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Research Article



Production of unsaturated fatty acids (omega 3,6,7,9) from algae *Lyngbya* and *Oedogonium* under the influence of nitrogen and phosphorus

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Abstract

Microalgae have a lot of promise for making nutritious food and feed additives. These photosynthetic show promise for the long-term production of lipids due to their capacity to transform carbon into high-value chemicals and their ability to be grown on a vast scale without interfering with crop cultivation. Particularly, microalgae serve as a substitute source of unsaturated fatty acids (UFAs), whose consumption is linked to a number of advantages for both human and animal health. The generation of PUFAs in microalgae has been improved in recent years through the investigation of several methods. Two algae were isolated from the Gharaf river in Thi-Qar (*Oedogonium*, *Lyngbya*). The study was conducted on the effect of different concentrations of nitrogen and phosphorus for each of the unsaturated fatty acids of the two algae. Nitrogen concentrations were (5,10,20) and for phosphorus (1,5,10). These (UFAs) are affected when these nutrients are added to the culture medium, and as the concentration of these nutrients increases, we notice a decrease in the proportions of these unsaturated fatty acids (Octadecatrienoic acid, omega 6, Oleic acid omega 9, Eicosapentanoic acid omega 3, Docosatetraenoic acid omega 3) in *Lyngbya* and with absence OA, Docosatetraenoic acid and presence (vaccenic acid, omega 7) in *Oedogonium*.

Keywords: Unsaturated Fatty Acids, Microalgae *Oedogonium*, *Lyngbya*, Nitrogen, Phosphorus.

Introduction

Microalgae have great potential for the production of nutrient-rich food and feed additives (Sepulveda et al., 2019). Due to their potential to convert carbon into high-value compounds and their ability to be produced on a large scale without interfering with crop agriculture, these photosynthetic bacteria offer promise for the long-term production of lipids (Van et al., 2012). In particular, polyunsaturated fatty acids (PUFAs), whose ingestion is associated with a range of benefits for both human and animal health, are substituted for in microalgae (Chew et al., 2017). In recent years, many techniques have been investigated in an effort to increase the production of PUFAs in microalgae (Kapoor et al., 2021).

Polyunsaturated fatty acids (PUFAs) are fatty acids (FAs) containing two or more double bonds in their acyl chain. Polyunsaturated fatty acids (PUFAs) are fatty acids (FAs) with two or more double bonds (Mason et al., 2020). Short-chain

polyunsaturated fatty acids (SC-PUFAs), which have 16 or 18 carbon atoms, and long-chain polyunsaturated fatty acids (LCPUFAs), which have more than 18 carbon atoms, are the two main groups of PUFAs (Saini et al., 2021). It is generally known that PUFA ingestion has multiple positive effects on human health including, increased metabolic rates, control of blood pressure and blood sugar levels, and defense against a wide range of disorders (Ghasemi et al., 2019).

As with other animals, humans have difficulty synthesizing several critical PUFAs, such as the precursors of AA and DHA, linoleic acid (LA-C18:2-6) and -linolenic acid (ALA-C18:3-3) (Khozin et al., 2011). Consequently, direct absorption of these chemicals from outside sources is required. Aquatic species (fish, mollusks, and crustaceans), animal-derived products (meat, milk, eggs), fungi, bacteria, and vegetable sources, such as certain plants and microalgae, are some of these sources (Simopoulos, 2016). The principal

microbial sources that might yield extremely useful lipids for nutraceutical (Makrides et al., 1997).

Modification of Growth Conditions to Promote PUFA Production

Lipid metabolism in microalgae may be managed by adjusting growth factors such temperature, light, CO₂ partial pressure, and nutrition availability.

The productivity of biomass and lipids may be increased by optimizing such factors, which can also boost growth rates.

