Chinese Journal of Oceanology and Limnology
Vol. 31 No. 6, P. 1163-1173, 2013
http://dx.doi.org/10.1007/s00343-013-2092-4

Effects of nutrients and light intensity on the growth and biochemical composition of a marine microalga *Odontella aurita**

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Received May 4, 2012; accepted in principle Jul. 5, 2012; accepted for publication Sep. 24, 2012

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Abstract Algal biotechnology has advanced greatly in the past three decades. Many microalgae are now cultivated to produce bioactive substances. *Odontella aurita* is a marine diatom industrially cultured in outdoor open ponds and used for human nutrition. For the first time, we have systematically investigated the effects of culture conditions in cylindrical glass columns and flat-plate photobioreactors, including nutrients (nitrogen, phosphorus, silicon, and sulfur), light intensity and light path, on *O. aurita* cell growth and biochemical composition (protein, carbohydrate, β-1,3-glucan, lipids, and ash). The optimal medium for photoautotrophic cultivation of *O. aurita* contained 17.65 mmol/L nitrogen, 1.09 mmol/L phosphorus, 0.42 mmol/L silicon, and 24.51 mmol/L sulfur, yielding a maximum biomass production of 6.1–6.8 g/L and 6.7–7.8 g/L under low and high light, respectively. Scale-up experiments were conducted with flat-plate photobioreactors using different light-paths, indicating that a short light path was more suitable for biomass production of *O. aurita*. Analyses of biochemical composition showed that protein content decreased while carbohydrate (mainly composed of β-1,3-glucan) increased remarkably to about 50% of dry weight during the entire culture period. The highest lipid content (19.7% of dry weight) was obtained under 0.11 mmol/L silicon and high light conditions at harvest time. Fatty acid profiles revealed that 80% were C_{14}, C_{16}, and C_{20}, while arachidonic acid and eicosapentaenoic acid (EPA) accounted for 1.6%–5.6% and 9%–20% of total fatty acids, respectively. High biomass production and characteristic biochemical composition profiles make *O. aurita* a promising microalga for the production of bioactive components, such as EPA and β-1,3-glucan.

Keyword: *Odontella aurita*; culture conditions; biomass; biochemical composition; eicosapentaenoic acid (EPA); β-1,3-glucan

1 INTRODUCTION

There have been great advances in algal biotechnology in the past three decades. Many microalgae such as *Chlorella pyrenoidosa*, *Dunaliella salina*, *Haematococcus pluvialis*, *Odontella aurita*, and *Spirulina platensis* are currently cultivated for the production of bioactive components, such as proteins, β-carotene, astaxanthins, eicosapentaenoic acid (EPA), and pharmaceutical formulations (Meiser et al., 2004; Del Rio et al., 2005; Raja et al., 2007; Hemaiswarya et al., 2011; Sarada et al., 2011).

O. aurita is a diatom that looks like a bobbin with a length of 10–95 μm and is widely distributed in the northern coasts of Japan and the European Atlantic coast (Chihara and Murano, 1997; Drebes and Schnepf, 1998). *O. aurita* was first recognized as a bloom-forming centric diatom, but is now industrially cultured in open ponds and used for human nutrition (Drebes and Schnepf, 1998). According to Guiheneuf et al. (2010), *O. aurita* can produce EPA up to about 28% of its total lipids. The isolation of fucoxanthin and sterol sulfate from this microalga has also been published

* Supported by the National High Technology Research and Development Program of China (863 Program) (Nos. 2009AA06440, 2013AA065805), the National Basic Research Program of China (973 Program) (No. 2011CB2009001), and the National Natural Science Foundation of China (No. 31170337)

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(Toume and Ishibashi, 2002; Moreau et al., 2006).

Long-chain polyunsaturated fatty acids (PUFAs), especially EPA, have profound benefits and functions in dietetics and therapeutics. They are believed to have a positive effect in the treatment of circulatory and inflammatory diseases (Calder, 2012). Presently, EPA is obtained from fish oil, but this has some significant problems as a food supplement. Pahl et al. (2010) reported that about 75% of native fish stocks in the United States were over-exploited. Therefore, microalgae are one of the most promising sources of PUFAs as they can be grown continuously (all year long), and their fatty acid profile and content can be manipulated by growth conditions (Chen, 2012).

β-1,3-glucan, also called chrysolaminarin, has been recognized to be the major storage carbohydrate in diatoms. It has an essentially linear β-D-(1→3)-linked backbone (DP 20–60) with some branching at positions 6 and 2 (Paulsen and Myklestad, 1978). β-1,3-glucan is well established as a fish immunostimulant, and has the ability to enhance the immune system of mammals. Commercial glucan products for use in aquaculture, such as yeast glucan, are available (Storseth et al., 2004). Many microalgae contain a food reserve in the form of β-1,3-glucan, and are interesting as sources of immunomodulatory glucan.

Although EPA and β-1,3-glucan are found in many species of microalgae, only a few strains are suitable for industrial production. The determinant is the growth of an algal strain. The growth rate and biochemical composition of microalgae can be substantially altered by manipulating cultivation conditions such as light, temperature, salinity, nutrient composition and concentration (Carvalho et al., 2009; Yeh and Chang, 2012). The optimization of cultivation conditions from the standpoint of both biomass and bioactive components has become highly important in industrial production.

Thus, for the first time, we assessed the effects of cultivation conditions on the growth of *O. aurita* in column and flat-plate photobioreactors. Variations in biochemical composition including proteins, lipids, carbohydrates, β-1,3-glucan and ash were analyzed at the same time. The fatty acid composition of *O. aurita* cultured in different nutrient conditions was also investigated.

