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Enhancing total fatty acids and arachidonic acid production by the red microalgae *Porphyridium purpureum*

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Abstract

Objectives: This study investigated the effect of aeration rate and light intensity on biomass production and total fatty acids (TFA) accumulation by *Porphyridium purpureum*. The red microalgae is also known to accumulate considerable amount of arachidonic acid (ARA).

Results: In artificial seawater medium, the highest yield of TFA (473.44 mg/L) was obtained with the aeration rate of 3 L/min and light intensity of 165 μmol/m^2s, whilst the highest yield of ARA (115.47 mg/L) was achieved with the aeration rate of 3 L/min and light intensity of 110 μmol/m^2s. It was found that higher aeration rate led to more biomass and TFA/ARA production. However, higher light intensity could contribute to biomass accumulation, but it was adverse for TFA and ARA biosynthesis.

Conclusion: By optimizing two operating factors (i.e., light intensity and aeration rate), TFA and ARA production by *P. purpureum* was significantly improved. This research provides a potential alternative means for producing ARA.

Keywords: Microalgae, *Porphyridium purpureum*, Aeration rate, Light intensity, Total fatty acids, Arachidonic acid

Background

Microalgae, exhibiting promising prospect for nourishment, medicine industry, biofuels production, and many other applications, have attracted global attention in recent decades (Fuentes et al. 2000; Ginzberg et al. 2000; Huo et al. 1997). Particularly, great potential of valuable polyunsaturated fatty acids (PUFAs) produced by photoautotrophic microalgae for large-scale microalgal industries have been found (Thompson 1996; Mendoza et al. 1999; Sukenik 1999). Among PUFAs, arachidonic acid (ARA) and eicosapentaenoic acid (EPA) are two of the most valuable extracts of microalgae. ARA is an important omega-6 polyunsaturated fatty acid (n-6 PUFA) that has been reported as one of the major fatty acids of brain cell phospholipids and a precursor of prostaglandins and leukotrienes (Koletzko and Braun 1991; Kromhout et al. 1985). EPA has been demonstrated effective for preventing and curing thrombosis and arteriosclerosis (Dyerberg 1986; De Bravo et al. 1991), and inhibiting the growth of a human lung carcinoma (Shinmen et al. 1989).

ARA is mainly produced by the microorganism *Mortierella* fungi and the heterologous expression of the ARA by *Escherichia coli* (Higashiyama et al. 2002; Bennett et al. 1987; Barclay et al. 1994) due to the fact that *Mortierella* fungi is more economically feasible, and *E. coli* is a good gene engineering carrier model and easy to be modified. Microalgae are photosynthetic, thus making them safer and environmentally friendly alternative source for ARA production. Furthermore, challenges, such as odor taste, in the traditional PUFA manufacturing techniques can be obviated using microalgae as raw material for PUFA production (Muradyan et al. 2004). Moreover, microalgae are

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totally natural compared to *E. coli*, and they possess other beneficial properties, such as non-toxicity, and less subjected to contamination and environmental fluctuations. In addition, microalgae could also produce other valuable products, such as proteins, pigments, and polysaccharides. In addition, the microalgae residue could be used in many fields, such as health care products and fodder. Therefore, the production of fatty acids by the microalgae is considered more advantageous.

Recently, much effort has been focused on controlling CO_2 flow rate to achieve higher fatty acids production considering the potential applications of algae in biodiesel industry (Tang et al. 2011; Cohen et al. 1988). However, although light intensity has been found to improve the fatty acids accumulation in microalgae, few reports have been published on the combined effect of light intensity and aeration rate on total fatty acids (TFA) and ARA productivity by microalgae.

The red microalgae *Porphyridium purpureum* is one of the very few microalgae species that could accumulate high concentrations of long-chain PUFAs, containing up to 36 % ARA and 17 % EPA of TFA (Ahern et al. 1983; Nichols and Appleby 1969; Jones et al. 1963). Previous efforts in enhancing ARA production by these microalgae usually established at the expense of growth limitation under sub-optimal conditions (Ahern et al. 1983; Nichols and Appleby 1969). The aim of the present study is to establish an efficient, economical, and environmental friendly method for ARA production by *P. purpureum*. The *P. purpureum* was cultivated in a simple, open, low-energy, organic carbon, and nitrogen sources free system for the fatty acids production, and the influence of aeration rate and light intensity on the yields of TFA and ARA production is investigated and presented.

Methods

Culture system

The microalgae, *P. purpureum,* CoE1 was screened and maintained by the authors' research group. Algae cells were cultivated in 1 L flasks containing 500 mL medium at 25 °C under continuous light illumination in a photoincubator. Four culture media reported to enhance *P. cruentum* growth, including Jones' ASW medium (Jones et al. 1963), KOCK medium (Koch 1952), Pringsheim medium II (Ernest and Pringsheim 1949), and F/2 medium (Oh et al. 2009), were screened for biomass production and fatty acids/ARA accumulation. The pH of the mediums was adjusted to 7.6 by Tris–HCl buffer. The medium was sterilized by autoclaving with a pressure of 1 kg/cm^2 for 20 min. The light intensities ranged from 110 to 220 μmol/m^{2}s and were provided by cool-white fluorescent lamps. The sterile air was constantly supplied at the aeration rate range of 0.5–3 L/min.

