

How Culture Medium pH Range Influence Phytoplankton Growth Performance and Biochemical Content

Shymaa S. Zaher* and Amr. M. Helal

National Institute of Oceanography and Fisheries, NIOF, Egypt
*Corresponding Author: Shymaasabry2@gmail.com

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ABSTRACT

The challenge today is to incorporate phytoplankton as a source of protein for food and feed to higher organisms in the food chain. The growth response and total biochemical content of three pure phytoplankton cultures to different ranges of pH in the synthetic medium were explored under controlled laboratory conditions. The tested species were *Chlorella vulgaris* Beyerinck [Beijerinck], *Nannochloropsis oculata* (Droop) D.J.Hibberd and *Scenedesmus quadricauda* (Turpin) Brébisson. Growth response was determined from acidic to alkaline conditions by chlorophyll values, total biochemical content, proteins, carbohydrates and lipids. The results indicated that the tested species showed different growth patterns and total biochemical content under the same incubation conditions. Generally, the highest biochemical content was total protein followed by carbohydrates and lipids. At pH (11) of the medium the highest protein peak of *Chlorella vulgaris* was (68.1mg l^{-1}) and *Nannochloropsis oculata* was (94.6mg l^{-1}). The maximum protein content of *Scenedesmus quadricauda* (86.7mg l^{-1}) recorded at pH (9) of the medium. The best pH values for growth of tested species were pH (7) for *Chlorella vulgaris* and pH (9) for *Nannochloropsis oculata* and *Scenedesmus quadricauda*. A major spot on the highly positive correlation between *Nannochloropsis oculata* optimum growth and its protein contents ($r= 0.87$) which indicate that pH (9) of the medium may be the best one in *Nannochloropsis oculata* large mass production. The highest carbohydrates and lipids content were detected at acidic conditions of the medium. This study assesses the best pH conditions needed for optimum growth of particular species to further applying in commercial mass production to get maximum protein yield.

INTRODUCTION

Phytoplankton as autotrophic microorganisms are the basic in food chain for feed to higher planktons as it provide others with energy and the main natural nutrients (**Barka and Blecker, 2016**). Microalgae usage in aquaculture and its internal biochemical content are primarily depends on its nutritional value, high mass production, ability to withstand fluctuating growth conditions, and being nontoxic, (**Muller-Feuga, 2013**);

(Roy and Pal, 2015). *Chlorella*, *Isochrysis*, *Nannochloropsis*, *Phaeodactylum*, *Chaetoceros*, *Pavlova*, and *Thalassiosira* are commonly used algae in mass production (Muller-Feuga, 2000). Certain species from different phytoplankton classes could overcome stress in natural environment, as they can exceed other microalgae classes, sometimes they vigorously grow and forming bloom generating secondary metabolites in the surrounding aquatic environment (Taheri *et al.*, 2017).

Influence of pH factor on the metabolic activity, biochemical constituents and ultrastructure of various algae was tested by (Sharma *et al.*, 2012, Safafar *et al.*, 2016). Also light intensity, temperature and minerals affect growth of cyanobacteria detected by (Katoli *et al.*, 2015). There is a relation between phytoplankton uptake of inorganic carbon during photosynthesis process and increase pH of the surrounding environment (Hansen, 2002). However, in the algal culture medium pH is considered one of the most important factors influence optimal algal growth, it controls algal metabolism and affects its bioactive products biosynthesis (Liu and Lee, 2000).

Chlorella spp. and *Scenedesmus* spp. are green phytoplankton commonly incorporated in food industries because of its high protein and lipid content, (Barka and Blecker, 2016). *Nannochloropsis* spp. of phytoplankton belongs to Eustigmatophyceae. Although its first record was in marine water (Vareltzis and Undeland, 2012) it commonly recorded in both fresh and marine environment. This species gained its importance from its high biochemical constituents and its possible mass production. A laboratory study about *Chlorella* spp. use as safe protein source for consumption and dietary supplementation and as natural food for some zooplankton species was tested by (Hegab *et al.*, 2019) and concluded that *Chlorella vulgaris* is used as food source for *Brachionus plicatilis* and *Heterocypris salina*. Zooplankton species grazing and ingestion rates were mainly depending on *Chlorella* densities. *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Nannochloropsis oculata* are known to be used in biodiesel production (Metsoviti *et al.*, 2019) and as feed for fish (Pulz and Gross, 2004).

