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ORIGINAL ARTICLE

# Influence of $Fe^{+2}$ on the biomass, pigments, and essential fatty acids of *Arthrospira platensis*

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**Abstract**
The effects of different ferrous sulfate ($FeSO_4$) concentrations on the fatty acid profile of the blue-green alga *Arthrospira platensis* cultured in Zarrouk's medium to produce biodiesel were studied. Different ferrous sulfate concentrations (0, 0.005, 0.01, 0.05, and 0.1 g/L) and 0.01 g/L were examined on the biochemical composition of the alga and fatty acids profile of *A. platensis*. Findings revealed that the highest and lowest yields of fatty acid were 0 and 0.1 g/L $FeSO_4$, respectively. It was also noticed an increase of palmitic acid, oleic acid, linoleic acid, γ-linolenic acid, and docosahexaenoic acid when ferrous sulfate was between 0.05 and 0.1 g/L, while these fatty acids decreased at low concentration. Ferrous sulfate at a concentration of 0.1 g/L exhibited an increase and best yields in the following: growth rate and the shortest doubling time, chlorophyll-a, phycocyanin, allophycocyanin, phycobiliproteins, and carotenoids. Thus, increasing the $FeSO_4$ concentration to 0.1 g/L has led to the increase in fatty acid individuals, which in turn, resulted in potential enhancement of the biodiesel production from *A. platensis.*

**Keywords** Blue-green algae · *Arthrospira platensis* · Biodiesel · Fatty acids · $FeSO_4$ · Bioenergy

## 1 Introduction

The global population is expected to increase by 9 billion in 2050 [1], which will lead to more fossil fuel consumption with increasing greenhouse gas emissions (GHGs) [2]. Therefore, seeking another energy source is a necessity for reducing GHGs emissions, minimizing the dependence on fossil fuels, and maintaining environmental sustainability [3, 4]. Recently, there has been considerable attention to microalgae as a source of third-generation liquid biofuels. The utilization of microalgae for biofuel production offers several benefits. Firstly, they can grow in different waters [5], including wastewater, and double their biomass in periods as short as 3.5 h. Secondly, microalgae can be cultivated without the need for herbicides or pesticides application [6] due to their ability to live on non-arable lands [7]. Thirdly, they are employed in wastewater treatment to remove nutrients such as phosphorus, nitrogen, carbon, and other toxic compounds [8, 9]. Finally, different bio-products other than biofuel can be produced from microalgae, such as cosmetic products, animal feed, and valuable pharmaceuticals [10, 11]. Biofuels produced from microalgae are generated via processes, including ethanol production by carbohydrates saccharification [12–15], biodiesel production by lipid transesterification [16, 17], and crude bio-oil production by thermochemical conversion [18]. Researchers further investigated microalgae as a gas fuel source, such as biohydrogen [19] and biogas [20].

Biodiesel is a renewable and biodegradable fuel produced from animal fats or vegetable oils reactions with short-chain alcohol (methanol or ethanol) through the transesterification process in the existence of a catalyst. Catalysts are incorporated at 1.5 h and 60 °C under 101,325 Pa, in order to reduce response time and rising conversion rate [21].

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Transesterification switches over fresh to dense microalgae lipids to decrease corrosive alkyl esters or the subatomic weight of fats, triacylglycerols, or safe unsaturated fats [22]. Several catalysts are employed in the process of transesterification such as alkaline, acid, enzymes, and heterogeneous inorganic catalyst [23]. In a comparison with conventional diesel, biodiesel can equilibrate the negative balance generated by the emission into the atmosphere. Further, it reduces carbon monoxide (CO), sulfur compounds ($SO_x$), and particulate matter (PM) emissions and having better lubricity and renewability [24]; however, it contributes to increasing nitrogen oxide ($NO_x$) emissions [25].

