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Effect of nutrients condition on the growth and lipid content of microalgae *Chlorella vulgaris* and *Chroococcus minor*

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Abstract

Two local microalgae are used in this study: *Chlorella vulgaris* as model of green algae and *Chroococcus minor* as model of blue green algae which used to test their ability to produce lipids as a source of biodiesel through starvation of some nutrients (-N) 100, (-N) 50%, (-P) 50%, (-N) 50 %+(-P) 50% and (-Fe) 50%, the study showed that the accumulation of lipids in *C.vulgaris* more efficient than *C.minor* , in *C.vulgaris* The highest lipid content was 41% from DW in treatment (-N) 100% while the lowest lipid content was 8% at control, carbohydrate content increased from 18% at control to 34% at (-N) 100% while the highest protein was 51% of DW in the control. In *C.minor* the highest lipid content was 31% in (-N) 100% while the lowest lipid content was 5% at control. The highest carbohydrate content was 28% of DW (-N) 100%, while the highest protein was 40% of DW in the control.

Keywords: **Microalgae, Nutrient, Lipid content, Biodiesel.**

Introduction

Human's continuous use for fossil fuels is unsustainable as they are limited resources of energy (Srivastava and Prasad, 2000), the combustion of fossil fuels is responsible for 73% of the CO_2 production (Veruma *et al*., 2010).their combustion will lead to generation of the energy-related emissions of greenhouse gases (GHG) (Gavrilescu and Chisti, 2005).

Greenhouse gases contributes not only to global warming but also increase turning the water pH gradually to more acidic, because oceans absorb about one-third of the CO_2 release per year by human activities (Mata *et al*., 2010). This pH decrease may cause the quick loss of marine ecosystem biodiversity such as coral reefs, a huge impact in the life in the ocean and thus on the life of the earth (Ormerod *et al*., 2002). The search for renewable energy sources that reduce CO_2 emissions becomes a matter of widespread attention; the solutions to meet the energy demand include solar energy either thermal or photovoltaic, hydropower, geothermal, water, wind and biofuel (Ragauskas *et al*., 2006; Demirbas, 2007).

Biofuel can be broadly defined as solid, liquid or gas fuel consisting of or derived from biomass (Pogaku *et al*., 2012), many studies have been conducted on biofuels for substituting fossil fuels and reduce the greenhouse gas emission (Bastianoni *et al.,* 2008). The most common biofuels are biodiesel and bio-ethanol, which can replace diesel and gasoline, respectively (Mata *et al*., 2010).

Three generations of biofuels have emerged. The First generation biofuel is based on edible plant parts (oilseeds, grains, etc.); they mainly correspond to ethanol-based fuels obtained from the fermentation of sugars (corn, beet, sugar cane, etc.), oleaginous plants (colza, palm, canola, etc.).The second generation refers to energy production from non-edible plants or non-edible parts of plants; are the cellulosic-based biofuels obtained from non-food crops materials (wood, leaves, straw, etc.), these biofuels include bio alcohols, bio-oil, bio hydrogen, wood diesel (Roman *et al*., 2007; Demirbas,2009). The third is based on energy production from microorganisms such as microalgae, yeast and fungi

Algae can provide several different types of renewable biofuels which include, methane produced by anaerobic digestion of the algal biomass, bio hydrogen, bioethanol and biodiesel derived from oil (Gavrilescu and Chisti, 2005; Kapdan and Kargi, 2006; Muthukumar *et al*., 2012; Zhu *et al*., 2016).

The study aims to identify the efficiency of both algae (Chlorella vulgaris and Chroococcus minor) isolated from local environment in following:

1-Assessment the lipids production from the studied algae by different cultivation production.

2 - Identified some fatty acids.

3- Determination of carbohydrates and proteins.

Materials and Methods

Sample Collection: Fresh water samples of algae were collected from the ponds in Al-Mustansiriya University. The samples were collected by sterile container (100 ml) which was marked with date and location of sampling then transported to laboratory immediately to be incubated under suitable condition (268 µE/m²/s, ,16:8 light: dark and 25± 2 C˚) (Sinigalliano *et al*., 2009) .

Algae Isolation and Purification: According to (Prescott, G.W and Stein, J.R) two methods were used for isolation and purification: streaking on plate agar; Chu-10 media solution solidified by 1.5 % agar-agar and sterilized by autoclave, after sterilization Chu-10 with 45-50 C˚ was poured. Into petri-dishes which left to solidify, sterile loop was used for streaking straight line. Then the plates were kept in a cooled illuminated incubator with light intensity about 268 µE/m²/s, 25± 2 C˚ and 16:8 lights: a dark period of 10 -14 days (Sinigalliano *et al*., 2009). The second method The serial dilution method by using ten test tubes, each one contains 9 ml Chu-10 nutrient solution, 1ml of algal culture was added to the first tube and shake carefully then 1ml from the first tube transported to the second tube and so on then incubated for two weeks . The same way again to the media culture for BG-11

Preparation and Sterilization of Media: Modified Chu-10 was used for the green algal growth (Kassim, T.I *et al*., 1999). BG-11 culture media for cyanobacteria (Rippka *et al*., 1979) and described their components in tables (1and 2), respectively, as It was prepared Stock solutions of each salt for macronutrients and stock solutions for micronutrients combined as follows:

