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Conditions optimising on the yield of biomass, total lipid, and valuable fatty acids in two strains of *Skeletonema menzelii*

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ABSTRACT

Polyunsaturated fatty acids (PUFA) and eicosapentaenoic acid (EPA) are essential for the health of aquaculture organisms and human beings. A total of 9 species of diatoms were screened and two strains of *Skeletonema menzelii* were selected for further study due to their high growth rates, PUFA and EPA contents. The culture conditions for the yield of biomass, total lipid, EPA and PUFA were optimised. The quickest growth rates (0.28–0.41 d^{-1}) were achieved with moderate supplement of nitrogen, phosphorus, iron and silicon. The accumulation of total lipid (17.85–22.70% of dry weight) benefitted from deprivation of nitrogen, phosphorus and silicon, but a moderate iron supplement. Highest PUFA (38.26–50.48% of total fatty acids) or EPA (14.26–18.39% of total fatty acids) contents were observed under high nitrogen and phosphorus supplement, moderate available iron but deprivation of silicon. These findings make tangible contributions to culture *S. menzelii* for commercial production of PUFA or EPA.

1. Introduction

The polyunsaturated fatty acids (PUFA) are defined as a set of long chain fatty acids with two or more double bonds (Brett & Müller-Navarra, 1997), which are critical for animals to maintain optimal growth rates, development, reproductive rates, survival and high food conversion efficiencies (Bell, Henderson, & Sargent, 1986). For human beings, PUFA have been proven to decrease the risk of cardiovascular disease and inflammatory disease, and improve brain development and mental health (Ruxton, Reed, Simpson, & Millington, 2004). In addition, they also have remarkable beneficial effects on pregnancy and infant nutrition (Koletzko et al., 2014). However, the PUFA are exclusively synthesized by plants or algaes, and only very few animals have very limited capability to convert linolenic acid to EPA (Brett & Müller-Navarra, 1997). The notable beneficial effects of PUFA have resulted in soaring demand for EPA- or DHA-enriched products (Boelen, van Dijk, Damsté, Rijpstra, & Buma, 2013). Marine fish oil is the richest source for EPA and used for commercial production of EPA as only raw material until now (Wen & Chen, 2003). However, the enormous demand for PUFA can easily lead to overfishing, which is even worse for the overexploited vulnerable fish stocks (Boelen et al., 2013). In addition, a number of people dislike the taste of fatty fish and alternatives are needed to cater to different appetites (Sinclair, 2000). Therefore, there is growing interests in the research into obtaining EPA from microalgae, due to their high PUFA content, rapid growth rate, CO_2 removal, and environmental favourability (Boelen et al., 2013).

The total content of lipids in microalgae have shown dramatic variation from approximately 1–85% of the dry weight between and within algal classes (Chisti, 2007). In addition, profile of fatty acids varies with taxa and can be used as a biomarker, or signature, for biochemical and energetic pathways in food webs (Taipale et al., 2013). Therefore, the selection of optimal candidates is the first step for PUFA or EPA scale-up production. In light of previous references, diatom is considered as the best oil producer among microalgae (Chen, 2012). Apart from species difference, lipid content and fatty acid profiles are also strongly affected by environmental conditions. For instance, nitrogen (Piorreck, Baasch, &

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Pohl, 1984), phosphorus (Pistocchi et al., 2005), iron (Yeesang & Cheirsilp, 2011) and silicon (Harrison, Thompson, & Calderwood, 1990) have been reported to be important factors affecting lipid content and fatty acid composition in a large amount of algae species. Therefore, species screening and condition optimising are essential steps to produce PUFA commercially, which is worthy of more investigations.

Based on the philosophy mentioned above, a total of nine species of diatoms were purified from coastal waters of Zhejiang, P.R. China and four of them were tested for the growth rates, total lipid, PUFA and EPA contents under different culture conditions. Two strains of diatom *Skeletonema menzelii* were chosen for further studies, due to their high growth rate and EPA content. Finally, the optimal conditions for production of PUFA or EPA of *S. menzelii* were established. The present study facilitates culturing *S. menzelii* for the production of PUFA and EPA.

2. Materials and methods

2.1. Species screening

The microalgae were sampled from Yushan Island (122°13′–122°18′E, 28°52′–28°57′N) and Zhujiajian Island (29°51′–29°53′N, 122°25′–122°27′E), Zhejiang, China. A total of nine species were identified with scanning electron microscopy, including *S. menzelii* Guillard, Carpenter & Reimann 1974, *Halamphora coffeaeformis* (Agardh) Levkov 2009, *Tropidoneis maxima* (Gregory) Cleve 1894, *Thalassiosira binata* Fryxell, *Chaetoceros diadema* (Ehrenberg) Gran 1897, *Chaetoceros muelleri* Lemmermann 1898, *Ceratoneis closterium* Ehrenberg 1839, *Melosira* sp., and *Achnanthees* sp. From all of the diatoms obtained, five strains were selected due to their easiness of culture. Afterwards, they were grown at the temperature of 17–25 °C and salinity of 25 psu and two strains of *S. menzelii* were chosen as target species due to their advantages in growth rate, EPA and PUFA content.

2.2. Conditions optimising

To obtain optimal nitrogen, phosphorus, iron, and silicon levels for yields of biomass, total lipid and fatty acids in *S. menzelii*, four experiments were performed. Only one factor was varied in each experiment. After 72 h starvation culture, the two strains SM-1 and SM-2 of *S. menzelii* were grown under various nitrogen (KNO_3) concentrations (0, 5, 10, 15, 20, 25, 30 mg/l) with 5 mg/l phosphorus (KH_2PO_4), 2 mg/l iron ($FeSO_4{\cdot}7H_2O$) and 10 mg/l silicon (Na_2SiO_4) in experiment one; various phosphorus (KH_2PO_4) concentrations (0, 0.5, 1, 1.5, 2, 2.5, 3 mg/l) with 50 mg/l nitrogen (KNO_3), 2 mg/l iron ($FeSO_4{\cdot}7H_2O$), and 10 mg/l silicon (Na_2SiO_4) in experiment two; various iron ($FeSO_4{\cdot}7H_2O$) concentrations (0, 0.1, 0.25, 0.5 and 1 mg/l) with 50 mg/l nitrogen (KNO_3), 5 mg/l phosphorus (KH_2PO_4), 10 mg/l silicon (Na_2SiO_4) in experiment three; and various silicon (Na_2SiO_4) concentrations (0, 0.5, 2, 4, 8 mg/l) with 50 mg/l nitrogen (KNO_3), 5 mg/l phosphorus (KH_2PO_4), and 2 mg/l iron ($FeSO_4{\cdot}7H_2O$) in experiment four. All chemicals used were of an analytic grade and each treatment was performed in an intelligent illumination incubator (GXZ-260C, Ningbo Southeast Instrument Company, China) in triplicate. The initial density was 1.3×10^5 cells/ml. Cultures were maintained under the following conditions: temperature of 25 °C, salinity of 25 psu, 12:12 h light and dark cycle, and light intensity of 60 μmol photons m^{-2} s^{-1}. Cells were harvested after seven-day culture for the analysis of growth, total lipid and fatty acids. The seven-day culture period was used as cells were in the exponential phase and high growth rates can be achieved during this stage.

2.3. Growth determination

The cell number was counted with an improved Neubauer haemocytometer (XB-K-25; Qiu Jing, Shanghai, China). Growth rate (μ) was determined from the following equation, $\mu = (\ln N_t - \ln N_0)/t$, where N_t is the cell number after t days culture, N_0 is the initial cell number, and t is the culture time.

2.4. Total lipid and fatty acid analysis

Algae were collected (4000*g*, 30 min, 4 °C) at the end of culture and then freeze-dried. Total lipids were extracted from 100 mg of algal powder according to the Bligh–Dyer method (Bligh & Dyer, 1959). Results were expressed as percentage of dry weight (DW).

Fatty acid methyl ester (FAME) preparation was carried out according to the method of Joseph and Ackman (1992). A mixture of 100 mg of fat matter and 1.5 ml 0.5 N methanolic NaOH (2.0 g NaOH in 100 ml methanol) was heated at 100 °C for 5 min. After reaching room temperature, 2 ml boron trifluoride 12% in methanol was added to the solution, which was then heated at 100 °C for 30 min. After cooling for 5 min at room temperature, 1.0 ml isooctane and 5 ml saturated NaCl solution were added. The solution was centrifuged for 5 min at 2000*g* and the upper layer was transferred to a new tube. Another 1.0 ml of isooctane was added to the first solution and the procedure was repeated. The two layers of isooctane were dried down under a steady stream of nitrogen and the residue was re-dissolved in 1.0 ml of hexane.

