



# Improvement of fatty acid composition by a strain of *Chaetoceros* sp. isolated from Moroccan seawater of Sidi Moussa for biodiesel production

El Bakraoui Houria<sup>1</sup> · Slaoui Miloudia<sup>1</sup> · Mabrouki Jamal<sup>2</sup> · Fatima El Aamri<sup>3</sup> · El Yadini Meryem<sup>2</sup>

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## Abstract

Microalgae have many characteristics that make them an excellent choice for use as a renewable energy source. Many recent studies have focused on obtaining lipids from microalgae, by stress conditions such as reducing the availability of nutrients in culture media, particularly nitrogen and phosphorus. This is a key step in the production of biodiesel feedstocks from microalgae that needs to be better understood. In this study, a strain of *Chaetoceros* sp. isolated from seawater off Morocco was investigated under different concentrations (0%, 50%, 100%, and 150%) of nitrogen and phosphorus for accumulation of fatty acids. Cell growth was observed over the course of 14 days, resulting in a final cell density of  $1.03 \times 10^7$  cells mL<sup>-1</sup> in the nitrogen starvation medium, with an initial NaNO<sub>3</sub> concentration of 112.5 g L<sup>-1</sup> and a maximal lipid level of 29.9% when subjected to complete nitrogen deprivation. For a 7.50 g L<sup>-1</sup> initial NaH<sub>2</sub>PO<sub>4</sub> concentration in the culture medium, a maximum cell density of  $1.07 \times 10^7$  cells mL<sup>-1</sup> was achieved with 21.82% lipid yield in the absence of phosphorus. According to these findings, a high concentration of phosphate and nitrate sources aided in the concentration of biomass. The current study also shows that, as the medium's phosphorus or nitrate content falls, the lipid content increases. Nutrient stress for *Chaetoceros* sp. cultivation was found to have a significant impact on biomass and lipid accumulation. It is suggested that wastewater with limited nutrients could be used as a medium for growth to stimulate lipid accumulation in this microalgae.

**Keywords** *Chaetoceros* sp. · Biodiesel · Stress conditions · Nitrogen · Phosphorus

## Introduction

The development of environmentally friendly, sustainable energy sources is the solution to the problem of the petroleum crisis and its impacts (Bi and He 2020; Zhao et al. 2022).

Biodiesel produced from renewable sources such as fats from animals and oils from plants have been developed and are economically viable (Santori et al. 2012). Biodiesel in its third generation relies on microalgae (Ananthi et al. 2021; Ferreira Mota et al. 2022). These species, unlike terrestrial energy crops, have high lipid productivity per unit area on marginal land, are able to recycle carbon from fossil resources with sunlight and other nutrients, and have a positive environmental impact (Li et al. 2020; Tang et al. 2010). Although microalgae lipids could be transformed into biodiesel, large-scale commercial production is limited by a variety of technological and economic restrictions (Chu et al. 2019). As a result, optimizing the productivity of microalgal biomass in addition to lipid production is critical for making this route competitive and economically viable (Joseph et al. 2017). These parameters can be affected by different culture conditions such as nitrogen and phosphorus stress followed by mode of cultivation, temperature, light intensity, light/dark period, salinity, and pH (Ji et al. 2021; Rehman et al. 2022; Viruela et al. 2021; Wu et al. 2021; Yaakob et al. 2021).

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✉ Mabrouki Jamal  
jamal.mabrouki@um5r.ac.ma

<sup>1</sup> Energy, Materials, and Sustainable Development (EMDD) Laboratory Higher School of Technology SALE, Center of Water, Environment, and Sustainable Development (CERN2D), Natural Resources, University Mohammed V in Rabat, Rabat, Morocco

<sup>2</sup> Laboratory of Spectroscopy, Molecular Modelling, Materials, Nanomaterials, Water and Environment, CERN2D, Mohammed V University in Rabat, Faculty of Science, AV IbnBattouta, BP1014 Rabat, Agdal, Morocco

<sup>3</sup> Institut National de Recherche Halieutique, 2 Boulevard Sidi Abderrahmane, Casablanca, Morocco

Microalgae have recently proved their efficiency in the absorption of nitrogen and phosphorus from wastewater as well as the generation of bioenergy (Wagner et al. 2021; Zhang et al. 2021; Greses et al. 2022). These macronutrients are required to promote the growth of algae; in addition, if they are given in an adequate ratio, they control metabolic activity (Su 2021). The variation in the concentration of these two nutrients in the culture medium of microalgae can influence the yield of lipids and fatty acids (Xin et al. 2010; Yaakob et al. 2021). Rios (2015) showed that increasing the quantity of nitrogen generates a rise in the amount of lipid accumulated in the microalgae *Desmodesmus* sp., but absolute removal of nitrogen from the culture medium caused the maximum accumulation of lipids with a percentage of 23%. Zarrinmehr et al. (2020) discovered a similar finding for the microalgal species *Isochrysis galbana*, where the content of saturated fatty acids was greater under nitrogen shortage, 75.79%, than under nitrogen sufficiency, 36.63%. Roopnarain (2014) found that decreasing phosphorus concentrations below 25% resulted in lipid storage in *Isochrysis galbana* U4. Under low amounts of phosphorus in the medium, the lipid content of *Scenedesmus* sp. improved. This species cultivated in phosphorus (50 mg/L) had a lipid composition of 22.3%, whereas the lipid yield was 42.5% in phosphorus (1 mg/L) (Yang et al. 2018).

