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Concurrent production of carotenoids and lipid by a filamentous microalga *Trentepohlia arborum*

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HIGHLIGHTS

- *T. arborum* could simultaneously synthesize high amounts of carotenoids and lipid.
- Carotenoids (mainly β-carotene) accounted for *ca.* 5% in the microalgal lipid.
- First report of lipid and fatty acid accumulation characteristics in *T. arborum*.

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ABSTRACT

During the study of *Trentepohlia arborum* it became clear that its cells are rich in lipids and carotenoids. Thus, lipid content, composition and fatty acids profiles in individual lipid classes, as well as pigment profiles, responding to different culture conditions, were further investigated. The results showed that the predominant carotenoids and lipid fraction in total lipid in this study was β-carotene and TAG, respectively. The lipid content increased significantly under high light while nitrogen-replete conditions induced the highest carotenoids content. However, only with a double stress of high light and nitrogen-deficiency it was possible to maximize the productivities of both carotenoids and lipids. Carotenoids (mainly β-carotene) accounted for *ca.* 5% of the microalgal lipid under the double stress. Data herein show the potential of *T. arborum* for the production of both lipids and carotenoids, and hence provide an appropriate way to produce different products from *T. arborum*.

1. Introduction

Interest in microalgae for the production of biofuels, food and feed has increased in recent decades (Hu et al., 2008). Because of the relatively low price of fossil fuels, microalgae based biofuel production is currently uneconomical; it seems to be more realistic to produce biomass from microalgae as food and feed or as intermediate target (Klok et al., 2014; Urreta et al., 2014).

Many microalgae can biosynthesize and accumulate high levels of lipids in form of glycerolipids, such as triacylglycerols (TAG), phospholipids (PLs) and glycolipids (GLs) (Thompson, 1996). TAG represent the main fraction of edible oils (Gunstone, 2011), and PLs are generally used as aquaculture feed and accessories of functional food (Chen et al., 2008), because of this, the potential application of algal lipids are determined by the relative contents of individual lipid classes. Several microalgae species contain fatty acid profiles rich in special high-value polyunsaturated fatty acids (Chen et al., 2008; Chen, 2012), or general fatty acid similar to other more common vegetable oils (Thompson, 1996; Draaisma et al., 2013), hence different algal lipids would be used for bulk or specific applications. Thanks to their high content in carotenoid and tocopherol, recently, algal lipids have been used to improve the stability of vegetable oils, (Gouveia et al., 2007; Limon et al., 2015). Therefore, the applications of algal lipid are greatly expanded.

According to some reports, the lipids and pigments contents and compositions, as well as the fatty acids profiles in individual lipid class, changes significantly with different culture conditions (Hu et al., 2008; Chen, 2012). It should be noted that so far almost only unicellular microalgal species have been studied. Very few previous reports are available in the literature concerning multicellular microalgae, especially filamentous microalgae, even though these are proved to be superior to unicellular microalgae in resistant to grazers and cost for harvest (Wang et al., 2014; Chen et al., 2015).

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Trentepohlia arborum, a filamentous microalga, commonly grows aerially or subaerially in tropical, subtropical and temperate regions (Nash et al., 1987; Rindi et al., 2005; Allali et al., 2013). The morphological study of *T. arborum* and other species in this genus demonstrated the presence of large and copious lipid bodies (LBs) in the cytoplasm (Ho et al., 1983). This suggested that this filamentous microalga might have ability to generate high levels of lipid (Liu et al., 2012; Chen et al., 2015). Physiological aspects such as pigments compositions and the formation process of lipid bodies in *Trentepohlia* have been thoroughly studied (Ho et al., 1983; Chen et al., 2015), whereas the lipid content and composition and fatty acid files in *T. arborum*, or any other species of this genus, have not yet been examined. In addition, large amount of carotenoids was observed in *T. arborum* (Chen et al., 2015), mainly stored in the form of lipid bodies (Ho et al., 1983). That is to say, this microalga could accumulate carotenoids and lipids simultaneously.

In this study, both lipids and carotenoids productivities in filamentous microalgae *T. arborum* under various culture conditions were investigated. Additionally, the lipid composition and fatty acid profiles of individual lipid class responding to different culture conditions were also investigated. This study result is critical to promote the practical application of *T. arborum* in the functional and health food industry.