Analysis of FAME was carried out with a Gas Chromatography–Mass Spectrometer (Shimadzu, QP2010, Kyoto, Japan), using an Agilent CP-SIL 88 fused silica capillary column (100 m * 0.25 mm ID * 0.2 μm film thickness). The conditions of gas chromatography analysis were as described below. Purified helium was used as a carrier gas with a head pressure of 210 kPa and a column flow of 1 ml/min. The temperature of the injector was set at 250 °C. FAME peaks were detected by flame ionisation detection at 255 °C. Then 1 μl of sample was injected at an initial column temperature of 80 °C, which was held for 1 min. The temperature was then raised at 4 °C/min to 100 °C, where it was held for 2 min, and then increased at 10 °C/min to 160 °C, where it was held for 61 min. Finally, the temperature was increased to 240 °C at a rate of 5 °C/min, where it was held for 21 min, thus giving a total runtime of 112 min. Peaks were identified using a commercial 52 FAME standard (GLC463, Nu-Chek Prep Inc., Elysian, MN, USA). Quantification of FA was based on peak areas of individual peaks identified, expressed as a percentage of the total peak areas for total FA.

2.5. Yields of PUFA and EPA

The daily yields of PUFA and EPA (mg/g DW d^{-1}) were estimated with the following equation:

$$Y = (e^{\mu} - 1) \times L \times F \times 1000$$

where e = the base of natural logarithm.

μ = growth rate (d^{-1}).
$e^{\mu} - 1$ = biomass change per g per day.
L = total lipid content (g/g DW).
F = PUFA or EPA content (% total fatty acids).

2.6. Data processing and Statistical analyses

SPSS (version 21) and Microsoft Office Excel (version 2013) were used for statistical analyses. A Kolmogorov–Smirnov test and a Levene's test were used to check normality and homogeneity of variance, respectively. One-way analysis of variance (ANOVA) and LSD multiple comparisons were conducted to test the effects

of various levels of nitrate, phosphonate, iron, or silicon on yields of biomass, total lipid and fatty acids. A confidence interval of 95% was set for all tests.

3. Results

3.1. Species selection

The growth rate, lipid, PUFA and EPA in 4 species were shown in Table S1. *S. menzelii* SM-1 ($0.69 \pm 0.03\ d^{-1}$) and SM-2 ($0.62 \pm 0.02\ d^{-1}$) located the first two places in the table in terms of growth rate ($P < 0.05$). *Thalassiosira binate* and *T. maxima* led others in total lipid, followed by *S. menzelii* SM-1 and SM-2. The PUFA of *S. menzelii* SM-1 (45.44 ± 1.15% total fatty acids) and SM-2 (47.08 ± 1.20% total fatty acids) was slightly lower than that in *Amphora coffeaeformis* (49.76 ± 1.23% total fatty acids), which had highest PUFA content ($P < 0.05$). The EPA contents of *S. menzelii* SM-2 and SM-1 were 27.74 ± 0.81% total fatty acids and 24.83 ± 0.75% total fatty acids, respectively, which were higher than that of other species ($P < 0.05$). Therefore, two strains of *S. menzelii* were selected for following study due to their quick growth rate, high production of EPA and relatively high PUFA and lipid contents.

3.2. Effects of nitrogen concentration on growth, total lipid and fatty acids in S. menzelii

The growth rate of both strains in *S. menzelii* rose with nitrate concentration (0–20 mg/l) ($P < 0.05$) (Fig. 1A). Above the concentration of 20 mg/l, the growth rate of SM-1 reached a plateau, and began to decrease, when the nitrate concentration exceeded 25 mg/l ($P < 0.05$). By contrast, the growth rate of SM-2 continued to increase until the concentration was 25 mg/l ($P < 0.05$) and a further increase in nitrate did not cause a decline in the growth rate ($P > 0.05$). Therefore, the optimal nitrate concentrations for growth of *S. menzelii* SM-1 and SM-2 were 20 mg/l and 25 mg/l, respectively. Nitrogen, one of the key nutrient components for algal growth, is generally thought to be limiting in marine systems (Elser et al., 2007). Therefore, growth of algae can be stimulated by adding extra nitrogen (Li, Gong, Rao, Liu, & Wang, 2005). The reason for decreased growth in *S. menzelii* SM-1 under highest nitrate level might be attributed to the toxicity of high nitrogen levels (Steffensen, 1976).

The cellular total lipid content was noticeably affected by various nitrogen concentrations ($P < 0.05$) (Fig. 2A). The total lipid content of both strains decreased from 20.32 ± 0.42% and 22.70 ± 0.39% to 11.20 ± 0.27% and 12.33 ± 0.36% of the dry weight, respectively ($P < 0.05$), as the potassium nitrate increased from 0 to 30 mg/l. Therefore, the maximum lipid content was obtained without extra nitrogen supplement ($P < 0.05$). Nitrogen concentration in the culture medium can regulate the degree of cellular lipid accumulation, and nitrogen deficiency has been regarded as the most effective approach to increase lipid content in algae (Brennan & Owende, 2010).

A total of 14 fatty acids were identified and measured, and the major fatty acids were C14:0, C16:0, C16:1$n-7$, C16:2$n-4$, C16:3, C20:5$n-3$ (EPA) (Table 1). For both strains of *S. menzelii* SM-1 and SM-2, C14:0 was the leading fatty acid in the cell, with the contents of 17.31–25.54% of total fatty acids and 15.40–21.99% of total fatty acids, respectively. The EPA content increased with the nitrogen concentration (0–25 mg/l), while further addition of nitrogen (30 mg/l) did not enhance EPA content ($P > 0.05$). The highest EPAs (14.20 ± 0.68% of total fatty acids for *S. menzelii* SM-1 and 15.02 ± 0.67% of total fatty acids for *S. menzelii* SM-2) were found at a nitrogen concentration of 25 mg/l, which were 96.13% (SM-1) and 65.78% (SM-2) higher than those with a nitrogen deficiency. The PUFA content in the strain *S. menzelii* SM-1

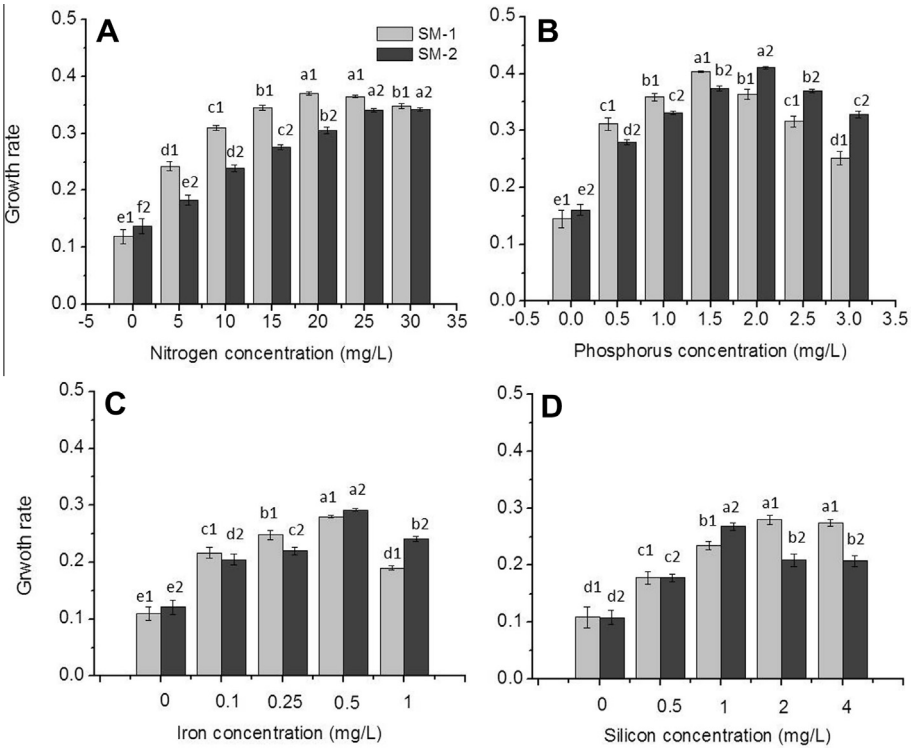

Fig. 1. Effects of different nutrient concentrations on the growth rate of *S. menzelii* SM-1 and SM-2. (A) Different nitrogen concentrations; (B) different phosphorus concentrations; (C) different iron concentrations; (D) different silicon concentrations. Different superscript letters indicate significant differences between various concentrations ($P < 0.05$, by one-way ANOVA and LSD test) and the numbers 1 and 2 represent the comparisons in *S. menzelii* SM-1 and SM-2, respectively. Data are the means ± SD ($n = 3$).