2 MATERIAL AND METHOD

2.1 Organism and culture medium

The *O. aurita* K-1251 used in this study was obtained from the Scandinavian Culture Collection of Algae and Protozoa at the University of Copenhagen and deposited in our laboratory. It was cultivated in artificial sea water (NaCl 0.35 mol/L, Na_2SO_4 24.41 mmol/L, KCl 7.87 mmol/L, $NaHCO_3$ 2.02 mmol/L, KBr 0.71 mmol/L, H_3BO_3 0.36 mmol/L, NaF 0.06 mmol/L, $MgCl_2·6H_2O$ 46.21 mmol/L, $CaCl_2·2H_2O$ 8.95 mmol/L, and $SrCl_2·6H_2O$ 80.26 μmol/L) enriched with nutrients based on modified L1 medium (Guillard and Hargraves, 1993) of the following composition: $NaNO_3$ 17.65 mmol/L, $NaH_2PO_4·H_2O$ 1.09 mmol/L, $Na_2SiO_3·9H_2O$ 0.42 mmol/L, trace elements solution ($MnCl_2·4H_2O$ 0.91 mmol/L, $ZnSO_4·7H_2O$ 76.5 μmol/L, $CoCl_2·6H_2O$ 42 μmol/L, $CuSO_4·5H_2O$ 9.8 μmol/L, $Na_2MoO_4·2H_2O$ 82.2 μmol/L, H_2SeO_3 10 μmol/L, $NiSO_4·6H_2O$ 10.3 μmol/L, Na_3VO_4 15 μmol/L and K_2CrO_4 10 μmol/L), vitamin solution (biotin 0.2 μmol/L, cyanocobalamin 0.37 μmol/L). The medium was autoclaved before use at 121°C for 20 min.

2.2 Experimental design

O. aurita was precultured in a cylindrical glass column (60 cm length, 6 cm diameter) in modified L1 medium. The cultures were transferred into new medium using 20%–30% of the total volume once every 6 days to maintain the algae at the logarithmic growth stage. For all experiments, 5–6-day-old culture was harvested by centrifugation, washed twice with artificial seawater, and inoculated into the different treatment mediums at approximately the same starting cell density according to the optical density of inoculum cultures at 750 nm wavelength.

The nutrient (nitrogen, phosphorus, silicon and sulfur) experiments were conducted in cylindrical glass columns (60 cm length, 3 cm diameter; total volume, 400 mL) with a 300 mL working volume. Modified L1 medium containing 17.65 mmol/L $NaNO_3$, 1.09 mmol/L $NaH_2PO_4·H_2O$, 0.42 mmol/L $Na_2SiO_3·9H_2O$ and 24.51 mmol/L Na_2SO_4 was used as a basic medium. For each single factor experiment, the nutrient concentrations were set as shown in Table 1.

The light path (LP) experiments were conducted with vertical flat-plate glass photobioreactors (120 cm length, 50 cm height). The photobioreactors varied only in LP: i.e., 3, 6, or 12 cm, while the working volumes were 14, 28, and 56 L, respectively. The culture medium was based on modified L1 medium.

The microalgal culture system is presented schematically in Fig.1. The culture conditions were as follows: continuous illumination was provided by

Table 1 Nutrient concentrations added to the different growth media of the *O. aurita* batch cultures

	Control	Nitrogen experiment	Phosphorus experiment	Silicon experiment	Sulfur experiment
NO_3^- (mmol/L)	17.65	3.53, 5.88, 11.76, 17.65	17.65	17.65	17.65
PO_4^{3-} (mmol/L)	1.09	1.09	0.36, 0.72, 1.09, 1.63	1.09	1.09
SiO_3^{2-} (mmol/L)	0.42	0.42	0.42	0.11, 0.21, 0.32, 0.42	0.42
SO_4^{2-} (mmol/L)	24.51	24.51	24.51	24.51	8.17, 12.25, 24.51, 48.94, 73.45