2. Materials and methods

2.1. Strains and culture

The filamentous algae *T. arborum* FACHB 1792 was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan, Hubei, China. The alga was maintained in solid BG11 medium containing 1% agar and cultured in liquid modified Bold's basal medium (BBM) under constant temperature of 25 °C and light irradiance of 50 μmol/m^2/s.

The BBM was prepared following Abe's report (Abe et al., 2007). The medium contained 1.0 g NH_4Cl, 175 mg KH_2PO_4,75 mg K_2HPO_4, 25 mg $MgSO_4{\cdot}7H_2O$, 25 mg NaCl, 50 mg EDTA, 30 mg KOH, 5 mg $FeSO_4{\cdot}7H_2O$ and 11 mg H_3BO_3 in 1 L of deionized (DI) water (pH adjusted prior to autoclaving to 8.0).

2.2. Experiment and sample

The alga strain was firstly grown in liquid BBM for 7 days and collected by gravity precipitation as follows; the cells were re-suspended in fresh BBM and then cultured in a column bioreactor (3 cm diameter) with continuous bubbling by compressed air (2% CO_2, v/v). Cultures of *T. arborum* were grown under different levels of nitrogen supplementation at a low light intensity of 35 μmol/m^2/s (LL) and at a higher light intensity of 150 μmol/m^2/s (HL). The nitrogen supplementation levels were set as follows: 0 (−N), 9.4 (+0.5N) and 18.7 (+1.0N) mmol/L ammonium chloride.

The algal cells were sampled every three days and then pelleted by filtration. The cells were lyophilized at −80 °C using a lyophilizer and were stored in a cool and dark place before analysis.

2.3. Biomass determination

Algae cells in 3–5 mL culture were collected using a pre-weighted nitrocellulose membrane filters (0.25 μm, NC membrane) under vacuum, and then dried to constant weight at 105 °C over 2 h. The biomass dry weight was calculated as the difference between the weight of the membrane filter with and without cells on it.

2.4. Morphology observation

Images were taken with an Olympus SZX16 stereomicroscope and an Olympus BX 51 microscope; both were equipped with a DP 72 digital camera. The filaments length, number of branches and the size of colonies were obtained by measuring 50–100 filaments or colonies using the Olympus microscope and the DP2-BSW Ver. 2.2 software (Olympus, Japan).

2.5. Analysis of pigment

Pigments in cells were extracted with dimethyl sulfoxide (DMSO) after grinding under liquid nitrogen protection. Total amounts of chlorophyll and carotenoids were estimated using a Varian 50 Bio UV–Visible spectrophotometer (Varion Inc., US). Then the chlorophyll and total carotenoids content were calculated with the following equations (Wellburn, 1994):

$$\text{Chlorophyll } a\ (C_a)(\mu g/mL) = 12.19(OD_{665}) - 3.45(OD_{649}) \quad (1)$$

$$\text{Chlorophyll } b\ (C_b)(\mu g/mL) = 21.99(OD_{649}) - 5.32(OD_{665}) \quad (2)$$

$$\text{Total carotenoids } (C_{x+c})(\mu g/mL) = (1000OD_{480} - 2.14C_a - 70.16C_b)/220 \quad (3)$$

$$\text{Car/Chl ratio} = \text{Total carotenoids}/(\text{Chlorophyll } a + \text{Chlorophyll } b) \quad (4)$$

The volumetric productivity of total carotenoids was calculated as follows:

$$\text{Volumetric productivity } (mg/L/d) = (C_t - C_o)/t \quad (5)$$

where the C_t and C_0 represented the volumetric content of carotenoids (mg carotenoids in 1 L algal culture, mg/L) at day t and day 0 respectively, and t represented the time of cultivation (days).

