Effects of iron on the biomass, chlorophyll a, total lipids, and fatty acids of Chaetoceros lorenzianus

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Abstract

Polyunsaturated fatty acid (PUFA) and eicosapentaenoic acid (EPA) are essential for the health of aquatic organisms and human beings. In order to optimize the culture conditions of Chaetoceros lorenzianus, effects of different iron sources (FeC$_6$H$_5$O$_7$, FeCl$_3$, FeSO$_4$) and different iron concentrations (0.10 mg L$^{-1}$, 0.25 mg L$^{-1}$, 0.50 mg L$^{-1}$, 0.75 mg L$^{-1}$, 1.00 mg L$^{-1}$, 1.25mg L$^{-1}$) on C. lorenzianus were studied. The results showed that different iron sources and different iron concentrations had significant effects on the biomass, chlorophyll A, total lipid and fatty acids of C. lorenzianus ($P<0.05$). Compared with FeSO$_4$ and FeC$_6$H$_5$O$_7$, FeCl$_3$ had better effects on the growth, chlorophyll a, total lipids, and n-3 PUFA (EPA and DHA) content of C. lorenzianus. The optimum concentration of FeCl$_3$ for the growth and synthesis of chlorophyll a in C. lorenzianus was 0.75–1 mg L$^{-1}$, and the optimum concentration for lipid accumulation was 0.25 mg L$^{-1}$. The secondary culture method can be used in large-scale culture, that is, the initial culture is performed under the optimal iron concentration (0.75mg L$^{-1}$ FeCl$_3$) for obtaining higher biomass, and then, the subsequent culture is performed under a low iron concentration (0.25 mg L$^{-1}$ FeCl$_3$) to accumulate higher total lipids.

Introduction

As primary producers in the food chain, diatoms play an important role in the energy flow and material cycling of aquatic ecosystems (Chisti 2007; Clemens and Pressman 2020). Diatoms have been widely used as a health food in recent years due to their high nutritional value, such as high contents of high-value natural active substances including carotenoids, astaxanthin, phycoerythrin, and polyunsaturated fatty acids (PUFAs), including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as their high growth rate, reproduction, and yield (Jiang et al. 2016; Malcata 2000). As producers of PUFAs, diatoms provide direct or indirect natural feed for fish, shrimp, crabs, and shellfish larvae or adults due to their rich and balanced nutrients and various bioactive substances, and they also provide abundant nutrients for the growth and development of aquatic animals (Kakhki et al. 2020; Berlinskysupa 2011; You et al. 2019). Therefore, diatoms have gained considerable research interest and have been widely used in many fields such as food, medicine, aquaculture, and bioenergy.

Chaetoceros lorenzianus (Bacillariophyta, Centricae, Biddulphiales, Chaetoceraceae, Chaetoceros) is a marine eukaryotic unicellular algae. Chaetoceros lorenzianus is box-type, with cilia at both ends of the shell. The length of the cilia is longer than the cell body, and the cell size is 8–20 µm (Zhai et al. 2017; Chen et al. 2019). C. lorenzianus is a newly isolated microalga. Preliminary studies have found that C. lorenzianus is a single-celled diatom with a high growth rate and rapid reproduction, is easy to culture, and is rich in a variety of unsaturated fatty acids. Its growth rate is faster than that of Chaetoceros mulleri, Phaeodactylum tricornutum, and Chlorella vulgaris, and the content of PUFAs is higher than that of Phaeopsis triangularis and C. vulgaris. C. lorenzianus can be used as high-quality feed for bivalves, shrimp, and crabs, such as larvae feed for Scapharca subcrenata, Sinonovacula constricta, and Litopenaeus vannamei in the seedling breeding process.
*C. lorenzianus* is a newly discovered high-quality microalgae, which means that the culture medium formula needed in the culture process needs to be further optimized to improve the growth rate and the content of nutrients in the cell, such as the content of unsaturated fatty acids (Chen et al. 2019; Yang et al. 2017). Iron plays an important role in the growth of phytoplankton and is one of the main factors limiting the growth of phytoplankton. The growth rate, chlorophyll biosynthesis, photosynthesis, and effective utilization of carbon, nitrogen, and phosphorus in phytoplankton are significantly affected by iron (Liao et al. 2009; Wang et al. 2018). Liang et al. found that *Nitzschia closterium* had the highest photosynthesis rate, the fastest growth, and the highest chlorophyll content when the Fe$^{3+}$ concentration was $1 \times 10^{-4}$ mol L$^{-1}$ (Liang et al. 2016). Marchetti found that a higher Fe$^{3+}$ concentration was helpful for fat accumulation in diatoms (Marchetti 2012). Jiang et al. found that the growth rate of *N. triangularis* and the contents of total lipids, PUFAs, and EPA were the highest at 0.5 mg L$^{-1}$ iron (Jiang et al. 2016). Herein, we studied the effects of iron on the growth, chlorophyll *a*, total lipids, and fatty acid composition in *C. lorenzianus*. We screened for the optimal source and concentration of iron in order to provide a foundation for the better exploitation and utilization of *C. lorenzianus*.

