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ORIGINAL ARTICLE

The effects of aeration and mixotrophy by acetate and pyruvate on the growth parameters in *Scenedesmus obliquus*

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Abstract

The mixotrophic growth of microalgae offers great potential as an efficient strategy for increasing biomass production. In this study, *Scenedesmus obliquus* was used to investigate the biomass growth conditions and biochemical parameters of algae cells in autotrophic and mixotrophic conditions with and without aeration. It was found that mixotrophic cultivation of algae cells by 50 mM acetate had a strong effect on increasing fresh weight, chlorophyll *a*, *b*, carotenoid, astaxanthin, protein, and lipid content (with 20, 4.6, 8.7, 60, 4.5, 4.8, and 18.4-fold, respectively) in comparison to the control cultures. Mixotrophy by pyruvate also increased the amount of all mentioned parameters. The best concentration of pyruvate was found to be 50 mM, but the effects of acetate were stronger. Aeration in a short time (2 h) had a different effect on mixotrophic cultures with acetate and pyruvate. It declined the effect of acetate on increasing the fresh weight and the amount of other biochemical parameters. Unlike the acetate treatment, aeration had an enhancement effect on increasing the fresh weight and biochemical parameters content in 50 and 100 mM pyruvate treatments. In the control cultures, the saturated acid, caprylic acid (C8:0) (40%), and the polyunsaturated eicosapentaenoic acid (EPA; C20:5 n3) (17.5%) showed higher percentages. Mixotrophy caused a decrease in the percentage of saturated fatty acids (SFAs) and, on the other hand, an increase in the percentage of unsaturated fatty acids (UFAs). EPA was the main fatty acid in the mixotrophic condition. Aeration in the phototrophic condition decreased the percentage of SFAs, while increasing that of UFAs. Aeration under mixotrophic conditions increased UFAs in both acetate and pyruvate-treated cultures. The results suggest that aeration could be an important factor in interaction with other algae growth conditions. It should be noted that acetate had generally better effects on the biomass quality of *S. obliquus* without aeration.

Keywords Aeration · Biomass · Lipid profile · Astaxanthin · Protein · FAMEs · Nile red

1 Introduction

In recent years, microalgae have received great attention for their potential effects as a bioresource that could be used for various purposes such as fish feed, human food, pharmaceutical products [1], and also as an alternative feedstock for the next generation of biofuels [2]. They can grow and be harvested almost continuously, reducing the seasonal problems of raw materials supply for different goals. The presence of many important biomolecules such as fatty acids, carotenoids, phycobilins, vitamins, sterols, and polysaccharides [1, 3] increases the value of these organisms.

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They are oxygenic photosynthetic organisms with simple growth requirements such as water, nutrients, and CO_2 coupled with sunlight, which are all available at low cost. They have important biotechnological properties, namely fast growth in a liquid medium and the ability to accumulate or secrete metabolites.

Algal lipid metabolism fascinates both scientists and entrepreneurs due to the large diversity of fatty acyl structures produced by algae. Algae have therefore long been studied as sources of genes for novel fatty acids and also due to their superior biomass productivity. Microalgae contain significant amounts of polyunsaturated fatty acids (PUFA) such as α-linolenic (ALA; 18:3n-3), eicosapentaenoic (EPA; C20:5n-3), and docosahexaenoic (DHA; C22:6n-3) acids. Because of this feature, selected microalgal strains rich in PUFA are used particularly for the aquaculture industry since omega-3 PUFA are required for the proper development of fish larvae and bivalves [4]. Recently, microalgae are being considered an important sources of PUFA for human nutrition.

Different factors such as the culture media, growing conditions, and nutrient compositions, in addition to gene transformation technology, can affect the growth and biochemical/physiological properties of microalgal cells [5, 6]. The mixotrophic culture of microalgae has recently received more attention. In this method, a dissolved organic carbon source (such as sugar, acetate, and malate) is supplemented in addition to inorganic carbon (CO_2) for growth. In comparison to the autotrophic condition, the presence of the organic carbon source reduces the culture's dependency on the lighting conditions [7]. The physiological and biochemical responses of different microalgae species to mixotrophy are quite different. Productivity is frequently increased significantly in the mixotrophy condition, making it a highly efficient method of producing microalgal biomass [8]. It has been proved that mixotrophy increases the specific growth rate in *Chlorella vulgaris* cells by induction of abiotic stress tolerance mechanisms [9]. Additionally, mixotrophy in *Asterarcys* sp. causes an increase in glycolysis, photosynthetic carbon fixation, and the pentose phosphate pathway [10].

Aeration is another factor that can influence the growth rates of some microalgae. It supports gas transfer, supplies CO_2, and creates more frequent access to light. Unfortunately, high rates of flows maintain a high level of mixing, but can also induce hydrodynamic stress that affects growth, cell damage, and eventually cell death [11–13]. There are several reports on the effects of aeration on algal growth and other biochemical parameters [14–16]. The effect of aeration on the biomass production and biochemical parameters of microalgae depends not only on the algal strain, but also on the culture conditions (e.g., temperature, salinity, and ionic strength of the medium). In some cases, aeration has not been effective on microalgae growth. Msuya and Neori [16] reported that on case nutrient concentrations are high enough, aeration per se is not essential for effective growth and biofiltration by seaweeds.