TABLE 1: Stock Solutions Component of Chu-10

A-macronutrient salt	Concentration g/L	B- micronutrient salt	Concentration g/L
Sodium Meta Silicate	5.8	EDTA. Na_2	1.00
$Ca(NO_3)_2.4H_2O$	57.56	H_3BO_3	2.86
K_2HPO_4	10	$MnCl_2.4H_2O$	1.81
$MgSO_4.7H_2O$	25	$ZnSO_4.7H_2O$	0.222
EDTA .Na_2	4.36	$NaMoO_4.5H_2O$	0.390
$FeCl_3.6H_2O$	3.15	$CuSO_4.5H_2O$	0.079
Na_2CO_3	20	$Co(NO_3)_2$.6H2O	0.0494

Ten ml was taken from stock solution No 1, one ml from stock solution No. (2,3,4,5,6 and 7) of macronutrient and mixed with 1 ml of No. 8 of micronutrient solutions then completed to one liter of distilled water.

TABLE 2: stock Solutions Component of BG-11

Macronutrient salt	Concentration g/L	Micronutrient salt	Concentration g/L
$NaNo_3$	150	H_3BO_3	2.86
K_2HPO_4	30	$MnCl_2.4H_2O$	11.8
$MgSO_4.7H_2O$	75	$ZnSO_4.7H_2O$	0.222
$CaCl_2$. H_2O	27.181	$(NH4)Mo_7O_{24}$. $4H_2O$	0.0124
Citric acid	6	$CuS0_4.5H_2O$	0.072
Ferric ammonium Citrate	6	$CO(NO_3)_2.6H_2O$	0.048
EDTA.Na_2	1		
Na_2CO_3	20		

Ten ml was taken from stock solution No. 1 and one ml from solution stocks No. (2,3,4,5,6,7 and 8) of macronutrient then mixed with 1 ml of stock solution No. 9 for micronutrient solutions .Then completed up to one liter of distilled water.

Adjusted pH for media Chu-10 and BG-11 to 6.4 and 7.5, respectively, by adding a few drops of sodium hydroxide or hydrochloric acid (0.01N) then sterilized in autoclave except K2HPO4 at 121Cº, 15bar/in^2 for 15min (Atlas *et al*., 1995).

Algae Cultivation for Biomass: A100 ml suitable media for both isolated algae and transfer 10 ml of isolated algae then incubated for 14 days, also transfer this culture growth to 1000 ml of culture media and incubated again for 14 days. Finally, the growth culture transmits to glass pools 4L dimensions (50 cm length, 40 cm width and 30 cm high) for biomass culture (Tredici, M.R. 2004).

Determination the Growth Curve

Growth curve was determined for the purpose of identifying growth phases. Then the deposition cultures at the beginning of stationary phase, on the *Chlorella vulgaris* harvested in the twelve day but *Chroococcus minor* in the ten day. Microalga concentration was determined daily by optical density (OD) measurement at 540 nm by UV-Vis spectrophotometer, all measurements of the study were triplicates. The growth rate (K) and doubling time (G) were calculated according to the following equation:

$$K = \frac{(\log OD_t - \log OD_0)}{t} \times 3.322$$ (Huang *et al*., 2002a)

$$G = \frac{0.301}{K}$$ (Huang *et al.,* 2002b)

t: time (day) OD $_{t:}$ product after (t) day OD_0: algae beginning of the experiment zero time

The growth curve was determined for the two studied isolated microalgae, in addition to the growth rate (K) and the doubling time (G) were calculated for each treatment of the study experiments (Li *et al*., 2008).

Stimulation of Algae to Produce Lipid : Nitrate represented as nitrogen source in this study that was used for algal isolated (*C.vulgaris* and *C.minor*). The nitrate $NaNO_3$ concentration is 14.3 g/250 ml for modified Chu-10 culture media and 37.5 g/250 ml for BG-11 culture media that considered to be the control for the experiments (Mutlu *et al*., 2011).

Potassium phosphate (K_2HPO_4) represented as phosphorus source in this study that was used in the media for both isolated algae. K_2HPO_4 its concentration 2.5g/250ml for modified Chu-10 media culture and 7.5g/250ml for BG-11 media culture that is considered to be the control for the experiments (Mutlu *et al*., 2011).

Iron Chloride and Ferric ammonium Citrate represented as Iron source in this study that was used in the media for both isolated algae. Iron Chloride its concentration 0.78g/250ml for modified Chu-10 media culture and Ferric ammonium Citrate its concentration 1.5g/250ml for BG-11 media culture that considered being the control for the experiments. The five treatment of nutrients this study used a (-N) 100%, (-N) 50%, (-P) 50%, (-N) 50 plus (-P) 50% and (-Fe) 50%.

Lipid Extraction: One gram of dry weight was put in thimble that was transferred to specific cylinder in the soxhlet then 250 ml of solvent (hexane) was put in the flask. After 3-4 hours, the solvent color in the cylinder change from green to colorless, extracted sample were dried by rotary evaporator at 40C^0 for few minutes. Samples were poured to clean plates and left in room temperature at 25 C^O overnight then samples were transported to test tubes to be analyzed (Fajardo *et al*., 2007; Al-Rubaie and Jawad, 2015).

Lipid Analysis: Samples were analyzed by gas chromatography model DANI 2015 in Ministry of Industry and Minerals / IBN SINA state company.