Nutrients

Both culture media composition (e.g., nutrient concentration and composition) and growth phase (since nutrients tend to decrease over time during algal growth) can affect lipid metabolism (Yates et al., 2014). Generally, the concentration of unsaturated fatty acids (UFAs) is higher during the exponential phase and tends to decrease in aged cultures (Abedi, 2014). This happens because plastidial membranes, which are actively biosynthesized during the exponential phase contain larger proportions of UFAs compared to other cellular membranes as well as lipid droplets (Almeyda et al., 2020). To our knowledge, nitrogen (N) limitation or depletion is the most common culturing manipulation used to enhance lipid production, although it usually causes a reduction in biomass and lipid yields (Krishnan, 2020). Two-stage cultivation systems—the first one of nutrient replenishment, to increase biomass, and the second one with low or null N supply, to enhance lipid production—can be effective to overcome this problem (Fakhry and El Maghraby, 2015) and are correlated to a strong increase of the neutral lipid fraction (especially triacylglycerols (TAGs). For example, two-stage cultivation was found to be effective in increasing lipid concentration in *Nannochloropsis* spp (Schreiber et al., 2017). Since PUFAs in phospholipids (PLs) are nutritionally more available than those esterified in TAGs (Pal et al., 2011), an efficient production process might be achieved by maximizing the proportion of PLs within cells and that of PUFAs within PLs (Su et al., 2010). This can be usually obtained by culturing microalgae under N limitation rather than incubating microalgal biomass in N-depleted media. In *Isochrysis aff. galbana* (clone T-Iso) and in *Nannochloropsis oceanica*, the proportion of EPA in PLs was, indeed, lower under total depletion of N, and a similar trend was observed for DHA in T-Iso (Guedes et al., 2010). N limitation likely led to a decrease in the production rates of ω-3 PUFA, stimulating TAGs' accumulation (Wang et al., 2019). Similar results were obtained for *P. purpureum*, where EPA was mostly concentrated under N replenishment than in the stationary phase, when an active cell division, and, thus, membrane lipid accumulation was still in progress (Breuer et al., 2012).

Phosphorus (P) limitation triggered an increase in the concentration of UFAs concerning the total FAs, since P depletion stimulated the activity of Δ⁶-desaturase and thus,

conversion from palmitic acid (PA-C16:0) to LA acid. Moreover, stimulation of the ω-6 pathway and inhibition of the enzyme Δ-17 desaturase under low P concentration selectively promoted the biosynthesis of AA, to the detriment of the EPA pool (Li et al., 2019). Similar results were obtained with the freshwater eustigmatophyte *Monodus subterraneus*, where EPA and LC-PUFAs were drastically reduced under P (K₂HPO₄) limitation in a dose-dependent way (Su et al., 2016).

The relative proportion of PUFAs over the total biomass, as well as PUFA composition, are dependent upon the growth phase. For example, EPAs were found to be more abundant during the late stationary phase, while the DHA-to-EPA ratio (which must be comprised between 1 and 2 for larval fish feed) decreased since no increase in DHA levels in *P. tricornutum*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana*, and *Rhodomonas salina* was observed (Boelen et al., 2017).

Materials and methods

Algae species was isolated from the Algarve River located in Thi-Qar and the studied algae is *Lyngbya* and *Oedogonium*. Modified BG11 was used for algal growth Table 1. A serial dilution method was used to isolate and purify algae in this study. For algae cultivation, 10 ml of the isolated culture medium was added to a beaker containing 100 ml of BG-11 and incubated for 14 days, then transferred to 1000 ml of medium and incubated for 14 days in culture. The growth curve of the studied algae was determined.

Cell growth was measured by determining the optical density (O.P. daily. Optical density 650 nm) was measured by using spectrophotometer UV-VIS (540 nm). All measurements of the study were triplicates. The growth rate (K) and doubling time

$$K = \frac{\log N_t - \log N_0}{t}$$

t: time.

N_t: the optical density of the algal mass after the passage of time t.

N₀: the optical density of the algal mass at the start of the experiment

$$G = \frac{0.301}{K}$$

Experiment design

In the current study, different concentrations of nitrogen and phosphorus were used to stimulate isolated algae to produce lipids that can be used as a food supplement and biodiesel treatment. Microalgae were harvested at the beginning of the

stationary phase. Each microalgae culture was centrifuged in a refrigerated centrifuge at 3000 rpm for 15 min, the supernatant removed but the organic precipitate washed with distilled water, then dried at 45 °C for 2 days. The dry weight of the extract was collected.