**Materials And Methods**

**Algal strain and laboratory scale-up**

The *C. lorenzianus* strain used in the experiment was isolated, purified, and preserved in the bait biological culture chamber of Ningbo University. All the test chemicals were analytically pure and of analytical grade. The seawater used was natural seawater from Xiangshan Port (the sand and dark precipitate in the water were filtered by absorbent cotton, boiled, and cooled). Each treatment was performed in an intelligent illumination incubator (GXZ-260C, Ningbo Southeast Instrument Company, China). The incubation conditions were as follows: temperature of (25 ± 1)°C, salinity of 25 psu, light intensity of 72 µmol photons m$^{-2}$ s$^{-1}$, pH of 8.10, light-dark cycle (L: D) = 12 h: 12 h, and closed-culture with shaking twice a day.

**Condition optimization**

Two experiments were conducted to obtain the optimal iron source and iron concentration for biomass, total lipid, and fatty acid production in *C. lorenzianus*. There was one variable in each trial. In experiment 1, *C. lorenzianus* was treated with iron starvation for 48 h and then supplemented with iron-deficient seawater. Culture medium 3 was used as the base culture medium (Table 1), and FeC$_6$H$_5$O$_7$, FeCl$_3$, and FeSO$_4$ were set as iron sources with effective iron concentrations of 0.5 mg L$^{-1}$. In experiment 2, after *C. lorenzianus* was treated with iron starvation for 48 h, the FeCl$_3$ selected in experiment 1 was used as the sole iron source, and the iron concentration gradient was set to 0.10, 0.25, 0.50, 0.75, 1.00, and 1.25 mg L$^{-1}$. All experimental groups were set up in parallel groups. After 7 d of culture, cells were collected for analysis of the growth, total lipids, and fatty acids. As the cells were in an exponential phase and grew fast, a 7-day culture period was chosen.
Table 1
Composition of 3# culture medium

<table>
<thead>
<tr>
<th>Nutrient composition</th>
<th>Mass concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO₃</td>
<td>100</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>10.0</td>
</tr>
<tr>
<td>MnSO₄</td>
<td>0.25</td>
</tr>
<tr>
<td>EDTANa₂</td>
<td>10.0</td>
</tr>
<tr>
<td>V₈₁</td>
<td>6×10⁻³</td>
</tr>
<tr>
<td>V₈₁₂</td>
<td>5×10⁻⁵</td>
</tr>
</tbody>
</table>

**Growth determination**

The cell number was counted with an improved Neubauer haemocytometer (XB-K-25; Qiu Jing, Shanghai, China). The growth rate ($\mu$) is determined by the following formula, $\mu = (\ln N_t - \ln N_0)/t$, $N_t$ is the cell number after $t$ days, $N_0$ is the initial cell number, $t$ is the days of cultivation.

**Determination of chlorophyll a**

Chlorophyll a was extracted from algal fluid by Hot Ethanol Method (Liang 2016). The chlorophyll extract is measured by UV-visible spectrophotometer at the wavelength of 665 nm and 750 nm; after acidification with 1 mol/L hydrochloric acid (50µL), the solution were measured at wavelengths of 665 nm and 750 nm. Calculation formula of chlorophyll a content: $\text{Chl-a} = 27.9 \times \left( E_{665} - E_{750} \right) - \left( A_{665} - A_{750} \right) \times \frac{V_e}{V_0}$, where Chl-a is the content of chlorophyll-a, $E_{665}$ and $E_{750}$ are the absorbance measured at the wavelength of 665 nm and 750 nm before hydrochloric acid acidification, $A_{665}$ and $A_{750}$ are the absorbance measured at the wavelength of 665 nm and 750 nm after hydrochloric acid acidification, $V_e$ is the constant volume of the extract (mL), $V_0$ is the volume of algal liquid (L).