Biomass concentration analysis

Algal biomass concentration was determined using the regression Eqs. (1–4) relating the optical density of the culture to the biomass dry weight (DW). The OD_{604nm} was measured with a Shimadzu UV-1750 spectrophotometer every 48 h during the cultivation and the DW was obtained by weighing the algal cells after washing two times with dH_2O and subsequently drying in an oven at 75 °C overnight until a constant weight is achieved.

$$W_{\mathrm{ASW}}(\mathrm{g/L}) = 2.4951 \times \mathrm{OD}_{604\mathrm{nm}} - 0.5121 \left(r^2 = 0.997\right) \quad (1)$$

$$W_{\mathrm{KOCK}}(\mathrm{g/L}) = 2.2808 \times \mathrm{OD}_{604\mathrm{nm}} - 0.5033 \left(r^2 = 0.9984\right) \quad (2)$$

$$W_{\mathrm{Pringshein\ II}}(\mathrm{g/L}) = 1.9341 \times \mathrm{OD}_{604\mathrm{nm}} - 0.2939 \left(r^2 = 0.9968\right) \quad (3)$$

$$W_{\mathrm{F/2}}(\mathrm{g/L}) = 1.7569 \times \mathrm{OD}_{604\mathrm{nm}} - 0.3922 \left(r^2 = 0.9979\right) \quad (4)$$

where W_{medium} (g/L) is the dry weight at different culture media, and OD_{604nm} is the absorbance of the suspension at 604 nm.

Lipids extraction

For the analysis of fatty acid content, the freeze-dried samples of algal biomass were extracted in a chloroform–methanol-water solution according to Bligh and Dyer's method (Bligh and Dyer 1959). Briefly, ~0.1 g lyophilized algal biomass was added to a solution consisting of 0.8 mL water, 2.0 mL methanol, and 1.0 mL chloroform, and the solution was intensely vibrated for 2 min. Thereafter, an additional 2.0 mL of chloroform and 2.0 mL water were added followed by vibrating for another 2 min. The solution was then centrifuged at 4500 rpm for 10 min. The substratum chloroform phase containing extracted lipids was transferred into a round-bottom flask, while the upper layer was again extracted with 2.0 mL of chloroform for two more times, and the chloroform phases were mixed together and heated in a nitrogen evaporator to remove the chloroform.

Esterification and analysis

In general, the fatty acids are linked to different lipid profiles during the biosynthesis (Merchuk et al. 1998);

however, only the fatty acid contents in the form of fatty acid methyl esters (FAMEs) were analyzed, which are enough for the aim of the present study.

FAMEs were prepared by direct esterification of the lipid in 2 mL 1 M KOH–methanol solution following the procedures described by Hartman and Lago (Hartman and Lago 1973) with modifications. Cyclohexane (5 mL, containing 0.15 g/L C17:0 ester as internal standard) was added to the solution, and the mixture was heated at 70 °C for 40 min with a reflux condenser. The mixture was cooled, and then extracted with 2 mL water, and the upper layer was separated for subsequent analysis.

FAME composition was measured utilizing a Shimadzu QP2010SE GC–MS instrument equipped with electron impact ionization (EI) detector and Rtx-5MS column (30 m × 0.25 mm × 0.25 μm). The running temperature was as follows: starting at 120 °C for 1 min, heating at 10 °C/min to 170 °C, maintained for 2 min, heating at 3 °C/min to 260 °C, and maintained for 5 min. The temperature of the injector and detector was kept constant at 260 °C, and the column flow rate and the split ratio were 13.9 mL/min and 5:1, respectively. The sample injection volume was 1 μL. The FAMEs were quantified by the internal standard method and calculated as follows:

$$\text{FA content} = \frac{\text{FA weight (mg)}}{\text{Algae powder weight (g)}} \quad (5)$$

$$\text{TFA or UFA content} = \frac{\text{TFA or UFA weight (mg)}}{\text{Algae powder weight (g)}}. \quad (6)$$

Results and discussion

Screening of the growth medium for *P. purpureum*

Different microalgae strains have different properties, and, therefore, can behave differently vis-à-vis the growth medium. Initially, the effect of culture medium on *P. purpureum* growth was investigated, and the results are presented in Fig. 1. The highest biomass concentration of 9.95 g/L was achieved after 22 days of cultivation in ASW medium, while a slightly lower biomass concentration was obtained when the algae was cultivated in Pringsheim II and KOCK medium (9.25 and 8.34 g/L, respectively). Unexpectedly, the growth rate of *P. purpureum* was rather slow in F/2 medium and the final biomass concentration obtained was only 2.58 g/L. However, it was observed that the microalgae growth was accompanied with autoflocculation of cells during cultivation in Pringsheim II medium. As the harvest of lipid requires certain level of biomass, it is imperative to maximize biomass production before lipid extraction to enhance the feasibility of ARA production. An appropriate medium