Morocco's potential for microalgae research has not yet been fully realized, despite the country's good climatic circumstances and unique geographic location: with the Mediterranean Sea to the north, and the Atlantic Ocean to the west (Hassi et al. 2023). So many studies have screened microalgae that can produce biodiesel (Asli et al. 2019; Benhima et al. 2018; El Arroussi et al. 2015, 2017). One such work was carried out by El Arroussi (2017), in which 57 strains isolated from the Moroccan coasts were evaluated for biodiesel production. Benhima (2018) studied the effect of nitrogen stress on lipid accumulation of *Dunaliella tertiolecta*. Despite these studies, there is still a lack of studies related to stress conditions and the production of biodiesel by microalgae isolated in Morocco.

In light of this, the aim of this study is to evaluate the effect of nitrogen and phosphorus stress conditions on growth, biomass production, and lipid accumulation as well as the fatty acid composition of the isolated marine water brown microalga, *Chaetoceros* sp., grown in f/2 medium.

## Materials and methods

### Algae source, culture medium, and conditions

The strain of the diatom *Chaetoceros* sp. studied in this research was isolated from seawater in the coastal area of Sidi Moussa Beach in the Town of Sale in Morocco. The seawater samples were collected and filtered before being

transported to 100-mL beakers. An optical microscope (Nikon ECLIPSE E200) was used to identify microalgae. To obtain a pure culture, serial dilution was used. A sterile loop was used to generate parallel streaks on the agar growth medium by placing a loop of sample culture from the highest dilution tubes on the agar growth medium. Parafilm was used to cover the Petri dishes, which were then incubated at 25 °C.

The diatom *Chaetoceros* sp. was obtained after 25 days of cultivation. Medium f/2 was employed in the maintenance of the culture of this species in 100-mL Erlenmeyer flasks.

The medium used for culture was f/2 medium (Guillard and Ryther 1962) with the composition presented in Table 1. The medium was sterilized in an autoclave at 121 °C for 15 min. The microalga was cultivated in 500-mL Erlenmeyer flasks under four different variations of nitrate (N) and phosphate (P) of the f/2 medium (0%, 50%, 100%, and 150% N or P) with an initial concentration  $1 \times 10^5$  cells/mL of inocula, and incubated at 25 °C with  $87.75 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  light intensity and a 12 h (light–dark) photoperiod. Aeration was provided by an air pump (Table 2).

The effects of nitrogen and phosphorus were evaluated out separately. We thus have two experiments, the first with varying starting nitrogen content and the second with varying initial phosphorus concentration in the culture medium.

### Measurement of cell growth parameters

Cell growth was determined by counting using a Malassez cell. In our case, the cells were mixed gently to dissociate the cell aggregates. The microalgae were then counted on six squares of 0.01  $\mu\text{L}$ , and the concentration is reported as the number of cells per mL.

During the exponential phase, the dry weight of the microalgae cells was measured. A volume of 10 ml was filtered by the use of a GF/F filter (Whatman GF/F) with a pore size of 0.7  $\mu\text{m}$ , and the cell concentration was determined (Elyakoubi et al. 2020; Zhu et al. 1997). The biomass was

**Table 1** The composition of the f/2 medium (Guillard and Ryther 1962)

Compound	Concentration (g. L <sup>-1</sup> )
NaNO <sub>3</sub>	75.0
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	5.0
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O	30.0
FeCl <sub>3</sub> ·6H <sub>2</sub> O	3.5
Na <sub>2</sub> EDTA·2H <sub>2</sub> O	4.36
CuSO <sub>4</sub> ·5H <sub>2</sub> O	9.8
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	6.3
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	22.0
CoCl <sub>2</sub> ·6H <sub>2</sub> O	10.0
MnCl <sub>2</sub> ·4H <sub>2</sub> O	180.0

**Table 2** Variations in the culture media used in the experimental design for NaNO<sub>3</sub>

Nitrogen variation	Initial concentration of NaNO <sub>3</sub> (g L <sup>-1</sup> )
0	0
50	37.5
100	75
150	112.5

washed three times with distilled water after the filtering phase. The filter was dried for 72 h at a temperature of 60 °C.