Optimization of the conditions of algal cultivation may lead to production of algal biomass in adequate microalgae quantity (Vasileva *et al.*, 2015). The major scope is using of algae as feed to higher planktons and other organisms in the food web. Microalgae proteins are one of the most important sources of protein to fill the gap in usual protein sources (Barka and Blecker, 2016). Micro algal protein content of *Spirulina paltensis* constitutes (50-65%) compared to fish protein (24%), (Moorhead *et al.*, 2011). *Chlorella vulgaris* protein constitutes (43-58% of its dry weight) differs by culture conditions (Safi *et al.*, 2014). Algal usage in different industries in cosmetics, food products enlarge the need for mass production of algae, so there is a growing interest for focus studies on the algal optimum condition needed for optimum growth. So, this study investigate the growth response and total biochemical content of three species of phytoplankton cultured in modified BG11 medium under pH shift from acidic to alkaline conditions. Moreover, large scale production of *Chlorella vulgaris* is involved.

MATERIALS AND METHODS

Indoor culture conditions of the tested species

The phytoplankton species obtained from faculty of girls, Botany Dep., Ain-Shams University. The tested algae were *Chlorella vulgaris* Beyerinck [Beijerinck], (1890),

Nannochloropsis oculata (Droop) D.J.Hibberd (1981), and *Scenedesmus quadricauda* (Turpin) Brébisson, (1835). The selected species belongs to two phytoplankton classes, Chlorophyceae represented by *Chlorella* and *Scenedesmus*, while *Nannochloropsis* is from Eustigmatophyte. Through a series of laboratory work the tested algae become well adapted and grew very well incubated in synthetic modified BG11 medium (Rippka *et al.*, 1979) under sterilized laboratory conditions. The room temperature was $25 \pm 1^\circ\text{C}$ and the intensity of light adjusted to $30\mu\text{Em}^{-2}\text{s}^{-1}$ under a day / night program of 14 h light and 10 h darkness. The algae were harvested in the exponential growth phase. The tested algae were grown under various pH values of 3, 5, 7, 9 and 11. pH was adjusted using 1N HCL and 1N NaOH solutions using pH meter model (WTWph91). Three replicates were made for each pH value for tested species. These experiments were done in 500 ml conical polycarbonate Erlenmeyer flasks kept under controlled laboratory condition. For each species definite samples were filtered on glass GF/F filter paper (whatman $0.7\ \mu\text{m}$). Chlorophyll *a* detection was used for determining algal growth. The concentrations of chlorophyll *a* were calculated according to the trichromatic equation (Apha, 1995).

Total Biochemical contents were detected for each micro algal species, Biuret method was used for total protein detection, using Biuret reagent. Bovine Serum Albumin (BSA) used as standard for protein. For each sample and standard Biuret reagent was added then incubated for 30 minutes at room temperature then measured by absorption spectroscopy at 540 nm, (David James and Hazel, 1994). Carbohydrate contents were measured at 480 nm according to Phenol sulphuric acid hydrolysis method as described by (Dubois *et al.*, 1956), employing glucose as standard. The total lipid content was estimated by the Sulphophosphovanillin procedure utilizing cholesterol as calibration standard, the absorbance of the characteristic pale pink color was measured at 525nm (Chabrol and Castellano, 1961).

Chlorophyll *a* and the total biochemical constituents were estimated using Kontron 930 UV visible Spectrophotometer. From the three tested species *Chlorella vulgaris* was selected for both indoor and large scale cultivation. Indoor experiments have been used modified BG11 medium under sterilized laboratory conditions.

Outdoor Culture conditions of *Chlorella vulgaris* for large scale production

The large scale cultivation of the tested species was carried out at Heliopolis University, Faculty of Engineering, the green house (STDF project), the water source is underground water well that separated through Reverse Osmosis (RO) device into saline and fresh water. The separated fresh water is used in *Chlorella vulgaris* large scale production. From 500 ml conical polycarbonate Erlenmeyer flasks to transparent plastic tank (70 L) as intermediary stage (plate 1), 3m^3 tanks in raceway, filled with fresh ground water and pure nutrient medium inoculated with culture of *Chlorella vulgaris* with 5% of the tank water volume. Continuous aeration with paddle wheel is supplied for algal oxygen demands and to keep the culture medium in suspension (plate 2). Keep the tanks in open air outdoor conditions and average temperature was $25\text{-}30^\circ\text{C}$ for 14 days to harvest the required algal concentrations. Time of harvest begin by detecting transparency with secchi disk when transparency value reached 2 cm the harvest begin. Special net mesh size of 5μ is used for harvest algae (plate 3). The harvested algae dried

at oven at 60°C to gain pure powder of *Chlorella*. For outdoor algal production the Amaral's medium was used (Pecegueiro do Amaral, 2012).



