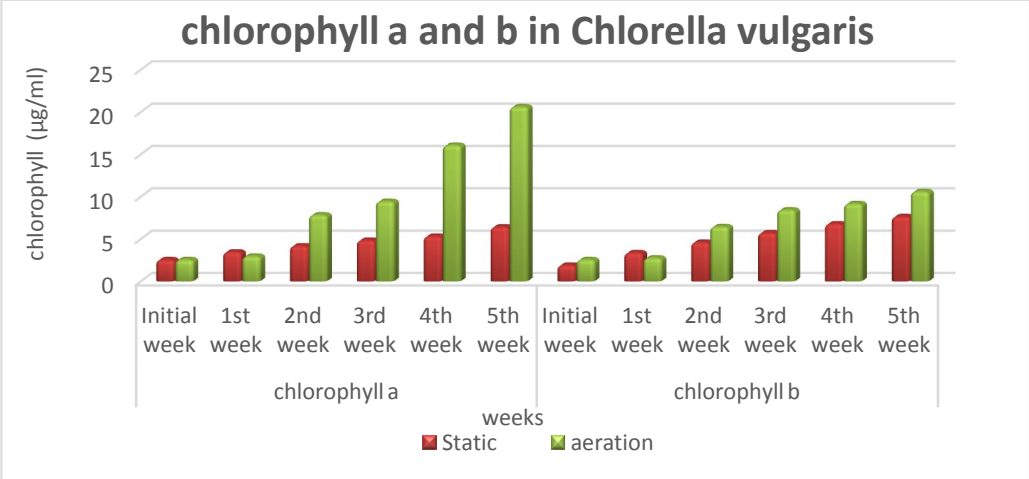

Fig. (4): Effect of Aeration on Chu-10 medium on growth (Chlorophyll a and b content) of *Chlorella vulgaris* (µg/ml)

4. Light Source

Chlorella vulgaris had better biomass production in sunlight than in the illuminated chamber. At the end of the 35 days incubation period, OD and CC had increased of the biomass concentration in the sunlight culture increased by 1.72 times and 1.76 times than of the non-aerated culture respectively Table (9 and 10).The growth was measured also by chlorophyll a and b estimation Figures (5). That show increase in the chlorophyll a and b content by 1.21 and 1.21 time than of the non-aerated culture respectively. It has been long known that direct sunshine is harmful to algal cultures. Under natural conditions, receiving direct rays of the sun rarely fall on an alga, and a few centimeters of interposed water are sufficient to reduce the harmful effects. In the natural habitats, algae grow predominantly in diminished light; hence cultures should be placed in the window, where direct sun light could be avoided **(Sharma *et al.*, 2012).**

Table (9): Effect of Light Source on Chu-10 medium on growth (O.D.) of *Chlorella vulgaris* (n.m.)

Light source / Weeks	Synthetic light	Natural light
Initial week	0.603±0.0	0.603±0.0
1st week	0.702±0.01	0.670±0.01
2nd week	0.798±0.014	0.697±0.07
3rd week	0.814±0.014	1.013±0.007
4th week	0.823±0.011	1.282±0.003
5th week	0.848±0.023	1.460±0.001
LSD	0.221	0.002

Each value is Mean ± SD (n= 3), 0ptical density (OD); * The mean difference is significant at the 0.05 level.

Table (10): Effect of Light Source on Chu-10 medium on growth (cell count CC) of *Chlorella vulgaris* (10^4cells/ml)

Light source / Weeks	Synthetic light	Natural light
Initial week	413.67±0.0	413.67±0.0
1st week	482.33±12.87	528.00±8.39
2nd week	502.25±10.33	604.92±11.91
3rd week	525.92±10.28	639.00±21.83
4th week	571.08±1.02	823.25±10.15
5th week	628.42±4.95	1107.42±9.68
LSD	0.000	0.000

Each value is Mean ± SD (n=3), Cell count (CC); * The mean difference is significant at the 0.05 level.

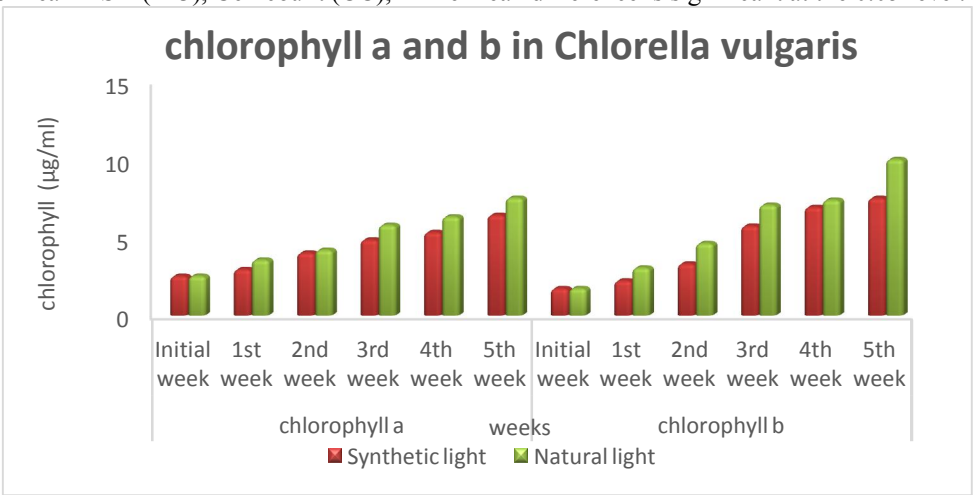

Fig. (5): Effect of Light Source on Chu-10 medium on growth (Chlorophyll a and b) of *Chlorella vulgaris* (µg/ml)

Conclusion

Microalgae *Chlorella vulgaris* has been identified as a feedstock for a diversity of applications such as biofuels, feed, and fertilizer. However major engineering obstacles are hindering its large-scale production. Once these obstacles are overcome algae could become a very useful crop which can be grown worldwide. The results of this study show that *Chlorella vulgaris* grows better on modified Chu-10 medium. The best Nitrogen source was ammonium nitrate (NH_4NO_3) at pH 10.It has been mentioned above that natural day light and aeration showed highest concentration of biomass and chlorophyll.

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