Scenedesmus is a ubiquitous organism and is a dominant species of microalga in freshwater lakes and rivers [17]. The normal plant chloroplast pigments such as chlorophylls *a* and *b*, *α* and *β*-carotene, lutein, zeaxanthin, violaxanthin, and neoxanthin together with astaxanthin and loroxanthin, as well as carotenoids that not found in higher plants, are all present in *Scenedesmus obliquus* [18]. Astaxanthin has been used as a pigmentation source for fish aquaculture and also as a powerful antioxidative reagent [19]. It is also considered one of the best candidates for biodiesel production among several microalgae species [2], with a suitable range of lipid content [20]. Therefore, studying the increase of biomass and its quality (considering the reduction of costs) can pave the way for the use of this alga in various fields.

Acetate and pyruvate are precursors of the lipid and carotenoid biosynthesis pathway. In this study, these two carbon sources were used in order to increase the biosynthesis of lipids and carotenoids in addition to increasing growth under the mixotrophic conditions. Since no study has been done on the interaction effects of aeration and mixotrophy, the current paper investigated the effects of the two carbon sources (acetate and pyruvate), with and without aeration, on the improvement of growth and the accumulation of pigments, proteins, and lipids in *S. obliquus*.

2 Material and methods

2.1 Materials

The microalga *S. obliquus* was collected from a fish culture pool and purified by solid culture. Chemical materials were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2 Methodolgy

2.2.1 Algae culture and treatment

Identification of the microalgal taxa was done based on morphological keys, and it was cultured in sterile Bold's Basal Medium (BBM) containing mM: NaNO3, 25.00; K2HPO4, 7.5; KH2PO4, 17.5; MgSO47H2O, 7.50; NaCl, 2.50; CaCl2.2H2 O, 2.50; EDTA Na, 17.1; acidified iron solution (FeSo4.7H2O H2SO4), 0.179; H3 BO3, 18.50; MnCl24H2O, 1.44; ZnSO47H2 O, 8.82; CuSO4 5H2O,

1.57; and $Co(NO_3)_2$ $6H_2O$, 0.494; at pH 6.8 and incubation temperature of 25 ± 3 °C, under white light with 60 µmol/m^2s intensity, on the surface of the flasks, on a 16:8 L/D cycle. The cells in the log growth phase, with an initial cell density of 7×10^6 cells mL^{-1}, were used in each experiment. The microalga was cultured under sterile conditions in Erlenmeyer flasks containing a 200-mL nutrient medium. To provide mixotrophic conditions, sodium acetate and sodium pyruvate were added to the medium to gain the concentrations of 0, 50, 100, and 200 mM. Aeration was done by bubbling atmospheric air (filtered through 0.22-µm cellulose acetate membranes, Orange Scientific, Belgium) at the bottom of the flasks at a speed of 70 mL per minute for 2 h a day. The cultures were harvested by centrifugation after 14 days of cultivation. Prior to the analysis, the obtained pellets were frozen and then stored at − 70 °C.

2.2.2 Biomass, pigments, and protein determination

Biomass was determined by filtering 20 mL of microalgal culture through a preweighed Whatman GF/C filter. Photosynthetic pigments, including chlorophyll *a*, *b*, and carotenoids, were determined spectrophotometrically at 666, 653, and 470 nm, respectively, after extraction with 96% methanol. The concentration of pigments was calculated and presented using the following equation [21]:

$$C_a = 15.65A_{666} - 7.340A_{653}$$

$$C_b = 27.05A_{653} - 11.21A_{666}$$

$$C_{x+b} = 1000A470 - 2.860C_a - 129.2\,C_b/245$$

$$\left(C_a = \text{chlorophyll } a,\ C_b = \text{chlorophyll } b,\ \text{and } C_{x+b} = \text{total carotenoid}\right)$$

The process of astaxanthin extraction and its content measurement was done based on Li et al.'s [22] suggestion. Protein quantification followed Bradford's [23] method. Bovine serum albumin was used as a standard to measure the protein content.

2.2.3 Lipid analysis

To determine neutral lipid content in algal biomass, 100 µL dimethyl sulfoxide (DMSO) was added to 20 µL of the algal cell suspension and shaken for 1 min. The mixture was heated in a microwave oven (100% power 1650 W) for 50 s; then, 20 µL of Nile red (0.25 mg L^{-1} in acetone) was added and diluted to 1 mL with distilled water. The mixture was shaken for 1 min before being heated for another 1 min. The samples kept in the dark (for 10 min) were transferred to a 2-mL vial with 1 mL of distilled water (total volume of the mixture was 2 mL) and analyzed by a fluorometer (Cary Eclipse, Varian, Italy) [24] with the excitation and emission bands 485 and 585 nm, respectively.