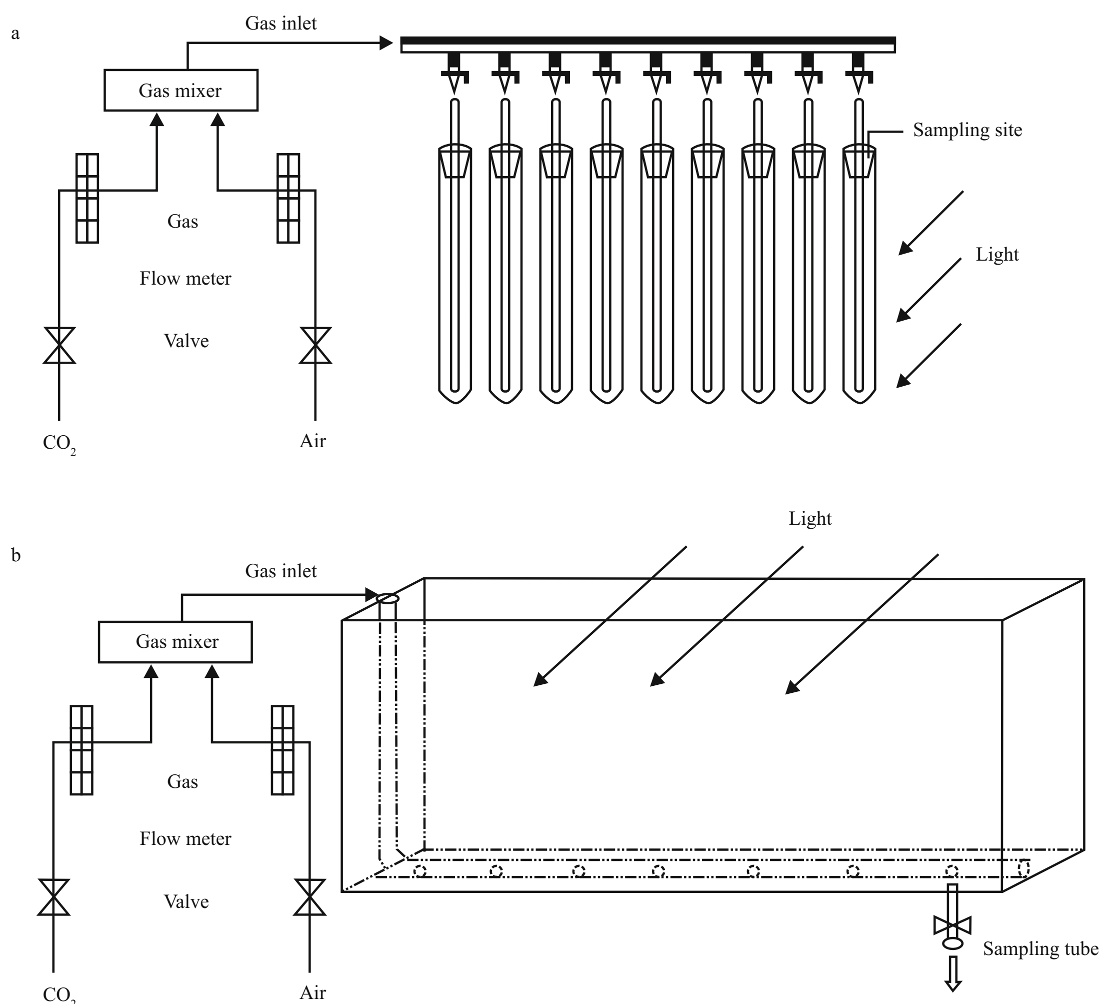

Fig.1 Schematic diagram of the culture systems (a. Cylindrical glass column; b. Vertical flat-plate photobioreactor)

The CO_2 concentration was adjusted by individual gas flow meters. External illumination was provided by a continuous, cool white fluorescent light from one side of the culture units.

cool white fluorescent lamps at the following intensities: 150 μmol photons/(m^2·s) (designated as low light for cylindrical glass columns; LL), 300 μmol photons/(m^2·s) (designated as high light for cylindrical glass columns; HL); 200 μmol photons/(m^2·s) (for flat-plate photobioreactors). Light intensity was measured on the surface of photobioreactors with a dual radiation meter (Apogee DRM-FQ, Logan, Utah, United States). Cultures were placed in a temperature-regulated room at 25°C and aerated by bubbling with a mixture of 1% CO_2 in air at a flow rate of approximately 0.3 vvm.

2.3 Analytical methods

2.3.1 Biomass measurement

A 5 mL culture suspension was filtered through pre-weighed GF/B filter paper. The filter paper was dried in the oven at 105°C to a constant weight for biomass measurement. The specific growth rate (μ) of

the cultures was calculated from the expression, $\mu=\ln N_t-\ln N_0/t$, where N_t=biomass at time 't', N_0=initial biomass at time '0' and t = time (days).

2.3.2 Lipids and carbohydrate

Biomass was harvested by centrifugation, freeze-dried and stored at -20°C prior to analysis. About 0.05 g algal powder was used for lipid content determination. The algal powder was placed in a Teflon-capped glass tube and extracted according to Khozin-Goldberg et al. (2005) by adding 2 mL 10% DMSO-methanol solution at 50°C for 10 min, and then incubating for 30 min at 4°C. After a 3 500 r/min centrifugation, the supernatant was transferred to a new vial. The residue was re-extracted with 4 mL 1:1 (v/v) diethyl ether and hexane for 1 h at 4°C, and centrifuged at 3 500 r/min. The supernatant was removed to the above vial. This process was repeated until the residue turned white. The methanol-extract was mixed with diethyl ether, hexane and water at a ratio of 1:1:1:1 (v/v/v/v). The organic phase was removed to another small glass bottle and nitrogen blown to a smaller volume. The concentrated solution was then transferred to a pre-weighed 1.5 mL Eppendorf tube, and dried with nitrogen to a constant weight. Thereafter, the total lipids were measured gravimetrically, and lipid content was calculated.

After lipid extraction, the residues were hydrolyzed with 4 mL of 0.5 mol/L sulfuric acid at 100°C for 4 h. Extracts were assayed quantitatively for carbohydrate by the phenol-sulfuric acid method described by Dubois et al. (1956).

2.3.3 β-1,3-glucan

β-1,3-glucan was extracted according to Granum and Myklestad (2002); 0.05 g algal power was extracted with sulfuric acid (0.05 mol/L, 5 mL) at 60°C for 10 min. Extracts were assayed quantitatively for β-1,3-glucan using the phenol-sulfuric acid method of Dubois et al. (1956).