**Total lipid and fatty acid analysis**

The content of total lipid was extracted from the algal powder by Bligh-Dyer method (Bligh et al. 1959). The fatty acid composition analysis adopts KOH methanol water method (Reiser et al. 1997). The gas chromatography-triple quadrupole mass spectrometry is used for analysis, results were carried out by comparing the relative retention time, mass spectrometry database (NIST14. L) and corresponding published mass spectrometry data with known standards. Calculation of relative percentage content of each component by area normalization method (Chen 2012; Ma et al. 2013).

**Data processing and Statistical analyses**

The data obtained are represented by “Mean ± SD”, and single factor analysis of variance (ANOVA) is performed by SPSS 22.0 statistical software. The differences between different test groups in growth rate
(K-Value), chlorophyll-a (Chl-a), total fat and relative content of fatty acids were examined by Duncan multiple comparison method (α = 0.05).

**Result**

**Effects of different iron sources on C. lorenzianus**

As shown in Fig. 1, different iron sources had significant effects on the growth rate (K-Value) of *C. lorenzianus* (*P* < 0.05). Under an effective iron concentration of 0.5 mg•L⁻¹, the growth rate of *C. lorenzianus* was the highest (0.47 ± 0.03 d⁻¹) when FeCl₃ was used as the sole source of iron, which was higher than that in the other groups (*P* < 0.05). The group with FeSO₄ was ranked second (0.35 ± 0.02 d⁻¹).

As shown in Fig. 2, different iron sources had significant effects on the content of chlorophyll a in *C. lorenzianus* (*P* < 0.05). Under an effective iron concentration of 0.5 mg•L⁻¹, the content of chlorophyll a in *C. lorenzianus* was the highest (510.57 ± 48.65 µg L⁻¹) when FeCl₃ was used as the sole source of iron, which was higher than that in any other group (*P* < 0.05). The group with FeSO₄ was ranked second (315.27 ± 34.85 µg L⁻¹), and the group with FeC₆H₅O₇ was ranked the lowest (156.02 ± 4.83 µg L⁻¹).

The effects of different iron sources on the total lipid content of *C. lorenzianus* are shown in Fig. 3. Different iron sources had significant effects on the content of total lipids in *C. lorenzianus* (*P* < 0.05). Under an effective iron concentration of 0.5 mg L⁻¹, the total lipid content of *C. lorenzianus* was the highest (11.82 ± 0.09%) when FeCl₃ was used as the sole source of iron. The group with FeSO₄ was ranked second and was not significantly different from the FeCl₃ group (*P* > 0.05). The FeC₆H₅O₇ group had the lowest lipid content (10.94 ± 0.04%).
Table 2
Effects of iron sources on fatty acid composition of *Chaetoceros lorenzianus*, expressed as % total fatty acids.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Iron source</th>
<th>FeC$_6$H$_5$O$_7$</th>
<th>FeCl$_3$</th>
<th>FeSO$_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td></td>
<td>14.09 ± 0.31$^a$</td>
<td>13.03 ± 0.79$^b$</td>
<td>13.61 ± 0.09$^{ab}$</td>
</tr>
<tr>
<td>C16:0</td>
<td></td>
<td>11.74 ± 0.28$^b$</td>
<td>10.84 ± 0.35$^c$</td>
<td>12.70 ± 0.61$^a$</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td></td>
<td>23.30 ± 0.37$^a$</td>
<td>21.15 ± 0.59$^b$</td>
<td>22.92 ± 0.56$^a$</td>
</tr>
<tr>
<td>C16:2n-6</td>
<td></td>
<td>4.64 ± 1.59$^a$</td>
<td>6.64 ± 0.71$^a$</td>
<td>4.92 ± 1.86$^a$</td>
</tr>
<tr>
<td>C16:3n-4</td>
<td></td>
<td>11.32 ± 0.48$^{ab}$</td>
<td>12.06 ± 0.72$^a$</td>
<td>10.63 ± 0.23$^b$</td>
</tr>
<tr>
<td>C18:0</td>
<td></td>
<td>5.43 ± 0.98$^a$</td>
<td>5.38 ± 0.32$^a$</td>
<td>6.09 ± 0.79$^a$</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td></td>
<td>4.76 ± 0.15$^a$</td>
<td>4.43 ± 0.46$^a$</td>
<td>4.84 ± 0.19$^a$</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td></td>
<td>2.57 ± 0.22$^a$</td>
<td>2.56 ± 0.41$^a$</td>
<td>2.56 ± 0.15$^a$</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td></td>
<td>2.63 ± 0.49$^a$</td>
<td>2.70 ± 0.51$^a$</td>
<td>1.87 ± 0.47$^a$</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td></td>
<td>17.99 ± 0.72$^b$</td>
<td>19.46 ± 0.27$^a$</td>
<td>18.32 ± 0.56$^b$</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td></td>
<td>1.53 ± 0.10$^b$</td>
<td>1.74 ± 0.02$^a$</td>
<td>1.54 ± 0.12$^b$</td>
</tr>
<tr>
<td>SFA</td>
<td></td>
<td>31.26 ± 1.41$^{ab}$</td>
<td>29.25 ± 0.75$^b$</td>
<td>32.41 ± 1.38$^a$</td>
</tr>
<tr>
<td>MUFA</td>
<td></td>
<td>28.06 ± 0.24$^a$</td>
<td>25.59 ± 0.96$^b$</td>
<td>27.76 ± 0.57$^a$</td>
</tr>
<tr>
<td>PUFA</td>
<td></td>
<td>40.68 ± 1.16$^b$</td>
<td>45.17 ± 1.67$^a$</td>
<td>39.84 ± 1.77$^b$</td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td></td>
<td>19.52 ± 0.62$^b$</td>
<td>21.20 ± 0.25$^a$</td>
<td>19.86 ± 0.67$^b$</td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td></td>
<td>9.84 ± 2.25$^a$</td>
<td>11.91 ± 0.73$^a$</td>
<td>9.35 ± 2.33$^a$</td>
</tr>
</tbody>
</table>