Lipid extraction was done according to the Fraser et al. [25] method. Fatty acid methyl esters (FAMEs) were prepared using a 3 mL solution of 5% (v/v) H_2SO_4 in methanol. The FAMEs, after extraction with 3 mL hexane, were analyzed by GC, based on Challagulla et al.'s [26] method. The methyl ester composition was determined using an Agilent N6890 series gas chromatograph (GC) equipped with a flame ionization detector (FID) and a DB-23 (60 m × 250 µm × 0.15 µm). The oven temperature was set at 150 °C for 1 min, heated at 2.9 °C min^{-1} up to 230 °C; then, it was held for 1 min, with a total run time of 30 min. One microliter of the sample was injected into GC. The FAMEs in the microalgal lipid sample were identified based on their retention time comparing it to the retention time of the peaks of known FAMEs in the standard solution (FAME Mix, C8:0–C24:0, Sigma Aldrich). The results were expressed as a percentage of total fatty acids.

3 Results

3.1 The effects of aeration and mixotrophy by acetate and pyruvate on fresh weight

The microalga *S. obliquus* responded positively to acetate with a considerable increase in fresh weight (Fig. 1a). Without acetate supplementation in culture, the *S. obliquus* fresh weight had average productivity of 0.9 mg mL^{-1}. Adding sodium acetate with 50 mM concentration increased fresh weight more than 20-fold. However, higher concentrations of acetate (100 and 200 mM) had less impact on fresh weight increase. Aeration for 2 h had no effect on the fresh weight of the control cultures, but in treated algae by acetate, aeration severely decreased the positive effects of acetate supply in cultures.

In addition, supplementing BBM with sodium pyruvate (50, 100, and 200 mM) led to a significant increase in microalgal fresh weight (Fig. 1b). The lowest pyruvate concentration (50 mM) caused the highest increase in fresh weight (about 8 times). Unlike the acetate treatment, aeration had an enhancement effect on fresh weight,

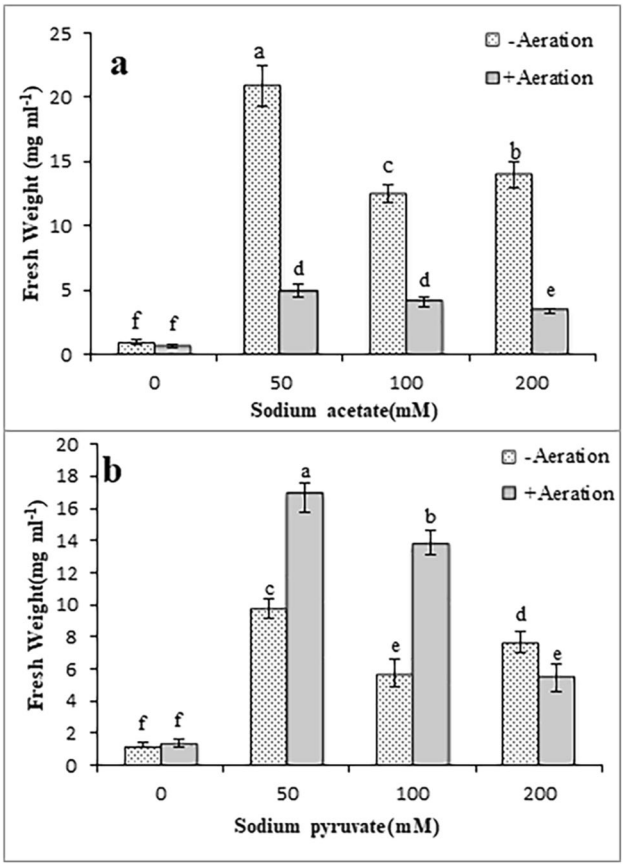

Fig. 1 The effects of aeration and mixotrophy by acetate and pyruvate on the fresh weight of *S. obliquus*. Values are means of three replications ± standard deviation. Different letters show a significant difference with $p < 0.05$ in one-way ANOVA and Duncan's tests

increasing in 50 and 100 mM pyruvate treatments. Aeration increased the fresh weight in treated cultures with 50 mM pyruvate up to 13-fold.

3.2 The effects of aeration and mixotrophy by acetate and pyruvate on photosynthetic pigments

The amount of chlorophyll *a*, *b*, and total carotenoids increased by adding acetate in all concentrations (Fig. 2a, b, and c). The highest increase was observed at 50 mM concentration with a 4.6-, 8.7-, and 60-fold increase in chlorophyll *a*, *b*, and total carotenoid contents in comparison to the control group, respectively. Sodium acetate with 200 mM concentration caused a decrease in chlorophyll *b*, but 50 and 100 mM concentrations stimulated its accumulation. Aeration declined the effect of acetate on increasing pigments contents. On the other hand, the amount of chlorophyll *b* slightly increased by aeration in 200 mM acetate treatment.