Pigment profiles were analyzed using HPLC (Waters 1525, US) equipped with a 996 photodiode array detector (Waters 2998, US) following the methodology described by Yuan et al. (2002). Separation of carotenoids was achieved by the following gradient procedure: 0% of B for 8 min; a linear gradient from 0% to 100% of B within 6 min; 100% of B for 40 min, at a flow rate of 1.0 mL/min. The mobile phase consisted of an eluent A (dichlor omethane:methanol:acetonitrile:water, 5.0:85.0:5.5:4.5, v/v) and

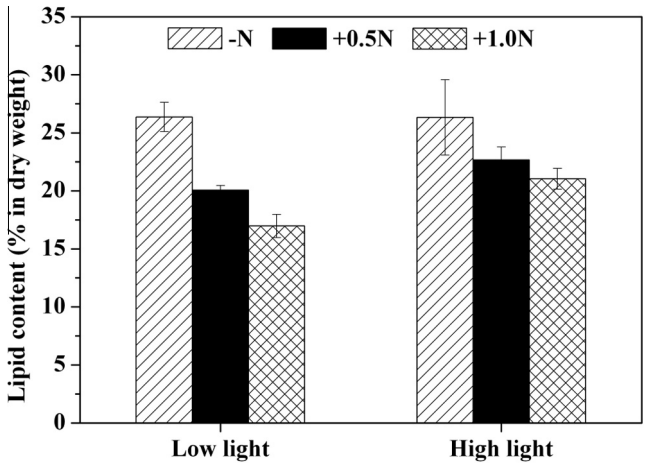

Fig. 1. The lipids contents of microalga *Trentepohlia arborum* grown in BBM containing 0 (−N), 9.4 (+0.5N) and 18.7 (+1.0N) mmol/L ammonium chloride, and were illuminated under 35 (low light) or 150 μmol photons/m^2/s (high light). Data are expressed as means ± SD of three replicates.

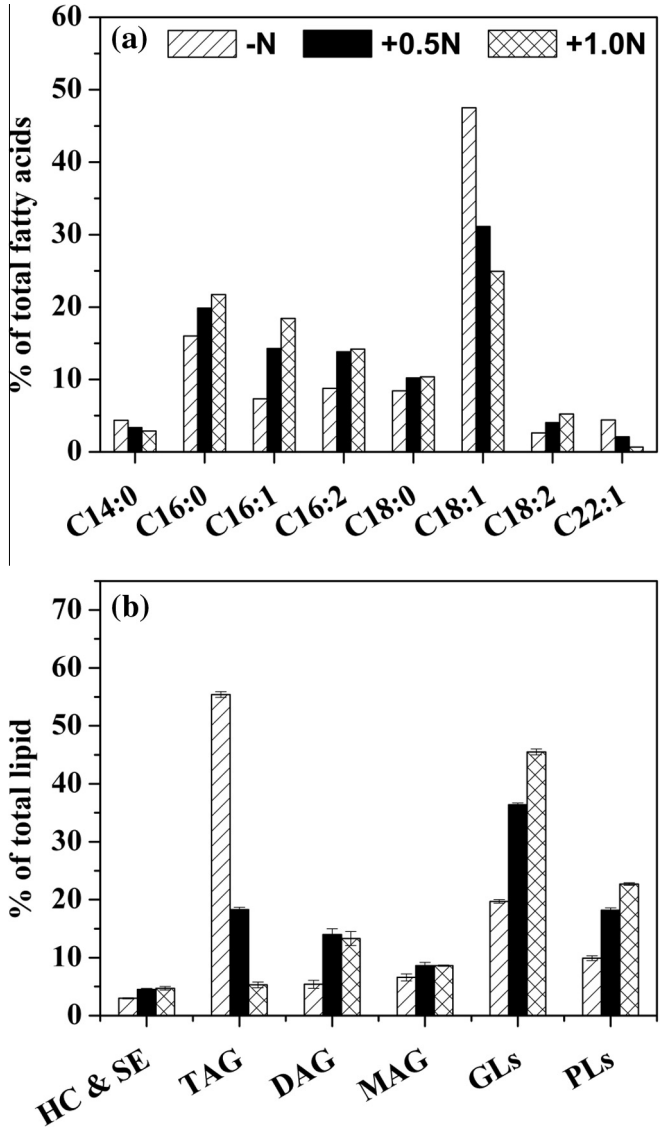

Fig. 2. The fatty acid profiles (a) and individual lipid class (b) in total lipids from microalga *Trentepohlia arborum* grown in BBM containing 0 (−N), 9.4 (+0.5N) and 18.7 (+1.0N) mmol/L ammonium chloride, and were illuminated under 35 μmol photons/m^2/s. Data are expressed as means ± SD of three replicates. HC and SE, hydrocarbon and sterol ester; TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; GLs, glycolipids; PLs, phospholipids.