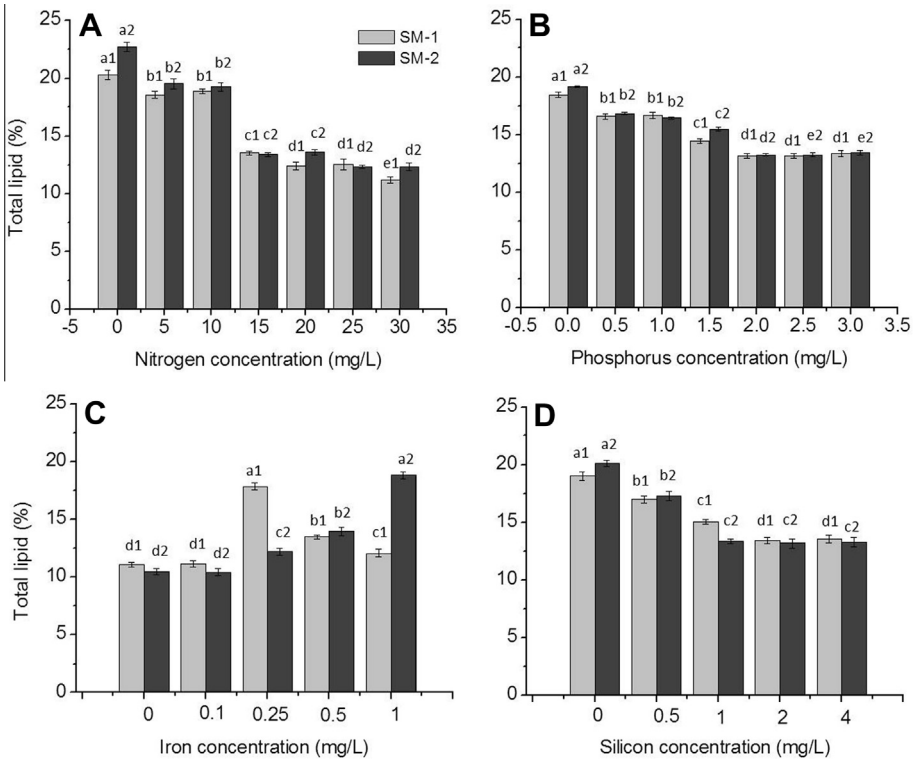

Fig. 2. Effects of nutrient concentrations on the total lipid contents of *S. menzelii* SM-1 and SM-2. (A) Different nitrogen concentrations; (B) different phosphorus concentrations; (C) different iron concentrations; (D) different silicon concentrations. Different superscript letters indicate significant differences between various concentrations within one strain ($P < 0.05$, by one-way ANOVA and LSD test) and the numbers 1 and 2 represent the comparisons in *S. menzelii* SM-1 and SM-2, respectively. Data are the means ± SD ($n = 3$).

increased with nitrogen and hit the highest value of 49.07 ± 0.93% total fatty acids ($P < 0.05$) when the nitrogen level was 15 mg/l, but a further ascending in nitrogen reduced PUFA content and the lowest value was found under the highest nitrogen concentration ($P < 0.05$). By contrast, high nitrogen did not decrease the PUFA content in the strain *S. menzelii* SM-2 and algae cultured under the highest nitrogen condition had the highest PUFA content ($P < 0.05$). The fatty acid composition can also be affected by the change of nitrogen concentration (Piorreck et al., 1984). The EPA content of *S. menzelii* increased with the increase of the nitrogen concentration, in accordance with the previous literature on *Phaeodactylum tricornutum* (Yongmanitchai & Ward, 1991).

3.3. Effects of phosphorus concentration on growth, total lipid and fatty acids in S. menzelii

The growth of *S. menzelii* SM-1 and SM-2 was highly affected by various phosphorus concentrations ($P < 0.05$) (Fig. 1B). The growth increased with phosphorus (0–1.5 mg/l for *S. menzelii* SM-1 and 0–2 mg/l for *S. menzelii* SM-2) and then declined after reaching its maximum (0.40 ± 0.00 d^{-1} for *S. menzelii* SM-1 and 0.41 ± 0.00 d^{-1} for *S. menzelii* SM-2). The optimal phosphorus concentrations for *S. menzelii* SM-1 and SM-2 were 15 and 20 mg/l, respectively. Phosphorus is another essential nutrient for algal growth and phosphorus limiting commonly occurs in the ocean. Thus, phosphorus enrichment increases biomass of algae (Elser et al., 2007). On the other hand, excess phosphorus inhibited the growth of *S. menzelii* in the present study. Inhibitory effect of high phosphorus was also observed in *Dinobryon* (Lehman, 1976). The possible explanation can be due to the toxicity of potassium added together with phosphorus rather than phosphate since no evidence of phosphate toxicity was found (Blindow, 1988; Lehman, 1976).

The cellular total lipid of both strains showed clear variations under different phosphorus concentrations ($P < 0.05$) (Fig. 2B). The total lipid declined from the peak (18.44% and 19.18% for *S. menzelii* and SM-2, respectively) as the phosphorus concentration increased (from 0 to 2 mg/l for *S. menzelii* SM-1 and 0 to 2.5 for *S. menzelii* SM-2; $P < 0.05$). The lowest total lipid content of the two strains occurred at the phosphorus concentration of 2 mg/l and further increase in phosphorus did not result in further decline in total lipid ($P > 0.05$). The promoting effect of phosphorus deficiency on total lipid was also reported in *P. tricornutum* and *D. tertiolecta*, in which phosphorus deficiency enhanced total lipid from 1.55% to 7.05% in *P. tricornutum* and from 2.82% to 4.88% in *D. tertiolecta* compared with phosphorus sufficient condition (Siron, Giusti, & Berland, 1989).

A total of 14 fatty acids were identified and measured, and the major fatty acids were C14:0, C16:0, C16:1$n-7$, C16:2$n-4$, C16:3, C20:5$n-3$ (EPA) (Table 2). The leading fatty acids in cells were C14:0 in *S. menzelii* SM-1 and C16:1$n-7$ in *S. menzelii* SM-2, accounting for 18.4–27.5% of the total fatty acids in the strain SM-1 and 18.92–23.72% of total fatty acids in strain SM-2. Different phosphorus concentrations also resulted in various fatty acid compositions for two strains of *S. menzelii* SM-1 and SM-2, especially in EPA, SFA, MUFA and PUFA ($P < 0.05$). The EPA content of both strains rose with phosphorus concentration (0–2.5 mg/l) and decreased when the phosphorus concentration reached 3 mg/l ($P < 0.05$). Thus, the largest amount of EPA (14.26 ± 0.58% of total fatty acids for *S. menzelii* SM-1 and 14.02 ± 0.63% of total fatty acids for *S. menzelii* SM-2) occurred at 2.5 mg/l phosphorus condition. As far as PUFA is concerned, the content in strain SM-1 increased with phosphorus and reached the peak (49.11 ± 0.96% of total fatty acids) at 3 mg/l phosphorus with a dip at 2.0 mg/l phosphorus ($P < 0.05$). In *S. menzelii* SM-2, small amount addition of phosphorus (0.5 mg/l) did not stimulate the PUFA content ($P > 0.05$).

Table 1
Effects of nitrogen concentration on fatty acid composition of *S. menzelii* SM-1 and SM-2, expressed as % total fatty acids.