The results showed that sodium pyruvate increased chlorophyll *a* and total carotenoid contents at all concentrations (Fig. 2d, e, and f). Chlorophyll *b* content increased only in 50 mM sodium pyruvate treatment. By applying aeration, control cultures had higher contents of chlorophyll *a*, *b*, and total carotenoid. Moreover, aeration in the treated algae with 50 and 100 mM pyruvate caused a significant increase in chlorophyll *a*, *b*, and total carotenoid. The effect of aeration on the increase of carotenoids was noticeable (more than six-fold in comparison to the control cultures without aeration).

3.3 The effects of aeration and mixotrophy by acetate and pyruvate on astaxanthin

Figure 3a shows changes to the astaxanthin content under mixotrophy with acetate. All concentrations of acetate induced astaxanthin accumulation in treated algae. However, the highest amount of astaxanthin was observed in 50 mM acetate with a 4.5-fold increase. Aeration without acetate caused a decrease in the astaxanthin content, but it along with 50 and 100 mM acetate increased the astaxanthin accumulation. In the treatment of 50 mM acetate with aeration, the astaxanthin content was 6.6-fold in comparison to the control group without aeration. Aeration had no significant effect on the astaxanthin content in 200 mM acetate treatment.

Pyruvate treatment raised the astaxanthin accumulation only in 50 mM concentration about 2-folds (Fig. 3b). Aeration had a significant effect on increasing the astaxanthin content in phototrophy and mixotrophy by 50 and 100 mM pyruvate, respectively.

3.4 The effects of aeration and mixotrophy by acetate and pyruvate on the protein content

The protein content considerably increased in the acetate-treated cultures (Fig. 4a). The greatest increase was observed in cultures treated with 50 mM acetate, with a 4.8-fold increase in comparison to the related control. Interestingly, aeration had opposite effects on the amount of protein in the phototrophic and mixotrophic conditions. It increased protein content in phototrophic and intensely decreased the amount of protein in mixotrophy cultures even less than the protein level in control cultures.

Mixotrophy with pyruvate and its interaction with aeration had similar effects on the protein content of *S. obliquus* (Fig. 4b). The amount of protein in mixotrophic cultures with 50, 100, and 200 mM pyruvate was about 6.3, 3.2, and 4 times more than in control cultures, respectively. Aeration increased the protein content in phototrophic cultures; however, in the mixotrophic conditions,