The cellular dry weight (g cell<sup>-1</sup>) was obtained using the Zhu and Lee (Zhu et al. 1997) formula, as shown in Eq. (1):

$$\text{Dry weight of cells} = \frac{\frac{\text{net weight}(W_1) - \text{net weight}(W_0)}{\text{filtered volume}}}{\text{cell concentration}} \quad (1)$$

W<sub>1</sub> is the total weight of the filter containing microalgae biomass after drying (g), W<sub>0</sub> signifies the weight of the dry filter alone (g), filtered volume (mL), and cell concentration (cells mL<sup>-1</sup>).

### Extraction of biomass oil

The biomass was harvested by adjusting the pH of each Erlenmeyer flask to 10.5 with NaOH (1 M), then drying at 60 °C for 72 h (Zhu et al. 1997).

Total lipid extraction from dry biomass was carried out using the Bligh and Dyer technique (Bligh and Dyer 1959). Dry cells (100 mg) were maintained in 3.5 mL of a chloroform/methanol/water (2/1/0.5 v/v/v) mixture. The mixture was centrifuged for 15 min at 4000 rpm after vortexing, and the organic phase was extracted and deposited in a tube that has been previously dried and weighed.

The lipid content was determined by using the following equation:

$$\text{Lipid content (\%)} = \frac{ML}{MA} \times 100 \quad (2)$$

where ML is the mass of the extracted lipids (corresponding to the difference in mass of the empty tubes and containing the dry lipids), and MA is the mass of dry algal biomass.

### Fatty acid characterization

Chloroform (500 µL) was used to resuspend the extracted lipids. A total of 100 µL of this mixture was combined with 800 µL of 10% boron trifluoride–methanol solution in a screw tube, and the combination was then heated to 100 °C in a water bath for 15 min. A quantity of 750 µL of the solvent (100 µL of heptadecane in 10 mL of hexane) and 1.5 mL of water were added after cooling, and the mixture

was vortexed for 2 min. The upper phase was retrieved with a Pasteur pipette, and 10 µL of it was injected into an Agilent gas chromatography (6850) system to characterize the methyl esters.

### Statistical analysis

This microalga was cultivated in triplicate ( $n = 3$ ) batch experiments. For statistical analysis, one-way analysis of variance (ANOVA) was employed (Graphpad Prism, version 8.0) to determine differences between the means of four levels of each treatment (nitrogen concentration/phosphorus concentration) on the final day of culture (day 14). The results are provided as mean value ± standard deviation (SD).

## Results and discussion

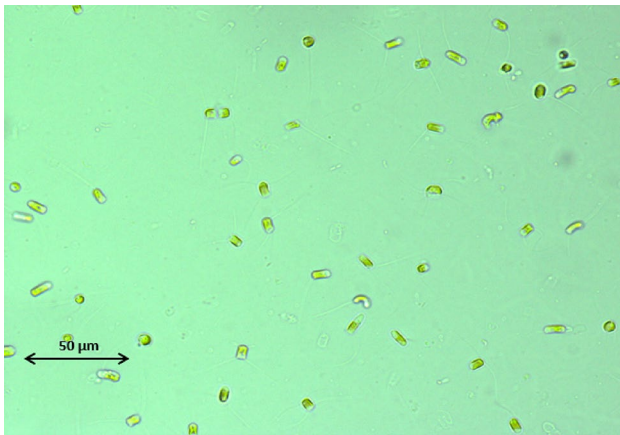
Stress conditions affect lipid accumulation in microalgae, by increasing or decreasing the proportion of lipid production or even the quality of the biodiesel produced (Ji et al. 2021; Rehman et al. 2022; Viruela et al. 2021; Wu et al. 2021; Yaakob et al. 2021). In this work, we investigate how varying the nitrate and phosphate concentration affects the lipid accumulation in a *Chaetoceros* sp. diatom isolated from seawater in the coastal area of Sidi Moussa Beach in the Town of Sale in Morocco. The results that we found are displayed below.

### Growth assessment

The intracellular chemical composition of different microalgae species varies. As a result, some species become P-limited in one habitat while others become N-limited in another (Harrison et al. 1990). Because cell development is typically restricted by deficiency of N and P, the continuation of cell division seen in this situation may be achieved by the provision of N and P from cell store reserves (Horiuchi et al. 2003). This is consistent with the species' capacity to absorb and store nitrogen and phosphorus, as well as its subsequent utilization of these reserves to promote cell development when external N and P in the surrounding media have been depleted.

The effect of these nutrients on our strain *Chaetoceros* sp. was studied. A photomicrograph of this species is shown in Fig. 1. From measurements of 30 distinct cells, an average length of  $9.03 \pm 1.45$  µm was determined. The growth curves for the four cultures were generated with varying initial concentrations of NaNO<sub>3</sub> and NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O are shown in Fig. 3 and 4, respectively.