In the past few decades, microalgae species have been utilized to produce biodiesel. For instance, *Chlorella vulgaris* [26], *Chlamydomonas reinhardtii* [27], *Scenedesmus dimorphus*, *Scenedesmus obliquus* [28, 29], *Nannochloropsis granulata*, *Nannochloropsis oculata* [30], *Scenedesmus dimorphus* [31], and *Caulerpa prolifera* [13] were evaluated for biodiesel production with lipid content 6, 49–52, 10, 30–50, 28.5, 45, 14.71 ± 0.26, and 10%, respectively. However, several challenges are facing microalgal technologies despite their participation in global energy demand. It is essential to understand the optimum growth condition of the selected microalgae to enable a large-scale production system. Such condition enhances lipid accumulation or promotes high biomass production, which leads to a reduction in the production cost. Several studies have shown an effective method to enhance lipid accumulation via sulfur and nitrogen limitation [32, 33].

*Arthrospira platensis* (formerly *Spirulina platensis*) is a species of microalga with high protein content. *A. platensis* was first isolated by Turpin in 1827 [34]. It has a spiral-shaped trichoma with 100–110 µm long and 8–10 µmm wide. It is a vital microalga as it contains high protein, pigments, gamma-linolenic acid (GLA), vitamins, fatty acids, and other valuable metabolites [35]. It is worth mentioning that five fatty acids are most abundant in the *A. platensis*, namely palmitic acid, palmitoleic acid, oleic acid, linoleic acid, and γ-linolenic acid [36, 37]. *Arthrospira platensis* is extensively cultured at a mass commercial scale and is considered as one of the most famous blue-green algae or cyanobacteria [38, 39]. Worldwide, the production of *Spirulina* has increased to over 89 thousand tons in 2016 [40]. It is widely utilized in many biotechnological applications such as biofertilizers, pharmaceuticals, pigments, human food supplements, omega-3-fatty acids, and animal feed additives [38, 41–44].

Recent studies have concluded that due to the high biomass and lipid productivity of *A. platensis*, it is considered one of the best feedstock for biodiesel production [39, 44–46]. As confirmed by literature, the extracted biodiesel from *A. platensis* is within the recommended specifications of the international standards of Europe (EN14214) and the USA (ASTM675103) [43, 44, 46]. For instance, the cultivation of *A. platensis* in an iron-enriched medium has been investigated by Cepoi et al. [47] who stated that the adaptation of the *Spirulina's* biomass to copper-containing effluents was more noticeable in Cu/Fe systems since in multicomponent systems, the process was more complex and varied based on the metal type, amount, and concentration. The spectra of iron hydroxides in *A. platensis* biomass differed from those of iron complexes put into Zarrouk's growth medium with ethylenediaminetetraacetic acid. The saturation limit of *A. platensis* trichomes with iron in the form of ferrihydrite in the culture medium was found to be 5 g/mL (0.09 mol/mL) Fe [48]. By utilizing Mössbauer spectroscopy, Wan et al. [36] identified the effects of iron on *Chlorella sorokiniana* growth and lipid synthesis, as well as the enzymes and metabolic pathways that may be altered in response to variations in iron levels in the environment. When compared to unsupplemented controls, the addition of iron up to $10^5$ mol $L^{-1}$ boosted ultimate cell densities by approximately twofold at 2.3 $10^7$ cells/mL, growth rate by twofold, and the length of the exponential phase by 5 days, while $10^3$ mol $L^{-1}$ iron was hazardous. The lipid content increased from 12% for unsupplemented cultures to 33% at $10^{-4}$% mol $L^1$ iron, with the maximum total lipid output of 179 mg $L^{-1}$. To the best of our knowledge, studies have not fully investigated the effects of different ferrous sulfate concentrations on the fatty acid profile, biomass, and pigments of the blue-green alga *Arthrospira platensis.* Thus, this study evaluated *A. platensis* for biodiesel production by cultivating it in various ferrous sulfate ($FeSO_4$) concentrations to find the optimal conditions. In addition, the assessment procedures involve studying the effects of the different cultivation conditions on some fatty acids profile, the growth rate, and the doubling time. Furthermore, the effect of $FeSO_4$ on the biochemical composition of the alga as a nutritional source was evaluated.