Determination of Protein and Carbohydrate: The protein determined according to Bradford, (1976) method and the carbohydrate according to Dubois *et al*., 1956 (Azeez, 2001; Al-Rekabi, 2003)

Statistical Analyses: The result express as mean ± sd. Data were analyzed by one way analysis of variance (ANOVA) followed by Fisher's test for multiple comparison, using stat view version 5.0. Different were considers significant when p <0.05.

Results and Discussion

The Effect Nitrogen Concentration on Growth Curve for *C. vulgaris*: Results showed the effect of different nitrogen percentage on *C.vulgaris* biomass growth. In control treatment spent in lag phase one days and then logarithmic phase began until the twelve day and then entered stationary phase which lasted until the eighteenth day and then observed the beginning of a decline in the number of cells (Figure 1).

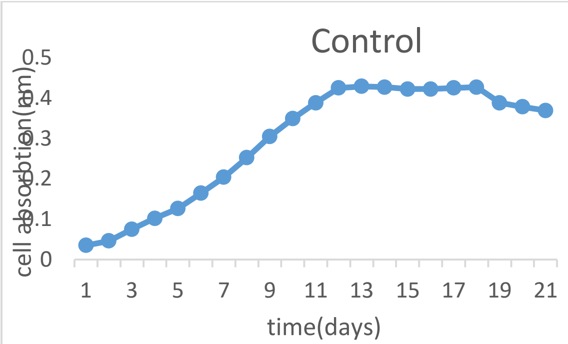

Fig. (1): Growth Curve of *C.vulgaris at* Control

In (-N) 100% treatment spent in lag phase four days and then logarithmic phase began until the seventh day and then entered stationary phase which lasted until the thirteenth day and then observed change in color from green to yellowish green figure (2).

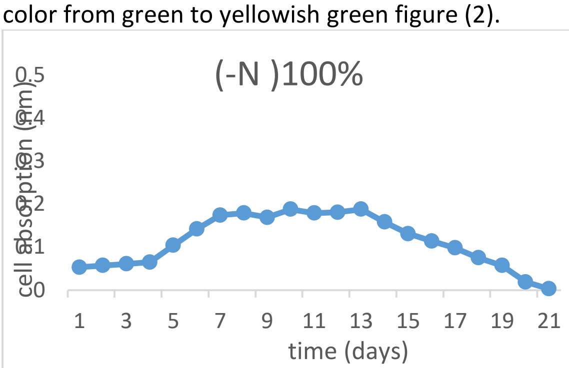

Fig. (2): Growth Curve of *C.vulgaris* at (-N) 100% Treatment

In (-N) 50% treatment spent in lag phase three days and then logarithmic phase began until the nine day and then entered stationary phase which lasted until the fifteenth day and then observed change in color figure (3).

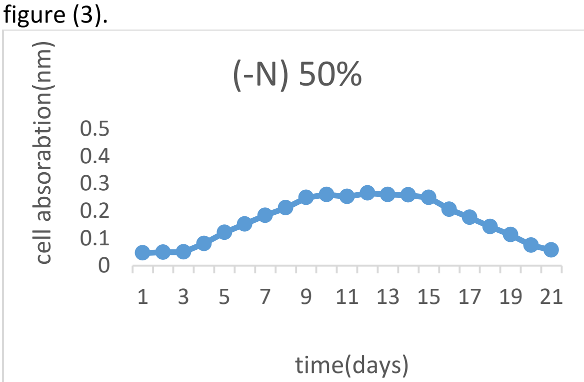

Fig. (3): Growth Curve of *C.vulgaris* at (- N) 50%Treatment

The algae spent in lag phase three days at (- P) 50%, treatment and then logarithmic phase began until the ninth day, then entered the stationary phase until the sixteenth figure (4)

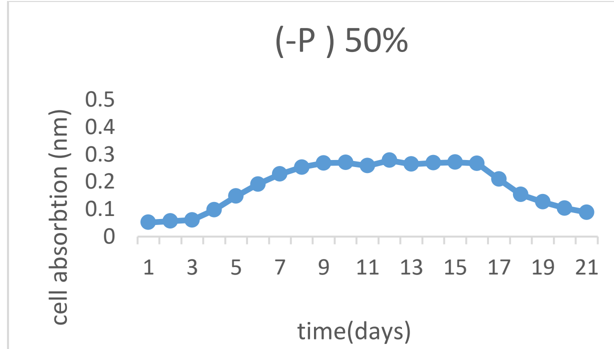

Fig. (4): Growth Curve of *C.vulgaris* at (-P) 50% Treatmen

Either at (-P) 50% plus (-N) 50% treatment spent in lag phase two days and then logarithmic phase began until the eighth day and entered the stationary phase until the fourteenth day figure (5).

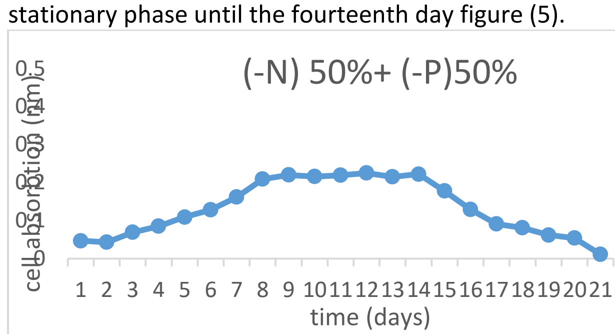

Fig. (5): Growth Curve of *C.vulgaris* at (-N) 50% + (-P) 50%Treatment

Finally, in (–Fe) 50% treatment spent in lag phase two days and then logarithmic phase began until the ten the day and then entered stationary phase which lasted until the seventieth day figure (6).