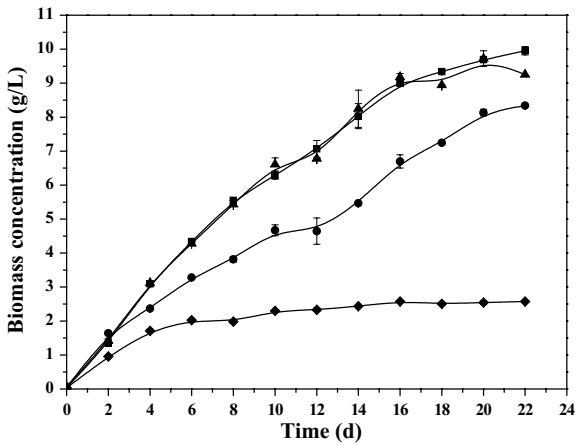

Fig. 1 Biomass production by *P. purpureum* under different culture media [ASW (*filled square*), KOCK (*filled circle*), Pringsheim II (*filled triangle*) and F/2 (*filled diamond*), and cultivation conditions: pH 7.6, 25 °C, 110 μmol/m^{2}s, 1 L/min aeration rate]

used for algae cultivation to accumulate as much as possible cells is the cornerstone for ARA production. As shown in Fig. 1, the cells of *P. purpureum* grew fast and stably in the ASW medium, the reason behind the fast and stable growth may be drawn from the fact that the ASW medium provided sufficient nutrition from simulated seawater ingredients providing the microalgae with the primal favorable growth environment. Thus, the ASW medium which contributed to higher biomass production is also believed promising for ARA production by *P. purpureum*.

The effect of aeration rate on biomass production and fatty acids/ARA accumulation by *P. purpureum*

The effect of aeration rate on *P. purpureum* growth, TFA content, and fatty acid yield was investigated in ASW culture medium, with the aeration rate range of 0.5–3 L/min. Higher aeration rate promoted the growth of *P. purpureum*, leading to final biomass yield of 13.07 g/L at the aeration rate of 3 L/min after 18 days of cultivation (Fig. 2). However, the biomass concentration obtained at the aeration rate of 0.5 L/min was only 5.55 g/L, implying that aeration rate has a significant effect on the growth of *P. purpureum.* These results are in agreement with the previously reported works. Merchuk et al. (1998) reported that the gas flow rate conduced to the accumulation of *Porphyridium* sp. cells. Obviously, aeration bubbles played an important role on mixing the culture, so that the mass transfer rate would be high enough for cells to exchange nutrients and extracellular metabolites during cultivation. As self-shadowing might occur, aeration bubbles contributed much for cells to get the chance to

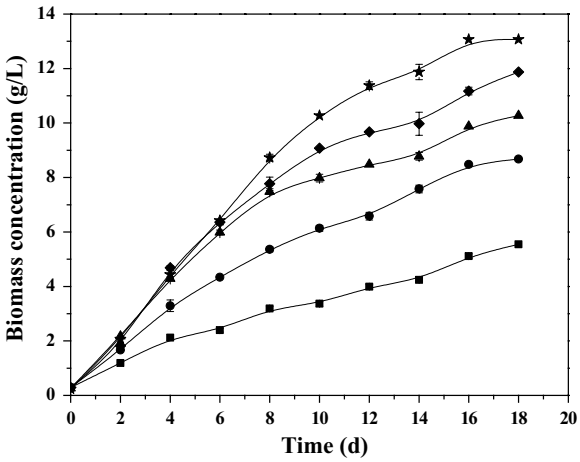

Fig. 2 *P. purpureum* growth under different aeration rates in ASW medium [0.5 L/min (*filled square*), 1 L/min (*filled circle*), 1.5 L/min (*filled triangle*), 2 L/min (*filled diamond*), and 3 L/min (*filled star*); cultivation conditions: 25 °C, 110 μmol/m^2s, pH 7.6]

access photons, which is the prerequisite for photosynthesis. However, higher aeration rate resulted in more intense evaporation and convection of culture medium (data not presented). The shear forces lead to cell disruption easily, which was also observed in the present work (Fig. 3a). The algae cells were bigger than the normal ones (Fig. 3b), and the process of cell disruption is apparently discernible as shown in Fig. 3a. Thus, aeration rate of 3 L/min was considered the highest aeration rate suitable for *P. purpureum* growth.

The highest TFA content of 43.62 mg/g was accumulated when the aeration rate was set at 1.5 L/min. The maximum TFA yield was 499.84 mg/L, achieved at the aeration rate of 3 L/min (Fig. 4). When *P. purpureum* was cultivated at the aeration rate of 2 L/min, the TFA yield was 399.76 mg/L, lower than 448.20 mg/L obtained at the aeration rate of 1.5 L/min. When the cells were cultivated at 0.5 and 1 L/min, much lower TFA yields of 136.97 and 216.70 mg/L, respectively, were acquired. Moreover, as the cultivation aeration rate rose from 0.5 to 3 L/min, the ARA production was immensely increased from 33.22 to 125.73 mg/L (Fig. 4), revealing that higher aeration rate played a positive role on ARA biosynthesis. Maximum volumetric content of TFAs and ARA was both obtained at cultivation with high aeration rate conditions possibly due to the constant supply of CO_2; which has been proven to play an important role in elevating the lipid content in algal cells (Oh et al. 2009; Akimoto et al. 1998). In addition, CO_2 was used for de novo fatty acid synthesis (Jiang et al. 2011). Furthermore, supplying CO_2 was favorable for the accumulation of polyunsaturated fatty acids as reported by Tang et al. (2011).