an eluent B (dichloromethane:methanol:acetonitrile:water, 25.0:28.0:42.5:4.5, v/v). The pigment extracts (20 μL) were injected onto a Beckman Ultrasphere C18 reversed phase column (5 μm; 250 * 4.6 mm) at 30 °C. The absorption spectra of carotenoids were displayed between 250 and 700 nm. The individual pigments in samples were identified by comparison of retentions times and spectrums with standards (Aladdin), and then the estimation of the quantity of the each carotenoid was carried out from the chromatogram peak areas.

2.6. Analysis of lipid and fatty acids

Total lipid was extracted with a solvent mixture of chloroform and methanol (1:2, v/v), and then analyzed using gravimetric method (Bligh and Dyer, 1959). For lipid class analysis, lipid samples were dissolved in chloroform to a concentration of 5 mg/mL. They were spotted onto thin-layer chromatography (TLC) plates coated with silica gel, and then developed in a solvent system of benzene:chloroform:acetic acid (150:60:2, v/v/v) for the first migration to 7 cm, followed by a solution of benzene:hexane (50:50, v/v) for the second migration to 10 cm. The lipid bands were stained by iodine vapor, and then each band was scraped off using a razor blade and to a tube containing 1 mL of chloroform and then lipid content of each band was determined gravimetrically. Alternatively, lipid class separation and analysis was carried out using a TLC coupled with a Flame Ionization Detector system (TLC–FID, MK-6, Iatron Laboratories, Inc., Japan) as described by Chen et al. (2012).

Total lipid and individual lipids were trans-methylated with 2% H_2SO_4 in methanol at 85 °C for 2.5 h. Then the fatty acids profiles were measured using gas chromatography (7890C, Agilent, US) equipped with a mass detector (5975A, Agilent, US). The fatty acid methyl esters were identified by comparison of retention times and mass spectrogram with standards (Sigma), and were quantified as follows:

$$W = \frac{m_S \times f_i \times A_i}{m \times A_S} \times 100\% \quad (6)$$

where W is the relative content of each fatty acid, presented as a percentage of total fatty acid; m_S is the mass of internal standard, f_i is the coefficient value of section i, A_i is the peak area of section i, m is the weight of sample and A_S is the area of standard.

2.7. Statistical analysis

Statistical analysis was carried out using SPSS 11.0 software (SPSS Inc., Chicago, US). ANOVA was performed to evaluate significance of individual differences with a probability threshold of 0.05, followed by a Post-Hoc Tukey test.

3. Results and discussion

3.1. Lipid accumulation and fatty acid profiles related to light intensity and nitrogen concentration

In order to examine the ability to produce lipid and valuable pigments, the microalga *T. arborum* was cultured in BBM medium

Table 1
Fatty acid profiling in individual lipid class of *Trentepohlia arborum*.[a]

Lipid class	Distribution (% total lipid)	Fatty acid composition (% in total fatty acid)												
		C14:0	C16:0	C16:1	C16:2	ΣC16	C18:0	C18:1	C18:2	ΣC18	C22:1	Σ SFA	Σ MUFA	Σ PUFA
TAG [b]	55.4	5.8	17	7.5	5.9	30.4	4.3	51.1	2	57.4	6.3	27.1	64.9	7.9
DAG	5.42	10.1	24.4	8.2	5.5	38.1	4.3	45.6	1.8	51.7	0.1	38.8	53.9	7.3
MAG	6.55	2.3	56.5	3.1	3.0	62.6	32.1	2.8	n.d.[c]	34.9	0.2	90.9	6.1	3.0
GLs	19.8	2.9	47.3	2.5	14.6	64.4	26.5	6.2	n.d.	32.7	n.d.	76.7	8.7	14.6
PLs	9.8	13.2	63.7	4.1	1.5	69.3	11.4	5.5	0.6	17.5	n.d.	88.3	9.6	2.1
Total lipid	100	4.4	18.1	7.3	7.7	33.1	8.4	47.5	1.6	57.5	4.4	30.9	59.2	9.3