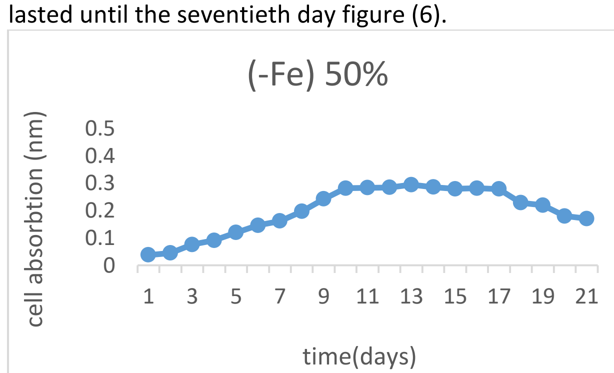

Fig. (6): Growth Curve of *C.vulgaris* at –Fe 50% treatment

The Effect Nitrogen Concentration on Growth Curve for *C.minor*: Results showed the effect of different nitrogen percentage on *C.minor* biomass growth .In control treatment spent in lag phase three days and then logarithmic phase began until the tenth day and then entered stationary phase which lasted until the seventeenth day and then observed the beginning of a decline in the number of cells figure (7).

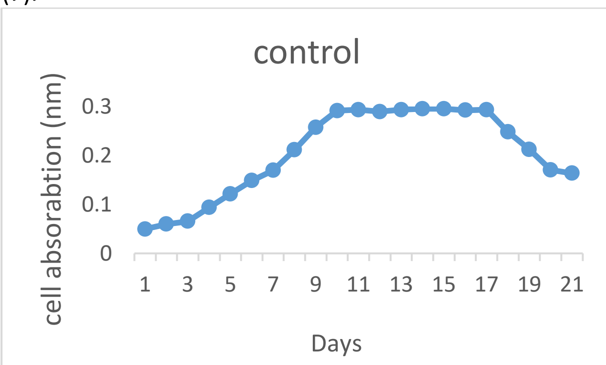

Fig. (7): Growth Curve of *C.minor* at Control

In N (-100%) treatment spent in lag phase four days and then logarithmic phase began until the eighth day and then entered stationary phase which lasted until the twelvfth day and then observed change in color from bluish green to yellowish green figure (8).

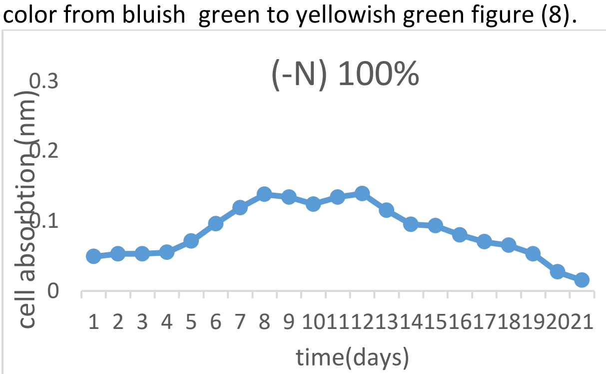

Fig. (8): Growth Curve of *C. minor* at (-N) 100% Treatment

In treatment (-N) 50% spent four days in lag phase and then logarithmic phase began until the ninth, then entered the stationary phase until the thirteen days and enter declaim phase figure (9).

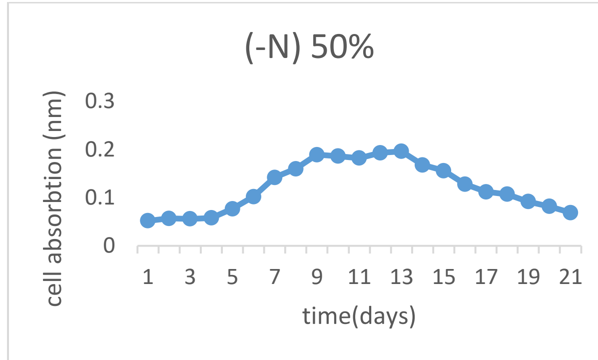

Fig. (9): Growth Curve of *C.minor* at (-N) 50% Treatment

The algae spent in treatment (–P) 50% lag phase three days and then logarithmic phase began until the ninth day, then entered the stationary phase until the fourteen days and enter declaim phase figure (10)

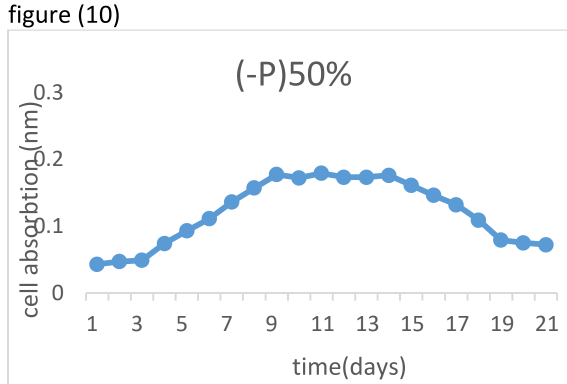

Fig. (10): Growth Curve of *C.minor* at (- P) 50% Treatment

Either at (- P) 50% plus (-N) 50% treatment spent in lag phase four days and then logarithmic phase began until the eighth day and then entered the stationary phase until the fourteen days and enter declaim phase figure (11).