2.3.4 Protein

Total nitrogen was determined by the Kjeldahl method with a Kjeltec 2003 analyzer unit (Foss Tecator AB, Hoganas, Sweden). Freeze-dried algal power (0.5 g) was weighed into a digestion tube and 12 mL of concentrated sulfuric acid and a catalyst/salt mixture consisting of 7.0 g K_2SO_4 and 0.8 g $CuSO_4 \cdot 5H_2O$ were added to the sample. The digestion tube was transferred to a heating block and digested for 60 min at 420°C. The digested sample was distilled with water, 40%-w/w aqueous sodium hydroxide solution and 4%-w/w aqueous boric acid solution. Determination of nitrogen was carried out by acid-base titration using 0.2 mol/L hydrochloric acid, and protein content was calculated as total nitrogen multiplied by 4.78 according to Lourenço et al. (2004).

2.3.5 Ash

Ash was determined gravimetrically according to The National Standard of the People's Republic of China (GB-T 17375-2008). Algal powder (0.5 g) was transferred into a pre-weighed crucible, and then heated to 575°C for 3 h in a muffle furnace. The ash weight was calculated as the difference between final weight and crucible weight.

2.3.6 Fatty acid analysis

Gas chromatography and mass spectrometry were used for fatty acid quantification according to Cohen et al. (1993). Transmethylation of fatty acids was performed by incubating 0.025 g biomass in a methanol: toluene mixture (90:10, v/v) containing 2% sulfuric acid at 80°C for 1.5 h. Heptadecanoic acid was added as an internal standard. Fatty acid methyl esters were identified by co-chromatography with authentic standards. Fatty acid content was calculated by comparing with the internal standard.

2.4 Statistical analysis

Statistical analysis was performed using SPSS (Version 17.0) statistical software. Data were considered significantly different at $P<0.05$ (one-way ANOVA, Tukey's HSD test).

3 RESULT

3.1 Effects of nutrient concentrations and light intensity on the growth of *O. aurita*

To examine the effects of initial nutrient concentrations and light intensity on the growth of *O. aurita*, different concentrations of nitrogen, phosphorus, silicon and sulfur were tested under LL and HL. As shown in Fig.2a and b, the biomass concentration increased with the initial nitrogen and phosphorus concentrations under LL. When the nitrogen concentration increased from 3.53 to 11.76 mmol/L and the phosphorus concentration increased from 0.36 to 1.09 mmol/L, the stationary