## 2 Material and methods

### 2.1 Algal strain and medium composition

A kit of the blue-green alga, *Arthrospira platensis*, was purchased from Suncoast Marine Aquaculture (St. Petersburg, Florida). *Arthrospira platensis* was grown in Zarrouk's medium [37]. To obtain the final medium, the solutions with the respective salts were sterilized by employing autoclaving for 15 min at a temperature of 121 °C. Then, they were mixed thoroughly [49]. For biomass production, *A. platensis* cells were inoculated at a concentration of 10% ($V_{inoculation}/V_{media}$) in 500-mL sterilized Erlenmeyer flasks containing 100 mL Zarrouk's medium at 32 ± 1 °C, pH 9 using cool white fluorescent tubes (35 µ$Em^2$ $s^{-1}$) with a

photoperiod cycle of 12:12 h light/dark. They were shaken twice a day to allow air and nutrients circulation [50].

## 2.2 The design of the experiment

To study the effect of iron (in the form of ferrous sulfate) on the biochemical composition of the alga and fatty acids profile, *A. platensis* was cultured in Zarrouk's medium supplemented with different iron concentrations (0, 0.005, 0.01, 0.05, and 0.1 g/L) and using 0.01 g/L as the control.

## 2.3 Biodiesel production

### 2.3.1 *Direct* in situ *transesterification*

*Arthrospira platensis* cells were centrifuged at 7500 rpm for 10 min. After that, samples were dried for 2 h at 55 °C and refrigerated at − 4 °C before analyses [51]. About 0.1 g was mixed with 8 mL of fresh reaction solution ($HCl/CHCl_3/CH_3OH$, 4:4:40 v/v/v) in Teflon-capped Pyrex tubes [52]. Then, suspended biomass was dehydrated for 1 h at 90 °C for the transesterification step. Then, the mixtures were cooled at room temperature. The upper extracted organic supernatant was collected, and the fatty acid methyl esters (FAMEs) were extracted twice. The final samples were then analyzed by gas chromatography [53].

## 2.4 Estimation of growth rate and doubling time

Using the UV–Vis spectrophotometer, the OD at 560 nm was converted to the cell density (cells/mL) according to the linear relationship between these two parameters and the culture medium [54]. The determination of the specific growth rate and doubling time was followed the past literature [55].

### 2.4.1 Chlorophyll

To determine the chlorophyll, Arnon's method [56] was adopted. The algal cells were placed in 1 mL of 80% acetone, and then they were centrifuged. The collected supernatant was analyzed by using UV–Vis spectrophotometer at a wavelength of 663 nm and 645 nm with the following equation:

$$\text{Chlorophyll}a(\text{mg/L}) = (12.7 \times \text{A663}) - (2.698 \times \text{A645}) \quad (1)$$

### 2.4.2 Carotenoids estimation

About 5 mL of *A. platensis* was centrifuged for 10 min at a speed of 4000 rpm. Samples were then washed with distilled water to remove any remaining salts. After that, about 5 mL of acetone was added until a white precipitate appeared. Then, samples were subjected to centrifugation for 15 min at a speed of 5000 rpm to separate the acetone extract. Following the steps reported by Jensen [57], the carotenoid content was determined with a spectrophotometer.

### 2.4.3 Phycobiliproteins estimation

The estimation was done by following the Bennett and Bogorad [58] method. About 10 mL of samples was centrifuged for 20 min at a speed of 4500 rpm. Then, samples were washed with distilled water. After that, samples were suspended in 10 mL phosphate buffer with further centrifugation at 4500 rpm for 10 min. The concentrations of phycocyanin, allophycocyanin, and phycobiliproteins (PBP) were determined in accordance with the equation from Devanathan and Ramanathan [59].

### 2.4.4 Total protein

Total protein was determined by the Bradford dye-binding assay method [60]. The main concept of this assay is relying on the binding of the dye Coomassie Blue G250 to protein. About 100 µL and 5 mL of Bradford dye reagent were added and mixed. Known dilutions of bovine serum albumin were prepared to calculate the protein.