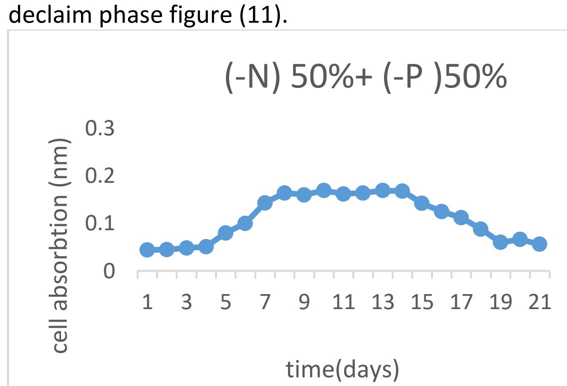

Fig. (11): Growth Curve of *C.minor* at (-N) 50% + (-P) 50%Treatment

Finally, in (- Fe) 50% treatment spent in lag phase three days and then logarithmic phase entered until the ninth day and then entered stationary phase which lasted until the sixteenth day figure (12).

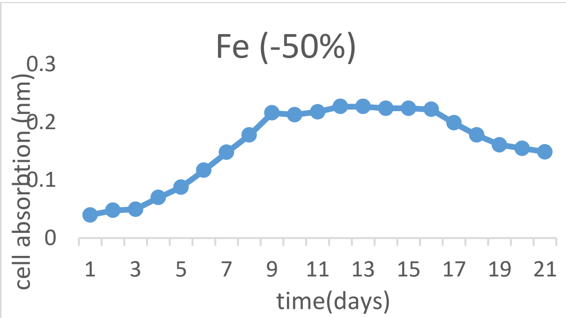

Fig. (12): Growth Curve of *C.minor* at (- Fe) 50% Treatment

The results showed that there a relationship between growth rates and doubling time for both algae in all treatment, in control at *C.vulgaris* the growth rate (k) was decrees from 0.33 day at control to 0.10 day at (-N)100%
as well as, show decreased in growth curve ether treatment as it recorded (0.15, 0.19 ,0.21 and 0.27) for each of the treatment (-N) 50% +(-P) 50%, (-N) 50% , (-P)50% and Fe (-50%) Respectively (table 3 and figure 13).

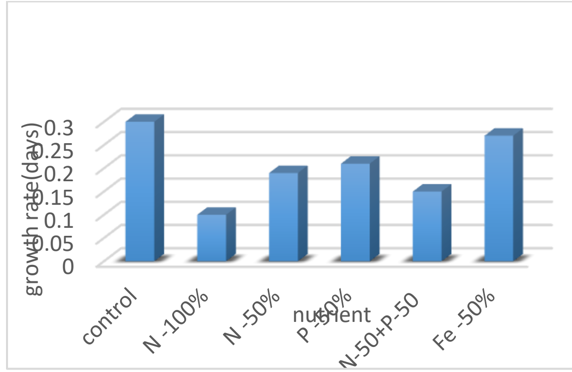

Fig. (13): Growth Rate *C.vulgaris* at Different Nutrient

The shorter doubling time rescored 1.0 at control while the longer doubling time at (-N)100% was rescored 4.6 , also show increased doubling time ether treatment as it recorded 3.4, 3 ,2 and 1.3 days For each of the treatment (-N) 50% +(-P) 50%, (-N) 50%, (-P) 50% and (-Fe)50% respectively .(Table 3 and figure 14).

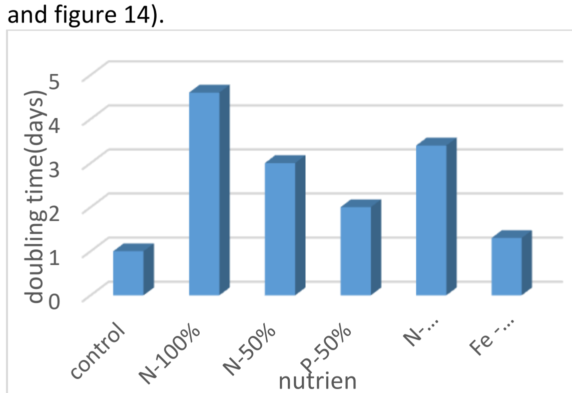

Fig. (14): Doubling Time of *C.vulgaris* at Different Nutrient

Table (3): Effect of Different Percentages of Nutrient on Growth Rate (K) and Doubling Time (G) for Both Algae (*Chlorella vulgaris* and *Chroococcus minor*)

Treatment	Growth rate (days) mean ± sd		Doubling time (days) mean ± sd	
	C.vulgaris	*C.minor*	*C.vulgaris*	*C.minor*
Control	0.33 ±0.021	0.25±0.030	1.0±0.265	1.4±0.26
(-N)100%	0.10±0.015 #	0.08±0.012#	4.6±0.265*$	5.4±0.22*$
(-N) 50%	0.19±0.021*#	0.15±0.015*#	3.0±0.265 $	3.5±0.26$
(-N) 50%+(-P)50%	0.15±0.015 #	0.12±0.026#	3.4±0.65 *$	4.2±0.24*$
(-P)50%	0.21±0.015 #	0.19±0.021#	2.0±0.265 *$	2.6±0.26*$
(-Fe) 50%	0.27±0.015 *#	0.22±0.020*#	1.3±0.289	1.7±0.26

* mean significantly differences between two algae, # mean significantly differences between two algae in growth rate and $ mean significantly differences between two algae in doubling time

In this study it is shown that the growth rate (k) of *C. minor* a decrease for all treatment, except control the growth rate was decreased from 0.25 at control to 0.08 (-N) 100% , as well as, show decreased in growth rate for rest treatment as it recorded 0.12,0.15,0.19 and 0.22 for each of the treatment (-N) 50% + (-P)50%, (-N) 50% , (-P) 50% and (-Fe) 50% Respectively (figure 15 and table 3).