SFA: sum of saturated fatty acid, MUFA: sum of monounsaturated fatty acid, and PUFA: sum of polyunsaturated fatty acids. Different superscript letters indicate significant differences between different nitrogen concentrations (P < 0.05, by one-way ANOVA and LSD test). Data are the means ± SD (n = 3).

The fatty acid composition of *C. lorenzianus* under different iron sources is shown in Table 2. Under an effective iron concentration of 0.5 mg L$^{-1}$, a total of 11 fatty acids were identified and measured in the three iron source media. There were three types of saturated fatty acids (SFAs): 14:0, 16:0, and 18:0; two types of monounsaturated fatty acids (MUFAs): 16:n-7, 18:n-9; and six types of PUFAs: 16:2n-6, 16:3n-4, 18:2n-6, 20:4n-6, 20:5n-3 (EPA), and 22:6n-3 (DHA). The leading fatty acids in cells were 14:0 (13.03–
14.09%), 16:0 (10.84–12.7%), 16:n-7 (21.15–23.3%), 16:3n-4 (10.63–11.32%), and EPA (17.99–19.46%). The results of the one-way analysis of variance (Table 2) showed that the relative contents of most fatty acids in the cells were significantly affected by iron source ($P < 0.05$). Under different iron sources, the contents of SFAs in *C. lorenzianus* ranged from 29.25–32.41%, and the FeCl$_3$ group was significantly lower than the FeSO$_4$ group. The contents of MUFAs ranged from 25.59–28.06% and were significantly lower in the FeCl$_3$ group than in the other two groups ($P < 0.05$). The contents of PUFAs in *C. lorenzianus* ranged from 39.84–45.17%, which were mainly contributed by 16:3n-4 and EPA, and were significantly higher in the FeCl$_3$ group than in the other two groups. The n-3 PUFAs synthesized by *C. lorenzianus* included EPA and DHA, the contents of which ranged from 19.52–21.2%. EPA and DHA synthesis was significantly higher in the FeCl$_3$ group than in the FeC$_6$H$_5$O$_7$ and FeSO$_4$ groups, and the maximum n-3 PUFA synthesis was detected when FeCl$_3$ was used as the sole source of iron. Under different iron sources, there was no significant difference in the n-6 PUFA contents, which ranged from 9.35–11.91% and included 16:2n-6, 18:2n-6, and 20:4n-6 (ARA).