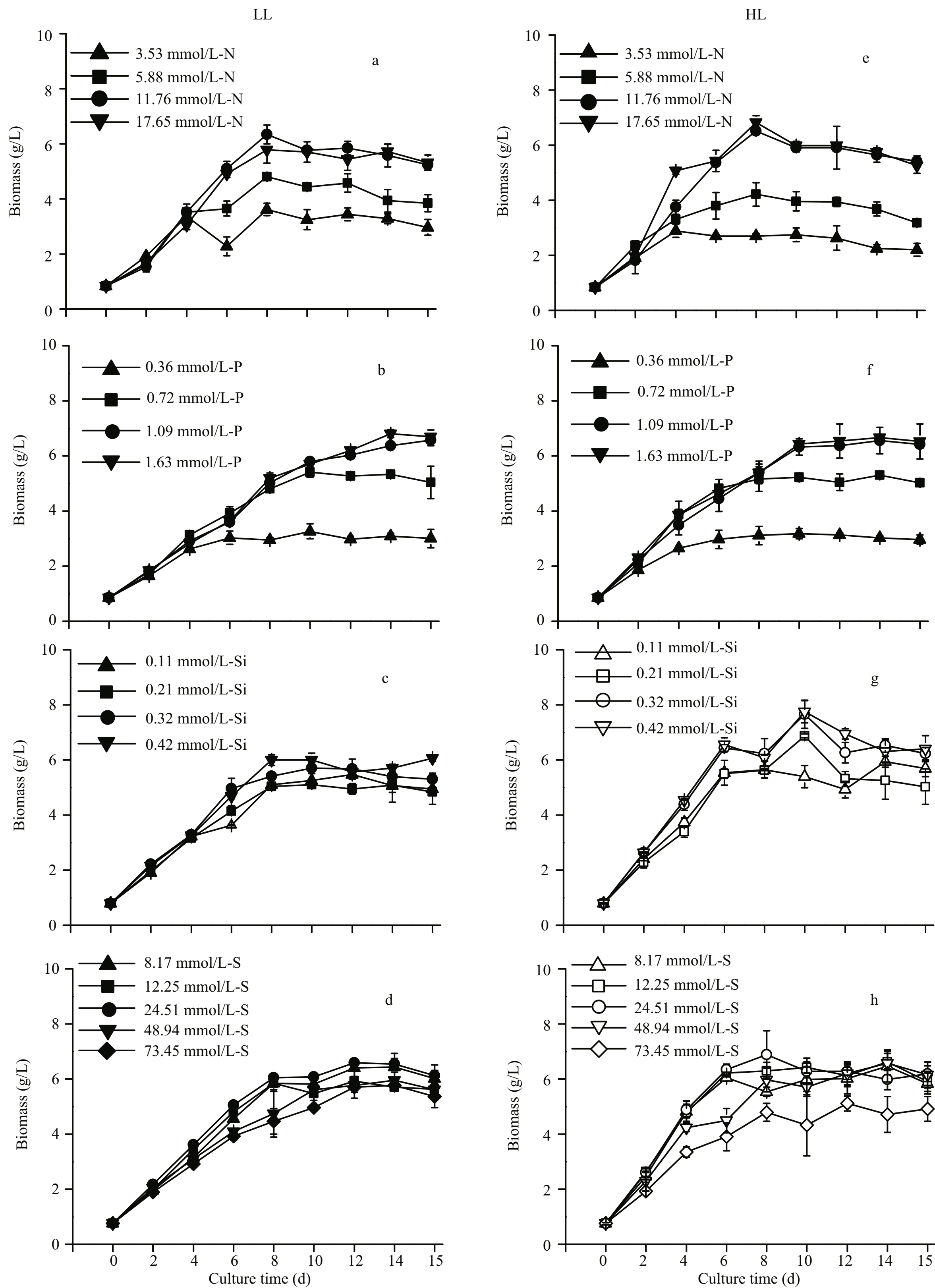

Fig.2 Effects of nutrient concentrations on the growth of *O. aurita*. Different nitrogen (a, e), phosphorus (b, f), silicon (c, g) and sulfur (d, h) concentration experiments were conducted under low light (LL, left) and high light (HL, right) simultaneously

All experiments were carried out in triplicate. Data are the means of three replicates with SD.

phase biomass concentration increased significantly (P<0.05) from 3.62 to 6.34 g/L and 3.26 to 6.58 g/L, respectively. Further increases of nitrogen concentration to 17.65 mmol/L and phosphorus concentration to 1.63 mmol/L had no significant effect (P>0.05) on the stationary phase biomass concentration. However, the chlorophyll *a* content was significantly higher in 17.65 mmol/L nitrogen compared with 11.76 mmol/L (unpublished data). Maximum biomass concentrations of 6.34 g/L and 6.81 g/L were obtained in 11.76 mmol/L nitrogen and 1.63 mmol/L phosphorus, respectively. As shown in Fig.2c, when the initial silicon concentration was in the range of 0.11–0.42 mmol/L, the stationary phase biomass concentration was between 5.1–6.1 g/L; the highest biomass concentration of 6.1 g/L was obtained in 0.42 mmol/L silicon. As shown in Fig.2d, the maximum biomass concentration obtained in 24.5 mmol/L sulfur was greater than that obtained in the other groups (P<0.05), while further increases of sulfur concentration to 48.94 and 73.45 mmol/L showed a significant negative (P<0.05) effect on biomass production.

To examine the influence of light intensity on growth and biochemical composition, two light intensities, 150 μmol photons/(m^2·s) (LL) and 300 μmol photons/(m^2·s) (HL), were tested with *O. aurita* cultures. As shown in Fig.2, with the increase in light intensity from LL to HL, the biomass concentration increased faster at the early stages (0–4 days), and a higher final biomass concentration was achieved (Fig.2). Maximum biomass concentrations of 6.82 g/L, 6.67 g/L, 7.75 g/L and 6.89 g/L were obtained under HL with 17.65 mmol/L nitrogen, 1.63 mmol/L phosphorus, 0.42 mmol/L silicon and 24.51 mmol/L sulfur concentrations, respectively.

3.2 Effect of LP on the growth of *O. aurita*

From our experiments with different starting nutrient concentrations, the medium that contained 17.65 mmol/L nitrogen, 1.09 mmol/L phosphorus, 0.42 mmol/L silicon and 24.51 mmol/L sulfur was found to be optimal for the growth of *O. aurita*. To assess the scale-up effect on growth and biochemical composition, and thus to confirm the production potential for large-scale commercial devices, *O. aurita* was grown in vertical flat-plate photobioreactors with different LP lengths. LP exerted a strong effect on the biomass concentration of *O. aurita*, with higher biomass concentrations obtained in the shorter LP reactors (Fig.3). The maximum biomass concentration obtained in the 3-cm LP photobioreactor was significantly higher than that obtained in the 6-cm and 12-cm LP photobioreactors (P<0.05). The biomass production of 3.8 g/L obtained in the 3-cm LP photobioreactor was 46% greater than the 6-cm LP and 110% greater than the 12-cm LP reactor.

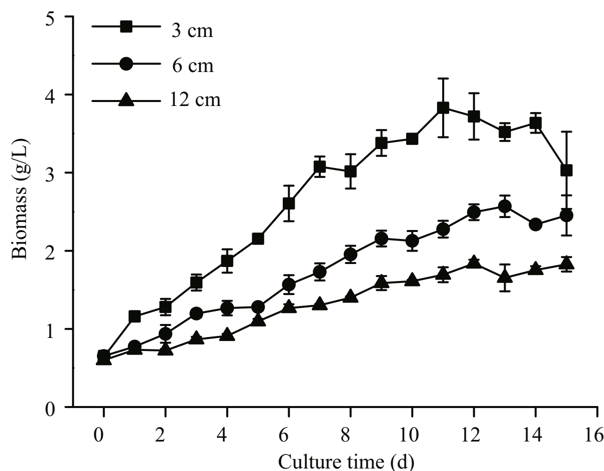

Fig.3 Growth profile of *O. aurita* grown under different light path lengths

Data are means of three replicates with SD.