Table 1. Components of BG11 culture medium

| Salt | Stock solution | Volume |
|-----------------|----------------|--------|
| Nano3 | 150(g/L) | 10 |
| K2HPO4 | 4.0 | 10 |
| Mgso4.7H2O | 7.5 | 10 |
| Cacl2.2H2o | 3.6 | 10 |
| Citric acid | 0.6 | 10 |
| Ferric citrate | 0.6 | 10 |
| EDTA-Na | 0.1 | 10 |
| Na2CO3 | 2.0 | 10 |
| H3BO3 | 61.0 | 1 |
| MnSo4.H2o | 169.0 | 1 |
| ZnSo4.7H2O | 287.0 | 1 |
| CuSo4.5H2O | 2.5 | 1 |
| NH4)6MO7O24.4H2 | 12.5 | 1 |

Fat extraction and analysis

Ultrasound-Assisted Extraction (UAE): The UAE principle consists of shaping sound waves in a liquid medium by

exposure to ultrasound (with a frequency of 20-50 kHz) and creating high and low-pressure cycles. During the high-pressure cycle, the tiny vacuum bubbles formed in the low-pressure cycle are destroyed intensively creating a phenomenon called cavitation. The high pressure and jets formed during mechanical cavitation destroy the cellular structure of the reactant and intensify mass transfer processes, facilitating lipid extraction. Samples were analyzed by sample GC mass system 4.6 mm × 5 µm injection flow 1 mL/min at UV.

Results and Discussion

The growth curve was estimated in terms of optical density (650) Fig.1 at pH 7 and temperature 30 °C, and the exponential growth phase began after the seven day and reached the stable phase after the fourteenth day to begin the decreasing phase after the seventeenth day Its relative growth constant was ($K = 0.0324$) and the rate of doubling time ($G = 13.35$) for *Lyngbya* and algae *Oedogonium* and the exponential growth phase began after the seven days and reached the stable phase after the fourteenth day to begin the decreasing phase after the sixteenth day $K = 0.0174$, $G = 12.73$ (Fig 2).

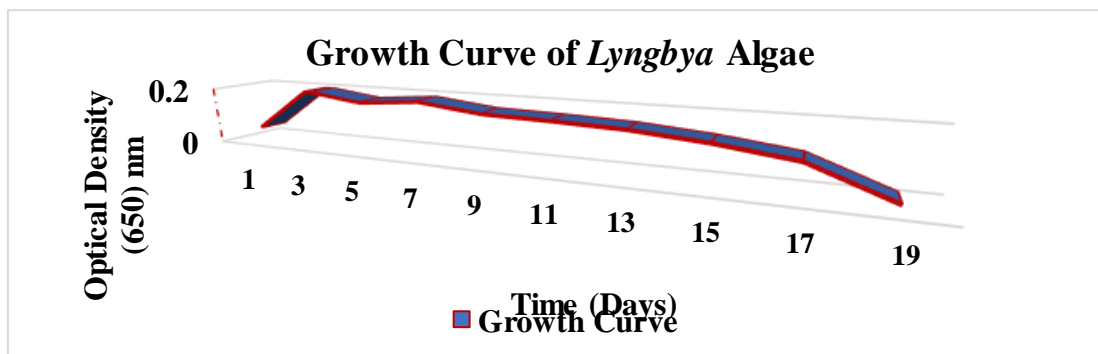


Fig. 1 Growth Curve of *Lyngbya* Algae

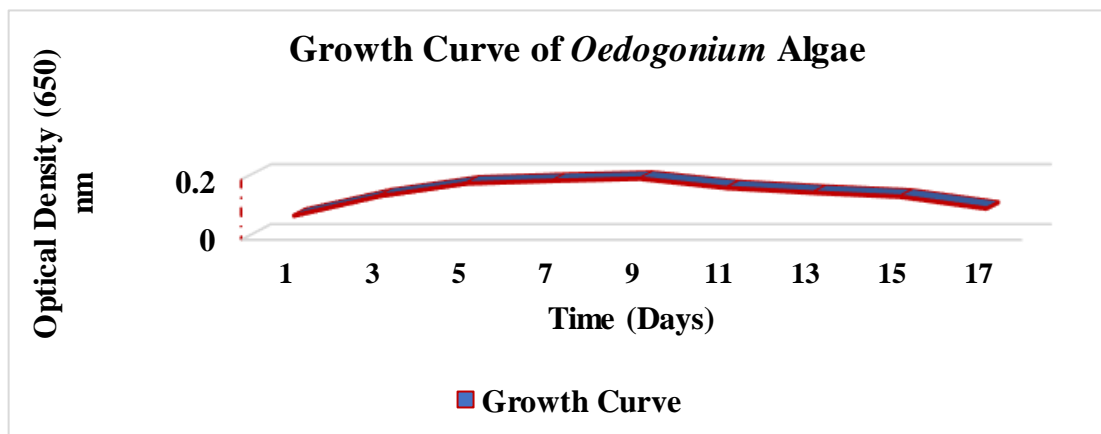


Fig. 2 Growth Curve of *Oedogonium* Algae

The unsaturated fatty acid content in the studied algae

The alga was investigated to study their lipids and essential omega fatty acids (3, 6, and 9) production capacity (*Lyngbya sp*) belonging to blue-green microalga.