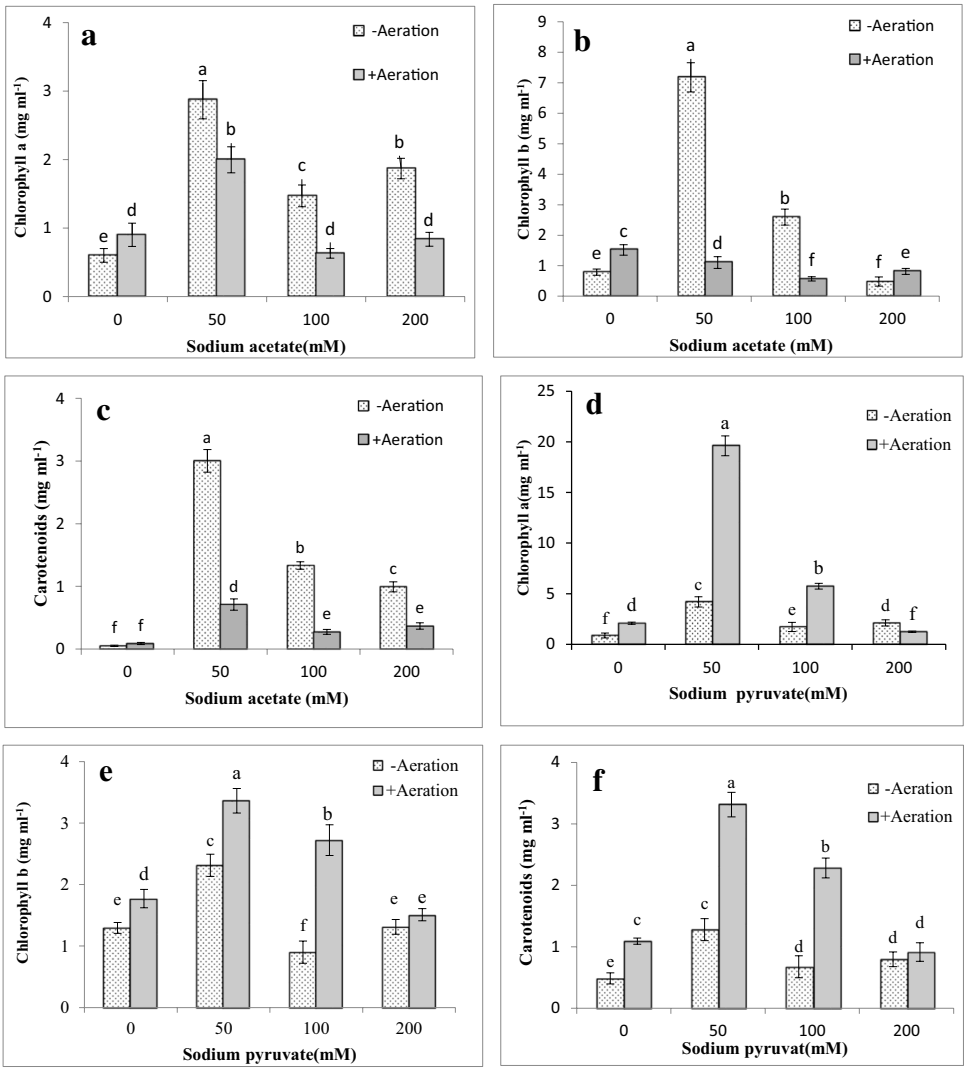

Fig. 2 The effects of aeration and mixotrophy by acetate and pyruvate on the photosynthetic pigments of *S. obliquus*. Values are means of three replications ± standard deviation. Different letters show a significant difference with $p < 0.05$ in one-way ANOVA and Duncan's tests

aeration decreased the positive effect of pyruvate. Aeration with 200 mM pyruvate caused a significant decrease in the protein content compared to the cases in the control group.

3.5 The effects of aeration and mixotrophy by acetate and pyruvate on the Nile red fluorescence determination of lipids

As shown in Fig. 5a, a significant increase in lipid accumulation was only observed in the mixotrophy condition. The maximum lipid content was detected in 50 mM acetate with an 18.4 times increase in comparison to the cultures in the control group. The higher concentrations of acetate had less effect on the increase of the lipid content. Aeration reduced the amount of protein in both phototrophic and mixotrophic conditions.

However, mixotrophy by pyruvate caused an increase in the lipid content (Fig. 5b). In this regard, the concentration of 50 mM of pyruvate was more effective with a fivefold increase compared to the control group. Aerated phototrophic cultures had less lipid content. On the contrary, aeration increased the number of neutral lipids in pyruvate-treated cultures. In 50 mM pyruvate with aeration treatment, the lipid content was 10.3-fold that of the control group without aeration.

3.6 The effects of aeration and mixotrophy by acetate and pyruvate on the lipid profile

Table 1 shows the influence of the two carbon sources on the fatty acids (FAs) content and profile in *S. obliquus* under the two aeration conditions. Under the non-aeration condition, saturated fatty acids (SFAs) had a higher percentage than unsaturated acids (UFAs). In control cultures, the saturated acid, caprylic acid (C8:0, 40%), and the polyunsaturated eicosapentaenoic acid (EPA; C20:5 n3, 17.5%) had a higher percentage than other FAs. In mixotrophic cultures,

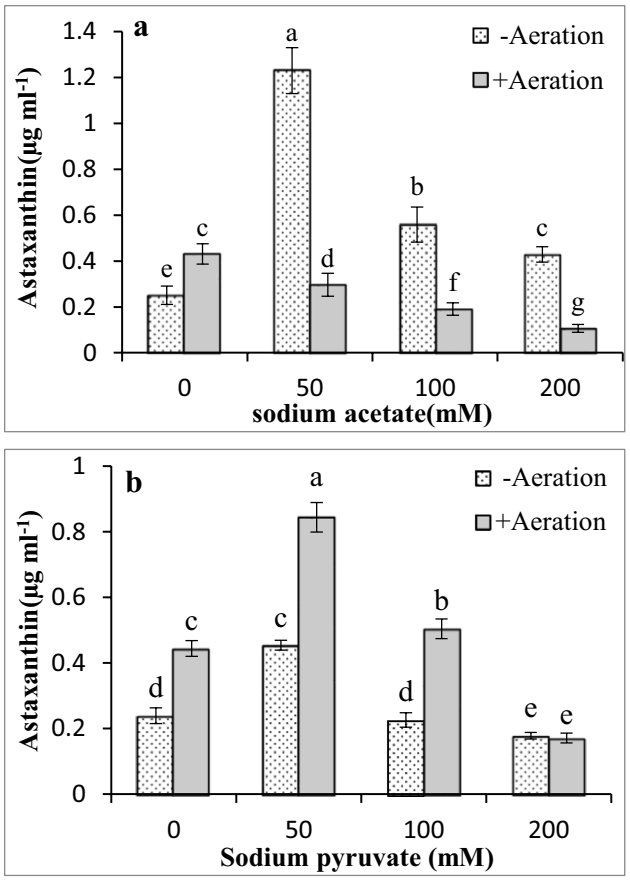

Fig. 3 The effects of aeration and mixotrophy by acetate and pyruvate on the astaxanthin content of *S. obliquus*. Values are means of three replications ± standard deviation. Different letters show a significant difference with $p < 0.05$ in one-way ANOVA and Duncan's tests