The effect of light intensity on biomass production and fatty acids/ARA accumulation by *P. purpureum*

It has been reported that light intensity has a significant effect on the growth and lipid production of *P. purpureum* (Koletzko and Braun 1991; Akimoto et al. 1998). The light intensities of 110, 165, and 220 μmol/m^2s with the aeration rates of 1 and 3 L/min were employed in the experiments of the current study. The maximum biomass concentration under high aeration rate (3 L/min) was much higher than that of low aeration rate (1 L/min) for all the light conditions tested (Fig. 5). The final biomass concentration at low aeration rate (1 L/min) was similar for all different light intensities evaluated, but the time for reaching the maximum concentration decreased as light intensity increased. For the algae cultured at high aeration rate (3 L/min), the log phase was shorter than

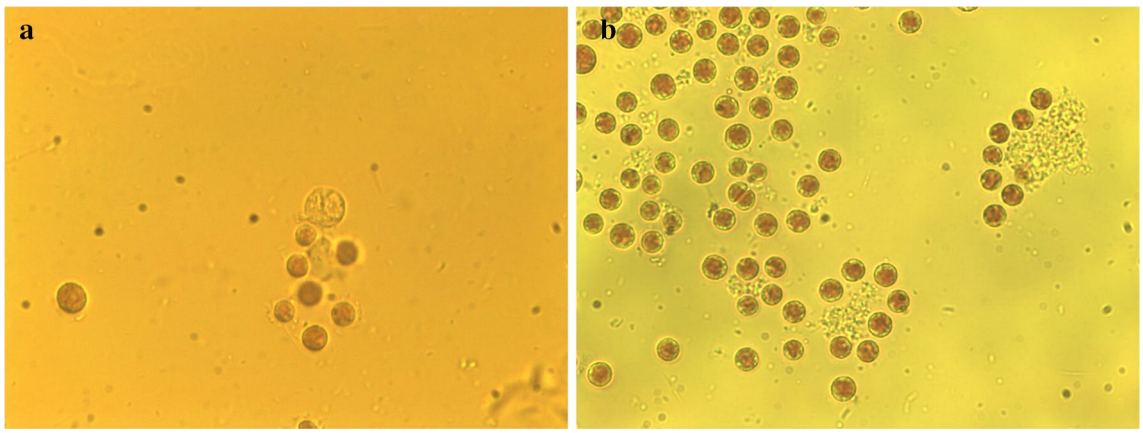

Fig. 3 Microscopic view of different *P. purpureum* cells (×400). **a** Algae cells grown in the aeration rate surpass 3 L/min, and **b** algae cells at the stationary phase under favorable culture conditions

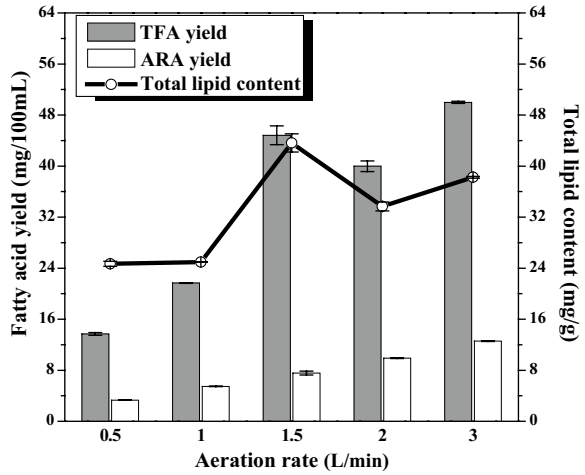

Fig. 4 Total fatty acid content and fatty acid yield accumulated in *P. purpureum* under different aeration rates in ASW medium

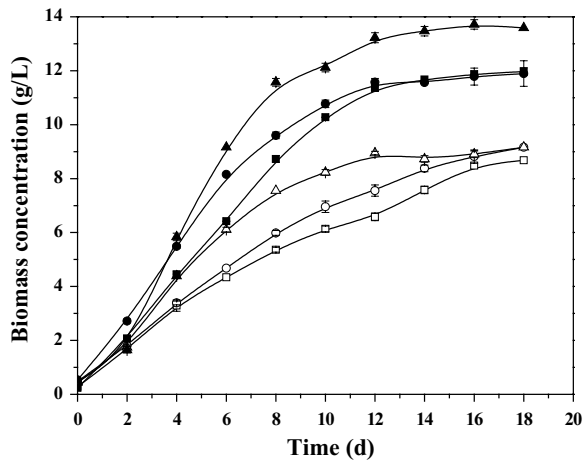

Fig. 5 Effect of light intensity on cell growth of *P. purpureum* at aeration rate of 1 and 3 L/min in ASW medium (1 L/min (*square*), 3 L/min (*filled square*) at 110 μmol/m^{2}s; 1 L/min (*circle*), 3 L/min (*filled circle*) at 165 μmol/m^{2}s; and 1 L/min (*triangle*), 3 L/min (*filled triangle*) at 220 μmol/m^{2}s) (Values are mean ± SD of two independent experiments)

the cases observed for low aeration rate. The highest algae cell yield reached 13.59 g/L at 220 μmol/m^{2}s, while the biomass accumulation at 110 and 165 μmol/m^{2}s was nearly equal but much lower than that achieved at the light intensity of 220 μmol/m^{2}s (Fig. 5).

Simultaneously, the effect of light intensity on TFA and ARA accumulation by *P. purpureum* was studied and the results are depicted in Fig. 6. In all cases, TFA kept accumulating as cultivation cycle extended, and the content notably increased between 7 and 10 days of cultivation. In earlier culture, the TFA content was almost the same at light intensities of 110 and 165 μmol/m^{2}s, and it was nearly half the level at 220 μmol/m^{2}s. In subsequent periods, the content showed inverse relation with light intensity, reaching 33.66 mg/g at 165 μmol/m^{2}s which was higher than that at low light intensity (110 μmol/m^{2}s). However, TFA accumulation was significantly inhibited by higher light intensity (220 μmol/m^{2}s). The highest TFA yield was obtained at light intensity of 165 μmol/m^{2}s (ca. 308.53 mg/L, Fig. 6a). However, due to higher growth rates (Fig. 5), cultivation at light intensity of 220 μmol/m^{2}s resulted in higher yield of TFA (ca. 219.04 mg/L, Fig. 6c) than that at 110 μmol/m^{2}s.