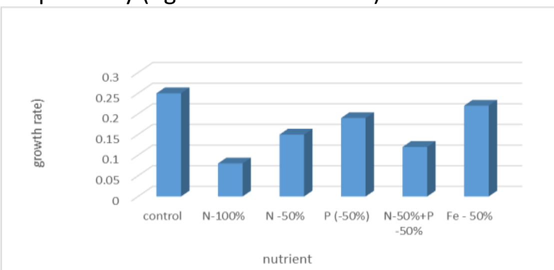

Fig. (15): Growth Rate of *C.minor* at Different Nutrient

The shorter doubling time rescored 1.4 day at control while the longer doubling time was at (-N) 100% was rescored 5.4 days ,also show increased doubling time ether treatment as it recorded 4.2, 3.5 ,2.6 and 1.7 days For each of the treatment (-P)50% +(-N) 50% ,(-N)50% ,(-P) 50%, (-Fe) 50% Respectively (Figure 16 and table 3).

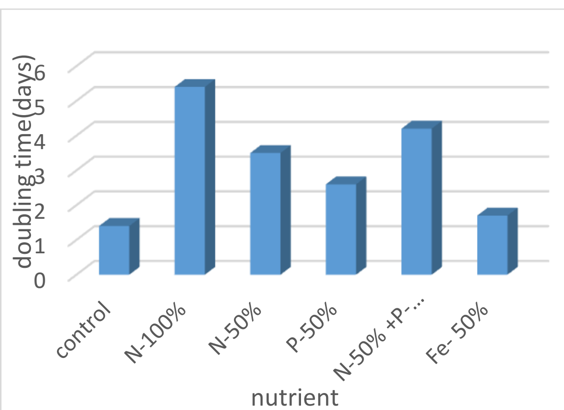

Fig. (4-16): Doubling Time of *C.minor* at Different Nutrient

The nitrogen nutrients major limitation to the growth of microalgae and biochemical composition (Valenzuela- Espinoza *et al*., 1999; Xu *et al*., 2001). Nitrogen is an essential constituent of all structural and functional proteins, amino acids, enzymes in the algal cells. Under deficiencies nitrogen cells usually change their metabolism to proceed total neutral lipid and carbohydrate content increase rapidly in algal cells as a protective mechanism for surviving in unfavorable conditions, and this mechanism is also termed the “lipid trigger” (Li *et al*.,2008).

Depending our results the growth rate was increased but doubling time was decreased at control due to growth medium most provide the inorganic element that constitute the algae cell .Nitrogen and Phosphor are the two most common nutrients limiting microalgae growth when nutrient availability particularly nitrogen has been documented to increase growth rate and doubling time decreases (Chisti, 2007; Li, 2008; Chen et *al*., 2011). Putt (2008) found the *C.vulgaris* cells can be doubled every eight hours during autotrophic growth and appropriate conditions.

Explanation of this situation cessation the growth with nitrogen limitations because without this nutrient these organisms cannot produce the amino acid and nucleic acid and protein necessary for growth and reproduction (Madigan *et al*., 2009). Another reason for growth inhibition, when starved of key nutrient, algae release autotoxin that inhibits cell division while photosynthesis continues to assimilate carbon and store it in the form lipids (Schnurr *et al*., 2013). And the cells continue growing and the nutrients will be consumed and led weak growth due to CO_2 converted to carbohydrates or lipids rather than into protein (Richardson *et al*., 1969).

This result agreement with a study by El-Kassas (2013) which reported under nutrient stresses the cell counts and biomass productivity of the *Picochlorum* sp. alga decreased as compared by control culture after 12 days.

The nitrogen deficient effect on microalgae growth rate and change to metabolic pathway have been observed in several spices of different microalgae such as *chlorella*, *nannochlorpsise* and *dunaliela* (Yeh *et al*., 2010; Sun *et al*., 2014) due to reducing of major and accessory photosynthetic pigment which leads to a color change of cells from blue-green to yellow-green (Grossman *et al*., 1993).

Iron is the most versatile and important trace element for biochemical catalysis. Iron-limited conditions are thought to alter cell physiology by reducing cell volume, chlorophyll content, and photosynthetic activity, and thus appear to impact cellular accumulation more than lipid accumulation per se. Specifically in *Phaeodactylum tricornutum*, the following enzymes were down-regulated during iron-starvation: β-carbonic anhydrase, phosphoribulokinase (PRK), two RuBisCO enzymes and a HCO_3^- transporter, likely resulting in decreased carbon fixation and cellular growth (Allen *et al*., 2008). High concentrations of iron are inhibitory to growth (Shrestha, 2014).