The content of *Lyngbya* alga varied in terms of unsaturated fatty acids (UFAs), as its content ranged from Octadecatrienoic acid, omega 6 (6.67)% and oleic acid (OA) (3.36%) and Eicosapentaenoic acid omega 3 (9.77)% and Docosatetraenoic acid omega 6(8.07)% of total fat When normal growth control And the content of *Oedogonium* alga varied in terms of unsaturated fatty acids (UFAs), as its content ranged from Octadecatrienoic acid, omega 6 (29.24)% and Eicosapentaenoic acid omega 3 (5.84)% and Vaccenic Acid, omega 7 (25.30)%.

The effect of nitrogen and phosphorus on the level of unsaturated fatty acid.

The most effective stimulus for inducing stress conditions in cells is nitrogen limitation, which increases lipid formation in microalgae ([Sulochana and Arumugam, 2020](#)).

We notice that when nitrogen decreases, the percentage of unsaturated fatty acids increases as follows; in *Lyngbya sp* Eicosapentaenoic acid omega 3 (11.07-2.54) %, Octadecatrienoic acid, omega 6 (8.67-4.78) %, Docosatetraenoic acid omega 6 (8.07-4.65) %, Oleic acid omega 9 (4.39-1.7) % and *Oedogonium sp* Octadecatrienoic acid, omega 6 (34.04-13.91) %, Vaccenic acid omega 7 (25.30-12.99) %, Eicosapentaenoic acid omega 3 (10.87-3.91) %. At shown in the [Table 2,3](#) below.

Table 2. Influence of Nitrogen on unsaturated fatty acids *Lyngbya*

| Isolates | N | RT | Area% | Compound name | Molecular formula | M.W |
|----------------|----|--------|-------|----------------------------------|-------------------|-----|
| <i>Lyngbya</i> | 0 | 24.107 | 8.67 | Octadecatrienoic acid, omega 6 | C18H34O2 | 292 |
| | | 24.106 | 4.39 | Oleic acid omega 9 | C18H34O2 | 292 |
| | | 21.853 | 11.07 | Eicosapentaenoic acid omega 3 | C23H38O2 | 346 |
| | | 19.91 | 8.07 | Docosatetraenoic acid omega6 | C23H38O2 | 346 |
| | 5 | 24.107 | 7.07 | -Octadecatrienoic acid, omega 6 | C18H34O2 | 292 |
| | | 24.106 | 3.30 | Oleic acid omega 9 | C18H34O2 | 292 |
| | | 21.853 | 9.47 | Eicosapentaenoic acid omega 3 | C23H38O2 | 346 |
| | | 19.91 | 6.57 | Docosatetraenoic acid omega6 | C23H38O2 | 346 |
| | 10 | 24.107 | 6.67 | -Octadecatrienoic acid, omega 6 | C19H32O2 | 292 |
| | | 24.106 | 3.39 | Oleic acid, omega 9 | C19H32O2 | 292 |
| | | 19.91 | 3.08 | Docosatetraenoic acid omega6 | C23H38O2 | 346 |
| | | 21.853 | 9.77 | Eicosapentaenoic acid omega 3 | C20H30O2 | |
| | 20 | 19.58 | 4.78 | - Octadecatrienoic acid, omega 6 | C19H32O2 | 292 |
| | | 21.853 | 2.54 | Eicosapentaenoic acid omega 3 | C20H30O2 | |
| | | 19.76 | 1.7 | Oleic acid, omega 9 | C18H34O2 | 292 |
| | | 19.91 | 4.65 | Docosatetraenoic acid omega6 | C23H38O2 | 346 |

In a study on lipid content, Hu and colleagues found that, in nitrogen-limited environments, microalgae had lipid contents that range from 10 to 20% ([Hu et al., 2008](#)). According to the same study, cyanobacteria under stress produced less than 10% of their cells' total lipid content.