Equally, a step-up in ARA content related to cultivation period was observed, and the highest ARA content was obtained after 14 days under both the light intensities of 110 and 165 μmol/m^{2}s. The accumulation rate was relatively low for the first 10 days at all light conditions evaluated and drastically, an increase in ARA content occurred within the following 4 days (ca. 9.86 mg/g, Fig. 6b) under the light intensity of 165 μmol/m^{2}s. However, it was noticed that prolonged cultivation cycle resulted in gradual decrease in ARA content, which might be due to apoptosis and the very high oxidation rate of ARA. Moderate light condition (165 μmol/m^{2}s) led to the highest ARA content, while high light condition (220 μmol/m^{2}s) provided the minimum. Similar observations were made on the ARA yield, whereby the maximum ARA production was obtained at moderate light intensity (165 μmol/m^{2}s) and lower aeration rate (1 L/min) tested (ca. 82.65 mg/L, Fig. 6d).

Table 1 summarizes the cellular fatty acid contents of *P. purpureum* cultivated under various light intensities and aeration rates in ASW medium. The major fatty acids found are as follows: C16:0 (Palmitic acid), C18:0 (Stearic acid), C18:2 (Linoleic acid), C20:3 (11, 14, 17-Eicosatrienoic acid), C20:4 n-6 (ARA), and C20:5 n-3 (EPA). Similar results were reported elsewhere. The fatty acids profile of *P. cruentum* was determined as a function of light intensity (Koletzko and Braun 1991; Akimoto et al. 1998). Cohen's et al. (1988) reported that there was a positive correlation between light intensity and fatty acid unsaturation. In the case of *P. cruentum*, the content of 20:5 acid decreased under higher light intensity (Harwood and Russell 1984), whereas the content of 20:4 acid and biomass concentration increased. Meanwhile, the production of unsaturated C-16 and C-18 fatty acids, which are prevalent in glycolipids, have been enhanced by illumination (Rosenberg and Pecker 1964; Constantopoulos and Bloch 1967). Moreover, prolonging illumination time benefited the generation of C20:4 acid, and moderate light/dark cycle was also essential for algae growth and total lipid accumulation (Oh et al. 2009). Merchuk et al. (1998) reported that low gas (air containing 3 % CO_2)

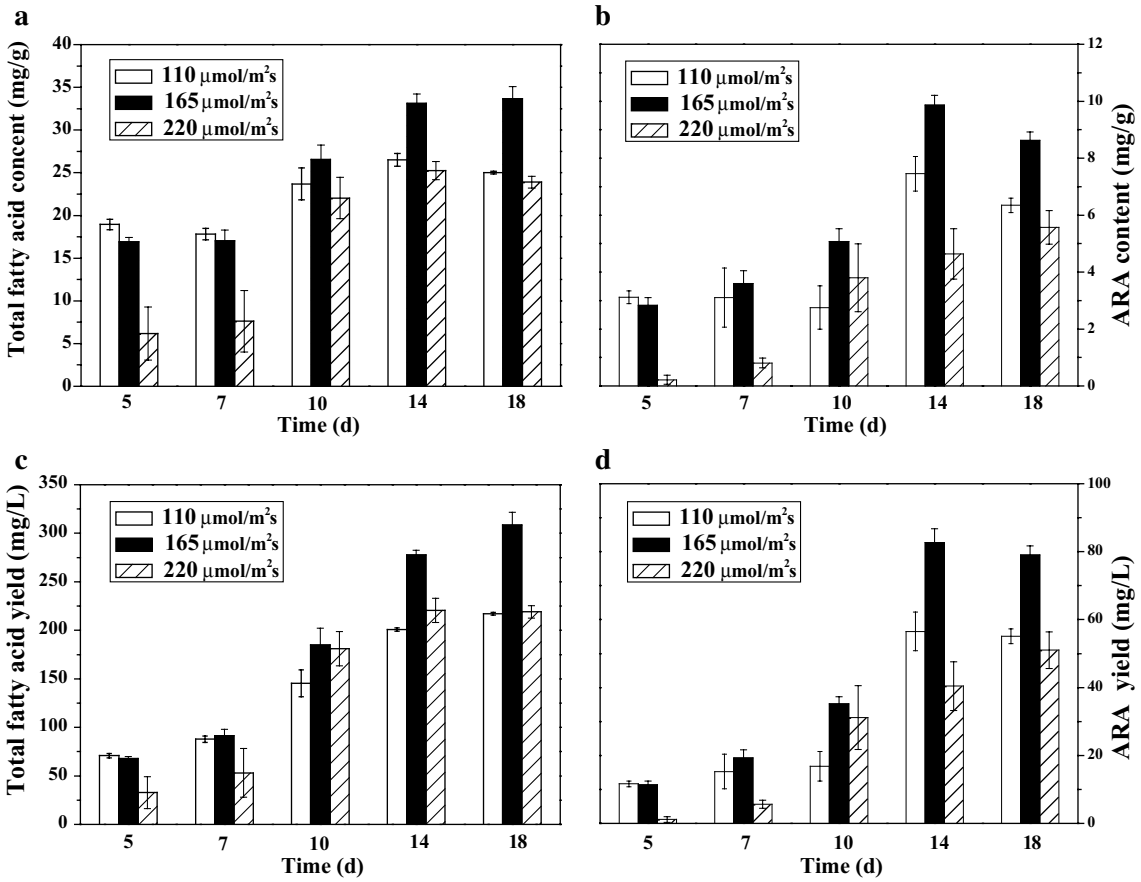

Fig. 6 Total fatty acids profiles (**a**) and arachidonic acid (**b**) content, and total fatty acids (**c**) and arachidonic acid (**d**) yield in *P. purpureum* cells at different light intensities. (Cultivation conditions: 25 °C, 1 L/min aeration rate, pH 7.6). (Values are mean ± SD of two independent experiments)