**Effects of different concentrations of iron on** *C. lorenzianus*

Different iron sources had significant effects on the growth rate (K-Value) of *C. lorenzianus* ($P < 0.05$) (Fig. 4). In the range of 0.10–1.25 mg L$^{-1}$, the growth rate of *C. lorenzianus* first increased and then remained unchanged with the increase in iron concentration. The maximum growth rate (0.49 ± 0.02) was reached when the iron concentration was 1.00 mg L$^{-1}$, but no significant difference in growth was detected among 0.50 mg L$^{-1}$, 0.75 mg L$^{-1}$, 1.25 mg L$^{-1}$, and 1.00 mg L$^{-1}$ ($P > 0.05$), while a significant difference was shown among 0.25 mg L$^{-1}$, 0.10 mg L$^{-1}$, and 1.00 mg L$^{-1}$ ($P < 0.05$). The growth rate reached its minimum in the 0.10 mg L$^{-1}$ group, which was 20.45% lower than that in the group (1.00 mg L$^{-1}$) that had the highest growth rate.

As shown in Fig. 5, different iron sources had significant effects on the content of chlorophyll a in *C. lorenzianus* ($P < 0.05$). In the range of 0.10–1.25 mg L$^{-1}$, the content of chlorophyll a in *C. lorenzianus* first increased and then remained unchanged with the increase in iron concentration (412.92–552.94 mg g$^{-1}$). The maximum content of chlorophyll a was reached when the iron concentration was 1.00 mg L$^{-1}$, which did not differ significantly from 0.75 mg L$^{-1}$ ($P > 0.05$) but did differ significantly from 1.25 mg L$^{-1}$, 0.50 mg L$^{-1}$, 0.25 mg L$^{-1}$ and 0.10 mg L$^{-1}$ ($P < 0.05$).

The total lipid content of *C. lorenzianus* was greatly influenced by different iron sources ($P < 0.05$) (Fig. 6). In the range of 0.10–1.25 mg L$^{-1}$, the content of chlorophyll a in *C. lorenzianus* first decreased and then remained unchanged with the increase in iron concentration. The total lipid content was the highest when the iron concentration was 0.25 mg L$^{-1}$, which was significantly higher than 0.50 mg L$^{-1}$, 0.75 mg L$^{-1}$, 1.00 mg L$^{-1}$ and 1.25 mg L$^{-1}$ ($P < 0.05$). The 1.00 mg L$^{-1}$ and 1.25 mg L$^{-1}$ groups had the lowest lipid contents, with a minimum value that was 20.17% lower than that of the group (0.25 mg L$^{-1}$) with the highest lipid content.
### Table 3
Effects of Iron concentration on fatty acid composition of *Chaetoceros lorenzianus*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Iron concentration (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>C14:0</td>
<td>21.21 ± 1.33&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:0</td>
<td>20.35 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:1n-7</td>
<td>29.6 ± 4.56&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:2n-6</td>
<td>1.94 ± 0.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C16:3n-4</td>
<td>7.35 ± 1.81&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.78 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>2.79 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>C18:1n-12</td>
<td>—</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>1.88 ± 0.39&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td>2.03 ± 0.28&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>7.65 ± 1.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C22:6n-3</td>
<td>0.4 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SFA</td>
<td>46.35 ± 0.62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MUFA</td>
<td>32.4 ± 3.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PUFA</td>
<td>21.25 ± 2.57&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td>8.05 ± 1.22&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fatty acid</td>
<td>Iron concentration (mg/L)</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td>0.10</td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td>5.85 ± 0.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

SFA: sum of saturated fatty acid, MUFA: sum of monounsaturated fatty acid, and PUFA: sum of polyunsaturated fatty acids. Different superscript letters indicate significant differences between different nitrogen concentrations (P < 0.05, by one-way ANOVA and LSD test). Data are the means ± SD (n = 3). “-” means not detected.