[a] The lipid was extracted from algae cultured in [LL−N].
[b] TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; GLs, glycolipids; PLs, phospholipids.
[c] n.d. not detected.

for 14 days with different levels of nitrogen supplement under two light intensities. Many lipid bodies (LBs) were observed in *T. arborum* cells grown in nitrogen stress conditions (Supplementary Material Fig. 1), meaning that the strain contains high amounts of oil. The total lipid content for *T. arborum* with different levels of nitrogen supply under low and high light intensities was analyzed (Fig. 1). Regardless of the light intensity considered, the lower the nitrogen supplement, the higher the total lipid content. In particular, the lipid content was *ca.* 55.3% higher in [LL−N] cultures than in [LL+1.0N] cultures. On the other hand, the alga accumulated *ca.* 13.0% and 23.9% more lipid under high light than low light in +1.0N and +0.5N cultures, whereas equal amount of lipid were observed under either two light intensities in −N cultures. Although both nitrogen deficiency and strong light could promote

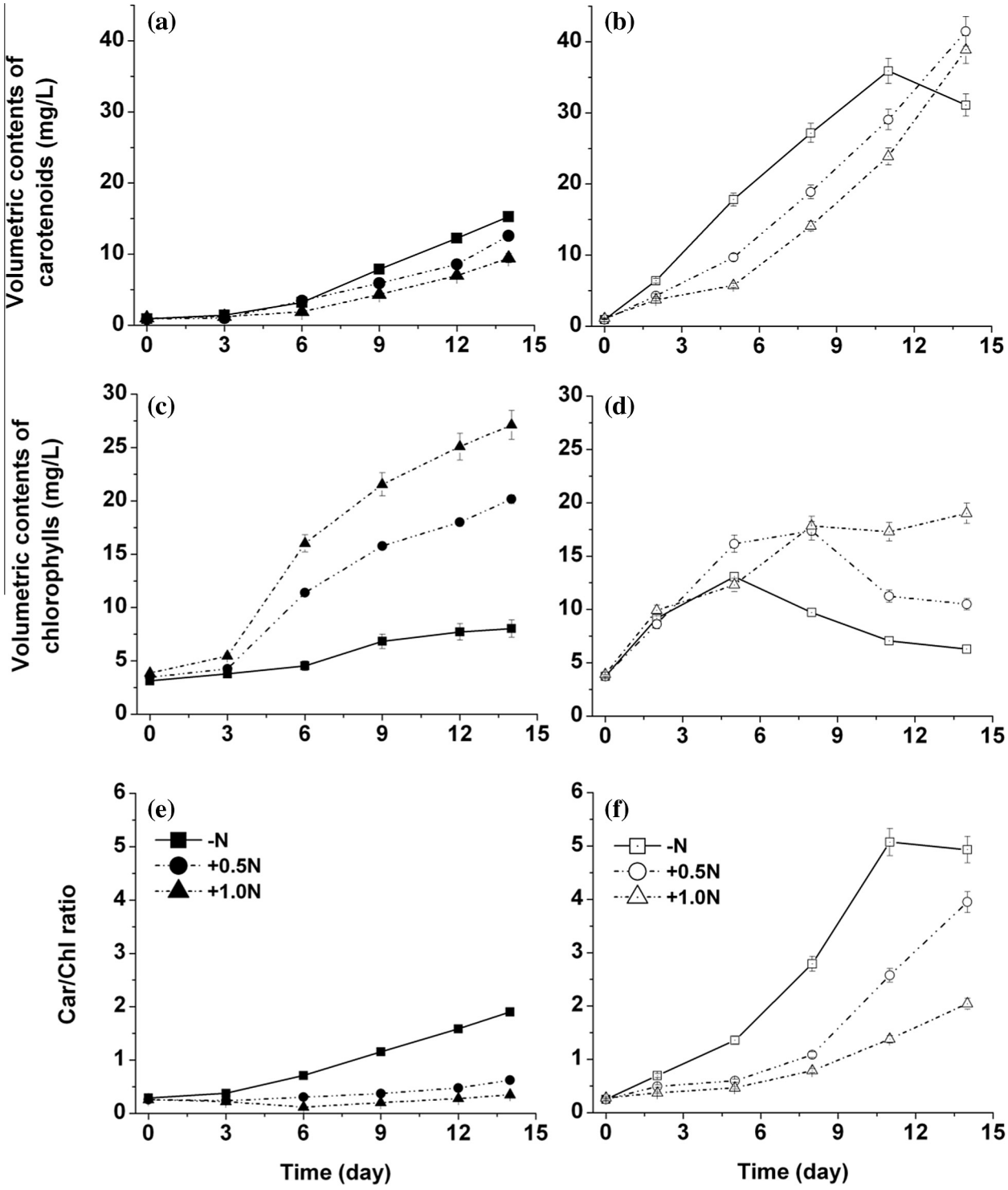

Fig. 3. Evolution over time of the volumetric content of carotenoids (a, b), chlorophyll (c, d) and carotenoids/chlorophyll ratio (e, f) in microalga *Trentepohlia arborum* grown in BBM containing 0 (−N, square), 9.4 (+0.5N, circle) and 18.7 (+1.0N, triangle) mmol/L ammonium chloride. Illumination was provided at 35 (closed symbols) or 150 μmol photons/m^2/s (open symbols).

lipid accumulation in *T. arborum*, there is no combined effect on lipid accumulation responding to double stress of high light and nitrogen deficiency. However, it seems clear that lipid accumulation in *T. arborum* was probably more sensitive to nitrogen starvation stress rather than strong light irradiance. Even though the lipid content in *T. arborum* is lower than that reported for the filamentous microalgae *Tribonema minus* (Wang et al., 2014), it is still comparable to filamentous algal *Spirogyra singularis* (Nabavi et al., 2013), and even much higher than *Spirulina platensis* (Zhang et al., 2015) and *Limnothrix* sp. (Economou et al., 2015).