Plates:

- (1): Transparent plastic tank of *Chlorella vulgaris* used as intermediate stage in the laboratory before mass production.
 (2): Tank of *Chlorella vulgaris* for large mass production showing the peddle wheel for continuous aeration
 (3): Net mesh size of 5 μ for algal harvest.

STATISTICAL ANALYSIS

Determination of algal growth as represented by chlorophyll *a* concentrations and total biochemical content were statistically analyzed using (StatSoft, 2011). Correlation and one way analysis of variance (ANOVA) were used to examine the independent effects as well as possible interactions.

RESULTS

1- Effect of different pH level on the tested phytoplankton species growth as representing by chlorophyll *a* concentrations

Growth response represented by chlorophyll *a* values of tested phytoplankton species were assessed during the present study under controlled laboratory conditions. The results showed that phytoplankton species recorded different growth pattern in spite of the similarity in the incubation conditions. Chlorophyll *a* concentrations of the phytoplankton species are illustrated in Figure (1).

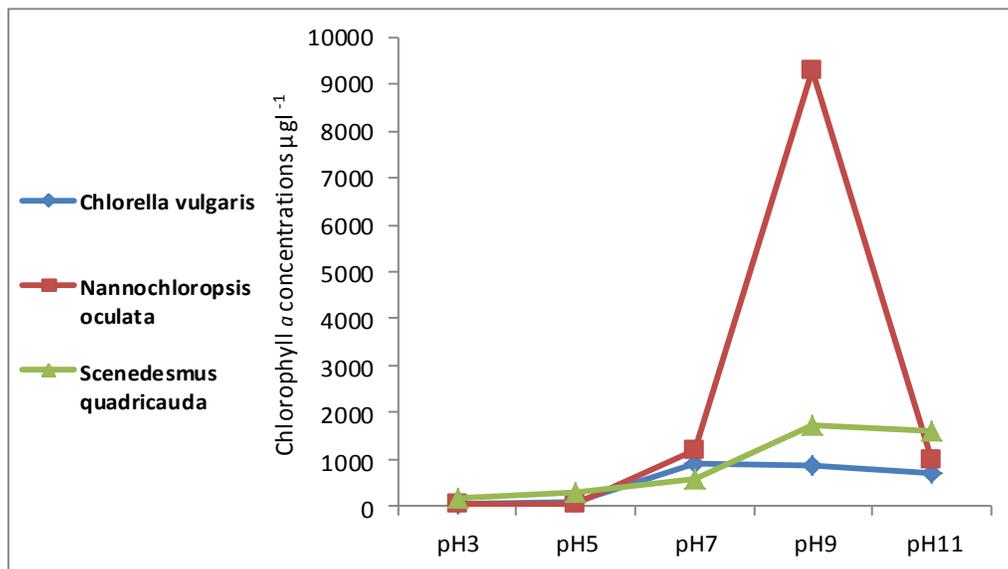


Figure (1): Chlorophyll *a* values ($\mu\text{g l}^{-1}$) of three phytoplankton species at different pH values.

Ia - Chlorella vulgaris

Chlorophyll *a* concentrations for *C. vulgaris* attained its lowest concentrations at pH 3 ($47.3 \mu\text{g l}^{-1}$) and the maximum one at pH 7 ($918 \mu\text{g l}^{-1}$). Gradual decrease in values was detected at pH (9) and till it reached ($695.2 \mu\text{g l}^{-1}$) at pH (11) of the medium. Chlorophyll *a* of *C. vulgaris* showed a positive correlation with total protein contents ($r= 0.629$) however strong negative correlation detected with carbohydrates ($r= -0.99$) and lipids ($r= -0.6$).

Ib- Nannochloropsis oculata

Growth of *N. oculata* showed considerably lower values of chlorophyll *a* at pH (3) and pH 5 ($47.1 \mu\text{g l}^{-1}$ and $51.2 \mu\text{g l}^{-1}$ respectively), while its highest peak was recorded at pH (9) when reached ($9284.1 \mu\text{g l}^{-1}$), then its value decreased to ($1005.07 \mu\text{g l}^{-1}$) at pH (11) of the medium. Special interest to the major peak of *N. oculata* recorded at pH (9) compared with the other tested species as shown in Figure (1). *N. oculata* growth as represented by chlorophyll *a* recorded high positive correlation with proteins and total biochemical contents ($r= 0.87$ & 0.83 respectively).