Fatty acid	Nitrogen concentration (mg/l)						
	0	5	10	15	20	25	30
Skeletonema menzelii SM-1							
C14:0	25.54±0.56^{a}	22.41±0.51^{b}	18.58±0.43^{c}	17.90±0.43^{cd}	18.22±0.45^{c}	17.31±0.37^{d}	17.69±0.35^{d}
C15:0	2.12±0.19^{c}	2.29±0.18^{bc}	1.62±0.15^{d}	2.33±0.19^{bc}	2.57±0.21^{b}	3.18±0.23^{a}	2.76±0.23^{b}
C16:0	11.87±0.37^{a}	10.47±0.35^{b}	9.07±0.34^{c}	9.44±0.34^{c}	9.49±0.35^{c}	9.76±0.34^{c}	9.95±0.36^{bc}
C16:1$n-7$	14.14±0.47^{d}	15.3±0.48^{c}	16.1±0.51^{c}	15.86±0.53^{c}	16.45±0.54^{bc}	17.04±0.56^{a}	16.73±0.54^{a}
C16:2$n-4$	17.27±0.54^{a}	15.98±0.48^{b}	16.28±0.58^{b}	15.11±0.47^{c}	14.99±0.42^{c}	13.10±0.41^{e}	13.85±0.42^{d}
C16:3	11.38±0.36^{f}	12.32±0.32^{e}	15.30±0.37^{b}	16.32±0.46^{a}	14.74±0.43^{bc}	14.25±0.41^{c}	13.59±0.38^{d}
C18:0	2.80±0.16^{b}	3.03±0.15^{ab}	2.87±0.15^{b}	3.16±0.15^{a}	2.72±0.14^{b}	2.99±0.18^{ab}	2.69±0.19^{b}
C18:1$n-9$	1.87±0.12^{b}	1.90±0.13^{b}	1.89±0.16^{b}	2.45±0.18^{a}	1.76±0.15^{b}	2.59±0.18^{a}	2.50±0.19^{a}
C18:2$n-6$	1.28±0.11^{ab}	1.38±0.12^{a}	1.23±0.11^{ab}	1.18±0.10^{b}	1.27±0.09^{ab}	1.05±0.08^{b}	1.18±0.11^{b}
C18:3$n-3$	0.38±0.05^{c}	0.41±0.04^{c}	0.51±0.05^{b}	0.62±0.06^{a}	0.56±0.05^{ab}	0.44±0.05^{c}	0.47±0.06^{bc}
C20:4$n-6$	0.43±0.05^{a}	0.41±0.04^{a}	0.20±0.02^{b}	0.17±0.01^{c}	0.23±0.02^{b}	0.13±0.02^{d}	0.23±0.02^{b}
C20:5$n-3$	7.24±0.48^{e}	10.41±0.52^{d}	11.51±0.57^{c}	11.42±0.57^{c}	13.20±0.65^{b}	14.20±0.68^{a}	13.96±0.64^{a}
C22:1$n-9$	0.84±0.09^{b}	0.91±0.08^{b}	0.80±0.08^{bc}	0.74±0.07^{c}	0.76±0.07^{c}	0.83±0.07^{b}	1.29±0.10^{a}
C22:6$n-3$	2.85±0.18^{c}	2.78±0.18^{c}	3.84±0.21^{a}	3.60±0.21^{a}	3.04±0.19^{c}	3.13±0.19^{bc}	3.31±0.18^{b}
SFA	42.33± 0.67^{a}	38.20±0.78^{b}	32.14±0.78^{e}	32.83±0.85^{d}	33.00±0.76^{d}	34.24±0.68^{c}	32.09±0.72^{e}
MUFA	16.85±0.54^{d}	18.11±0.56^{c}	18.79±0.57^{b}	19.05±0.57^{b}	18.97±0.56^{b}	20.46±0.59^{a}	20.52±0.62^{a}
PUFA	40.83±0.86^{f}	43.69±0.85^{e}	49.07±0.93^{a}	48.12±0.92^{b}	48.03±0.92^{b}	45.30±0.84^{d}	47.39±0.79^{c}
Skeletonema menzelii SM-2							
C14:0	21.99±0.56^{a}	19.45±0.45^{b}	18.56±0.45^{c}	16.24±0.43^{d}	16.50±0.46^{d}	16.23±0.42^{d}	15.40±0.39^{e}
C15:0	3.08±0.15^{a}	3.07± 0.15^{a}	2.83±0.14^{ab}	3.13±0.16^{a}	2.40±0.15^{b}	2.57±0.14^{b}	2.48±0.14^{b}
C16:0	14.45±0.47^{a}	13.04±0.47^{a}	12.92±0.43^{b}	12.70±0.45^{b}	8.84±0.43^{c}	9.12±0.35^{c}	8.93±0.37^{c}
C16:1$n-7$	20.06±0.73^{a}	18.47±0.72^{a}	16.88±0.71^{b}	15.45±0.56^{c}	18.33±0.70^{a}	18.23±0.71^{a}	18.01±0.68^{a}
C16:2$n-4$	13.41±0.43^{c}	13.50±0.45^{c}	14.60±0.43^{b}	14.34±0.42^{b}	13.78±0.42^{c}	14.71±0.44^{b}	16.45±0.56^{a}
C16:3	8.57±0.41^{e}	10.24±0.45^{d}	11.20±0.44^{c}	13.45±0.42^{b}	13.74±0.41^{b}	13.50±0.41^{b}	14.89±0.46^{a}
C18:0	4.08±0.21^{a}	4.07±0.21^{a}	3.74±0.19^{b}	2.25±0.16^{c}	2.54±0.16^{c}	2.36±0.18^{c}	2.41±0.17^{bc}
C18:1$n-9$	2.72±0.19^{a}	2.65±0.17^{a}	2.34±0.14^{b}	2.30± 0.17^{b}	1.64±0.14^{c}	1.01±0.08^{e}	1.34±0.09^{d}
C18:2$n-6$	1.86±0.09^{a}	1.85±0.09^{a}	1.70±0.08^{ab}	1.59±0.09^{b}	1.18±0.09^{c}	1.18±0.09^{c}	1.06±0.08^{c}
C18:3$n-3$	0.55±0.04^{b}	0.55±0.0 4^{b}	0.51±0.05^{b}	0.83±0.06^{a}	0.52±0.05^{b}	0.49±0.05^{c}	0.42±0.04^{c}
C20:4$n-6$	0.63±0.05^{a}	0.55±0.06^{b}	0.51±0.06^{b}	0.23±0.02^{c}	0.21±0.02^{c}	0.15±0.01^{d}	0.21±0.02^{c}
C20:5$n-3$	4.43±0.34^{e}	8.91±0.38^{d}	9.06±0.43^{d}	11.65±0.53^{c}	14.87±0.58^{a}	15.02±0.67^{a}	13.38±0.65^{b}
C22:1$n-9$	1.22±0.11^{a}	1.22±0.12^{a}	1.12±0.11^{ab}	1.00±0.09^{b}	0.71±0.07^{d}	0.93±0.08^{c}	1.06±0.09^{b}
C22:6$n-3$	3.15±0.14^{e}	3.73±0.12^{c}	3.43±0.13^{d}	4.64±0.12^{a}	4.54±0.12^{a}	4.50±0.13^{a}	4.07±0.13^{b}
SFA	43.60±0.78^{a}	39.63±0.76^{b}	38.04±0.75^{c}	34.32±0.75^{d}	30.27±0.72^{e}	30.28±0.71^{e}	29.21±0.71^{f}
MUFA	24.01±0.67^{a}	22.44±0.68^{b}	20.35±0.64^{c}	18.75±0.63^{d}	20.68±0.68^{c}	20.17±0.65^{c}	20.31±0.64^{c}
PUFA	32.39±0.89^{f}	38.93±0.93^{e}	41.61±0.94^{d}	46.93±0.89^{c}	49.05±0.93^{b}	49.55±0.91^{b}	50.48±0.89^{a}

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 ($n = 3$).

Afterwards, PUFA increased with phosphorus and plateaued to the maximum of 44.19 ± 0.92% of total fatty acids at 2.5 mg/l phosphorus concentration. The enhanced content of EPA and PUFA by enrichment of phosphorus was also observed in other diatoms, such as *Skeletonema costatum*, *Achnanthes brevipes*, *Cylindrotheca fusiformis*, and *Pseudonitzschia* sp. (Pistocchi et al., 2005). However, the optimum concentration of phosphorus for EPA production varies from species to species, such as 88.9 mg/l for *P. tricornutum* (Yongmanitchai & Ward, 1991), but 2.5 mg/l for *S. menzelii* in present study.

3.4. Effects of iron concentration on growth, total lipid and fatty acids in S. menzelii

The growth rates of both strains of *S. menzelii* were affected by iron with the same pattern (Fig. 1C), in which growth rate increased with iron concentration (0–0.5 mg/l) and further addition of iron (1 mg/l) led to decrease of growth ($P < 0.05$). Thus, the concentration of 0.5 mg/l was optimal for growth in both strains. As an essential trace element for the growth of microalgae, iron deficiency affects physiological performances, which is represented by low level of phytoplankton photosynthesis and intrinsic photochemical energy conversion (Kolber et al., 1994). The growth of diatoms in the major high-nitrate low-chlorophyll regions of the world's oceans is limited by the availability of dissolved iron in the ambient surface seawater. Iron enrichment could improve the reproduction and biomass of the diatom, and even diatom bloom (Tsuda et al., 2003).