The effect of iron concentration on the fatty acid composition of *C. lorenzianus* ranged from 0.10 to 1.25 mg L<sup>-1</sup>, as shown in Table 2. A total of 11 fatty acids were detected when the iron concentration was 0.10–0.75 mg L<sup>-1</sup>, whereas a total of 12 fatty acids were detected when the iron concentration increased to 1.00–1.25 mg L<sup>-1</sup>, which was characterized by increased MUFAs (18:n-12) compared with the above 11 fatty acids. The main fatty acids in *C. lorenzianus* were 14:0 (19.36–22.54%), 16:0 (15.82–20.35%), 16:n-7 (28.48–33.03%), 16:3n-4 (7.35–10.45%), and EPA (7.24–10.46%). The one-way analysis of variance (Table 3) showed that iron concentration had a significant effect on the relative contents of most fatty acids in *C. lorenzianus* (P < 0.05). Under different iron concentrations, the content of SFAs ranged from 39.85–46.35% and was the highest in the 0.10 mg L<sup>-1</sup> group (46.35 ± 0.62%), which was significantly higher than that in the other groups. The content of SFAs showed no significant difference (P > 0.05) among these groups (31.26–34.84%). The content of PUFAs ranged from 21.25–28.16%, and the 1.00 mg L<sup>-1</sup> group had the highest PUFA content (28.16 ± 1.12%), which was significantly higher than that of the 0.10 mg L<sup>-1</sup>, 0.25 mg L<sup>-1</sup>, and 0.50 mg L<sup>-1</sup> groups. When the iron concentration increased to 1.00–1.25 mg L<sup>-1</sup>, the relative content of n-3 PUFAs increased to 10.45–11.05%. This was mainly contributed by EPA (9.89–10.46%), which was significantly higher than other groups (7.62–9.08%).

**Discussion**

Iron is one of the most important trace mineral elements in the body. It is an essential element necessary for maintaining normal physiological and biochemical functions in algae and plays an important role in the utilization of nitrogen, the synthesis of chlorophyll, and the metabolism of algal cells. It is one of the main factors limiting the growth and reproduction of algal cells and plays an important role in marine systems (William et al. 1997). The results of this study showed that different iron sources play an important role in the growth, chlorophyll *a* synthesis, total lipid accumulation, and fatty acid synthesis of *C. lorenzianus*. In terms of the growth rate of *C. lorenzianus*, FeCl<sub>3</sub> with an iron valence of + 3 was significantly better than FeC<sub>6</sub>H<sub>5</sub>O<sub>7</sub> and FeSO<sub>4</sub>, which have iron valences of + 2. Xia et al. (2010) found that the growth rate of *Isochrysis galbana* was significantly better than FeSO<sub>4</sub> (+ 2) in Fe(NO) and FeCl<sub>3</sub>, which had an iron valence of + 3. During microalgae culture, the added iron source determines the morphology of Fe in the water to a certain extent. There are many forms of Fe in seawater, such as Fe<sup>3+</sup>,
Fe$^{2+}$, and colloid hydration oxide. Microalgae can utilize various of iron source, and the most important method of utilization is to convert other forms of Fe into Fe$^{2+}$ and Fe$^{3+}$ and then absorb and utilize them. The conversion efficiency of different forms of Fe will affect the utilization efficiency of Fe by microalgae (Shaked et al. 2005; Morel et al. 2008). FeC$_6$H$_5$O$_7$ is in the form of a soluble colloidal iron complex in water, which is stable and easily absorbed and utilized by algae. However, during the experiment, it was found that FeC$_6$H$_5$O$_7$ easily formed flocculent material when dissolved, which may lead to slow growth and reproduction in *C. lorenzianus*. Fe$^{2+}$ in FeSO$_4$ is dissolved iron, which in theory can be directly absorbed and utilized by algae (Xing et al. 2006). However, as Fe$^{2+}$ is easily oxidized to colloidal iron or granular iron, which causes its available Fe content to decrease, the growth of *C. lorenzianus* is affected.

In the photosynthesis of green plants such as algae, iron is the activator of one or more enzymes in the process of chlorophyll a synthesis. When iron is deficient, the chloroplast structure is destroyed, resulting in the formation of chlorophyll a. Chlorophyll a plays a role in capturing photoelectrons and is an important catalyst for photoreaction. Its content can directly reflect the accumulation of substances in algal cells (Katz et al. 1978; Yasushi et al. 1997). The results of this experiment showed that in terms of the effects of different Fe forms on chlorophyll *a* synthesis in *C. lorenzianus*, FeCl$_3$ was significantly superior to FeC$_6$H$_5$O$_7$ and FeSO$_4$, which was more favorable to chlorophyll *a* synthesis in *C. lorenzianus* and thus more conducive to its growth and reproduction. Liang et al. (2016) found that *Skeletonema menzeli* had a significantly better growth rate in culture medium supplemented with FeCl$_3$ than in culture medium supplemented with other iron sources (FeC$_6$H$_5$O$_7$ and FeSO$_4$). Li et al. (2009) also found that Fe$^{3+}$ was more conducive to the synthesis of chlorophyll in *Haematococcus pluvialis*.