Ic- Scenedesmus quadricauda

Chlorophyll *a* concentrations of *S. quadricauda* showed different pattern of growth as it recorded higher values compared with other species. At pH (3) of the medium chlorophyll *a* value was ($174.9 \mu\text{g l}^{-1}$), while it recorded ($604.6 \mu\text{g l}^{-1}$) at pH (7), and then it reached the highest value of ($1750.9 \mu\text{g l}^{-1}$) at pH (9) of the medium. Growth of *S. quadricauda* was weakly correlated with protein and total biochemical contents but correlate negatively with carbohydrates and lipids (-0.61 & -0.54).

ANOVA analysis indicated significant differences attributed to the effect of pH shift on chlorophyll *a* between different species.

2- Effect of different pH level on phytoplankton total biochemical contents

The total biochemical contents of the selected species were detected at different pH range of the medium under laboratory conditions as shown in Figures (2, 3 and 4). Generally the highest biochemical content was total proteins followed by carbohydrates and lipids. "There were statistically significant differences between group means as determined by one-way ANOVA in both of *C. vulgaris* ($F(4, 20) = 6.71, p = 0.0013$) and *S. quadricauda* ($F(4, 20) = 6.66, p = 0.0014$). However ANOVA results showed non-significant difference in *N. oculata* species ($F(4, 20) = 1.68, p = 0.19$). For all tested species when medium tend to acidic conditions especially at pH (3) the highest carbohydrates content were recorded.

2a- *Chlorella vulgaris*

Total biochemical contents of *C. vulgaris* (Figure 2) showed more or less similar concentrations at pH (3,5 and 7) with average of (56.8 mg l^{-1}), then it slightly increased to (67.9 mg l^{-1}) at pH (9) reached its highest concentration of (74.2 mg l^{-1}) at pH (11) of the medium. The detected protein contents of *C. vulgaris* showed slightly gradual increase to a certain extent as pH increase from pH 3 (45.6 mg l^{-1}) till reaching the highest values at pH 11 (68.2 mg l^{-1}) of the medium. Carbohydrates contents of *C. vulgaris* were in the same range at pH (7, 9 and 11) while it reached its highest concentrations when the medium tends to the acidic condition (9.5 and 10 mg l^{-1}) at pH (5 and 3) respectively. Lipid contents of *C. vulgaris* were the lowest one ranged from (1.25 to 2 mg l^{-1}) at pH (11) and pH (3) respectively. Positive correlation was recorded between carbohydrates and lipids ($r = 0.67$). Total biochemical contents showed strong positive correlation with *Chlorella* proteins ($r = 0.96$) and strong negative correlation with its lipids contents ($r = -0.905$).

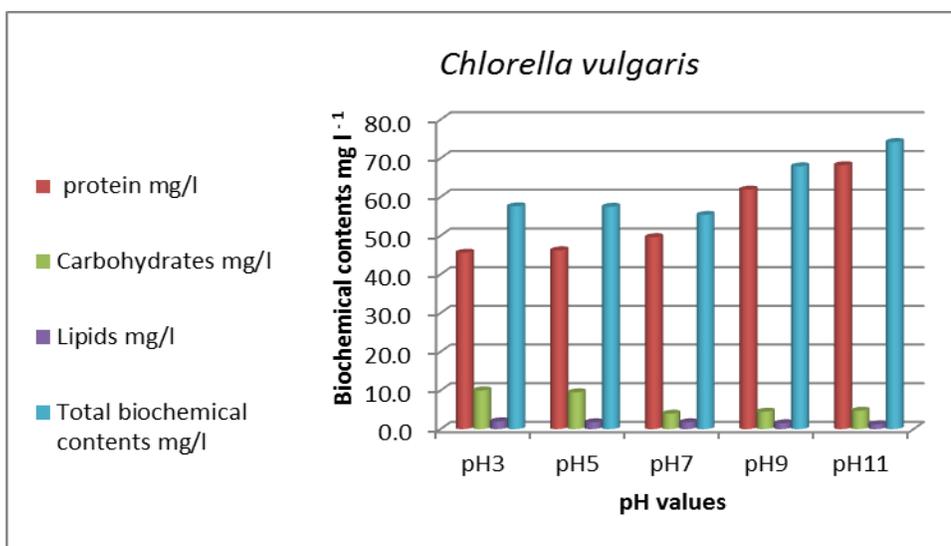


Figure (2): Total biochemical contents (mg l^{-1}) of *Chlorella vulgaris* under various pH levels.