3.3 Variations in the biochemical composition of *O. aurita*

Table 3 shows the variations in the biochemical composition of *O. aurita* cultured in different conditions of nutrient deficiency and light intensity. Under LL conditions, the protein content decreased from 34.1% of dry weight (DW) at inoculation time to 9.5% DW in low nitrogen (L-N, 3.53 mmol/L nitrogen group in Fig.2a), 26.6% DW in low phosphorus (L-P, 0.36 mmol/L phosphorus group in Fig.2b), 17.2% DW in low silicon (L-Si, 0.11 mmol/L silicon group in Fig.2c), 14.3% DW in low sulfur (L-S, 8.17 mmol/L sulfur group in Fig.2d) and 15.6% in the control group at harvest time, whereas the carbohydrate content increased from 20.7% DW at inoculation time to 54.2% (L-N), 36.4% (L-P), 50.5% (L-Si), 51.9% (L-S) and 53.9% (control) DW at harvest time. The β-1,3-glucan content showed the same trend as the total carbohydrate content, which had a remarkable increase during the entire culture period. The ratio of β-1,3-glucan/carbohydrate increased from 38% at inoculation time to above 80% at harvest time in all nutrient conditions. Compared with the large variations in protein and carbohydrate, the lipid content showed smaller increases, from 11.2% at

inoculation time to 15.4% (L-N), 16.3% (L-P), 15.9% (L-Si), 14.1% (L-S) and 12.8% (control) DW at harvest time, whereas the ash content decreased from 20.4% at inoculation time to 13.9% (L-N), 15.4% (L-P), 10.8% (L-Si), 13.5% (L-S) and 10.7% (control) DW at harvest time. Increased light intensity (HL) resulted in higher lipid and ash content, and lower carbohydrate and β-1,3-glucan content, but it had no significant effect on the protein content of *O. aurita*.

3.4 Effects of nutrient concentrations on the fatty acid composition of *O. aurita*

Table 4 shows the fatty acid compositions of *O. aurita* cultured in different nutrient conditions. In general, about 80% of the total fatty acids (TFAs) were C_{14}, C_{16} and C_{20} fatty acids, with myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1) and eicosapentaenoic acid (C20:5) being the main components. Over the entire batch culture, the saturated fatty acids (SFAs) content increased from 20.5% to 25.4%–39% and the monounsaturated fatty acids (MUFAs) increased from 23.5% to 31.7%–35.8% of the total fatty acids, while PUFAs decreased from 33.8% to 15.4%–24.7% of the TFAs. The major PUFAs were arachidonic acid (AA, 1.6%–5.6% of TFAs) and EPA (9%–20% of TFAs), with the highest proportion of EPA on Day 0 (20% of TFAs) and the highest proportion of AA in nutrient-sufficient conditions at harvest time (5.6% of TFAs). As shown in Table 3, *O. aurita* has similar fatty acid compositions at different nutrient concentrations. However, compared with nutrient-deficient conditions, *O. aurita* had lower SFAs content and a higher proportion of AA (5.6% of TFAs) and EPA (12.9% of TFAs) in the nutrient-sufficient conditions at harvest time. Compared with LL, cells grown under HL contained higher proportions of SFAs at harvest time.

4 DISCUSSION

The principle of algal biotechnology for achieving high growth rate is to ensure an abundant supply of nutrients. According to Droop (1975), when the nutrient supply cannot match the metabolic needs of the algal population, the growth rate decreases and growth ceases. Yongmanitchai and Ward (1991) studied the growth of *Phaeodactylum tricornutum* under different culture conditions. The highest algal growth was observed at nitrogen concentrations of 11.8 to 17.7 mmol/L, with the former value resulting in a maximum biomass production of 2.7 g/L. The phosphorus requirements for optimal growth differ considerably from species to species. Studies of diatoms and green algae under defined laboratory conditions revealed that phosphorus concentrations below 1.6 μmol/L were limiting, and those of about 0.65 mmol/L were inhibitory (Chu, 1943). In the present study, the biomass production of *O. aurita* increased with phosphorus concentration, and a high biomass production above 7.0 g/L was achieved under 1.09 and 1.63 mmol/L phosphorus. These results were inconsistent with Yongmanitchai and Ward (1991), who indicated that phosphorus concentrations in the range of 0.29 to 2.87 mmol/L had little effect on the biomass production of *P. tricornutum*, with 3.2 g/L biomass production obtained under both 1.44 and 2.87 mmol/L phosphorus. Compared with the obvious influence of nitrogen and phosphorus, any of the silicon and sulfur concentrations we tested may satisfy the growth requirements of *O. aurita* as they did not show clear distinctions. The negative effect of the highest concentrations of sulfate we examined probably derived from the resulting high salinity.

Light is another important factor affecting the microalgal growth rate. As indicated in Fig.2, the irradiance conditions we set had a limited effect on the stationary biomass production of *O. aurita*. This may indicate that 150 μmol photons/(m^2·s) was enough for the growth of *O. aurita*. However, the alga grew faster at the early stages under high light (Table 2). This may be due to the low cell density at the early stages, which enables the cells to receive more irradiance under high light conditions. The fast growth characteristics of *O. aurita* mean that cell density increases quickly. The mutual shading effect caused by high cell density in later growth stages results in limited light penetrating the cultures. Thus, irradiance had a limited effect on the final stationary biomass production. The responses of different algae to variations in irradiance distinguish them from each other. For example, Qian and Borowitzka (1993) showed that *P. tricornutum* was capable of maintaining higher cell division rates in higher light intensities, which allowed *P. tricornutum* to have a growth rate of 2.19 g/L.