Fig. 2a shows the fatty acid profiles of alga *T. arborum* in different nitrogen supply cultures at low light. It is apparent that fatty acids in all testing cultures were mainly composed of oleic acid (18:1), palmitic acid (16:0) and palmitoleic acid (16:1). As shown in Fig. 2a, the proportion of oleic acid (18:1), myristic acid (14:0) and erucic acid (22:1) decreased dramatically responding to nitrogen supply increase. In particular, the relative content of oleic acid in +1.0N culture was only half of that in −N cultures. On the contrary, the proportion of palmitic acid (16:0), palmitoleic acid (16:1), as well as other minor fatty acids, increased as the nitrogen supplement was incremented.

3.2. Lipid composition related to culture conditions, and fatty acid distribution in individual lipid class

Although polar lipids, especially GLs and PLs, could be used as functional food or feed (Schneider, 2001), TAG is more acceptable as cooking oil, due to its oxidative stability. Therefore, the information about lipid composition in total lipid is critical for determining the uses of lipid produced from this microalga. As shown in Fig. 2b, different levels of nitrogen supplement induced dramatic change on the relative contents of individual lipids. TAG was the predominant fraction (*ca.* 55.4% in total lipid) in lipid from −N cultures, but to be a secondary fraction in lipids from +0.5N and +1.0N cultures. The proportions of TAG among total lipid increased 3.5-fold and 10.5-fold as the nitrogen was reduced to 0.5N and −N, respectively, while the proportions of GLs and PLs decreased in a smaller range (Fig. 2b). The overall increase in TAG under nitrogen deficiency (−N) is most likely due to the up expression of *de novo* FAs biosynthesis, in addition to the conversion of polar lipids (PLs and GLs) into TAG triggered by nitrogen starvation, which has been reported in other green algae (Recht et al., 2014; Xia et al., 2015). A visible increase in relative contents of DAG, MAG and other lipid fractions following an increase of nitrogen supply was also observed.