2b- *Nannochloropsis oculata*

Total biochemical contents of *N. oculata* (Figure 3) attained its highest concentrations (102.36 mg l^{-1}) at pH (9), this value related to high protein contents which confirmed by highly positive correlation ($r= 0.99$), while the lowest total biochemical contents of (42.15 mg l^{-1}) recorded at pH (5) with lowest protein values. The tested species *N. oculata* was greatly affected by the pH shift in the growth medium attained protein contents of (59.8 and 57.4 mg l^{-1}) at pH (3) and (11) respectively. This species recorded sharp significant reduction of protein yield (32.7 mg l^{-1}) at pH (5) and the highest protein peak of (94.6 mg l^{-1}) was recorded at pH (9). A major interest in carbohydrates content of *N. oculata* attained the same carbohydrates level (11.1 mg l^{-1}) at the two extremes of acidic and basic conditions. The lowest value of (5.75 mg l^{-1}) was recorded at pH (9) of the medium. Lipid values were approximately in the same range at different pH values in average of (1.8 mg l^{-1}). There is no significance difference between biochemical contents of *N. oculata* due the course of this study.

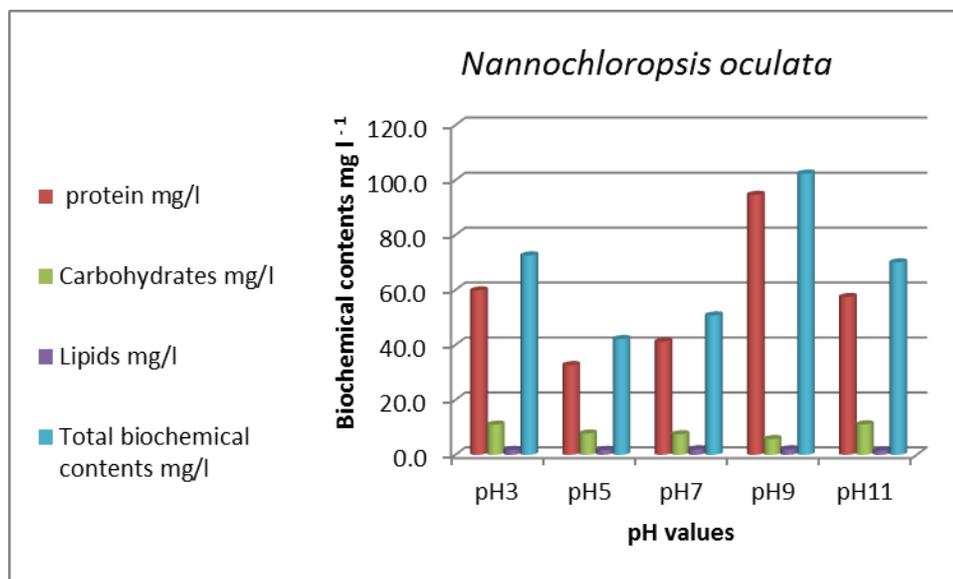


Figure (3): Total biochemical contents (mg l^{-1}) of *Nannochloropsis oculata* under various pH levels

2c- *Scenedesmus quadricauda*

The total biochemical contents of *S. quadricauda* (Figure 4) recorded the highest average value of (78.61 mg l^{-1}) compared with other species. The maximum value was recorded when the medium tends to alkaline conditions at pH (9) while the minimum of (67.69 mg l^{-1}) was recorded towards the acidic conditions at pH (3) of the medium. *S. quadricauda* maximum protein content of (86.8 mg l^{-1}) was recorded at pH (9) and the minimum of (57.2 mg l^{-1}) was recorded at pH (3). The maximum carbohydrate content of (8.5 mg l^{-1}) was at pH (3) and the minimum of (6 mg l^{-1}) was at pH (9). Lipid contents of *S. quadricauda* take the same trend as those of carbohydrates achieving its highest level of (2.0 mg l^{-1}) at pH (3) and the lower of (1.0 mg l^{-1}) at pH (7).

S. quadricauda showed highly positive correlation between its total biochemical contents and protein ($r= 0.99$) also between carbohydrates and lipids contents ($r= 0.89$). However

negative correlations were detected for carbohydrates and protein & its total biochemical contents ($r = -0.74$ & -0.67 respectively).