Nitrogen limitation is the most efficient stimulus for creating stress conditions in cells and therefore enhances lipid accumulation in microalgae ([Sulochana and Arumugam, 2020](#)).

The same study reported that stress conditions in cyanobacteria caused less than 10% lipid content production in cells. Under nutrient stress conditions, microalgae will change the metabolism of fatty acids towards the synthesis and accumulation of triacylglycerol which consists of up to 80% of the total lipid content in the cell, The results are consistent with the current study ([Ratomski, 2021](#)). When nitrogen is limited in the cultivation medium, microalgae will accumulate large amounts of lipids but due to the lack of nitrogen the cells will not produce sufficient amounts of proteins which results in lower biomass production Chlorophyll content also changes

under the nutrient stress conditions, which is the indicator of energy accumulated in ATP is generated (Rai et al., 2015).
 photosynthesis and photochemical processes during which the

Table 3. Influence of phosphorus on unsaturated fatty acids *Oedogonium*.

| Isolates | N | RT | Area% | Compound name | Molecular formula | M.W |
|-------------------|----|-------|-------|---|-------------------|-----|
| <i>Oedogonium</i> | 0 | 14.99 | 10.87 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 14.21 | 25.30 | vaccenic acid, omega 7 | C18H34O2 | |
| | | 19.91 | 34.04 | 6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | 5 | 14.99 | 8.45 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 19.91 | 31.04 | 6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | | 14.21 | 20.30 | vaccenic acid, omega 7 | C18H34O2 | |
| | 10 | 19.91 | 29.24 | 6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | | 14.99 | 5.84 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 14.21 | 16.76 | vaccenic acid, omega 7 | C18H34O2 | |
| | 20 | 19.91 | 13.98 | 6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | | 14.99 | 3.91 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 14.21 | 12.99 | vaccenic acid, omega 7 | C18H34O2 | |

Table 4. Influence of phosphorus on unsaturated fatty acids *Oedogonium* sp.

| Isolates | p | RT | Area% | Compound name | Molecular formula | M.W |
|-------------------|----|-------|-------|--|-------------------|-----|
| <i>Oedogonium</i> | 0 | 14.99 | 6.44 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 19.91 | 30.84 | ,6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | | 14.21 | 25.30 | Vaccenic Acid Omega 7 | C18H34O2 | |
| | 1 | 14.99 | 6.03 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 19.91 | 29.9 | ,6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | | 14.21 | 23.34 | Vaccenic Acid Omega 7 | C18H34O2 | |
| | 5 | 19.91 | 27.24 | 6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | | 14.99 | 5.84 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 14.21 | 20.76 | Vaccenic Acid Omega 7 | C18H34O2 | |
| | 10 | 19.91 | 10.78 | 6,9,12-Octadecatrienoic acid, methyl ester, (GLA) omega 6 | C19H32O2 | 292 |
| | | 14.99 | 2.91 | 5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (EPA) | C21H32O2 | 316 |
| | | 14.21 | 15.45 | Vaccenic Acid Omega 7 | C18H34O2 | |

The current study is similar to that of Aljbory, who studied two locally isolated microalgae (*Chlorella vulgaris* Beijerinck and *Nitzschia palea*) to test their ability to produce biodiesel by catalyzing different nitrogen concentration treatments (0, 2, 4, 8 g/l), and the effect of nitrogen concentration on the amount of initial product (carbohydrates, protein), as well as the quantity and quality of fat.

Results revealed that nitrogen starvation led to elevated lipid production in *C. vulgaris* and *N. palea* Fat content increased from 6.6% to 40% and from 40% to 60% dry weight (DW) (Hassan et al., 2013).