The fatty acids profiling includes length of carbon chain and degree of insaturation for individual lipid classes of alga *T. arborum*. The characteristic of fatty acid profiles was different in each lipid class (Table 1). TAG, the largest fraction in total lipid, was mainly composed of oleic acid (18:1) and palmitic acid (16:0), minor amount of linoleic acid (18:2) and hexadecadienoic acid (16:2) were also present. Furthermore, TAG contained *ca.* 27.1%, 64.9% and 7.9% of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), respectively. The smaller fraction of total lipid-PLs and GLs mainly contained short-chain fatty acids ($\leqslant$C16) and palmitic acid (16:0) in particular (Table 1). It should be noted that the fatty acid profile of TAG was similar to that of commercial olive oils (Limon et al., 2015), and therefore makes the TAG from alga *T. arborum* suitable to human consumption.

3.3. Pigments content and carotenoids composition related to light intensity and nitrogen supply

Fig. 3 shows how the volumetric content of chlorophyll and carotenoids changes over time in *T. arborum* cultures under different conditions. After 14 days of cultivation, the volumetric contents of carotenoids were *ca.* 9.4-folds, 14.0-folds and 16.8-folds the initial ones, for +1N, +0.5N and −N supplements respectively (Fig. 3a). In addition, the volumetric content of carotenoids in cells increased more rapidly at each nitrogen level with high light than if subjected to low light intensities (Fig. 3a and b). It is noteworthy that the volumetric content of carotenoids peaked (over 35.9 mg/L) after 11 days under double stress of high light and nitrogen deficiency (Fig. 3b). The volumetric carotenoids productivity reached 3.18 mg/L/day, which is higher than what previously reported for the algal genus *Trentepohlia* in aquatic cultivation (Abe et al., 1999; Chen et al., 2015). However, at the last day of cultivation, the carotenoids content unexpectedly reduced slightly in [HL−N] cultures, which suggested that a severe double stress might trigger the degradation of the accumulated. This phenomenon was also observed for *Trentepohlia* sp. in nature habitats, because of photo-bleaching induced by long exposure to high light intensities (Ortega-Morales et al., 2013). The nitrogen deficiency had a significant positive effect on carotenoids accumulation in *T. arborum* and other species in the same genus (Tan et al., 1993; Abe et al., 1998), which might depend on high expression levels of enzymes involved in *de novo* β-carotene synthesis, resembling that for *Haematococcus pluvialis*, under nitrogen deficiency (Recht et al., 2014).

The way chlorophyll was accumulated by *T. arborum*, particularly under high light intensity, differed significantly from how carotenoids were accumulated. As shown in Fig. 3d, the volumetric content of chlorophyll firstly increased and then began to decease linearly from day 5 and day 8 with −N and +0.5N supplements, respectively. The decline of chlorophyll content is a general response to nitrogen deficiency in green algae (Xia et al., 2015; Ikaran et al., 2015), since nitrogen is a major element in chlorophyll molecules and unavailable of this nutrient could prevent chlorophyll synthesis. Due to the combined effects of carotenoids accumulation and chlorophyll degradation, the Car/Chl ratio therefore increased much faster at high light than that at low light. Correspondingly, an obviously visible color variation from green to bright orange occurred in [LL−N] cultures, while still being green or yellow–green in [LL+0.5N] and [LL+1.0N] cultures (Supplementary Material Fig. 1). Moreover, the cells cultured at high light exhibited orange or reddish color with every testing nitrogen levels (Data not shown).

The pigment compositions of *T. arborum* cells under different cultivation conditions are shown in Table 2. The orange biomass contained 13.19 mg of total carotenoids per gram of dry biomass, in which the major carotene profile was β-carotene (Table 2). Somewhat differently, the green biomass contained less total carotenoids (6.17 mg/g dry biomass), but was dominated in zeaxanthin (Table 2), which was considered as a new high value byproducts, such as coloring agents for human consumption, feed additives to enhance the pigmentation of fish, poultry and eggs, and also color enhancement in cosmetics and pharmaceutical products (Jin and Melis, 2003). Microalga *T. arborum* could generate an

Table 2
Pigments compositions in the green and orange cells of *Trentepohlia arborum*.