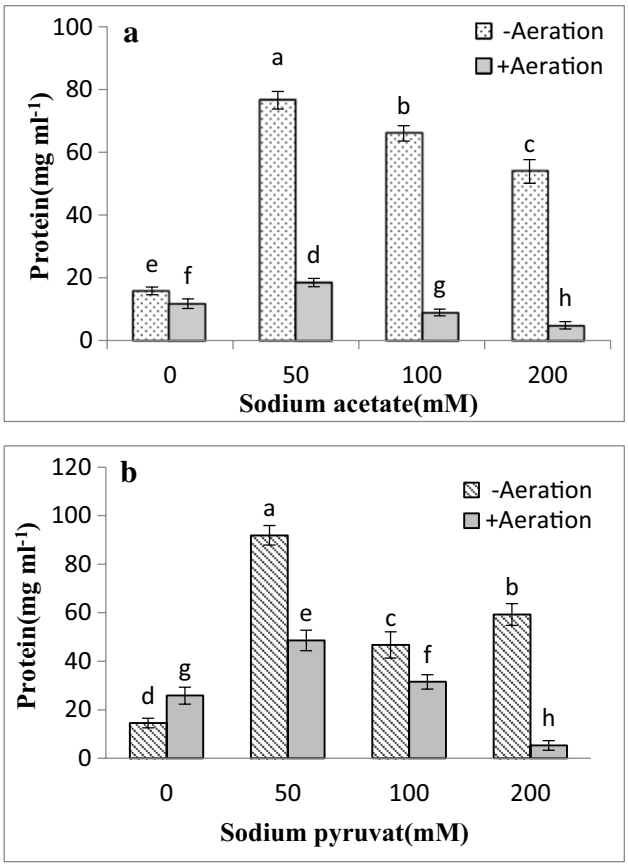

Fig. 4 The effects of aeration and mixotrophy by acetate and pyruvate on the protein content of *S. obliquus*. Values are means of three replications ± standard deviation. Different letters show a significant difference with $p < 0.05$ in one-way ANOVA and Duncan's tests

the percentage of SFAs decreased, whereas the percentage of UFAs increased. Arachidic acid (C20:0, 10.4% of the total FAs) was only observed in aerated control and acetate-treated culture. Despite the decrease in the total amount of SFAs under mixotrophic conditions, the amount of some individual SFAs such as stearic acid increased in acetate and pyruvate-treated cultures. On the other hand, caprylic acid (C8:0) severely decreased by adding acetate and pyruvate in cultures without aeration.

Interestingly, aeration had a notable effect on FAs profiles. Aeration is a phototrophic condition that caused a severe decrease in SFAs and an increase in UFAs. The maximum amount related to EPA with 60.8% (the highest percentage between all treatments) of the total FAs. However, DHA (3.4% of total FAs) as a UFA was detected in these samples. Aeration under the mixotrophic conditions increased UPAs in both acetate and pyruvate-treated cultures. In acetate-treated cultures, EPA was the main FA, but DHA was absent in the profile. The result of aeration was very interesting in the pyruvate treatment. The amount of all SFAs except stearic acid (C18:0) was greatly reduced. Stearic acid and linolenic acids (C18:3 n3) were the main SFA and UFA in lipid extract of aerated pyruvate treatments. In addition, this treatment had the highest percentage (9%) of DHA between all treatments.

4 Discussion

Biomass production in the mixotrophic condition was higher than in the phototrophy condition, which is consistent with the other results found on *S. obliquus* and other microalgae in mixotrophic conditions [27, 28]. However, in some algae, using an organic carbon source has no effect on algal growth. For example, Åberg and Fries [29] reported that c-sources such as glucose, sodium acetate, or glycolic acid did not change the yield of *Vaucheria dichotoma*. Such observed advantages of mixotrophy over phototrophy are likely due, in part, to the fact that photosynthesis consumes CO_2 derived from the oxidation of an external carbon source. On the other hand, in the mixotrophy conditions, cells can use two

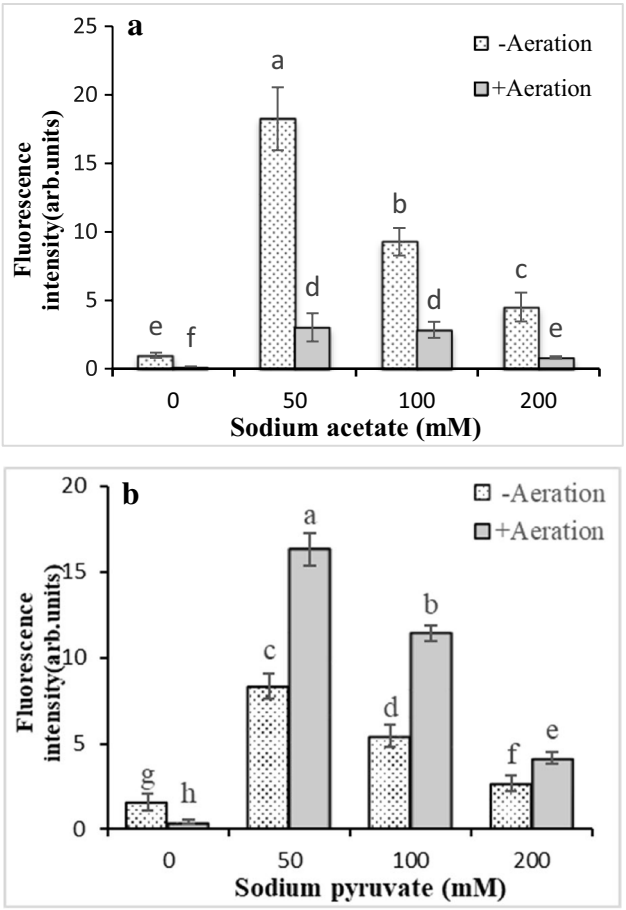

Fig. 5 The effects of aeration and mixotrophy by acetate and pyruvate on the Nile Red fluorescence determination of lipids of *S. obliquus*. Values are means of three replications ± standard deviation. Different letters show a significant difference with $p < 0.05$ in one-way ANOVA and Duncan's tests