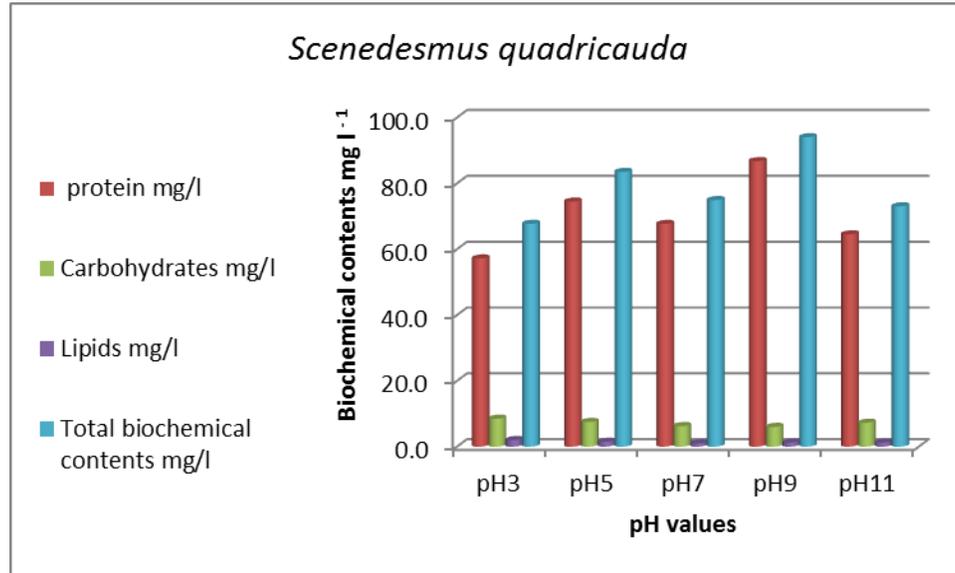


Figure (4): Total biochemical contents (mg l⁻¹) of *Scenedesmus quadricauda* under various pH levels.

Total biochemical contents percentage (%) of the tested species differed from species to another showed special variation at different pH levels. Figure (5) represents the percentage (%) of total proteins, total carbohydrates and total lipids at pH (7) of the medium as control.

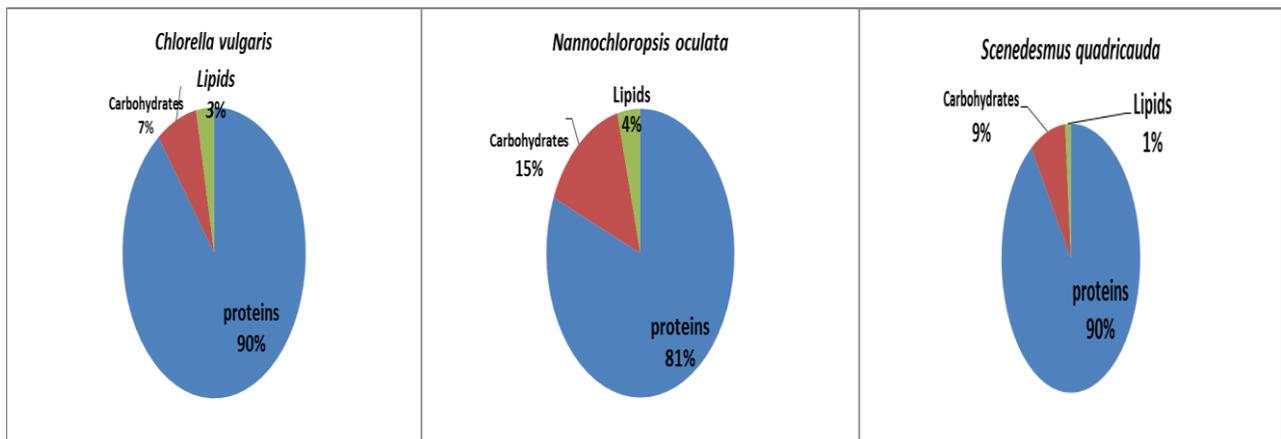


Figure (5): Biochemical contents percentage (Proteins, Carbohydrates and Lipids) of the three tested species at control conditions, pH (7) of BG11 medium under laboratory conditions.

For *C. vulgaris* proteins were 90% while carbohydrates represent 7% and only 3% for lipids. *N. oculata* protein percent attained 81% while carbohydrates were 15% with 4% lipids contents. For *S. quadricauda* protein percentage was 90% while carbohydrates represent 9% with minor lipids of 1%.

3- Outdoor Cultivation of *Chlorella vulgaris* for large scale production

The present results confirmed that *C. vulgaris* contain high total biochemical contents and high protein contents moreover under stress conditions especially when pH shifts to acidic conditions *C. vulgaris* can accumulate more carbohydrates and lipids content. For these reasons, this species was selected for cultivation for large mass production. In large scale mass production, commercial medium was used instead of the synthetic medium. This includes modification in the medium components by adding commercial fertilizers as urea and phosphoric acid as considerably low cost components. The implementation of this technique has achieved promising results in commercial farms (personal communication).