flow rate had a positive effect on biomass and polysaccharide production by *Porphyridium* sp. Muradyan et al. (2004) reported that an increase in the CO_2 concentration from 2 to 10 % for 1 day was enough to provoke an increase in the total fatty acids on dry weight basis by 30 % in the green algae *Dunaliella salina* (known to be susceptible to CO_2). Feeding microalgae with CO_2 enhance the fatty acids' elongation and desaturation. Furthermore, moderate CO_2 level (10 % CO_2) enhanced the biomass concentration of both *Scenedesmus obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2; however, high content of TFA and PUFAs was accumulated under high CO_2 levels (30–50 %) (Tang et al. 2011).

At lower aeration rate (1 L/min), the cellular content of ARA was always higher than that of EPA (5.57–8.62 mg/g relative to 2.23–3.25 mg/g biomass). The highest cellular TFA and ARA contents were both obtained at 165 μmol/m^2s (33.66 and 8.62 mg/g, respectively). When the light intensity was raised from 110 to 165 μmol/m^2s, a sharp increase in cellular ARA content by 37 % was achieved. However, it decreased drastically by 35 % at higher light intensity (220 μmol/m^2s). On the other hand, a step-up in TFA content by 35 % was obtained, as the light intensity was raised from 110 to 165 μmol/m^2s, whereas it decreased by 29 % when light intensity was further raised to 220 μmol/m^2s. In the case of high aeration rate (3 L/min), the cellular ARA and TFA contents were higher than that obtained under lower aeration rate (1 L/min). However, slight differences in the EPA content were observed at both aeration rates. Meanwhile, there was a little variation in ARA and TFA contents when the light intensity was raised from 110 to 165 μmol/m^2s. The maximum ARA content (9.59 mg/g) was obtained at low light intensity (110 μmol/m^2s), whilst the maximum TFA content (39.82 mg/g) was achieved at 165 μmol/m^2s. Higher light intensity (220 μmol/m^2s) led to marked decline of both ARA and TFA contents, but it took shorter time for the cells to approach the stationary phase (Fig. 4). Thus, all levels of fatty acid contents detected at 18 days were higher than that detected at 14 days of cultivation under light intensity of 220 μmol/m^2s. At higher light intensity, the double bond index (DBI) of cellular fatty acids decreased gradually. Whereas, the content ratio of C20:4 acid to C20:5 acid increased with increased light intensity

Table 1 Fatty acid contents in *P. purpureum* under different light intensities and aeration rates

Aeration rate	1 L/min			3 L/min			
Light intensity (μmol/m^{2}s)	110	165	220	110	165	220[a]	220[b]
Fatty acid content (mg/g)							
C16:0	9.16	13.51	9.94	11.90	13.32	8.68	10.90
C18:0	1.19	2.04	1.37	1.49	2.32	1.01	1.32
C18:2	3.20	4.02	2.87	7.65	8.38	4.16	5.64
C20:3	0.53	0.66	0.52	1.49	1.26	1.15	1.64
C20:4	6.28	8.62	5.57	9.59	9.03	4.41	5.72
C20:5	3.25	2.83	2.23	3.72	2.60	2.31	2.80
Others[c]	1.33	1.98	1.40	2.30	2.90	1.92	2.25
Total	24.94	33.66	23.90	38.15	39.82	23.63	30.28
DBI[d]	1.39	1.15	1.08	1.82	1.53	1.43	1.46
20:4/20:5 ratio	1.93	3.05	2.50	2.58	3.47	1.91	2.05

Values are mean ± SD of two independent experiments

[a] Cultivated at 3 L/min for 18 days

[b] Cultivated at 3 L/min for 14 days

[c] Summary of 14:0, 15:0, 16:1, 18:1, 18:3, and 20:2

[d] Double bond index of cellular fatty acids (DBI = Unsaturated fatty acids/saturated fatty acids)

from 110 to 165 μmol/m^{2}s; however, the ratio decreased with further increase of light intensity to 220 μmol/m^{2}s. High aeration rate together with low light intensity conditions contributed to ARA biosynthesis in *P. purpureum* cells.

Due to similar growth rates (Fig. 5) and cellular fatty acid contents (Table 1), algae cultivation under 110 and 165 μmol/m^{2}s light intensity resulted in similar contents of both TFA (ca. 458.90 mg/L at 110 μmol/m^{2}s; 473.44 mg/L at 165 μmol/m^{2}s, Fig. 6) and ARA (115.47 mg/L at 110 μmol/m^{2}s; 107.49 mg/L at 165 μmol/m^{2}s, Fig. 7), accumulation from total biomass (i.e., volumetric content). However, cultivation under light intensity of 220 μmol/m^{2}s, both TFA and ARA volumetric contents were rather low due to low cellular contents (Table 1) in spite of the high growth rate (Fig. 5).