### 2.4.5 Total carbohydrates

*Arthrospira platensis* was centrifuged at 5000 rpm for 30 min at 4 °C. Utilizing glucose as a standard, the Dubois et al. [61] method was employed to determine the carbohydrate content. A mixture of 1 mL of sample, 5% phenol, and 5 mL of 96% $H_2SO_4$ were added to the test tube. The tube was water bathed for 20 min at 25–30 °C. The total carbohydrate was determined by following the standard graph prepared by Nordin et al. [62].

## 2.5 Determination of fatty acid composition

To analyze the washed FAMEs, gas chromatography (Shimadzu, Japan) equipped with a flame ionization detector using the SP-2480 column was used. Furthermore, the carrier gas used in this study was helium. The temperatures at the injector port and the detector were 280 °C and 330 °C, respectively. The initial column temperature was started at 150 °C and gradually increased by 10 °C $min^{-1}$ until it reached 300 °C. The FAMEs for the selected algae were identified by comparing the standard fatty acids retention time and sample peaks [63]. Five fatty acids (palmitic acid, oleic acid, linoleic acid, γ-linolenic acid, and docosahexaenoic acid) were used as the standard materials.

### 2.6 The analysis of fatty acid by gas chromatography

The fatty acid methyl esters (FAMEs) of *A. platensis* were examined by comparing the peak retention times with the standard one [63]. The gas chromatography was equipped with a flame ionization detector. The carrier gas in the SP-2480 column was helium. The injector temperature was 280 °C, and the initial column temperature was set at 150 °C, which raised to 300 °C at the rate of 10 °C $min^{-1}$.

## 3 Results and discussion

### 3.1 *The effect of $FeSO_4$ concentrations on growth rate and doubling time of A. platensis*

The growth rate (K) increased to 0.135 and 0.141 under the treatments of 0.05 g/L and 0.1 g/L, respectively, and decreased to 0.120 and 0.118 under the treatments 0.005 g/L and 0 g/L, respectively, as compared with 0.131 in 0.01 g/L (control). Figure 1 shows the growth rate (K) with and doubling time (d) of *Arthrospira platensis* at different $FeSO_4$ concentrations. The maximum growth induction of 7.092% was recorded in 0.1 g/L, while the maximum growth inhibition of 9.923% was found in the 0 g/L treatment (Fig. 1). In 0.005, 0.01, and 0.05 g/L treatments, the doubling times (G) were 2.511, 2.297, and 2.228 days, respectively. The shortest doubling time was 2.136 days with the 0.1 g/L, while the longest was 2.547 days with the 0 g/L treatment (Fig. 1).

The different $FeSO_4$ concentrations caused different biomass growth production for *A. platensis*. The growth rate was increased when iron concentration increased; meanwhile, the doubling time was decreased. The findings of this study agreed with Cheng et al. [64], who showed that increasing the concentration of iron in the growth medium stimulated the growth rate of *Chlamydomonas reinhardtii*. The mean cell abundance in the 2×treatment (Fe = 92.4 µM) was observed to be significantly higher than the cell abundance in the 1/2×treatment (Fe = 23.1 µM) on day 16 and significantly higher than the 1×treatment (Fe = 46.2 µM) on day 23. Moreover, Glaesener et al. [65] concluded that *C. reinhardtii* cells displayed an increase in cell growth as the iron concentration in the medium increased.

### 3.2 *The effect of $FeSO_4$ concentrations on carbohydrate and protein content of A. platensis*

The treatment of *A. platensis* with $FeSO_4$ concentrations of 0.05 and 0.1 g/L caused an increase in the total carbohydrate content, while decreasing $FeSO_4$ concentrations to 0 and 0.005 g/L caused a decrease in the carbohydrate content. Figure 2 illustrates the carbohydrate and protein contents of *Arthrospira platensis* with respect to different $FeSO_4$ values. As compared with the 37.077 mg/L carbohydrates in control (0.01 g/L $FeSO_4$), the highest carbohydrate content (41.732 mg/L) was recorded with 0.1 g/L, and the lower carbohydrate content (34.435 mg/L) was recorded with 0 g/L (Fig. 2).