Algal growth requires an appropriate iron concentration, and the growth of algae will be inhibited when the iron concentration is too high or too low (Wang 2006). An insufficient Fe content in the ocean will cause a decrease in phytoplankton biomass (Hutchins et al. 2002). FeCl$_3$ supplemented with 11.64 µmol L$^{-1}$ was the most suitable medium for *Phaeodactylum tricornutum*. A concentration below or above this level was not conducive to algal growth (Wang et al. 2011). *Amphora* sp. could grow well in an iron concentration of 0–1.5 mg L$^{-1}$, with the optimum iron concentration being 0.5 mg L$^{-1}$ (Zhou et al. 2008). This study showed that the growth of *C. lorenzianus* and the synthesis of chlorophyll *a* were inhibited when the iron concentration was too high or too low. In the range of 0.10–1.25 mg L$^{-1}$, *C. lorenzianus* grew normally, whereas a low concentration of iron ($\leq$ 0.25 mg L$^{-1}$) was not conducive to growth. When the iron concentration is higher than 1.00 mg L$^{-1}$, the growth rate will decrease. The optimal iron concentration is 0.5–1.00 mg L$^{-1}$.

Microalgae are considered a potential biodiesel raw material, but high breeding costs present a bottleneck for the industrialization of microalgae biodiesel. Moreover, microalgae contain rich PUFAs. Improving the oil and PUFA production of microalgae through biological pathways is key to resolving this problem (Lu et al. 2018; Senthil et al. 2010). The formation of the double bond of fatty acids is catalyzed by fatty acids desaturase (FADs), which has three conserved histidine clusters, which combine with Fe ions to form the active center of the enzyme (Wei and Zhang 2000). Therefore, the content of iron in the medium
may also affect the activity of FADs in microalgae, impact the physiological and biochemical processes of microalgae, and then influence the composition of microalgae lipid synthesis. The results of this study showed that different iron sources could significantly affect the synthesis of total lipids by *C. lorenzianus*. The effect of FeCl$_3$ on the total iron source was significantly superior to that of FeSO$_4$ and FeC$_6$H$_5$O$_7$. In the culture medium, FeCl$_3$ or FeSO$_4$ could improve the ability of *C. lorenzianus* to synthesize total lipids and increased the content of total lipids in the cells. The addition of FeCl$_3$ or FeSO$_4$ in the culture medium improved the ability of *C. lorenzianus* to synthesize total lipids and increased the total lipid content in *C. lorenzianus*. FeCl$_3$ improved the synthesis of PUFAs in *C. lorenzianus*. Compared with FeSO$_4$ and FeC$_6$H$_5$O$_7$, the content of PUFAs in *C. lorenzianus* increased by 11.04% and 13.38%; the DHA content increased by 13.73% and 12.98%; and the content of EPA increased by 8.17% and 6.22%, respectively. Therefore, FeCl$_3$ as the iron source in the culture medium is not only conducive to improving the content of total lipids in *C. lorenzianus* but is also conducive to improving the content of PUFA in cells, especially EPA and DHA, as well as enhancing the nutritional value. Xia et al. (2010) studied the effects of different iron sources on the growth and lipid accumulation of *Phaeodactylum tricornutum*, *Chlorella*, and *Isochrysis galbana* and found that Fe$^{3+}$ was more conducive to lipid accumulation in these species. In *Chlorella vulgaris*, Liu et al. (2008) also found that Fe$^{3+}$ had a promoting effect on growth and lipid accumulation. Regarding the effect of Fe$^{3+}$ in promoting the growth and lipid accumulation of algae, it may be that Fe$^{3+}$ promotes the expression of genes related to growth and reproduction and increases the cell concentration (Chen et al. 2009). By contrast, Fe$^{3+}$ can influence the expression of some or several key genes in the lipid synthesis of microalgae and increase the lipid content (Xia et al. 2010; Behrenfeld et al. 2006). At present, the mechanism by which Fe$^{3+}$ influences the growth and lipid accumulation of microalgae is still unclear and deserves further study.