All of the kinetics exhibit the typical S-curve form of microorganism's growth: from the beginning to day 3, there



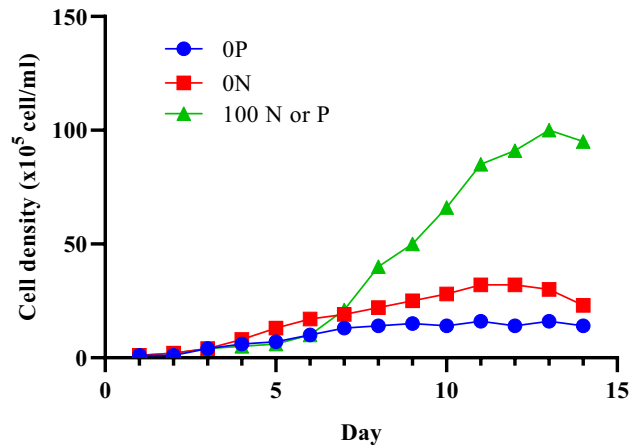
**Fig. 1** Microscopical observation of the microalgae *Chaetoceros* sp. used in the research ( $\times 40$ )

is a phase of adaptation to the medium (latent period), during which the species displayed identical cell densities in cultures rich and deficient N. Following that, the N-depleted cultures had a higher cell density than the N-replete cultures until day 7, while after that the cell concentration decreased to the lowest level for the rest of the trial of nitrogen effect. From day 3 to day 11 the exponential growth phase (log phase) appears. The stationary phase begins after day 11.

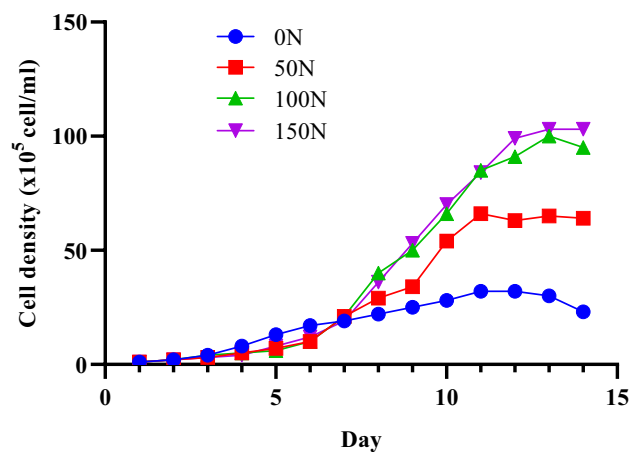
Figure 4 shows that, until the fifth day, the cell density of the P-depleted cultures was similar to the P-repleted cultures, but after that, this density became the lowest when compared with the other levels of phosphorus content in the f/2 medium.

Under conditions both rich and deficient in nitrate (N) and phosphate (P), a rise in dry weight concentration was found in all trials. But when the species was deprived of nitrogen, it grew significantly differently than when deprived of phosphorus. Under depletion, the cell density initially rose, confirming prior research findings (Benvenuti et al. 2015; Rios et al. 2015; Van Vooren et al. 2012; Yaakob et al. 2021; Zarrinmehr et al. 2020). This is due to the fact that new biomass is generated in the absence of nitrogen and phosphorus, indicating that the cells are still photosynthetically active to some extent and that the photosynthetic efficiency period is very specific to a species (South et al. 1987). The maximum rise under deprivation was attained on day 11 for either P deprivation or N deprivation, but the increase under N deprivation was twice as large as that under P deprivation (Fig. 2).

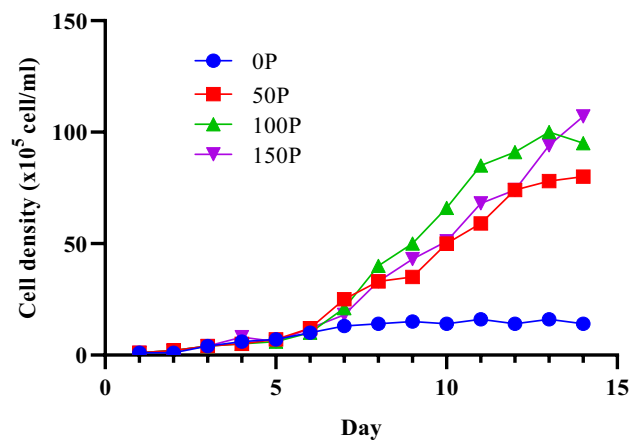
As seen in the graphs (Figs. 3, 4), increasing the concentrations of  $\text{NaNO}_3$  and  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  increased the growth rate. Low growth was seen during nitrogen deprivation (0% N), and cells appeared bleached, with a maximum cell density of  $3.2 \times 10^6$  cells/mL. On day 11, the culture with 50% nitrogen reached a high cell number concentration of  $6.6 \times 10^6$  cells/mL. The rest of the 100% and 150% N