Pigment	mg/g dry biomass	
	Green cells [a]	Orange cells [b]
Neoxanthin	0.12 ± 0.04	0.06 ± 0.02
Lutein	0.10 ± 0.01	0.08 ± 0.02
Zeaxanthin	3.88 ± 0.07	0.56 ± 0.04
Chlorophyll *a*	13.18 ± 0.11	1.23 ± 0.05
Chlorophyll *b*	5.51 ± 0.06	1.49 ± 0.03
β,β-Carotene	1.72 ± 0.01	10.41 ± 0.02
β,ε-Carotene	0.35 ± 0.01	2.08 ± 0.03
Total carotenoids	6.17 ± 0.03	13.19 ± 0.02

[a] Algae grown in [LL+1.0N] cultures for 14 days.
[b] Algae grown in [HL−N] cultures for 14 days.

Table 3
Carotenoids, lipid and biomass production of *Trentepohlia arborum* at different light intensity and ammonium concentrations for 14-days-cultivation.[a]

Culture conditions		Yield (g/L)	Content (mg/g)		Productivity (mg/L/day)	
Ammonium (mmol/L)	Light (μmol/m^2 s)	Biomass	Carotenoids	Lipid	Carotenoids	Lipid
0	35	1.62 ± 0.04	9.44 ± 0.03	263.7 ± 12.6	1.09 ± 0.00	30.51 ± 1.46
0	150	2.32 ± 0.06	13.40 ± 0.11	263.3 ± 32.6	2.22 ± 0.02	43.63 ± 5.40
9.4	35	1.94 ± 0.12	8.12 ± 0.10	200.6 ± 4.0	1.13 ± 0.01	27.80 ± 0.55
9.4	150	3.54 ± 0.59	11.71 ± 0.07	226.6 ± 11.1	2.96 ± 0.02	57.30 ± 2.81
18.7	35	1.53 ± 0.21	6.17 ± 0.09	169.7 ± 10.1	0.67 ± 0.01	18.55 ± 1.10
18.7	150	4.36 ± 0.65	8.92 ± 0.13	210.4 ± 9.0	2.78 ± 0.04	65.52 ± 2.80

[a] Data are expressed as means ± SD of three replicates.

amount of zeaxanthin (3.88 mg/g dry biomass) comparable to the highest value of zeaxanthin reported among algal species, *Dunaliella salina zea1* (Jin et al., 2003).

3.4. Co-production of lipid and carotenoid

The contents and productivities of carotenoids and lipid under tested conditions were calculated and summarized in order to make a concise evaluation of the potential of *T. arborum* as a carotenoids and lipid source in Table 3. Both carotenoids and lipid contents were the highest under double stress of high light and nitrogen deficiency and reached to 13.40 and 263.3 mg per gram of dry biomass, respectively. The lipid extracted in [HL−N] appeared bright yellow. Pigment analysis showed that this algal lipid contained high concentration of total carotenoids (*ca.* 5% in total lipid), which were mainly composed of β-carotene (Table 2). According to the previous reports, carotenoids from microalgae had excellent antioxidative ability (Abe et al., 2007; Gouveia et al., 2007; Limon et al., 2015), the lipid from *T. arborum* shall have an antioxidant potential in terms of special edible oil.

On the other hand, besides lipid and carotenoids accumulation, biomass production is also the important factor which should be considered. Table 3 showed that biomass yield can be notably enhanced by increment of nitrogen in the nutrient solution. For instance, increasing the nitrogen content from a condition of nitrogen deficiency (−N) to a condition of nitrogen repletion (+1N) induced a *ca.* 1.9-fold higher biomass yield under high light. Under this same condition, the contents of carotenoids and lipid slightly decreased, but their productivities significantly increased. Therefore, an appropriate way to produce different kind of products from *T. arborum* has been demonstrated.

4. Conclusion

The filamentous microalga *T. arborum* could simultaneously synthesize high amounts of carotenoids and lipid under double stress of high light and nitrogen deficiency. The predominant carotenoids and lipid fraction in total lipid from selected culture conditions was β-carotene and TAG, respectively. Fatty acid profiles were different in individual lipid class. These findings indicate good carotenoids and lipid production characteristics of *T. arborum*, making of it an interesting source of functional material for either the food or feed industry, in particular considering its potential as functional edible oil.

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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.biortech.2016.05.017.

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