Table 5. Influence of phosphorus on unsaturated fatty acids *Lyngbya sp*

| Isolates | p | RT | Area% | Compound name | Molecular formula | M.W |
|----------|----|--------|-------|---------------------------------|-------------------|-----|
| Lyngbya | 0 | 24.107 | 8.67 | Octadecatrienoic acid, omega 6 | C19H32O2 | 292 |
| | | 24.106 | 5.39 | Oleic acid omega 9 | C18H34O2 | 292 |
| | | 14.958 | 10.07 | Eicosapentaenoic acid omega 3 | C23H38O2 | 346 |
| | | 14.958 | 9.07 | Docosatetraenoic acid omega6 | C23H38O2 | 346 |
| | 1 | 24.107 | 7.6 | Octadecatrienoic acid, omega 6 | C19H32O2 | 292 |
| | | 24.106 | 5.05 | Oleic acid omega 9 | C18H34O2 | 292 |
| | | 14.958 | 8.87 | Eicosapentaenoic acid omega 3 | C23H38O2 | 346 |
| | | 14.958 | 7.67 | Docosatetraenoic acid omega6 | C23H38O2 | 346 |
| | 5 | 24.107 | 6.67 | Octadecatrienoic acid , omega 6 | C19H32O2 | 292 |
| | | 24.106 | 3.39 | Oleic acid , omega 9 | C19H32O2 | 292 |
| | | 19.91 | 9.88 | Docosatetraenoic acid omega6 | C23H38O2 | |
| | | 21.853 | 9.77 | Eicosapentaenoic acid omega 3 | C20H30O2 | |
| | 10 | 24.107 | 2.78 | Octadecatrienoic acid , omega 6 | C19H32O2 | 292 |
| | | 24.106 | 2.87 | Oleic acid , omega 9 | C18H34O2 | 292 |
| | | 21.853 | 4.65 | Eicosapentaenoic acid omega 3 | C20H30O2 | 346 |
| | | 19.91 | 5.09 | Docosatetraenoic acid omega 6 | C23H38O2 | |

In a number of cases, phosphate deprivation was reported to be more efficient than nitrogen starvation, such as the case in the two-step strategy of *Ankistrodesmus falcatus* where lipid productivity was higher than that in the nitrogen limitation method (Park et al., 2018). Additionally, reports on the effects of phosphate starvation on algal lipid productivity, based on the experimental setup, seem to be strain dependent. For instance, the lipid accumulation of *Phaeodactylum tricornotum*, *Pavlova lutheri*, *Chaetoceros*, and *Dunaliella salina* enhanced under phosphorus starvation (Chauhan, 2022).

We note that the proportions of unsaturated fatty acids change according to the concentrations used for phosphorus (1,5,10) the quantity of these acids decreases decrease (UFAs) from to in *Lyngbya* Octadecatrienoic acid (8.67 to 2.78) and Eicosapentaenoic acid (10.07 to 4.65) and Oleic acid (5.39 to 2.87), Docosatetraenoic acid (9.07 to 5.09) and *Oedogonium* the (UFAs) decline to Vaccenic acid (from 25.30 to 15.45) and Eicosapentaenoic acid from (6.44 to 2.91) and Octadecatrienoic acid (30.84 to 10.78).

The results agree with the present study with Chu who prepared five types of media containing different concentrations of nitrogen and phosphorus or their mixture to culture *Chlorella vulgaris*. This resulted in a maximum fat yield of 58.39 mg/l/day. This study demonstrates that phosphorus plays an important role in lipid production by *C. vulgaris* under nitrogen-deficient conditions, and demonstrates the possibility of combining phosphorus removal from

wastewater with biodiesel production via microalgae (Chu et al., 2013).

Similar to Shen studied the effects of nitrogen and phosphorus concentrations on growth, nutrient uptake, and lipid accumulation in freshwater microalgae *Scenedesmus sp.* In nitrogen (2.5 mg L⁻¹) or phosphorus (0.1 mg L⁻¹) limitation conditions, *Scenedesmus sp.* lipids could accumulate up to 30% and 53%, respectively, of the algal biomass (Xin et al., 2010).

Conclusions

Microalgae have received widespread interest owing to their unique properties in producing large amounts of lipids and fast growth rates. However, algal strains exhibit conflicting features in terms of the conditions required for maximal lipid and biomass production but phylogeny and cultivation conditions determine the cellular lipid content. Lipid accumulation correlates with some limiting factors (often N, P), which in turn may result in a lower biomass yield. Production of essential fatty acids from microalgae is, therefore, a compromise between maximizing the lipid content by modulation of growth conditions without lowering biomass production, or a matter of harvesting cells at the right moment. It was found that cultivating in nitrogen and phosphorus depletion media will result in the accumulation of lipid in microalgal cells. As in two algae *Oedogonium* and *Lyngbya*, in the presence of the nutrients nitrogen and phosphate, amounts of fat, especially unsaturated, reduce their proportions.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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