Iron affected the total lipid content of both strains of *S. menzelii* in different patterns (Fig. 2C). For the strain SM-1, minor addition did not enhance the total lipid content ($P < 0.05$) and a medium concentration (0.25 mg/l) brought about the highest lipid production (17.85 ± 0.29% of dry weight). Afterwards, lipid content declined with iron concentration ($P < 0.05$). In contrast, the total lipid in the strain of SM-2 rose along with the increase of iron concentration, with the highest total lipid of 18.81 ± 0.28% at the largest iron concentration of 1 mg/l ($P < 0.05$). In the reference to previous studies, the total lipid content in marine microalgae *Chlorella vulgaris* could be stimulated by excess iron addition and highest value for iron concentration at 0.67 mg/l was 7 times as high as that in iron depleted medium (Liu, Wang, & Zhou, 2008). A similar result was also documented in freshwater microalgae *Botryococcus* spp. (Yeesang & Cheirsilp, 2011).

In summary, 11 fatty acids were identified from *S. menzelii* SM-1 and SM-2 cultured under various iron levels (Table 3). C16:0 was the leading fatty acid in both strains (18.25–28.98% of total fatty acids in *S. menzelii* SM-1 and 17.81–26.66% in *S. menzelii* SM-2), followed by C14:0 (15.74–24.20% of total fatty acids in *S. menzelii* SM-1 and 15.32–22.26% of total fatty acids in *S. menzelii* SM-2). The EPA content in *S. menzelii* SM-1 increased with iron concentration, although there was no significant difference between 0.1 and 0.25 mg/l or between 0.5 and 1 mg/l ($P > 0.05$), with the highest EPA content of 16.67 ± 0.54% of total fatty acids at the iron concentration of 1 mg/l. However, higher iron concentrations led to a decline in EPA of strain SM-2, compared with 0.5 mg/l the EPA ($P < 0.05$), the highest EPA content of 18.39 ± 0.48% of total fatty

Table 2
Effects of phosphorus concentration on fatty acid composition of *S. menzelii* SM-1 and SM-2, expressed as % total fatty acids.

Fatty acid	Phosphorus concentration (mg/l)						
	0	0.5	1	1.5	2	2.5	3
Skeletonema menzelii SM-1							
C14:0	27.50 ± 0.76^{a}	25.80 ± 0.73^{b}	19.56 ± 0.56^{d}	18.40 ± 0.53^{e}	21.06 ± 0.43^{c}	18.72 ± 0.42^{e}	20.47 ± 0.43^{c}
C15:0	2.01 ± 0.15^{a}	1.59 ± 0.13^{c}	1.86 ± 0.13^{b}	1.64 ± 0.14^{c}	1.37 ± 0.14^{d}	1.33 ± 0.14^{d}	1.36 ± 0.13^{d}
C16:0	10.93 ± 0.54^{a}	9.78 ± 0.43^{b}	9.74 ± 0.32^{b}	9.22 ± 0.31^{c}	9.24 ± 0.31^{c}	9.24 ± 0.30^{c}	9.04 ± 0.30^{d}
C16:1*n* − 7	22.56 ± 0.74^{a}	20.96 ± 0.78^{b}	16.25 ± 0.56^{c}	15.27 ± 0.57^{d}	15.04 ± 0.54^{d}	14.49 ± 0.56^{e}	14.68 ± 0.61^{e}
C16:2*n* − 4	14.43 ± 0.56^{e}	15.99 ± 0.58^{d}	18.82 ± 0.67^{ab}	19.31 ± 0.67^{a}	17.80 ± 0.64^{c}	17.33 ± 0.71^{c}	18.22 ± 0.73^{b}
C16:3	9.12 ± 0.37^{f}	10.05 ± 0.39^{e}	16.01 ± 0.53^{a}	15.13 ± 0.48^{b}	13.05 ± 0.54^{d}	12.61 ± 0.45^{d}	14.40 ± 0.47^{c}
C18:0	2.21 ± 0.15^{d}	2.38 ± 0.16^{d}	2.92 ± 0.18^{b}	2.74 ± 0.19^{c}	2.92 ± 0.18^{b}	3.99 ± 0.21^{a}	3.03 ± 0.22^{b}
C18:1*n* − 9	1.02 ± 0.09^{e}	1.27 ± 0.09^{d}	1.95 ± 0.14^{a}	1.68 ± 0.12^{b}	1.90 ± 0.12^{a}	1.93 ± 0.13^{a}	1.51 ± 0.12^{c}
C18:2*n* − 6	0.37 ± 0.03^{e}	0.49 ± 0.05^{d}	1.06 ± 0.06^{b}	1.18 ± 0.09^{a}	1.04 ± 0.08^{b}	0.92 ± 0.09^{c}	0.88 ± 0.08^{c}
C18:3*n* − 3	1.50 ± 0.12^{a}	1.27 ± 0.10^{b}	0.36 ± 0.06^{d}	0.45 ± 0.05^{b}	0.37 ± 0.03^{d}	0.37 ± 0.03^{d}	0.36 ± 0.02^{d}
C20:4*n* − 6	0.19 ± 0.01^{a}	0.15 ± 0.01^{b}	0.20 ± 0.02^{a}	0.17 ± 0.02^{b}	0.18 ± 0.02^{ab}	0.20 ± 0.02^{a}	0.17 ± 0.01^{b}
C20:5*n* − 3	3.72 ± 0.36^{f}	6.09 ± 0.38^{e}	7.21 ± 0.41^{d}	9.23 ± 0.46^{c}	11.35 ± 0.52^{b}	14.26 ± 0.58^{a}	11.77 ± 0.61^{b}
C22:1*n* − 9	1.94 ± 0.19^{a}	1.53 ± 0.16^{c}	1.05 ± 0.09^{d}	2.12 ± 0.19^{a}	1.78 ± 0.18^{b}	1.68 ± 0.17^{b}	0.80 ± 0.07^{e}
C22:6*n* − 3	2.50 ± 0.16^{c}	2.65 ± 0.18^{c}	3.01 ± 0.21^{b}	3.46 ± 0.23^{a}	2.90 ± 0.24^{b}	2.93 ± 0.24^{b}	3.31 ± 0.25^{a}
SFA	42.65 ± 0.87^{a}	39.55 ± 0.83^{b}	34.08 ± 0.78^{d}	32.00 ± 0.74^{e}	34.59 ± 0.72^{c}	32.28 ± 0.75^{e}	33.9 ± 0.74^{d}
MUFA	25.52 ± 0.72^{a}	23.76 ± 0.67^{b}	19.25 ± 0.63^{c}	19.07 ± 0.62^{c}	18.72 ± 0.61^{c}	18.10 ± 0.59^{d}	16.99 ± 0.60^{e}
PUFA	31.83 ± 0.87^{e}	36.69 ± 0.88^{d}	46.67 ± 0.92^{c}	48.93 ± 0.93^{b}	46.69 ± 0.94^{c}	48.62 ± 0.94^{b}	49.11 ± 0.96^{a}
Skeletonema menzelii SM-2							
C14:0	21.61 ± 0.58^{a}	21.54 ± 0.57^{a}	19.63 ± 0.54^{b}	19.99 ± 0.53^{b}	18.52 ± 0.47^{c}	18.14 ± 0.47^{c}	17.73 ± 0.42^{d}
C15:0	0.64 ± 0.07^{c}	0.60 ± 0.06^{c}	1.13 ± 0.12^{b}	1.50 ± 0.13^{a}	0.62 ± 0.06^{c}	0.67 ± 0.07^{c}	0.65 ± 0.06^{c}
C16:0	11.25 ± 0.36^{b}	10.87 ± 0.36^{c}	14.54 ± 0.41^{a}	10.98 ± 0.39^{bc}	11.27 ± 0.38^{b}	11.20 ± 0.39^{b}	10.80 ± 0.32^{c}
C16:1*n* − 7	23.72 ± 0.65^{a}	23.61 ± 0.64^{a}	20.23 ± 0.62^{b}	19.93 ± 0.61^{b}	19.16 ± 0.59^{c}	18.92 ± 0.58^{c}	19.22 ± 0.61^{c}
C16:2*n* − 4	9.81 ± 0.39^{d}	10.02 ± 0.39^{d}	12.11 ± 0.41^{c}	12.18 ± 0.41^{c}	13.23 ± 0.43^{b}	13.38 ± 0.43^{b}	13.90 ± 0.44^{a}
C16:3	10.96 ± 0.41^{b}	10.10 ± 0.40^{d}	10.43 ± 0.41^{c}	10.49 ± 0.41^{c}	11.53 ± 0.47^{a}	10.66 ± 0.42^{bc}	11.40 ± 0.47^{a}
C18:0	3.24 ± 0.23^{a}	3.12 ± 0.24^{a}	2.90 ± 0.21^{b}	2.51 ± 0.23^{c}	3.19 ± 0.25^{a}	2.42 ± 0.21^{c}	2.39 ± 0.20^{c}
C18:1*n* − 9	0.82 ± 0.08^{c}	0.85 ± 0.08^{c}	0.53 ± 0.04^{d}	0.88 ± 0.08^{c}	1.08 ± 0.09^{b}	1.16 ± 0.09^{b}	1.33 ± 0.11^{a}
C18:2*n* − 6	0.88 ± 0.08^{b}	0.86 ± 0.09^{b}	0.66 ± 0.05^{c}	1.11 ± 0.10^{a}	1.00 ± 0.09^{a}	0.86 ± 0.09^{b}	1.07 ± 0.11^{a}
C18:3*n* − 3	0.19 ± 0.02^{d}	0.50 ± 0.03^{b}	0.68 ± 0.06^{a}	0.28 ± 0.02^{c}	0.23 ± 0.02^{cd}	0.23 ± 0.02^{cd}	0.28 ± 0.03^{c}
C20:4*n* − 6	0.16 ± 0.01^{d}	0.11 ± 0.01^{e}	0.59 ± 0.05^{a}	0.21 ± 0.02^{c}	0.31 ± 0.03^{b}	0.14 ± 0.01^{d}	0.18 ± 0.01^{cd}
C20:5*n* − 3	8.32 ± 0.43^{e}	8.64 ± 0.43^{d}	8.62 ± 0.42^{d}	11.46 ± 0.48^{c}	11.35 ± 0.51^{c}	14.02 ± 0.63^{a}	12.71 ± 0.63^{b}
C22:1*n* − 9	4.20 ± 0.24^{c}	4.51 ± 0.26^{b}	3.10 ± 0.21^{f}	5.06 ± 0.26^{a}	3.91 ± 0.26^{d}	3.30 ± 0.21^{f}	3.57 ± 0.24^{e}
C22:6*n* − 3	4.20 ± 0.26^{c}	4.57 ± 0.26^{b}	4.85 ± 0.27^{a}	3.42 ± 0.25^{d}	4.60 ± 0.27^{b}	4.90 ± 0.27^{a}	4.77 ± 0.27^{a}
SFA	36.74 ± 0.76^{b}	36.13 ± 0.78^{c}	38.20 ± 0.79^{a}	34.98 ± 0.74^{d}	33.60 ± 0.71^{e}	32.43 ± 0.70^{f}	31.57 ± 0.72^{g}
MUFA	28.74 ± 0.74^{a}	28.97 ± 0.76^{a}	23.86 ± 0.69^{d}	25.87 ± 0.67^{b}	24.15 ± 0.63^{c}	23.38 ± 0.65^{e}	24.12 ± 0.64^{c}
PUFA	34.52 ± 0.87^{e}	34.80 ± 0.86^{e}	37.94 ± 0.89^{d}	39.15 ± 0.90^{c}	42.25 ± 0.91^{b}	44.19 ± 0.92^{a}	44.31 ± 0.91^{a}