**Fig. 2** Nitrogen and phosphorus absence's impact on cell growth of *Chaetoceros* sp.



**Fig. 3** Growth curve of *Chaetoceros* sp. grown on f/2 medium with different initial  $\text{NaNO}_3$  concentrations



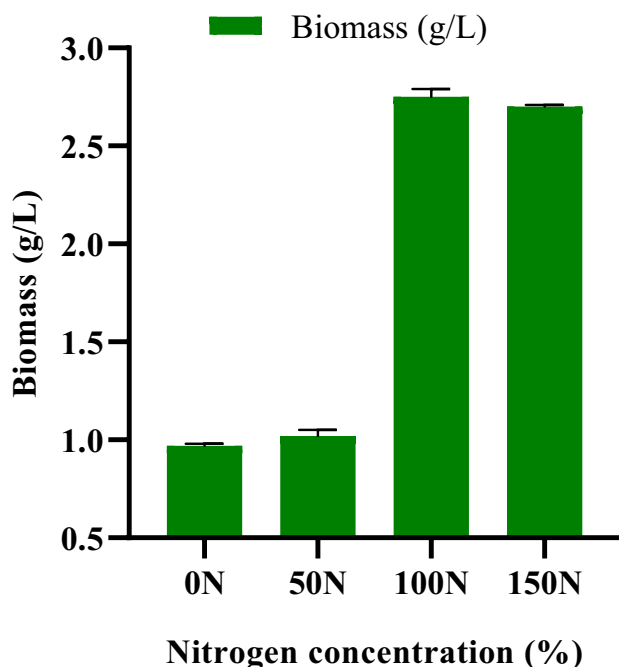
**Fig. 4** Growth curve of *Chaetoceros* sp. grown on f/2 medium with different initial  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  concentrations

cultures had the maximum cell density, with  $1.0 \times 10^7$  cells per mL and  $1.03 \times 10^7$  cells per mL, respectively.

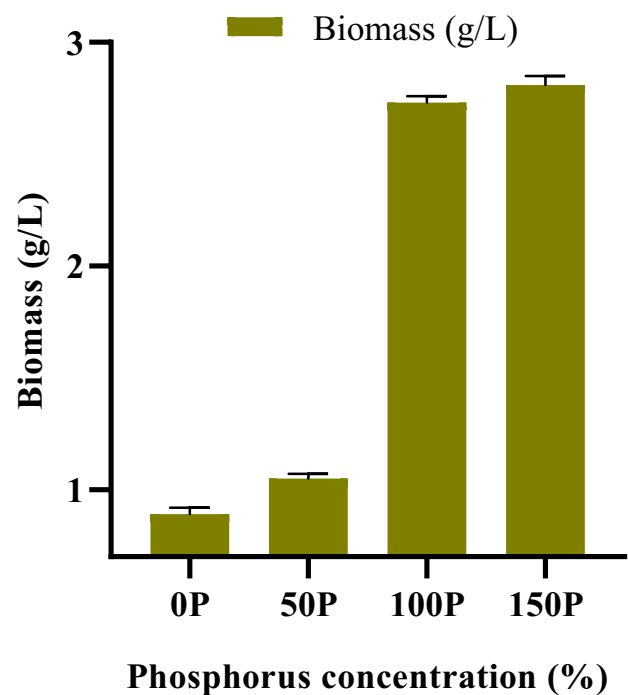
During the experiment, the effect of changing the phosphorus content on the cell density of the strain was shown in each case. In contrast to the other levels of 50%, 100%, and 150% P, deprivation of P (0%) resulted in the lowest cell density of  $1.6 \times 10^6$  cells/mL, yielding concentrations of  $8.0 \times 10^6$ ,  $1.0 \times 10^7$ , and  $1.07 \times 10^7$  cells/mL, respectively.

The findings of the current investigation show that a high concentration of nitrogen and phosphorus sources promoted the biomass increase (Figs. 5, 6). This is consistent with Yeesang and Cheirsilp'. When the microalgae *Botryococcus* spp. was subjected to nitrogen-depletion conditions, they reported a decrease in biomass (Yeesang and Cheirsilp 2011). Roopnarain (2014) had also reported the same conclusion regarding *Isochrysis galbana* U4, under phosphorus limitation and starvation conditions.

The microalga *Chaetoceros* sp. isolated from seawater cannot survive without nitrogen and phosphate, and its growth is related to the concentration of these two components in the culture medium. Many microalgal strains have been reported to show an increase in biomass concentration after early nitrogen and phosphorus deprivation (Yaakob et al. 2021; Benvenuti et al. 2015; Roopnarain et al. 2014; Breuer et al. 2012; Pruvost et al. 2011). This rise in biomass concentration might be justified by the buildup of storage molecules such as triglycerides (TAGs) (Msanne et al. 2012; Pal et al. 2011).