Discussion: light intensity—one of the key factors affecting the biomass production and fatty acids/ARA accumulation by *P. purpureum*

In algae cultivation of the current work, higher light intensity resulted in more biomass accumulation, which was in agreement with previous works reported about the effects of light intensity on cell growth and the fatty acid content in algae cells (Koletzko and Braun 1991; Akimoto et al. 1998; Velea et al. 2011). Akimoto et al. (1998) revealed a clear correlation between light intensity and cellular fatty acid content. The authors found that ARA was the main polyunsaturated fatty acid under sub-optimal growth conditions of light intensity, while cellular EPA content decreased sharply. The variations of algal EPA and ARA contents under different growth conditions were also observed in the present study. As presented in Table 1, double bond index (DBI) decreased more obviously as light intensity increased, which indicated that higher light intensity inhibited the synthesis of polyunsaturated fatty acids in *P. purpureum* cells. More interestingly, the ratio of two main polyunsaturated fatty acids, ARA to EPA peaked in middle light condition (165 μmol/m^{2}s), suggesting that *P. purpureum* is more favorable for ARA biosynthesis rather than EPA at moderate light condition. Moreover, when *P. purpureum*

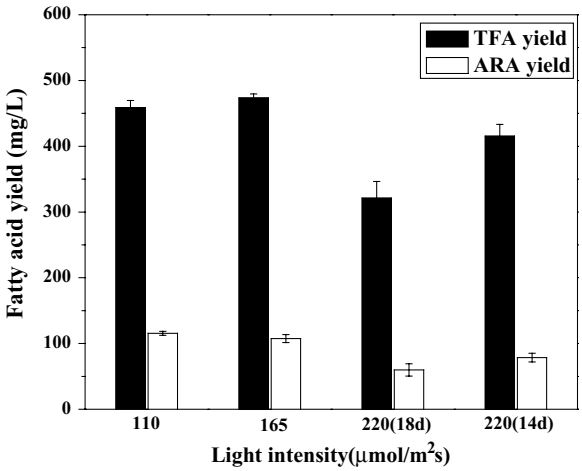

Fig. 7 Volumetric contents of TFAs and ARA in *P. purpureum* after 18-days of cultivation under different light intensities. (Cultivation conditions: 25 °C, 3 L/min aeration rate). (Values are mean ± SD of two independent experiments)

was cultured with a moderate aeration rate (1 L/min), light intensity played a prominent role in synthesis of fatty acids. The maximum cellular contents of TFAs were achieved at moderate light condition, while satisfying ARA content was obtained at low light condition under high aeration rate (Table 1). It was reported that high light intensity and low gas flow rates contributed to higher productivities of both biomass and polysaccharides in *Porphyridium* sp. (Merchuk et al. 1998). Particularly, high light intensity was a key factor for extracellular polysaccharides production by *P. purpureum* (Liqin et al. 2008). Therefore, the inhibition of fatty acids synthesis caused by high light intensity was likely due to the carbon source consumption for the synthesis of polysaccharides. However, further studies are necessary to reveal this mechanism. Oh et al. (2009) reported that the lipids production was only partially or non-growth related with the cell growth process.

In the present study, the cell growth rate and fatty acids production upon aeration rate indicated that higher cell quantity and large amount of fatty acids could be produced under moderate light conditions, whereas fatty acids synthesis was discontinued once approaching the stationary phase and the content decreased during the apoptosis phase (Table 1; Figs. 6, 7).

Conclusion

This work revealed that high aeration rate with moderate light intensity is promotive factor for biomass and ARA production by *P. purpureum*; and this provide a promising technical support for the production of ARA. However, additional investigations are needed, including studies on the lipid profile, and highlight tolerance mechanisms by *P. purpureum,* that may further enhance the production of valuable PUFA by this algae. Furthermore, *P. purpureum* produces not only fatty acids, but also several other high value biochemicals, such as polysaccharides and phycoerythrin. Comprehensive applications of microalgae biomass, combining several targeted products or services, would be the mainstream of microalgae biotechnology-based industries.

Abbreviations

PUFA: polyunsaturated fatty acid; ARA: arachidonic acid; EPA: eicosapentaenoic acid; TFA: total fatty acid; DW: dry weight; OD: optical density; FAME: fatty acid methyl ester; UFA: unsaturated fatty acid; DBI: double bond index; EI: electron impact ionization.

Authors' contributions

GMS and XHZ designed the experiments, GMS, JYC, and XYG performed the experiments, GMS drafted the manuscript, ZL, XHZ, YS, and YHL contributed to the discussion, and ZL and XHZ gave important feedback on draft versions of several sections and improved the manuscript by critical revision. XHZ and LL supervised the research and wrote the final version of the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

Ahern TJ, Katoh S, Sada E (1983) Arachidonic acid production by the red algae *Porphyridium cruentum*. Biotechnol Bioeng 25(4):1057–1070

Akimoto M, Shirai A, Ohtaguchi K, Koide K (1998) Carbon dioxide fixation and polyunsaturated fatty acid production by the red algae *Porphyridium cruentum*. Appl Biochem Biotechnol 73(2–3):269–278

Barclay W, Meager K, Abril J (1994) Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms. J Appl Phycol 6(2):123–129

Bennett PR, Rose MP, Myatt L, Elder MG (1987) Preterm labor: stimulation of arachidonic acid metabolism in human amnion cells by bacterial products. Am J Obstetrics Gynecology 156(3):649–655