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 phosphorus concentrations ($P < 0.05$, by one-way ANOVA and LSD test). Data are the means ± SD ($n = 3$).

acids occurred at the iron level of 0.5 mg/l. The PUFA content in *S. menzelii* SM-1 rose from 28.49 ± 0.76% of total fatty acids to 36.99 ± 0.89 % of total fatty acids when the iron concentration increased from 0 to 0.25 mg/l. After that, there was a decrease, with a slight increase at 1 mg/l iron concentration ($P < 0.05$). Meanwhile, the highest PUFA content (38.26 ± 0.86% of total fatty acids) in strain SM-2 was found at 0.5 mg/l iron condition, since further enrichment reduced PUFA content to 35.69 ± 0.83% of total fatty acids ($P < 0.05$). To the best of our knowledge, there is little documented on the effects of iron supplement on fatty acid composition of algae, referring to PUFA and EPA content.

3.5. Effects of silicon concentration on growth, total lipid and fatty acids in S. menzelii

The growth rate of *S. menzelii* SM-1 increased with silicon concentration (0–2 mg/l) and did not show further increase when silicon concentration was 4 mg/l (Fig. 1D). The effect of silicon on the growth of *S. menzelii* SM-2 was different from SM-1. The growth rate of SM-2 began to decrease when the silicon concentration was above 1 mg/l, following an increase in the silicon concentration of 0–1 mg/l ($P < 0.05$). Thus, the optimal silicon concentrations for growth rate of *S. menzelii* SM-1 and SM-2 were 2 mg/l and 1 mg/l, respectively. The silicon is particularly important for diatoms, as it participates in the formation of cell wall, cell division, synthesis of protein, DNA and photosynthetic pigments (Werner, 1977). Silicon is a major limiting nutrient for diatom growth (Nelson & Treguer, 1992). The optimal growth can be obtained with a moderate silicon supplement (at the silicon concentration of 1.5–2 mg/l), in agreement with another diatom *Amphora exigua* (0.8–1.6 mg/l) reported by Zhou (2008), although the half-saturation constant of silicon for diatom growth varies with species (Martin-Jézéquel, Hildebrand, & Brzezinski, 2000).

The cellular total lipid content of both strains demonstrated the same trend with change of silicon concentrations in the culture medium (Fig. 2D). The total lipid declined along with the increase of the silicon concentrations (0–2 mg/l) and the highest silicon concentration did not induce further decrease ($P > 0.05$). Algae cultured under silicon deficiency had the highest total lipid content (19.02 ± 0.37% for strain SM-1 and 20.12 ± 0.25% for strain SM-2), while the lowest total lipid content of two strains (13.43 ± 0.27% and for 13.20 ± 0.39% for strain SM-1 and strain SM-2) was found when the silicon concentration was 2 mg/l. The promoting effect of silicon limitation on total lipid was also found in *Cyclotella cryptica*, in which the lipid fraction increased from 30% to 42% within 6 h of silicon deficiency (Shifrin & Chisholm, 1981). This can be interpreted that silicon deficiency increases the transcription of nuclear genes responsible for the formation of lipid biosynthetic enzymes (Roessler, 1988). However, silicon starvation with the same treatment time (6 h) did not induce a significant change of the lipid content in *Thalassiosira pseudonana* (Harrison et al., 1990). That indicates the effect of silicon on lipid content of microalgae is species-dependent.

The various silicon concentrations resulted in different fatty acid profiles for *S. menzelii* SM-1 and SM-2 (Table 4). A total of 11 fatty acids were identified and measured, with the major fatty

Table 3
Effects of iron concentration on fatty acid composition of *S. menzelii* SM-1 and SM-2, expressed as % total fatty acids.