**Fig. 5** Biomass production of *Chaetoceros* sp. under different initial NaNO<sub>3</sub> concentrations



**Fig. 6** Biomass production of *Chaetoceros* sp. under different initial NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O concentrations

### Measurement of lipids

One of the major aims of a microalgae-based oil production process is to achieve a high lipid yield per area (resulting from the biomass areal productivity and lipid content), as this has a significant impact on production costs. In outdoor culture, the chosen microalgal strain should be very productive, have a high lipid content in its natural state and/or be able to respond to nutrient deficiency with a significant accumulation of lipids, be robust enough to withstand mixing-induced shear stress, and be flexible enough to respond to inevitable changes in the physicochemical characteristics of an outdoor environment. In this study, the isolated microalgae *Chaetoceros* sp. was evaluated in the laboratory for oil production capacity under nitrogen and phosphate stress conditions (Table 3).

Using the Bligh and Dyer technique, lipids were obtained from the biomass collected from each culture of the experiment after 14 days. Tables 4 and 5 present the results. The lipid content in the 0% N culture is the maximum among the lots. Other cultures had a range of lipid content from 17.55% (37.5 g/L NaNO<sub>3</sub>) to 19.2% (112.5 g/L NaNO<sub>3</sub>) and 17.25% (2.5 g/L NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O) to 18.95% (7.5 g/L NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O).

The influence of modifying the N and P concentrations in the f/2 medium on lipid depends on the microalgal species. The lipid content of the isolated strain increased gradually with increasing nitrogen and phosphorus concentrations, although this rise was smaller than the yield achieved in



**Table 3** Variations in the culture media used in the experimental design for  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 

Phosphorus variation	Initial concentration of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (g. L <sup>-1</sup> )
0	0
50	2.5
100	5
150	7.5

**Table 4** The impact of varying nitrogen level on the biomass and lipid content of *Chaetoceros* sp. on the 14th day of culture

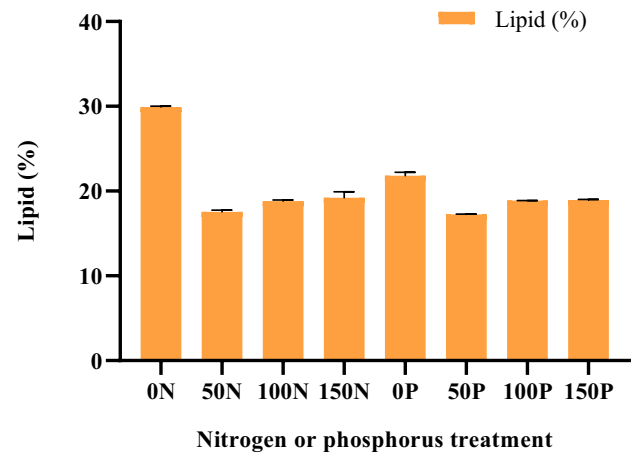
Variation of $\text{NaNO}_3$ (g/L)	Biomass (g/L)	Lipid percentage (%)
0	0.97 ± 0.01	29.90 ± 0.13
37.5	1.02 ± 0.03	17.55 ± 0.21
75	2.75 ± 0.04	18.80 ± 0.15
112.5	2.70 ± 0.01	19.20 ± 0.71

**Table 5** The impact of varying the phosphorus concentration on the biomass and lipid content of *Chaetoceros* sp. on the 14th day of culture

Variation of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (g/L)	Biomass (g/L)	Lipid percentage (%)
0	0.89 ± 0.03	21.82 ± 0.41
2.5	1.05 ± 0.02	17.25 ± 0.03
5	2.73 ± 0.03	18.87 ± 0.02
7.5	2.81 ± 0.04	18.95 ± 0.07

the two nitrogen and phosphorus deprivation conditions. It was increased dramatically in the stationary phase, reaching 29.90% and 21.82% respectively.

Biomass, as well as lipid content, are significant factors in the cost-effective generation of biofuel from microalgae (Bi and He 2020; Zhao et al. 2022). The findings of this study show that, in contrast to the lipid content, a high concentration of nitrogen or phosphorus sources promoted biomass concentration. The current study also shows that, as the nitrate or phosphorus concentration in the medium falls, the lipid content increases (Fig. 7). Algal cells acquire carbon metabolites in the form of lipids in nitrogen-deficient circumstances, according to Yeesang and Cheirsilp (2011). Rios (2015) demonstrated that increasing the amount of nitrogen causes an increase in lipid accumulation in the microalgae *Desmodesmus* sp., while 100% removal of nitrogen from the culture medium generated the greatest accumulation of lipids (23%). Zarrinmehr et al. (2020) reported a similar discovery for the microalgal species *Isochrysis galbana*, where the amount of saturated fatty acids was higher