The doubling time was increased from 1.3 to 3 days and 1.8 to 3.3 days in *C.vulgaris* and *C.minor* respectively, due to Phosphate deficiency has been demonstrated to result in accumulation of lipids content and reduction in cell growth in (Belotti *et al*., 2013).

The Effect of Different Percentages of Nutrients on Lipids: The harvesting of the biomasses for both isolated algae were done in the end of exponential phase for lipid, carbohydrate and protein analysis.

This study shows increased lipids content all treatment when compare with control, in table (4) showed the lipid content for *C.vulgaris* was Increased from 8% at control to 41% (-N)100% treatment and the other treatment lipid contents have shown varying proportions, and they were 30%, 24%, 20% and 12% at the treatment (-N) 50%+(-P) 50%, (-N) 50%,(-P) 50% and (-Fe)50% respectively Figure (17) .

The same trend showed increased lipids content all treatment when compared with control for *C.minor* but observed lipids content lower than C. *vulgaris*. Increased lipids content from 5% at control to 30 % at (-N)100% treatment and the other treatment lipid contents have shown varying proportions 21%, 16%, 19% and 7% at the treatment (-N) 50+(-P) 50% , (-N)50%,(-P) 50% and (-Fe50%) respectively,(Figure18 and table 4).

Table (4): Effect of Different Nutrient Concentration on Lipids Content (%) for *C.vulgaris* and *C.minor*.

Treatment	Growth rate (days) mean ± sd		Doubling time (days) mean ± sd	
	C.vulgaris	*C.minor*	*C.vulgaris*	*C.minor*
Control	0.33 ±0.021	0.25±0.030	1.0±0.265	1.4±0.26
(-N)100%	0.10±0.015 #	0.08±0.012#	4.6±0.265*$	5.4±0.22*$
(-N) 50%	0.19±0.021*#	0.15±0.015*#	3.0±0.265 $	3.5±0.26$
(-N) 50%+(-P)50%	0.15±0.015 #	0.12±0.026#	3.4±0.65 *$	4.2±0.24*$
(-P)50%	0.21±0.015 #	0.19±0.021#	2.0±0.265 *$	2.6±0.26*$
(-Fe) 50%	0.27±0.015 *#	0.22±0.020*#	1.3±0.289	1.7±0.26

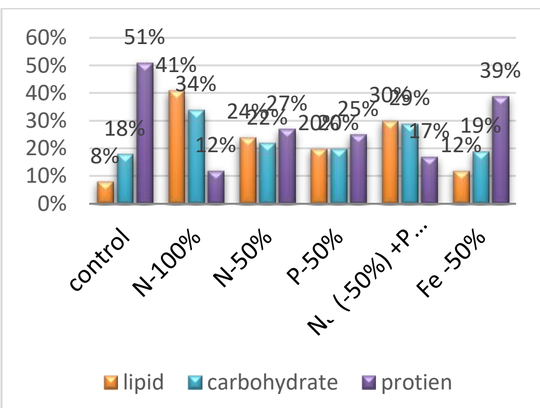

Fig. (17): Total Lipid, Carbohydrate and Protein of *Chlorella vulgaris* at Different Nutrient Concentration

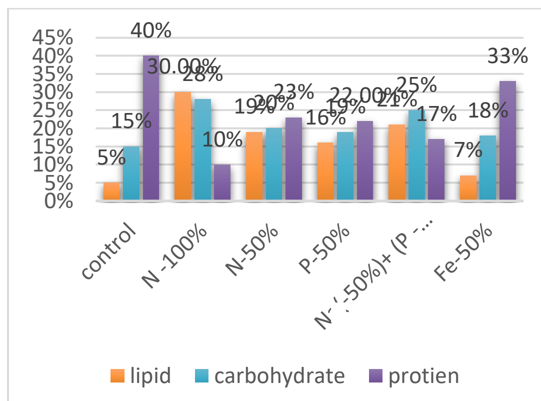

Fig. (18): Total Lipid, Carbohydrate and Protein of *Chroococcus minor* at Different Nutrient Concentration.

The growth density was measured to follow-up phases of growth from zero time (daily) to twenty days to determine the best phase that depending for harvesting, in this study show different harvesting time for both isolated algae in the treatment because observed different growth curve and growth rate.

Effect of Nutrient Concentration on Protein and Carbohydrate Content for Both Algae *C.vulgaris* and *C. minor*

The effects of limitation of nitrogen, phosphorus and iron on the biochemical composition of both microalgae *C.vulgaris* and *C.minor*. Increased accumulation of lipids, carbohydrate but observed decrease in protein content per cell for all treatment.

Current results showed, the effect of different percentages of nutrients on *C.vulgaris* that the highest percentage of protein at the control and the lowest percentage at -N 100% treatment reaching from 51% to 12%.

On the other hand, the effect of different percentages of nutrients on *C.minor* that the highest percentage of protein at the control 40% and the lowest percentage at -N 100% treatment Reaching from 10%. Table (5) and figure (18). Protein content of both studied microalgae was decreased sharply among treatments in contrast with control. The protein content was higher in *C. vulgaris* than in *C.minor* Significant differences were recorded among treatments at both studied microalgae.

Table (5): Effect of Different Nutrient Concentration on Protein Content (%) for *C.vulgaris* and *C.minor*.