energy sources (light and carbon source) for metabolism. Baldisserotto et al. [30] observed that the 6-day mixotrophic cells of *Neochloris oleoabundans* were in large number, but lighter and smaller than the control cells.

Interestingly, the *S. obliquus* biomass yield on sodium acetate was higher than sodium pyruvate. It shows that the type of carbon source used by algae has a particular effect on cell metabolism. Both acetate and pyruvate are respiratory intermediates but probably their roles in other metabolism pathways may explain the differences between the results related to acetate and pyruvate application. In all the investigated parameters, except for protein and chlorophyll content, acetate function was found better than pyruvate. Increasing the concentration of pyruvate can cause induction of Krebs cycle reactions. As a result, the amount of α-ketoglutarate and amino acids derived from it, such as glutamate that is a chlorophyll biosynthesis precursor, increased, and it can be an explanation for more increase in chlorophyll content in pyruvate treatment in comparison to the acetate treatment. Furthermore, the role of pyruvate as a precursor in some amino acid biosynthesis can explain a better effect of pyruvate on protein accumulation. The reason for the much greater increase in lipid and carotenoid content in mixotrophy by acetate compared to pyruvate is that acetate is a substrate in fatty acid biosynthesis in chloroplasts; then, it stimulates carbon flow toward fatty acid metabolism. Given that fatty acids are the main component of lipid membranes, a further increase in biomass is reasonable in the acetate treatment. On the other hand, acetate is a substrate for the mevalonate pathway that can export extra isopentenyl pyrophosphate (IPP; terpenoids precursor) produced by chloroplast and induce carotenoid biosynthesis.

Aeration caused an increase in the chlorophylls and carotenoid content without a significant effect on fresh weight in the phototrophic conditions. It showed that increasing CO_2 by aeration induced chlorophyll and carotenoid biosynthesis. Shene et al. [31] also reported that in the absence of the pH control, changes in air CO_2 levels up to 1.34% had no effect on the biomass concentration. Mechanisms such as active transport of CO_2 [32] and induction of carbonic anhydrase activity cause algae to be able to get enough CO_2 in non-aeration conditions [33]. In this study, the protein, lipid, and astaxanthin contents were reduced by aeration. This decrease could be due to the reduction in the number of cells under hydrodynamic stress caused by aeration. A reduction in the lipid content of *Chlorella sorokiniana* with aeration was reported by Chen [14]. On the other hand, Mohsenpour and Willoughby [34] showed that with the aeration of the culture with the atmospheric air, CO_2 was absorbed and the pH reduced. The reduction in pH can be due to a decrease in growth parameters such as lipid and protein in algae that are sensitive to pH changes.

An interesting result in this study was the different interaction effects of aeration with acetate and pyruvate mixotrophy. Aeration intensely decreased the positive influence of mixotrophy by acetate and, inversely, increased the pyruvate function, especially in 50 mM concentration. Acetate is more volatile than pyruvate, probably aeration caused the removal of a part of acetate from culture, which led to a decrease in the acetate effect. Aeration significantly increased chlorophyll content in pyruvate-treated cultures, implying that aeration can cause an induced pyruvate effect by increasing photosynthesis. These results showed that aeration has important interactions with other factors in the culture medium, and optimization is crucial for the successful scale-up of microalgal cultivation systems.

According to our results, SFAs' production is favored under phototrophy conditions, while high PUFA (EPA) contents are mainly produced under mixotrophy conditions, especially by pyruvate. However, Baldisserotto et al. [30] reported that mixotrophy by glucose increased SFAs and MUFAs, while PUFAs decreased in *Neochloris*

Table 1 Fatty acid profile (% of total) of *Senedesmus obliquus* grown in the mixotrophic condition by 50 (mM) acetate or pyruvate with and without aeration