The increased $FeSO_4$ concentrations of 0.05 and 0.1 g/L caused a significant reduction in the protein content, while the decreased $FeSO_4$ concentrations of 0 and 0.005 g/L induced the increase of the protein content. The highest protein content (57.300 mg/L) was recorded with 0 g/L, and the lowest protein content (34.861 mg/L) was recorded with 0.05 g/L compared with 39.414 mg/L protein, which

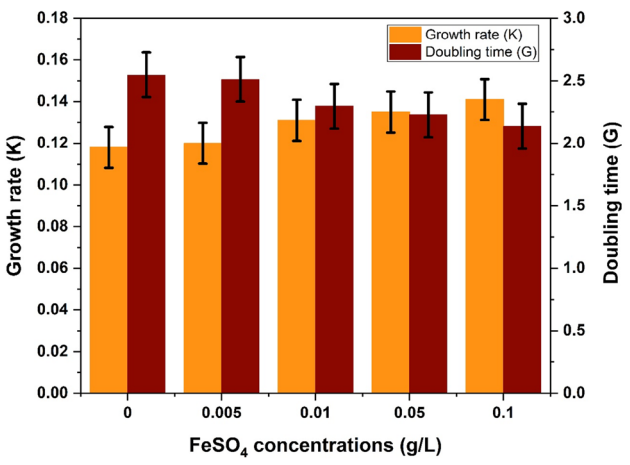


**Fig. 1** Growth rate (K) with LDS 0.009 and doubling time (d) with LSD 0.015 of *Arthrospira platensis* at different $FeSO_4$ concentrations ($p < 0.05$)

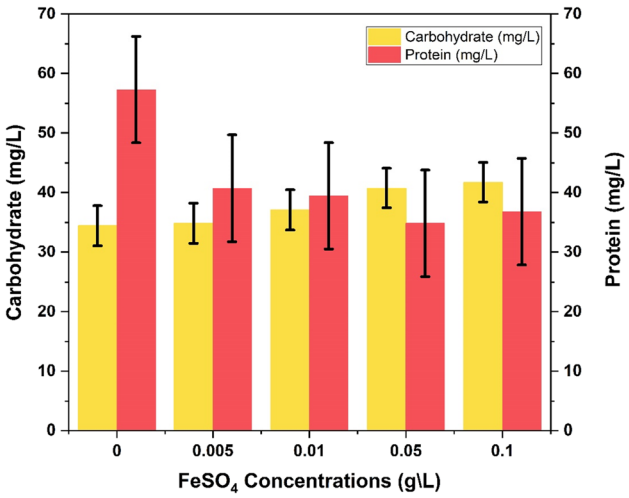


**Fig. 2** Carbohydrate content (LSD 1.679) and protein content (LSD 1.326) of *Arthrospira platensis* with respect to different $FeSO_4$ concentrations

was recorded in 0.01 g/L (control) (Fig. 2). In this study, *A. platensis* exhibited a decline in the total protein contents when the concentrations of $FeSO_4$ increased. This result is in accordance with the findings of Das et al. [66], who demonstrated that the carbohydrate content in *Tetraselmis* sp. increased with the increase of iron concentrations, while the increased iron in the culture decreased the protein content in the marine microalgae *Tetraselmis* sp. and *Chlorocystis* sp. Marrez and his colleagues have conducted a study on *A. platensis* cultured in different growth media. They concluded that the protein and carbohydrate of the selected algae were between 49.5 and 59.8% and between 12.4 and 22.8%, respectively [67]. Another study has reported that *A. platensis* has a protein content ranging between 50.2 and 58.6% [68]. Jaruwan et al. [69] reported that *A. platensis* has a lipid content between 4 and 16%. These variations in the lipid content may be due to different extraction methods, culture system, as well as other environmental conditions, or available nutrient limitations [38, 39, 70, 71].