Nutrient	*C.vulgaris*	*C.minor*
Control	51	40
(-N) 100%	12	10
(-N) 50%	27	23
(-P) 50%	25	22
(-N) 50% + (-P) 50%	17	17
(-Fe)50%	39	33

Reduced protein content under nitrogen and phosphor starvation due to the nitrogen in charge to manufacture structure of amino acids, enzymes, proteins, and thus will affect the translation process RNA into proteins which led to reduced number of ribosomes (Ahmed and Hellebust, 1990; Kamalanathan *et al*., 2016; El-Kassas, 2013. A study by Mutlu *et al*., (2011) on *C. vulgaris* found that decreased of protein content from 50.8% at control to 20.3% ,13.01% ,21% , 38% at treatment (-N)50%, (-N)100%,(-N plus P) 50% and (-P)50% treatment, respectively .another study on *Spirulia platensis* found lowest protein content Reached 5.6% under nitrogein starvation when compare with control it was 67% (Uslu *et al*., 2011).

It is also shown in this study, that an increased carbohydrate content in *C.vulgaris* when exposed decreased P and N nutrient in each treatment, it observed the effect of different percentages of nutrients on the carbohydrate content, it is highest present at treatment (–N)100% As it recorded 34% (figure 17) table (6)

On the other hand in *C.minor* that an increased carbohydrate content when exposed decreased P and N nutrient in each treatment, it observed the effect of different percentages of nutrients on the carbohydrate content, it is highest present at treatment N-100% as it recorded 28% (table 6 and figure 18)

Table (6): Effect of different nutrient on carbohydrate content (%) for *C.vulgaris* and *C.minor*.

Nutrient	*C.vulgaris*	*C.minor*
Control	18	15
(-N) 100%	34	28
(-N) 50%	22	20
(-P) 50%	20	19
(-N) 50% + (-P) 50%	29	25
(-Fe)50%	19	18

On the other hand, Carbohydrate content of both microalgae was increased among treatments. The study found that there are alternative paths to convert carbohydrates into TAG, under nitrogen starvation all structures of carbon emitted during metabolism may be directed to lipid and carbohydrates that increase the carbohydrate content of the algae cells. (Afify *et al*., 2010; Sibi *et al*., 2016). Another study by Markou *et al*., 2012 showed that under phosphorus-limitation, carbohydrate content increased from 11to 67%in *Arthrospira platensis*. Heraud *et al*., (2005) reported that nitrogen deficiency thereby increasing the relative carbohydrate content in algal cells phosphorus starvation reduces chlorophyll a and protein content thereby increasing the relative carbohydrate content in algal cells (El-Kassas, 2013).

Phosphate deficiency has been demonstrated to result in accumulation of astaxanthin and an overall reduction in cell growth (Sherstha, 2014). Cyclohexamide has also been used to block protein generation and increase carbohydrate levels (Bra´nyikova 2011). A study on the green algae *Pichochlorum* Sp. Show an increasing carbohydrate content by nearly 30.27%, 62.38% in the cultures grown in media supplemented with -50% and -100% $H_2PO_4 \bullet 2H_2O$ (El-Kassas,2013).

The net accumulation of carbohydrates and starch can be affected by several chemical and physical factors such as the type of light and light intensity medium composition, growth conditions, autotrophic and heterotrophic (Choixa *et al*., 2012).

The Fatty Acid: Two fatty acids were analyzed using the gas Chromatography (GC), Stearic acid and Oleic acid , under stress condition of pH (pH9)two fatty acids (oleic, stearic and) appeared, This agreed with study of Thompson, (1996) fatty acids found in Chlorophyceae, C16:0, C18:1, C18:0 were reported as the most common types.

Singh and Mallick (2014) demonstrated the relative percentage of saturated fatty acids was maximum in green as well as blue-green algae species under control and all the treated conditions. Oleic acid content was increased, and the relative percentage of linoleate and linolenate decreased against control microalgae under the treated conditions.

The area that spent in the Colum by the time of the standard of the fatty acid was 14154.342 microvolt/second [mV.s] and 4489.562 [mV.s] for Oleic acid and Stearic acid respectively.

The result of GC show that the area of the control treatment at *C.vulgaris* was 134.710 [mV.s] and 1007.513 [mV.s] for Oleic acid and Stearic acid respectively while the (-Na) 100% was 636.349[mV.s] and 3464.012[mV.s] for Oleic acid and Stearic acid respectively and in pH 9 treatment was 527.578[mV.s] and 6876.272 [mV.s] for Oleic acid and Stearic acid respectively.

Also the result shows the area of oleic acid and steric acid was 271.289 [mV.s] and 671.584 [mV.s] respectively for control to *C.minor* but in Na (-100%) treatment the area was 956.621[mV.s] and 2065.918 for Oleic acid and Stearic acid respectively, also the area in pH 9 was 194.708 [mV.s] and 747.024 [mV.s] for Oleic acid and Stearic acid respectively

Table (4-10): Oleic Acid and Stearic Acid Count in both Stadied Alga

Treatment	Oleic acid count [mV.s]		Stearic acid count[mV.s]	
	C.vulgaris	*C.minor*	*C.vulgaris*	*C.minor*
Stander for fatty acid	14154.342	14154.342	4489.562	4489.562
Control	134.710	271.289	1007.513	617.548
(-Na) 100%	636.349	956.621	3464.012	2065.918

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