Fatty acid	Iron concentration (mg/l)					Iron concentration (mg/l)				
	0	0.1	0.25	0.5	1	0	0.1	0.25	0.5	1
	SM-1					SM-2				
C14:0	24.20 ± 0.76^{a}	20.66 ± 0.68^{b}	18.95 ± 0.65^{c}	18.80 ± 0.67^{c}	15.74 ± 0.51^{d}	22.26 ± 0.67^{a}	19.14 ± 0.63^{b}	18.70 ± 0.62^{c}	15.32 ± 0.57^{d}	15.36 ± 0.56^{d}
C15:0	2.85 ± 0.25^{a}	2.31 ± 0.24^{b}	1.81 ± 0.19^{d}	2.13 ± 0.21^{c}	1.78 ± 0.19^{d}	2.62 ± 0.21^{b}	2.59 ± 0.23^{b}	1.89 ± 0.19^{d}	2.39 ± 0.21^{c}	3.26 ± 0.27^{a}
C16:0	9.47 ± 0.32^{d}	10.93 ± 0.33^{c}	9.45 ± 0.32^{d}	11.56 ± 0.34^{b}	14.01 ± 0.37^{a}	12.71 ± 0.46^{b}	8.24 ± 0.41^{d}	9.33 ± 0.43^{c}	12.98 ± 0.47^{b}	13.67 ± 0.49^{a}
C16:1$n-7$	28.98 ± 0.76^{a}	25.08 ± 0.75^{b}	22.70 ± 0.72^{c}	22.93 ± 0.71^{c}	18.25 ± 0.69^{d}	26.66 ± 0.76^{b}	28.09 ± 0.78^{a}	23.70 ± 0.75^{c}	20.37 ± 0.68^{d}	17.81 ± 0.61^{e}
C16:2$n-4$	2.89 ± 0.23^{b}	2.84 ± 0.23^{b}	3.05 ± 0.24^{a}	2.12 ± 0.23^{c}	1.77 ± 0.17^{d}	2.66 ± 0.26^{c}	3.18 ± 0.28^{a}	3.01 ± 0.29^{b}	2.38 ± 0.24^{d}	2.12 ± 0.21^{e}
C16:3	10.55 ± 0.35^{d}	12.60 ± 0.47^{b}	15.86 ± 0.43^{a}	11.79 ± 0.33^{c}	9.74 ± 0.34^{e}	12.88 ± 0.43^{c}	14.11 ± 0.47^{b}	15.65 ± 0.50^{a}	13.04 ± 0.48^{c}	9.94 ± 0.38^{d}
C18:0	1.38 ± 0.13^{d}	1.42 ± 0.14^{d}	2.70 ± 0.21^{c}	2.99 ± 0.24^{b}	4.25 ± 0.26^{a}	1.23 ± 0.13^{e}	1.66 ± 0.16^{d}	2.66 ± 0.21^{c}	3.36 ± 0.26^{b}	4.15 ± 0.29^{a}
C18:1$n-9$	4.67 ± 0.25^{e}	5.97 ± 0.31^{d}	7.30 ± 0.32^{c}	8.52 ± 0.31^{b}	10.30 ± 0.42^{a}	6.30 ± 0.26^{d}	6.69 ± 0.21^{c}	7.21 ± 0.21^{b}	7.32 ± 0.23^{b}	10.05 ± 0.31^{a}
C18:2$n-6$	0.54 ± 0.06^{c}	0.67 ± 0.06^{b}	0.72 ± 0.07^{b}	0.75 ± 0.07^{b}	2.24 ± 0.18^{a}	0.50 ± 0.05^{d}	0.75 ± 0.06^{c}	0.71 ± 0.06^{c}	0.84 ± 0.07^{b}	2.19 ± 0.16^{a}
C20:5$n-3$	12.30 ± 0.43^{c}	15.08 ± 0.53^{b}	14.98 ± 0.45^{b}	16.48 ± 0.52^{a}	16.67 ± 0.54^{a}	10.32 ± 0.33^{e}	12.89 ± 0.35^{d}	14.79 ± 0.37^{c}	18.39 ± 0.48^{a}	16.49 ± 0.42^{b}
C22:6$n-3$	2.21 ± 0.21^{d}	2.38 ± 0.22^{c}	2.38 ± 0.23^{c}	3.03 ± 0.26^{b}	5.07 ± 0.29^{a}	2.03 ± 0.18^{e}	2.67 ± 0.21^{c}	2.35 ± 0.23^{d}	3.40 ± 0.27^{b}	4.95 ± 0.27^{a}
SFA	37.86 ± 0.87^{a}	35.38 ± 0.85^{c}	33.01 ± 0.84^{d}	35.48 ± 0.84^{bc}	35.73 ± 0.86^{b}	38.83 ± 0.88^{a}	31.62 ± 0.76^{e}	32.58 ± 0.78^{d}	34.05 ± 0.79^{c}	36.44 ± 0.81^{b}
MUFA	33.65 ± 0.83^{a}	31.05 ± 0.78^{b}	30.10 ± 0.76^{c}	30.45 ± 0.80^{c}	28.55 ± 0.76^{d}	32.96 ± 0.82^{b}	34.78 ± 0.83^{a}	30.91 ± 0.75^{c}	27.69 ± 0.73^{d}	27.86 ± 0.73^{d}
PUFA	28.49 ± 0.76^{e}	33.57 ± 0.83^{d}	36.99 ± 0.89^{a}	34.07 ± 0.78^{c}	35.72 ± 0.82^{b}	28.21 ± 0.76^{e}	33.60 ± 0.81^{d}	36.51 ± 0.84^{b}	38.26 ± 0.86^{a}	35.69 ± 0.83^{c}

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 iron concentrations within one strain ($P < 0.05$, by one-way ANOVA and LSD test). Data are the means ± SD ($n = 3$).

Table 4
Effects of silicon concentration on fatty acid composition of *S. menzelii* SM-1 and SM-2, expressed as % total fatty acids.

Fatty acid	Silicon concentration (mg/l)					Silicon concentration (mg/l)				
	0	0.5	1	2	4	0	0.5	1	2	4
	SM-1					SM-2				
C14:0	15.85 ± 0.47^{c}	16.71 ± 0.51^{b}	16.76 ± 0.53^{b}	16.54 ± 0.53^{b}	17.74 ± 0.57^{a}	14.03 ± 0.45^{d}	14.98 ± 0.46^{c}	16.29 ± 0.52^{a}	15.61 ± 0.53^{b}	15.62 ± 0.53^{b}
C15:0	1.82 ± 0.17^{c}	2.51 ± 0.19^{b}	2.46 ± 0.21^{b}	2.50 ± 0.20^{b}	2.94 ± 0.21^{a}	1.78 ± 0.14^{d}	2.26 ± 0.21^{c}	2.44 ± 0.21^{b}	2.48 ± 0.23^{b}	3.08 ± 0.26^{a}
C16:0	9.56 ± 0.34^{c}	9.49 ± 0.35^{c}	10.13 ± 0.37^{b}	10.47 ± 0.38^{a}	10.42 ± 0.37^{a}	7.90 ± 0.35^{d}	8.68 ± 0.37^{c}	11.93 ± 0.40^{b}	12.02 ± 0.41^{b}	12.73 ± 0.42^{a}
C16:1$n-7$	21.61 ± 0.76^{c}	21.57 ± 0.74^{c}	23.49 ± 0.78^{b}	23.98 ± 0.78^{a}	23.95 ± 0.76^{a}	23.89 ± 0.67^{b}	23.97 ± 0.65^{b}	22.30 ± 0.64^{c}	25.02 ± 0.67^{a}	25.04 ± 0.65^{a}
C16:2$n-4$	2.99 ± 0.21^{a}	2.45 ± 0.22^{b}	2.38 ± 0.21^{c}	2.32 ± 0.20^{c}	1.89 ± 0.17^{d}	3.57 ± 0.23^{a}	3.38 ± 0.23^{b}	2.99 ± 0.21^{c}	2.67 ± 0.22^{d}	2.63 ± 0.21^{d}
C16:3	17.89 ± 0.56^{a}	16.71 ± 0.55^{b}	15.38 ± 0.56^{c}	14.41 ± 0.51^{d}	13.64 ± 0.48^{e}	17.79 ± 0.69^{a}	16.79 ± 0.65^{b}	15.87 ± 0.62^{b}	13.02 ± 0.50^{d}	12.06 ± 0.42^{e}
C18:0	2.28 ± 0.21^{d}	2.87 ± 0.20^{c}	3.87 ± 0.23^{a}	3.59 ± 0.25^{b}	3.65 ± 0.27^{b}	1.20 ± 0.11^{e}	1.62 ± 0.13^{d}	2.60 ± 0.19^{c}	3.28 ± 0.21^{b}	3.87 ± 0.23^{a}
C18:1$n-9$	4.56 ± 0.29^{b}	4.55 ± 0.30^{b}	4.58 ± 0.30^{b}	6.37 ± 0.31^{a}	6.59 ± 0.32^{a}	6.19 ± 0.33^{d}	6.57 ± 0.33^{c}	7.18 ± 0.35^{b}	7.20 ± 0.36^{b}	9.88 ± 0.39^{a}
C18:2$n-6$	3.43 ± 0.21^{a}	3.37 ± 0.20^{a}	2.31 ± 0.18^{b}	1.64 ± 0.15^{c}	1.12 ± 0.10^{d}	2.72 ± 0.21^{a}	1.05 ± 0.09^{b}	0.88 ± 0.07^{c}	0.93 ± 0.08^{c}	0.66 ± 0.05^{d}
C20:5$n-3$	17.12 ± 0.65^{a}	17.01 ± 0.58^{a}	16.38 ± 0.54^{b}	15.89 ± 0.54^{c}	15.80 ± 0.53^{c}	17.22 ± 0.67^{a}	17.18 ± 0.68^{a}	14.28 ± 0.52^{b}	14.20 ± 0.46^{b}	11.37 ± 0.41^{c}
C22:6$n-3$	2.89 ± 0.21^{a}	2.76 ± 0.21^{a}	2.26 ± 0.19^{b}	2.29 ± 0.19^{b}	2.26 ± 0.18^{b}	3.61 ± 0.27^{a}	3.55 ± 0.28^{a}	3.43 ± 0.29^{b}	3.58 ± 0.27^{a}	3.31 ± 0.27^{c}
SFA	29.51 ± 0.79^{d}	31.58 ± 0.81^{c}	33.22 ± 0.80^{b}	33.10 ± 0.82^{b}	34.75 ± 0.83^{a}	24.91 ± 0.74^{d}	27.54 ± 0.76^{c}	33.37 ± 0.79^{b}	33.45 ± 0.81^{b}	35.30 ± 0.82^{a}
MUFA	26.17 ± 0.78^{c}	26.12 ± 0.76^{c}	28.07 ± 0.76^{b}	30.35 ± 0.83^{a}	30.54 ± 0.81^{a}	30.38 ± 0.78^{c}	30.44 ± 0.8^{c}	29.38 ± 0.76^{d}	32.22 ± 0.78^{b}	34.92 ± 0.81^{a}
PUFA	44.32 ± 0.87^{a}	42.30 ± 0.86^{b}	38.71 ± 0.85^{c}	36.55 ± 0.86^{d}	34.71 ± 0.84^{e}	45.00 ± 0.87^{a}	41.92 ± 0.84^{b}	37.45 ± 0.81^{c}	34.33 ± 0.82^{d}	29.78 ± 0.81^{e}