### 3.3 *The effect of $FeSO_4$ concentrations on chlorophyll a content of A. platensis*

The chlorophyll-a (Chl-a) content in the control (0.616 mg/L), showed that a decrease in $FeSO_4$ concentration to 0 and 0.005 g/L affected *A. platensis*. Figure 3 describes the influence of $FeSO_4$ concentrations on the average of chlorophyll and carotenoid contents of *Arthrospira platensis.* The maximum reduction of Chl-a was 0.461 mg/L, while the maximum induction was 0.683 mg/L (Fig. 3).

The results showed that limiting $FeSO_4$ inhibited photosynthetic pigments like Chl-a, PC, APC, PBP, and carotenoids. Iron is an important component in protein complexes that contributes to photosynthesis and acts as a redox cofactor [72]. The cyanobacterial photosynthetic system photosystem I (PSI) has considerable amounts of iron. In addition, intermediary electron transport complex cytochrome also contains iron. Therefore, with iron deficiency, these pigments can be synthesized in small amounts [73]. Researchers have further investigated the relationship between chlorophyll biosynthesis and iron. They concluded that iron deficiency leads to chlorosis as Fe is considered the most important element for the growth of spirulina with its ability as a limiting factor in pigment production [74, 75].

### 3.4 *The effect of $FeSO_4$ concentrations on phycocyanin, allophycocyanin, and phycobiliproteins content of A. platensis*

The comparison with the control, which supported 0.186 mg/L PC, 0.194 mg/L APC, and 0.381 mg/L PBP, showed that the increasing $FeSO_4$ concentration to 0.05 and 0.1 g/L affected *A. platensis* by increasing the PC, APC, and PBP concentrations. Figure 4 shows the influence of ferrous sulfate concentrations on the average of phycocyanin, allophycocyanin, and phycobiliproteins contents of *Arthrospira platensis*. The maximum induction values of APC (0.274 mg/L) and PBP (0.578 mg/L) were recorded in the presence of 0.1 g/L $FeSO_4$, while the maximum PC value (0.267 mg/L) was recorded with 0.05 g/L $FeSO_4$. On the other hand, the minimum reduction values of PC, APC, and PBP were 0.140, 0.155, and 0.296 mg/L with $FeSO_4$ concentration of 0 g/L (Fig. 4).

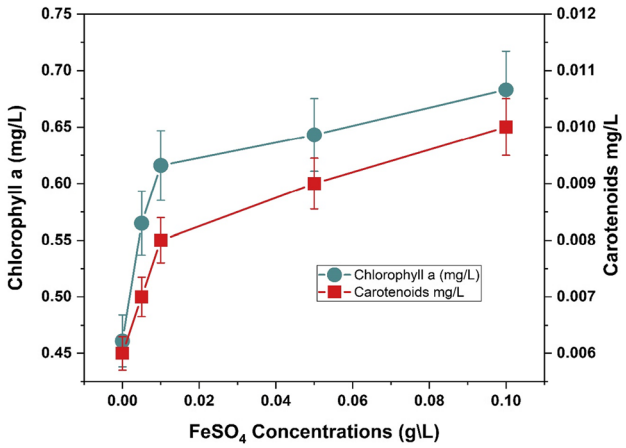


**Fig. 3** Effect of different $FeSO_4$ concentrations on the average of chlorophyll content (LSD 0.048) and carotenoids content (LSD 0.003) of *Arthrospira platensis*