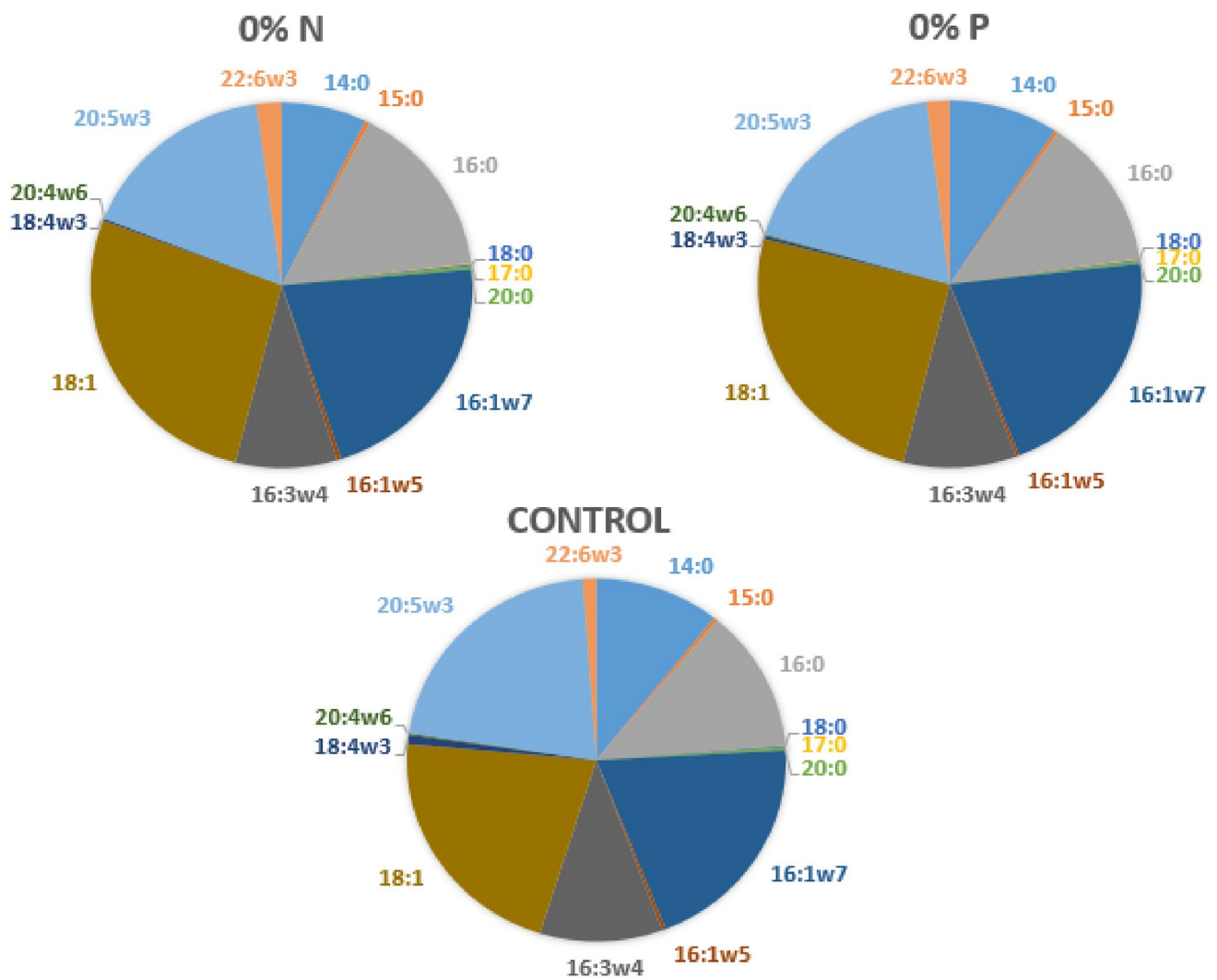
**Fig. 7** Lipid content of *Chaetoceros* sp. grown on modified f/2 medium with  $\text{NaNO}_3$  concentration (0–150N) and  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  concentration (0P–150P)

in nitrogen deficiency (75.79%) than in nitrogen sufficiency (36.63%). *Scenedesmus* sp. lipid content increased in the presence of low phosphorus levels in the medium. This species exhibited a lipid content of 22.3% when grown in 50 mg/L phosphorus, but the lipid yield was 42.5% when grown in 1 mg/L phosphorus (F. Yang et al. 2018).