Fatty acid	Control + aeration	Control-aeration	50 mM A.S + aeration	50 mM A.S-aeration	50 mM P.S + aeration	50 mM P.S-aeration
C8:0	8.1 ± 1.9	40.0 ± 5.6	17.3 ± 2.45	23.5 ± 3.09	0.14 ± 0.02	24.1 ± 3.98
C10:0	1.7 ± 0.4	5.4 ± 1.09	2.9 ± 0.25	4.9 ± 1.23	ND	3.4 ± 0.34
C10:1	1.0 ± 0.3	3.7 ± 1.8	1.6 ± 0.7	2.8 ± 1.0	ND	2.2 ± 0.9
C11:0	2.5 ± 0.56	5.6 ± 1.35	1.9 ± 0.21	3.5 ± 0.53	0.11 ± 0.03	1.8 ± 0.11
C11:1	ND	2.6 ± 1.5	ND	ND	ND	ND
C12:0	0.9 ± 0.04	ND	ND	ND	ND	1.4 ± 0.23
C13:0	2.0 ± 1.3	3.7 ± 1.9	1.8 ± 1.2	ND	0.15 ± 0.06	1.9 ± 0.9
C14:0	ND	3.6 ± 0.76	ND	7.0 ± 0.98	ND	ND
C14:1	ND	ND	2.2 ± 0.54	ND	0.18 ± 0.03	1.8 ± 0.7
C16:0	1.6 ± 0.09	ND	ND	ND	ND	ND
C18:0	6.2 ± 0.99	5.1 ± 1.04	6.0 ± 0.94	5.6 ± 0.84	51.6 ± 5.26	7.9 ± 1.06
C18:3(n3)	1.4 ± 0.3	2.7 ± 0.78	5.3 ± 1.08	3.4 ± 0.69	36.0 ± 6.54	1.3 ± 0.07
C18:3(n6)	2.9 ± 0.56	ND	4.5 ± 0.87	3.7 ± 0.87	ND	5.1 ± 1.76
C20:0	1.5 ± 0.067	ND	2.2 ± 0.52	10.4 ± 2.43	ND	ND
C20:1	1.2 ± 0.24	ND	2.5 ± 0.99	ND	3.0 ± 1.05	ND
C20:5(n3) (EPA)	60.8 ± 8.65	17.5 ± 2.93	51.2 ± 5.92	20.6 ± 4.86	ND	ND
C21:0	ND	ND	ND	ND	ND	48.7 ± 8.45
C22:2	ND	4.6 ± 0.76	ND	10.4 ± 1.75	ND	ND
C22:6(n3)(DHA)	3.4 ± 0.92	ND	ND	ND	9.0 ± 1.07	ND
C24:0	ND	4.8 ± 0.99	ND	4.1 ± 1.05	ND	ND
SFA	24.5	68.2	32.1	59	52	89.2
USFA	70.7	31.1	67.3	40.9	48	10.4

ND, not detected; *DHA*, docosahexaenoic acid; *EPA*, eicosapentaenoic acid; *SFA*, saturated fatty acids; and *USFA*, unsaturated fatty acids. Values are means of three replications ± standard deviation. Different letters show a significant difference with $p < 0.05$ in one-way ANOVA and Duncan's tests

oleoabundans. In this study, five doses of xylose were tested to determine the effects and mechanisms of the carbon source on microalgae in a mixotrophic mode. At the optimal xylose dosage of 4 g/L, the highest lipid content (38.61%) and productivity (139.55 mg/L/day) were reported [35]. The differences observed in SFA, MUFA, and PUFA proportions among the mixotrophic treatments indicated that the type of carbon source can have a significant effect on the fatty acid profile. Since the fatty acid profiles rich in SFAs and MUFAs are the most interesting profiles for biodiesel purposes, the untreated algae profile is suitable for biodiesel production, but the mixotrophic conditions that caused a significant increase of PUFAs are not suitable in this regard. Instead, mixotrophy, particularly pyruvate, can be a good way to increase the amount of EPA in this alga, and an increase in EPA consumption can result in improved general health and well-being [13]. Aeration induced UFAs in both the phototrophic and mixotrophic conditions. Aeration causes the amount of oxygen to increase in the culture medium, and, as a result, conditions for the formation of unsaturated bands in FAs are provided. Li et al. [15] also reported that aeration increased almost all classes of the essential fatty acids (EFAs)-enriched lipids at the onset of the stationary phase.

Our results showed mixotrophy caused a decrease in the percentage of SFAs and an increase in the percentage of UFAs. EPA was the main fatty acid in the mixotrophic condition. Aeration in the phototrophic condition decreased the percentage of SFAs and increased that of UFAs. Aeration under the mixotrophic conditions increased UFAs in both acetate and pyruvate-treated cultures. Our results suggested that aeration can be an important factor in the interaction with other algae growth conditions.

5 Conclusions

The results of this study showed that, in the phototrophy condition, aeration had no significant effect on biomass (fresh weight); however, the other measured biochemical parameters except the total lipid increased in the aerated samples. Interestingly, the effect of aeration in the mixotrophy condition depended on the carbon source used. In mixotrophy by acetate, aeration considerably decreased the

positive effect of acetate on increasing the biomass, chlorophyll, carotenoid, protein, and lipid contents, although, in mixotrophy by pyruvate, aeration increased the enhancement effect of pyruvate in the growth parameters. This experiment showed that a simple factor such as aeration can have a profound and different effect on the amount of organic carbon source used in the mixotrophic cultures. It can be argued that the use of acetate or pyruvate as a carbon source is not cost-effective; however, given the sharp 20-fold increase in algal biomass, its use could be economically justified.

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Declarations

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