Nutrient availability has a significant impact on the biochemical profiles of microalgae. When nutrients are used up, stress conditions may occur, and microalgae can alter their metabolic paths from protein synthesis to either lipid or carbohydrate synthesis. Spoehr and Milner (1949) first reported that N-deficiency induced an obvious accumulation

Table 2 The specific growth rate of *O. aurita* in different nutrient conditions under low light (LL) and high light (HL)

Nutrients condition	Light intensity	Specific growth rate			
		Day 4	Day 8	Day 12	Day 15
17.65 mmol/L-N[a]	LL	0.337±0.016	0.249±0.026	0.161±0.021	0.127±0.012
	HL	0.464±0.011	0.269±0.016	0.169±0.029	0.126±0.008
1.09 mmol/L-P[b]	LL	0.306±0.021	0.221±0.013	0.162±0.011	0.136±0.011
	HL	0.350±0.026	0.230±0.023	0.167±0.021	0.134±0.026
0.42 mmol/L-Si[c]	LL	0.354±0.012	0.253±0.012	0.163±0.010	0.136±0.012
	HL	0.437±0.011	0.255±0.026	0.181±0.016	0.140±0.021
24.51 mmol/L-S[d]	LL	0.391±0.023	0.260±0.013	0.181±0.011	0.140±0.010
	HL	0.467±0.019	0.277±0.036	0.177±0.013	0.140±0.022

Data are means±SD. [a]: group from Fig.2a, e; [b]: group from Fig.2b, f; [c]: group from Fig.2c, g; [d]: group from Fig.2d, h

Table 3 Variations in the biochemical composition of *O. aurita* cultured in different nutrient conditions under low light (LL) and high light (HL)

Time	Nutrient condition	Biochemical compositions (% of dry weight)									
		Lipid		Protein		Total carbohydrate		β-1,3-glucan		Ash	
		LL	HL	LL	HL	LL	HL	LL	HL	LL	HL
Day 0	Inoculum	11.2	11.2	34.1	34.1	20.7	20.7	7.8	7.8	20.4	20.4
Day 15	L-N[a]	15.4	16.4	9.5	9.2	54.2	50.8	50.4	45.3	13.9	20.1
	L-P[b]	16.3	19.0	26.6	28.3	36.4	31.0	29.2	27.5	15.4	16.5
	L-Si[c]	15.9	19.7	17.2	17.9	50.5	46.4	43.1	42.3	10.8	12.8
	L-S[d]	14.1	15.5	14.3	13.6	51.9	48.4	45.4	43.4	13.5	13.0
	Control[e]	12.8	15.9	15.6	15.3	53.9	50.4	50.1	47.2	10.7	12.3

Data are means (n=2). [a] L-N: low nitrogen (3.53 mmol/L nitrogen group in Fig.2a and e); [b] L-P: low phosphorus (0.36 mmol/L phosphorus group in Fig.2b and f); [c] L-Si: low silicon (0.11 mmol/L silicon group in Fig.2c and g); [d] L-S: low sulfur (8.17 mmol/L sulfur group in Fig.2d and h); [e] Control: nutrient-sufficient conditions (17.65 mmol/L nitrogen, 1.09 mmol/L phosphorus, 0.42 mmol/L silicon and 24.51 mmol/L sulfur)

of lipids in *C. pyrenoidosa*. These findings were subsequently extended by other investigators to many other microalgal species. Reitan et al. (1994) reported that P-limitation leads to increased lipid accumulation in *P. tricornutum*, *Chaetoceros* sp. and *Pavlova lutheri*, but a decrease of lipid content was also observed in some species, such as *Nannochloris atomus* and *Tetraselmis* sp. Silicon deficiency induced a change in partitioning of newly assimilated carbon to lipid accumulation in *Cyclotella cryptica* (Roessler, 1988). Otsuka (1961) studied the effects of sulfur deprivation on *Chlorella ellipsoidea*, and demonstrated that it enhanced total lipid content. Nutrient deficiency also induced an increase in the ACCase synthesis rate in *C. cryptica* and *Isochrysis galbana* (Roessler, 1988; Sukenik and Livne, 1991). ACCase, which catalyzes the conversion of acetyl-CoA to malonyl-CoA, exerts an intense regulation of fatty acid synthesis in microalgae. Changes in enzymatic activity may be partially responsible for the accumulation of lipids in *O. aurita*.

Storage carbohydrates are another component accumulated during photosynthesis, particularly during nutrient limitation. Myklestad and Haug (1972) found that large amounts of carbohydrates, represented mainly by β-1,3-glucan, accumulated in *Chaetoceros affinis* under nitrate-deprivation conditions. Guerrini et al. (2000) showed that the carbohydrate concentration of *Achnanthes brevipes* increased under both nitrogen and phosphorus starvation treatments, and the activation of several enzymes involved in carbohydrate metabolism was observed under phosphorus limitation. The synthesis of lipids or carbohydrates under nutrient-limited conditions may be species-specific. The results in Table 3 indicate that carbohydrates (mainly β-1,3-glucan) are the major storage materials in *O. aurita*.

The fatty acid profiles of *O. aurita* cultured in different conditions were consistent with results from other diatoms (Liang et al., 2005). EPA ratios of