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37(8):911–917

Cohen Z, Vonshak A, Richmond A (1988) Effect of environmental conditions on fatty acid composition of the red algae *Porphyridium cruentum*: correlation to growth rate. J Phycol 24(3):328–332

Constantopoulos G, Bloch K (1967) Effect of light intensity on the lipid composition of *Euglena gracilis*. J Biol Chem 242(15):3538–3542

De Bravo M, De Antueno R, Toledo J, De Tomas M, Mercuri O, Quintans C (1991) Effects of an eicosapentaenoic and docosahexaenoic acid concentrate on a human lung carcinoma grown in nude mice. Lipids 26(11):866–870

Dyerberg J (1986) Linolenate-derived Polyunsaturated Fatty Acids and Prevention of Atherosclerosis. Nutr Rev 44(4):125–134

Ernest G, Pringsheim O (1949) The growth requirements of *Porphyridium cruentum*: with remarks on the ecology of brackish water algae. J Ecol: 57–64

Fuentes MR, Fernández GA, Pérez JS, Guerrero JG (2000) Biomass nutrient profiles of the microalgae *Porphyridium cruentum*. Food Chem 70(3):345–353

Ginzberg A, Cohen M, Sod-Moriah UA, Shany S, Rosenshtrauch A, Arad SM (2000) Chickens fed with biomass of the red microalgae *Porphyridium* sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. J Appl Phycol 12(3–5):325–330

Hartman L, Lago R (1973) Rapid preparation of fatty acid methyl esters from lipids. Lab Pract 22(6):475

Harwood J, Russell N (1984) Lipids in Plants and Microbes. Allen and Unwin, London

Higashiyama K, Fujikawa S, Park EY, Shimizu S (2002) Production of arachidonic acid by *Mortierella* fungi. Biotechnol Bioprocess Eng 7(5):252–262

Huo J-Z, Nelis HJ, Lavens P, Sorgeloos P, De Leenheer A (1997) Determination of E vitamers in microalgae using high-performance liquid chromatography with fluorescence detection. J Chromatogr A 782(1):63–68

Jiang L, Luo S, Fan X, Yang Z, Guo R (2011) Biomass and lipid production of marine microalgae using municipal wastewater and high concentration of CO_2. Appl Energy 88(10):3336–3341

Jones RF, Speer HL, Kury W (1963) Studies on the growth of the red algae *Porphyridium cruentum*. Physiol Plant 16(3):636–643

Koch W (1952) Untersuchungen an bakterienfreien Masenkulturen der einzelligen Rotalge *Porphyridium cruentum* Naegeli. Archiv für Mikrobiologie 18(1–4):232–241

Koletzko B, Braun M (1991) Arachidonic acid and early human growth: is there a relation? Ann Nutr Metab 35(3):128–131

Kromhout D, Bosschieter EB, Coulander CdL (1985) The inverse relation between fish consumption and 20-year mortality from coronary heart disease. N Engl J Med 312(19):1205–1209

Liqin S, Changhai W, Lei S Effects of light regime on extracellular polysaccharide production by *Porphyridium cruentum* cultured in flat plate photobioreactors. In: Bioinformatics and Biomedical Engineering, 2008. ICBBE 2008. The 2nd International Conference on, 2008. IEEE, pp 1488–1491

Mendoza H, Martel A, Del Río MJ, Reina GG (1999) Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. J Appl Phycol 11(1):15–19

Merchuk J, Ronen M, Giris S, Arad SM (1998) Light/dark cycles in the growth of the red microalgae *Porphyridium* sp. Biotechnol Bioeng 59(6):705–713

Muradyan E, Klyachko-Gurvich G, Tsoglin L, Sergeyenko T, Pronina N (2004) Changes in lipid metabolism during adaptation of the *Dunaliella salina* photosynthetic apparatus to high CO_2 concentration. Russian J Plant Physiol 51(1):53–62

Nichols B, Appleby R (1969) The distribution and biosynthesis of arachidonic acid in algae. Phytochem 8(10):1907–1915

Oh SH, Han JG, Kim Y, Ha JH, Kim SS, Jeong MH, Jeong HS, Kim NY, Cho JS, Yoon WB (2009) Lipid production in *Porphyridium cruentum* grown under different culture conditions. J Biosci Bioeng 108(5):429–434

Rosenberg A, Pecker M (1964) Lipid Alterations in *Euglena gracilis* Cells During Light-induced Greening*. Biochem 3(2):254–258

Shinmen Y, Shimizu S, Akimoto K, Kawashima H, Yamada H (1989) Production of arachidonic acid by *Mortierella* fungi. Appl Microbiol Biotechnol 31(1):11–16

Sukenik A (1999) Production of eicosapentaenoic acid by the marine eustigmatophyte *Nannochloropsis*. Chemicals from microalgae: 41–56

Tang D, Han W, Li P, Miao X, Zhong J (2011) CO_2 biofixation and fatty acid composition of Scenedesmus obliquus and Chlorella pyrenoidosa in response to different CO_2 levels. Bioresour Technol 102(3):3071–3076

Thompson GA (1996) Lipids and membrane function in green algae. Biochim Biophys Acta 1302(1):17–45

Velea S, Ilie L, Filipescu L (2011) Optimization of *Porphyridium purpureum* culture growth using two variables experimental design: light and sodium bicarbonate. UPB Sci Bull Series B 73(4):81–94