DISCUSSION

Microalgae response to stress in the surrounding medium are greatly fluctuated and pH shift consequently affect phytoplankton growth performance and its biochemical profile. pH of the medium is largely affected on CO₂ solubility, availability and its role on the essential nutrients in algal metabolism are considered (**Chen and Durbin, 1994**). The suitable pH of the growth differs from species to another. Chlorophyll *a* concentrations of the tested species grown in BG11 attained the maximum values (at pH from 7 to 9) from neutral to slightly alkaline conditions, then its values tended to decrease by shifting the medium to acidic or basic conditions. (**Falkowski and Raven, 1997**) observed inhibition in growth of *Chlorella* as represented by chlorophyll *a* concentrations at pH (11) of the medium, at high alkaline pH, almost no carbon is accessible for the algae because carbonate ion (CO₃²⁻) is the dominant form of inorganic carbon and the bicarbonate ion (HCO₃⁻) is the form utilized by the microorganisms (**Azov, 1982**). Limitation in CO₂ supply which potentially reduces the phytoplankton growth, influence the inter-speciation of inorganic carbon (as CO₂, HCO₃⁻, CO₃²⁻) may be from reasons of inhibition of phytoplankton growth at high pH values (**Barka and Blecker, 2016**). *Nannochloropsis* species attained its optimum growth values as detected by chlorophyll *a* at pH (9). (**Hu and Gao, 2006**) recorded the optimum pH for *Nannochloropsis salina* ranged from (7.5 to 8.5), while in other experiment doing on *Nannochloropsis salina* by (**Safafar et al., 2016**) the growth at pH (6.3), was significantly lower than at higher pH values. The same results recorded by (**Schmidt and Hansen, 2001**) when the marine phytoplankton cultures grown in f/2 medium showed obvious inhibition in growth at higher pH more than those caused by high nutrients (N and P). *Scenedesmus quadricauda* achieved its highest value of growth as represented by chlorophyll *a* concentrations at pH (9) of the medium, the same results obtained by (**Bakuei et al., 2015**) who recorded high growth of *Scenedesmus* in alkaline conditions at pH 8 of the medium. As photosynthesis process of algae is deeply affected by pH of the medium, alkaline condition preferred for algal cultivation (**Bakuei et al., 2015**).

In the present study, high protein contents was detected at pH (11) for *Chlorella* (91.9%) however high values were detected at pH (9) for *Nannochloropsis* (92.4%) and *Scenedesmus* (92.2%), it seems that these pH values stimulate protein production in comparison with its lower value at pH (3). Strong positive correlation of *Nannochloropsis oculata* growth with total proteins contents ($r= 0.87$) indicated that pH

(9) of the growth medium of this species is the best one to produce higher protein content, so the result of this study recommended pH (9) as the best value for growth of *N. oculata* in large scale production to obtain optimum protein contents. However, further studies must be performed on commercial medium used in algal mass production.

Safafar et al. (2016) recorded higher biochemical contents in *C.vulgaris* and *C.minor* were at pH (9). Protein contents in plankton have been relatively less studied compared with its lipids. Proteins are the basic organic components in cells except during storage of carbohydrates and lipids. Microalgae contain high proteins contents, *Chlorella vulgaris* contain 51-58%, (**Enyidi, 2017**) *Scenedesmus obliquus* contain 48-55% (**López et al., 2010**). Protein content in *Nannochloropsis salina* are 39% (**Safafar et al., 2016**). Protein from microalgae as natural source can be used as an animal protein replacement e.g *Chlorella* protein, which in turn may have positive effect on the environment (**Jones, 2016**).

All the tested species recorded its highest carbohydrates contents at pH (3) of the medium, for *Chlorella* (17.3%), *Nannochloropsis* (15.1%) and *Scenedesmus* (12.5%). The highest lipid contents of *Chlorella* (3.4%) and *Scenedesmus* (2.9%) were at pH (3) of the medium while, *Nannochloropsis* high lipid contents (4.1%) was recorded at pH (5) of the medium. The high carbohydrate and lipid contents of the tested species recorded in acidic condition of pH may be a mode of adaptation of these algae, since some algae adapt to the medium acidic condition by accumulating of more lipids. *Dunaleilla acidophila* accumulating glycerol to adapt to acidic conditions and avoid osmotic imbalance (**Fuggi et al., 1988**) storage of lipids also detected in *Chlamydomonas* and *pinnilaria braunii* var. *amplicephala* under highly acidic conditions (**Tatsuzawa et al., 1996**). Maintenance of neutral intracellular pH in acidic conditions of medium require excessive energy to pump protons out of the cell (**Terry and Abadía, 1986**) so, algae need to store carbohydrates, this explain the high lipid and carbohydrates content of the tested species under acidic condition of the medium.