**Table 6** Fatty acid values (mean ± SD) for *Chaetoceros* sp.

Fatty acids	Control (100% nitrogen and phosphorus)	0% nitrogen	0% phosphorus
14:0	11.62 ± 0.73	8.67 ± 0.21	10.75 ± 0.29
15:0	0.35 ± 0.04	0.45 ± 0.03	0.37 ± 0.01
16:0	13.83 ± 0.18	18.54 ± 0.15	15.40 ± 0.16
17:0	0.05 ± 0.01	0.12 ± 0.01	0.10 ± 0.01
18:0	0.15 ± 0.01	0.19 ± 0.01	0.14 ± 0.01
20:0	0.27 ± 0.03	0.39 ± 0.02	0.25 ± 0.06
<b>Total SFAs</b>	<b>26.25 ± 0.91</b>	<b>28.35 ± 0.15</b>	<b>27.01 ± 0.40</b>
16:1w7	21.65 ± 0.01	25.57 ± 0.13	24.33 ± 0.38
16:1w5	0.34 ± 0.01	0.42 ± 0.03	0.31 ± 0.02
16:3w4	11.39 ± 0.66	10.32 ± 0.25	11.08 ± 0.56
18:1w9	23.50 ± 0.05	32.20 ± 0.07	29.19 ± 0.04
18:4w3	0.87 ± 0.03	0.20 ± 0.02	0.30 ± 0.02
20:4w6	0.17 ± 0.02	0.05 ± 0.02	0.15 ± 0.01
20:5w3	23.37 ± 0.75	20.27 ± 0.07	21.75 ± 0.34
22:6w3	1.25 ± 0.03	2.54 ± 0.16	2.24 ± 0.23
<b>Total PUFAs</b>	<b>60.88 ± 0.13</b>	<b>66.00 ± 0.28</b>	<b>65.03 ± 0.53</b>

Cultures in 100% N and P, 0% N and 0% of P. Results are presented as the percentage of fatty acids with SFAs saturated fatty acids and PUFAs polyunsaturated fatty acids



**Fig. 8** By comparing the results with the control (*f/2* medium without modification), gas chromatography analysis was used to assess changes in the fatty acid content of *Chaetoceros* sp. The mean values are plotted ( $n = 3$ ), and each fatty acid is represented as a percentage of total fatty acids

### Fatty acid characterization

Using gas chromatography analysis, the fatty acid composition of *Chaetoceros* sp. under various concentrations of nitrogen and phosphorus was determined. In comparison with control (100% nitrogen and phosphorus in *f/2* media), the compositions of the fatty acids are shown in Table 6 and Fig. 8. For saturated fatty acids, the experiment with total nitrogen starvation showed the largest percentage ( $28.35 \pm 0.15\%$ ) compared with phosphorus starvation ( $27.01 \pm 0.40\%$ ), and *f/2* medium without modification ( $26.25 \pm 0.91\%$ ) as the lowest concentration. These stress conditions also affect the concentration of polyunsaturated fatty acids, increasing their percentage compared with the control condition ( $60.88 \pm 0.13\%$ ). Chin (2023) reported that high oleic acid concentrations will balance fuel characteristics such as viscosity, ignition quality, lubricity, and

combustion heat. In the current study, at nitrogen starvation, oleic acid content showed an increase from  $23.50 \pm 0.05\%$  to  $32.20 \pm 0.07$ , indicating that the strain *Chaetoceros* sp. isolated from seawater in the coastal area of Morocco can produce high-quality biodiesel under nitrogen stress condition. The dominant fatty acids under nitrogen and phosphorus starvation conditions are palmitic acid (16:0), palmitoleic acid (16:1w7), oleic acid (18:1w9), and eicosapentaenoic acid (20:5w3, EPA).

In the future, microalgae are projected to act as a source of long-chain n-3 polyunsaturated fatty acids such as EPA as well as a green energy source, e.g., biodiesel production in biorefinery systems coupled with wastewater treatment (El Bakraoui et al. 2022; Kim et al. 2022; Tokushima et al. 2016). Diatoms are well known for being extremely prolific microalgae that store a lot of neutral lipids in their cells under various stress conditions (Tokushima et al. 2016). In

this experiment, *Chaetoceros* sp. accumulated a high oleic acid under nitrogen starvation, but eicosapentaenoic acid was decreased compared with the control culture, although its concentration remains high compared with other polyunsaturated fatty acids (22:6w3). Hence, this strain isolated from seawater off Morocco could be a suitable candidate for biodiesel production as well as a source of long-chain n-3 polyunsaturated fatty acids.

## Conclusions

Limiting the amount of nitrogen and phosphorus in *Chaetoceros* sp. cultures was found to have a significant impact on cell development and fatty acid accumulation. The current research shows that growing the isolated strain *Chaetoceros* sp. autotrophically in a nitrogen-depleted growth medium is the most effective way to improve the lipids, resulting in an 11.1% increase compared with control. In addition, nitrogen-depleted culture increased oleic acid from 26.87% to 38.69% of fatty acids, indicating that the lipids from this species can be used to obtain a high quality of biodiesel.

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**Data availability** No life science threat was practiced in this research.

## Declarations

**Conflict of interest** The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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