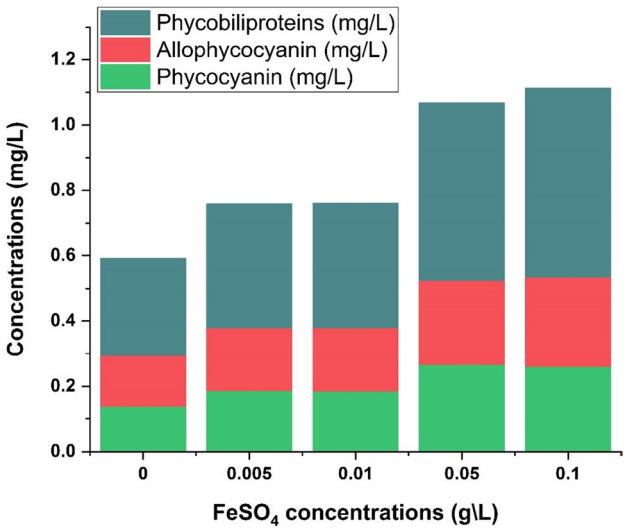


**Fig. 4** Effect of different $FeSO_4$ concentrations on the average of phycocyanin, allophycocyanin, and phycobiliproteins content (LSD value 0.042, 0.034, and 0.085, respectively) of *Arthrospira platensis*

### 3.5 *The effect of $FeSO_4$ concentrations on carotenoids content of A. platensis*

As compared to control (0.01 g/L), which contain 0.008 mg/L of carotenoids, the data showed that carotenoids content increased to 0.009 and 0.010 mg/L at high $FeSO_4$ concentrations (0.05 and 0.1 g/L), respectively (Fig. 3).

### 3.6 *The effect of $FeSO_4$ concentrations on the fatty acid profile*

The effect of different $FeSO_4$ concentrations (0, 0.005, 0.01, 0.05, and 0.1 g/L) on the content of the individual fatty acids is recorded in Table 1 which describes the influence of different ferrous sulfate values on palmitic acid, oleic acid, linoleic acid, gamma-linolenic acid, and docosahexaenoic acid in *Arthrospira platensis*. According to the literature, the selected five fatty acids are the most abundant in *A. platensis* [76, 77]. The chromatograms of gas chromatography (GC) for palmitic acid, oleic acid, linoleic acid, γ-linolenic acid, and docosahexaenoic acid in *A. platensis* revealed that spirulina at higher iron concentrations has a major percent of fatty acids. The maximum content induction of palmitic acid, oleic acid, linoleic acid, γ-linolenic acid, and docosahexaenoic acid was 1.217, 2.060, 0.279, 19.213, and 44.469 mg/g, respectively, with $FeSO_4$ concentration of 0.1 g/L, and their maximum reduction values were 0.234, 0.172, 0.026, 1.699, and 0.998 mg/g, respectively, with $FeSO_4$ concentration of 0 g/L as compared to 0.366 mg/g (palmitic acid), 0.302 mg/g (oleic acid), 0.086 mg/g (linoleic acid), 3.237 mg/g (γ-linolenic acid), and 11.896 mg/g (docosahexaenoic acid) in 0.01 g/L $FeSO_4$ (control).