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 silicon concentrations within one strain ($P < 0.05$, by one-way ANOVA and LSD test). Data are the means ± SD ($n = 3$).

acids being C14:0, C16:0, C16:1$n-7$, C16:3, C20:5$n-3$ (EPA) in both strains. Among these fatty acids, the largest two components were C16:1 n-7 (21.57 ± 0.74–23.98 ± 0.78% of total fatty acids in strain SM-1 and 22.30 ± 0.64–25.04 ± 0.65% of total fatty acids in strain SM-2) and EPA (15.80 ± 0.53–17.12 ± 0.65% of total fatty acids in strain SM-1 and 11.37 ± 0.41–17.22 ± 0.67% of total fatty acids in strain SM-2). The general pattern of PUFA content versus silicon concentration in both strains was that higher silicon levels resulted in lower PUFA content, although there was no significant difference between 0 and 0.5 mg/l or 2 and 4 mg/l ($P > 0.05$). The highest EPAs were 17.12 ± 0.65% of total fatty acids for strain SM-1 and 17.22 ± 0.67% of total fatty acids for strain SM-2 under silicon deficiency. This was inconsistent with the finding in *Chaetoceros calcitrans* and *T. pseudonana*, in which the percentage of EPA remained relatively constant under silicon starvation (Harrison et al., 1990). PUFA decreased with silicon concentration in both strains, with highest values of 44.32 ± 0.87% of total fatty acids (SM-1) and 45.00 ± 0.87% of total fatty acids (SM-2) occurring under silicon deficiency ($P < 0.05$) in the present study. This finding was consistent with that in *P. tricornutum*, in which silicon starvation also resulted in a slight increase in PUFA (from 42.13% to 43.48% of total fatty acids) (Liao, Li, Chen, & Zheng, 2000). However, PUFA of *Chaetoceros gracilis* declined with decreasing silicate availability (Mortensen, Børsheim, Rainuzzo, & Knutsen, 1988). These various effects of silicon on EPA or PUFA content might be due to interspecific differences.

3.6. Maximum yield of biomass, total lipid, PUFA, and EPA in S. menzelii

To the best of our knowledge, little research on the yield of biomass, total lipid, EPA and PUFA in *S. menzelii* has been documented. The maximum yield of biomass, total lipid, EPA, and PUFA of *S. menzelii* has to be compared with a close species and *S. costatum* was chosen, due to its quick growth, high EPA level and wide employment in biochemical studies (Blanchemain & Grizeau, 1996; Guihéneuf, Mimouni, Ulmann, & Tremblin, 2008). The maximum growth rate was 0.41 ± 0.00 d^{-1} of *S. menzelii* SM-2 at 2 mg/l phosphorus, which was much lower than that in *S. costatum* reported by Lourenço, Barbarino, Mancini-Filho, Schinke, and Aidar (2002) (1.54–1.73 division d^{-1} equaling to the growth rate of 1.06–1.20 d^{-1}) or by Oh et al. (2008) (maximum growth rate of 1.58 d^{-1}). One of the reasons that lead to the big gap in growth rate between *S. menzelii* and *S. costatum* is that the growth rates reported by Lourenço et al. (2002) or Oh et al. (2008) were from exponential growth phase, while the results in the present study were average values of a 7 day growth, which included the lag phase separate from the exponential phase. In addition, the 60 μmol photons m^{-2} s^{-1} of light intensity was employed in present study, which might not meet the maximum growth demand of *S. menzelii*, while the growth rates were obtained under saturated light level in Lourenço et al. (2002) or Oh et al.'s (2008) studies. The maximum total lipid was 22.7 ± 0.39% in *S. menzelii* SM-2 under the nitrogen deficiency condition. This value was also much lower than that in *S. costatum* (36.90 ± 1.94–41.42 ± 2.18% of total fatty acids) (Chen, 2012). In addition to species difference, the gap may also be caused by the culture duration because *S. menzelii* were collected for lipid analysis after 7-day culture in present study, while the culture duration in Chen's study was up to 40 days. Low content of total lipid (11.2%) was also observed in 7-day cultured *S. costatum* (Pistocchi et al., 2005). The maximum EPA content was 18.39 ± 0.48% in *S. menzelii* SM-2 at 0.5 mg/l iron concentration, which was remarkably higher than that in *S. costatum* reported by Chen (2012) (0.31 ± 0.01–7.47 ± 0.36%) and by Guihéneuf et al. (2008) (8.3–18.7%). The maximum PUFA content was 50.48 ± 0.89% of total fatty acids in *S. menzelii* SM-2 at 30 mg/l nitrogen concentration, which was higher than that in *S. costatum* reported by Chen (2012) (7.83–22.17% of total fatty acids) and Guihéneuf et al. (2008) (16.6–35.5% of total fatty acids). The differences in EPA and PUFA between *S. menzelii* and *S. costatum* may be down to the interspecific difference to a large extent.

It is important to note that the aforementioned maximum contents of EPA and PUFA are relative values. An absolute production is preferable in terms of commercial culture. Therefore, the yields of EPA and PUFA in both strains under various conditions were calculated based on growth rate, total lipid and fatty acids content (Table S2). The maximum yield of EPA (7.89 ± 0.08 mg/g DW d^{-1}) and PUFA (35.20 ± 0.48 mg/g DW d^{-1}) in *S. menzelii* SM-1 were detected at levels of 10 mg/l nitrogen and 1.5 mg/l phosphorus, respectively. Meanwhile, *S. menzelii* SM-2 showed highest EPA yield (8.31 ± 0.11 mg/g DW d^{-1}) at 2.5 mg/l phosphorus level and highest PUFA yield (28.43 ± 0.24 mg/g DW d^{-1}) at 2 mg/l phosphorus level. Therefore, the optimal culture condition and strain can be picked up depending on the priority. For instance, if EPA is in the primary need, *S. menzelii* SM-2 should be the ideal strain and 2.5 mg/l phosphorus level should be the optimal condition while *S. menzelii* SM-1 should be cultivated at 1.5 mg/l phosphorus level to meet PUFA demand. Furthermore, these yields of EPA and PUFA were considerably high when compared with fish. For instance, the yields of EPA and PUFA in Atlantic salmon *Salmo salar* were only 0.12–0.20 mg/g DW d^{-1} and 1.62–2.05 mg/g DW d^{-1} fed with various diets (Hatlen, Berge, Odom, Mundheim, & Ruyter, 2012).

4. Conclusion

PUFA are in great demand due to their significant role in the health of animals and human beings. Diatom is considered the ideal option for PUFA supplier. The present study screened a diatom species with quick growth and high PUFA/EPA content, S. *menzelii* from nine marine diatoms. Furthermore, the optimal conditions for growth, total lipid, PUFA, and EPA yield were investigated for the first time. The maximum growth rate, contents of total lipid, PUFA, and EPA were detected at levels of 2 mg/l phosphorus, 0 mg/l nitrogen, 0.5 mg/l iron, and 30 mg/l nitrogen concentration, respectively. Meanwhile, the maximum yields of PUFA and EPA were found at 1.5 mg/l phosphorus level and 2.5 mg/l phosphorus level, respectively. The findings of this study provide an essential foundation for culturing this species to produce PUFA/EPA.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.08.073.

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