Table 4 Fatty acid composition of *O. aurita* in different culture conditions

Time	Nutrient condition	Fatty acid composition (% of total fatty acid)											
		14:0	16:0	16:1	16:2	18:0	18:1	18:2	20:4	20:5	SFA	MUFA	PUFA
Day 0	Inoculum	10.4	8.2	21.9	7.4	1.9	1.6	2.1	4.3	20.0	20.5	23.5	33.8
Day 15	HL-N[a]	18.1	19.2	30.2	2.6	1.7	3.5	1.9	2.2	9.0	39.0	33.7	15.7
	LL-N[b]	14.0	17.6	32.9	2.8	0.6	3.9	1.9	2.2.	9.6	32.2	36.8	16.5
	HL-P[c]	16.9	18.0	34.5	3.6	1.8	1.3	0.9	1.6	9.3	36.7	35.8	15.4
	HL-Si[d]	15.3	13.3	30.0	3.4	1.8	3.8	2.1	3.8	11.8	30.4	33.8	21.1
	HL-S[e]	18.8	12.5	35.3	3.9	0.5	0.5	0.4	3.2	12.5	31.8	35.8	20.0
	Control[f]	15.7	8.6	29.6	5.2	1.1	2.1	1.0	5.6	12.9	25.4	31.7	24.7
		Fatty acid composition (% of dry weight)											
		14:0	16:0	16:1	16:2	18:0	18:1	18:2	20:4	20:5	TFAs		
Day 0	Inoculum	0.71	0.56	1.49	0.50	0.13	0.11	0.14	0.29	1.36	6.78		
Day 15	HL-N[a]	1.57	1.66	2.62	0.22	0.14	0.31	0.17	0.19	0.78	8.66		
	LL-N[b]	1.22	1.54	2.87	0.24	0.05	0.34	0.17	0.19	0.84	8.69		
	HL-P[c]	1.53	1.63	3.13	0.32	0.16	0.12	0.08	0.14	0.85	9.06		
	HL-Si[d]	1.41	1.22	2.76	0.31	0.17	0.35	0.19	0.35	1.09	9.21		
	HL-S[e]	1.52	1.00	2.84	0.31	0.04	0.04	0.03	0.26	1.00	8.04		
	Control[f]	1.17	0.64	2.22	0.39	0.08	0.16	0.07	0.42	0.97	7.50		

[a] HL-N: High light and low nitrogen (3.53 mmol/L nitrogen group in Fig.2e); [b] LL-N: Low light and low nitrogen (3.53 mmol/L nitrogen group in Fig.2a); [c] HL-P: High light and low phosphorus (0.36 mmol/L phosphorus group in Fig.2f); [d] HL-Si: High light and low silicon (0.11 mmol/L silicon group in Fig.2g); [e] HL-S: High light and low sulfur (8.17 mmol/L sulfur group in Fig.2h); [f] Control: High light and nutrient-sufficient conditions (17.65 mmol/L nitrogen, 1.09 mmol/L phosphorus, 0.42 mmol/L silicon and 24.51 mmol/L sulfur).

0.1%–43.2% of TFAs have been observed in various species of microalgae (Basova, 2005). Brand (1998) reported that in combined cultivation of *O. aurita* and *Chondrus crispus*, the biomass yield and PUFAs in each species rose by 44% when compared with monocultures. In the present study, the EPA content was lower than the results of Brand (1998) (25.6%) and Guiheneuf et al. (2010) (27%–28%), which could be related to the different culture conditions or strains of *O. aurita*. Under optimum growth conditions, algae contain mostly polar lipids associated with the chloroplast and other membranes. Under stress conditions, the biomass of algal cells decreases sharply. However, many algae alter their lipid biosynthetic pathways to the formation and accumulation of neutral lipids (Hu et al., 2008). Lynn et al. (2000) showed that neutral lipids increased in *Stephanodiscus minutulu* under silicon, nitrogen and phosphorus limited conditions, with a concomitant decrease in polar lipids. One interesting finding is that neutral lipids are often composed primarily of SFAs and MUFAs, which provide more energy upon oxidation than PUFAs and possibly may allow more efficient packaging of storage lipids (Roessler, 1990). In the present study, the proportion of SFAs and MUFAs increased during cultivation with a concomitant decrease in EPA, possibly because of a decrease in polar lipids and an increase in neutral lipids.

The microalgal culture system is another important aspect of algal biotechnology. Vertical flat-plate photobioreactors have various advantages for mass production (Zhang et al., 2002; Zhang and Richmond, 2003). The LP of flat-plate photobioreactors is a critical factor for the light regime to which cells in a reactor are exposed. Similar to the results of Zhang et al. (2002), the cell concentration of *O. aurita* was higher in the 3-cm LP photoreactor, which indicated that a short LP was more suitable for biomass production. As there is a higher area density in the longer LP photobioreactor, more cells are exposed to a given photon flux, and thus the irradiance per cell is lower (Zhang and Richmond, 2003). Significant differences in productivity were found between the tubular culture units and the flat-plate photobioreactors. At constant external illumination, small changes in culture depth are known to affect the productivity of microalgal cultures (Kobayashi and Fujita, 1997). In

the same light path and illumination, the area of microalgal cell exposure was larger in the cylindrical columns compared with the flat-plate photobioreactors because of the shape differences in the two culture units. Consequently, the irradiance per cell and the frequency of microalgal cell exposure to light were higher in the tubular culture units. Thus, higher biomass production was achieved in the tubular culture units.

5 CONCLUSION

This work represents the first systematic investigation into the effects of culture conditions on the growth and biochemical composition of *O. aurita*. A modified L1 medium consisting of 17.65 mmol/L nitrogen, 1.09 mmol/L phosphorus, 0.42 mmol/L silicon and 24.51 mmol/L sulfur was found to be optimal for photoautotrophic cultivation of *O. aurita*. Carbohydrates, mainly composed of β-1,3-glucan, were found to be the major storage materials produced under stress conditions. However, lipid and EPA production was relatively low. Thus, additional studies on the fatty acid distribution of *O. aurita* are needed. Understanding the effects of environmental conditions on fatty acid positional distribution will provide valuable information concerning the pathways of conversion from one type of lipid to another.

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