The maximum induction of the total fatty acids was 76.371% in the 0.1 g/L treatment, while the maximum reduction of the total fatty acids was 80.304%, which was recorded with 0 g/L of $FeSO_4$. In the desaturated molecule, the iron created a reactive complex with oxygen. However, oxygen reacted with carbon in the fatty acid chain and changed the single bonds to double bonds [78] Low iron concentration resulted in the decrease of the chlorophyll concentration, which has led to low biomass and lipid content [69]. With the increased iron concentration, reactive oxygen such as hydrogen peroxide and other oxidant compounds could be formed. In light of this, an interaction between lipids and other biochemical components and oxidant compounds may occur. Therefore, due to iron stress, the synthesis of lipid may be produced to counteract the reactive oxygen species [70]. A study conducted by Mostafa and his co-worker has concluded that *A. platensis* showed 89.6%, 6.2%, and 2.11% of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), respectively, as they have grown the algae indoor and enriched them with Zarrouk's medium [39]. However, another study stated that the PUFAs of *A. platensis* might reach up to 30% of SFAs [38]. Other researchers have also concluded that lipid content in *Botryococcus* spp. and *Scenedesmus obliquus* increased when supplemented with high iron concentration [79, 80]. Moreover, treating the alga *Chlorella vulgaris* with ferrous sulfate (35 µM $FeSO_4.7H_2O$) resulted in a decrease in SFAs with percentages of 3% lower than the corresponding control (17 µM ferrous sulfate) [81]. In 2019, another study was done by Sahin, and his coworker illustrated the growing diatoms under higher iron concentrations. The study has revealed a dramatic increase in lipid content. In *Nitzschia* sp., there was an increase between $37.7 \pm 0.77\%$ of the total lipid and between $37.44 \pm 0.54\%$ of the total lipid in *Nanofrustulum shiloi*. As a result, they were about 2.7 and 1.7 times higher than those obtained with the standard f/2 ($13.8 \pm 0.42\%$) and BG11 ($21.71 \pm 0.34\%$) media, respectively [82]. Additionally, *C. vulgaris* with ferrous sulfate (35 µM after 12 days of incubation) revealed the highest lipid content, whereas the corresponding control showed a lowering in the lipid content. This decrease may be shifted towards the biosynthesis of the unsaturated fatty acids (UFAs) and vice versa. The study has reported that the predominant saturated and unsaturated fatty acids of *C. vulgaris* were C16:0 and C18: 2, respectively [83].

**Table 1** Effect of different $FeSO_4$ concentrations on some fatty acids content of *Arthrospira platensis,* 0.01 (control) ($p < 0.05$). Significant differences were found between control and all treatments except 0.005 g/L in palmitic acid, oleic acid, and γ-linolenic acid, as well as 0 g/L in palmitic acid

| $FeSO_4$ concentrations (g/L) | Fatty acid concentration mg/g | | | | |
|---|---|---|---|---|---|
| | Palmitic acid | Oleic acid | Linoleic acid | γ-linolenic acid | Docosahexaenoic acid |
| 0 | 0.234 | 0.172 | 0.026 | 1.699 | 0.998 |
| 0.005 | 0.235 | 0.251 | 0.039 | 2.923 | 8.108 |
| 0.01 | 0.366 | 0.302 | 0.086 | 3.237 | 11.896 |
| 0.05 | 0.751 | 0.934 | 0.16 | 9.015 | 26.72 |
| 0.1 | 1.217 | 2.06 | 0.279 | 19.213 | 44.469 |
| LSD | 0.158 | 0.123 | 0.033 | 1.112 | 1.025 |

## 4 Conclusions

Recently, there has been considerable attention among the scientific community with regard to microalgae to produce third-generation liquid biofuels. The present study examined the potential of *A. platensis* to produce biodiesel. Therefore, five concentrations of $FeSO_4$ (0, 0.005, 0.01 (control), 0.05, and 0.1 g/L) were utilized. The fatty acid profile of the *A. platensis* revealed that palmitic, oleic acid, linoleic acid, γ-linolenic acid, and docosahexaenoic acid were most prevalent. It was also found that the maximum-minimum yields of fatty acid individuals were achieved with 0.1–0 g/L $FeSO_4$, respectively. The study indicated that when the concentrations of $FeSO_4$ were between 0.05 and 0.1 g/L, palmitic acid, oleic acid, linoleic acid, γ-linolenic acid, and docosahexaenoic acid were increased, and at low concentrations, these fatty acids decreased. The maximum induction rate of the total fatty acids was obtained in the modified media with 0.1 g/L $FeSO_4$. The results revealed that the highest growth rate and the shortest doubling time were recorded with the $FeSO_4$ concentration of 0.1 g/L. The best concentration used to increase the yields of chlorophyll-a, phycocyanin, allophycocyanin, and phycobiliproteins is 0.1 g/L $FeSO_4$, and carotenoids is 0.1 g/L $FeSO_4$. Finally, increasing the $FeSO_4$ concentration to 0.1 g/L has led to the increase in fatty acid individuals, hence, the potential enhancement of the biodiesel production from the point of view of quality and quantity.

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## Declarations

**Conflict of interest** The authors declare no competing interests.



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