Phytoplankton compositions in case of cultured medium differ from those grown in natural habitat, during the exponential growth phase protein is the dominant while carbohydrates, mainly soluble sugars, stored during stationary phase, (**Platt and Irwin, 1973**), total protein content values differ from 13% and 59% of the phytoplankton dry weight for cultured (**Strickland, 1968**) and natural habitat (**Platt and Irwin, 1973**). The previous authors indicated that the variations are basically related to the physicochemical factors of the environment and to a lesser extent on the algal growth stage. (**Xia et al., 2014**) assessed the high protein content of *Scenedesmus* sp. also its high lipid content and biomass productivity. Microalgae species e.g *Scenedesmus* sp. and *Chlorella* sp. are almost being produced for biofuel synthesis, and after lipid extraction the by-product obtained can be readily valorized for its high protein content. (**López et al., 2010**) determined that for most microalgae the protein contents vary widely from 30 to 55% depending on the cell disruption pre-treatment method applied the strain and the growth medium nature.

Identifying the optimum growth conditions for algal growth lead to production of enough mass production of microalgae. (**Vasileva et al., 2015**) then usage the dried form of these algae in synthetic feed lead to determination of the so called, techno functional properties of protein, this term describe the protein properties that is plus their nutritional function

are able to have important role in food formulation. These properties are deeply involved in enhancing the organoleptic characteristics of processed food, including texture, viscosity, palatability and mouth feel. This described by (Amita and Ahluwalia, 2010) who compared native and chemically modified *Spirulina* proteins for their functional properties. The author found that *Spirulina* protein solubility in water decreased considerably when treated with succinic anhydride, acetic anhydride and formaldehyde. Foam stability was found to be higher with methylation and acetylation. Maximum viscosity was obtained with the succinic anhydride modified protein fraction followed by acetylation.

As phytoplankton considered a very good source of protein that have beneficial nutritional properties and in turn its use in fish feed need mass production so, studying all the optimum condition needed for optimum growth and maximum protein yield are recommended. Also, microalga mass production needs to be encouraged by public authorities to overcome food shortages for the increased world population. Studying the algal product status from the point of view its toxicology, microbiology and its biochemical contents must be assessed before its complete integration as basic components into processed foods and feed.

Selection of *C. vulgaris* for cultivation and mass production mainly due to it is one of the most studied and commercially produced algae known to have several biochemical components with extensive range of applications in different fields (Ru *et al.*, 2020).

C. vulgaris employed for human consumption has high proteins, vitamins, minerals, and polyunsaturated fatty acids (Becker, (2007);Liu and Hu, (2013);Chacón-Lee and González-Mariño, (2010) ;Petkov and Garcia, (2007). *C. vulgaris* protein constitutes (43-58%) while, lipid content forms 5-58%, (Safi *et al.*, 2014), moreover carbohydrate content can reach up to (12–55%) dry weight especially when cultured under unfavorable conditions like, limited nitrogen conditions (Choix *et al.*, 2012).

Algal usage for food and feed need large mass production but face high cost so, selection of commercial medium drop the costs for algal large scale production. Considerable rise in algal mass production costs is the primary reason for restricting further applications of these microalgae (Wijffels and Barbosa, 2010).

Economical microalgal mass production need significant reduction in cost in order to be more competitive and sustainable (Zittelli *et al.*, 2013). Commercial medium production depends on replacement of high cost medium components by another considerably low cost components as verified by (Abou El-Kheir *et al.*, 2016) who succeeded in modification of Zarouk medium for large scale *Spirulina* production by using urea and phosphoric acid as commercial fertilizers. The same technique was used in *Chlorella* large scale production (Hasanein *et al.*, 2018) . In this field, ongoing studies and techniques are recommended for developing commercial low costs medium for outdoor large scale algal production.

CONCLUSION

Based on the results it is clear that, the habituation behavior of the three tested alga seems to be different at various pH range. The best pH values for chlorophyll *a* production were pH (7) for *Chlorella vulgaris* and pH (9) for *Nannochloropsis oculata* and *Scenedesmus quadricauda*. There is a limitation in growth of the tested phytoplankton species at

elevated levels of pH medium. High protein contents for *C. vulgaris* obtained at pH (11) while, for *N. oculata* and *S. quadricauda* were at pH (9). Generally, the highest carbohydrates and lipids contents were at acidic conditions of the medium. These experiments depend on particular species grown as well as on definite growth medium, which could bring useful data for full scale mass production. The tested algal species need more intensive researches on economic commercial culture conditions to cope with challenge in algal mass production.

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