We found that iron concentration had a significant effect on total lipid synthesis in *C. lorenzianus* (Jiang et al. 2016; Mekhalfi et al. 2014). The content of total lipids in *P. tricornutum* was the highest when the iron concentration was about 0.5 mg L$^{-1}$, and the total lipid content decreased when the iron concentration was higher or lower than this concentration (Jiang et al. 2016). The addition of FeC$_6$H$_5$O$_7$ (11.64 µmol L$^{-1}$) to the medium of *P. tricornutum* was most suitable. A concentration below this level was not conducive to algal growth, while a concentration above this level was not conducive to lipid accumulation (Wang et al. 2011). The optimal iron concentration for the growth of *C. lorenzianus* was found to be 0.5–1.00 mg L$^{-1}$, and the optimal iron concentration for lipid production was 0.25 mg L$^{-1}$. Before reaching the optimum iron concentration, the total lipid content of *C. lorenzianus* gradually increased with increased iron concentration. Beyond this optimum concentration, the synthesis of total lipids was inhibited in *C. lorenzianus*. Therefore, the secondary culture method can be used in large-scale culture, that is, the first culture is performed under the optimal iron concentration to obtain a higher biomass, following which culture is performed under a low iron concentration to accumulate higher total lipids. However, this remains to be further studied. Behrenfeld et al. (2006) found that Fe played a key role in the growth and oil accumulation of microalgae in water with high nitrogen concentrations or in nutrient-deficient waters. The result showed that phosphorus concentration also had a significant effect
on the fatty acid synthesis of *C. lorenzianus*. The fatty acids of *C. lorenzianus* increased with increased iron concentration, and the contents of PUFA and n-3 PUFA showed an increasing trend. At a phosphorus concentration of 1 mg L$^{-1}$, the PUFA content of *C. lorenzianus* was higher than that of the other groups, and the n-3 PUFA (EPA and DHA) content was significantly higher than that of the other groups. Increased EPA and DHA is beneficial to increasing the nutritional added value of *C. lorenzianus* (Bhattacharjya et al. 2020).

In conclusion, different sources and concentrations of iron play a key role in growth and chlorophyll *a* and lipid accumulation in *C. lorenzianus*. Compared with FeSO$_4$ and FeC$_6$H$_5$O$_7$, FeCl$_3$ had better effects on the growth, chlorophyll *a*, total lipids, and n-3 PUFA (EPA and DHA) content of *C. lorenzianus*. The optimum concentration of FeCl$_3$ for the growth of *C. lorenzianus* and the synthesis of chlorophyll *a* in *C. lorenzianus* was 0.75–1 mg L$^{-1}$, and the optimum concentration for lipid accumulation was 0.25 mg L$^{-1}$. The secondary culture method can be used in large-scale culture, that is, the initial culture is performed under the optimal iron concentration (0.75 mg L$^{-1}$ FeCl$_3$) for obtaining higher biomass, and then, the subsequent culture is performed under a low iron concentration (0.25 mg L$^{-1}$ FeCl$_3$) to accumulate higher total lipids.

**Declarations**

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**Authors’ contributions**

Zuhao Zhang: performed the experiment and wrote the paper.

Ruibing Peng: conceived and designed the experiment.

Xinyi Xia: contributed significantly the experiment.
Pingping Liu: contributed significantly the experiment.

Si Chen: contributed significantly the experiment.

Ran Xia: contributed significantly the experiment.

Xiamin Jiang: contributed significantly to analysis and manuscript preparation.

Maowang Jiang: contributed to the conception of the study.

Jianyuan Lin: revised the paper.

**Data availability**

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

**Competing interests** The authors declare no competing interests.

**References**


Figure 1

Effects of different iron sources on the growth rate of *Chaetoceros lorenzianus*. Different superscript letters denote significant differences between groups at the same time (P < 0.05). Data are the means ± SD (n = 3). The same as below.
Figure 2

Effects of different iron sources on the content of chlorophyll \( a \) of *Chaetoceros lorenzianus*.
Figure 3

Effects of different iron sources on the content of total lipid of *Chaetoceros lorenzianus.*
Figure 4

Effects of iron concentrations on the growth rate of *Chaetoceros lorenzianus.*
Figure 5

Effects of iron concentrations on the content of chlorophyll a of Chaetoceros lorenzianus.
Figure 6

Effects of iron concentrations on the content of total lipid of *Chaetoceros lorenzianus*. 