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Outdoor mass culture of the marine microalga *Pavlova viridis* (Prymnesiophyceae) for production of eicosapentaenoic acid (EPA)

Mei LI [a], Changwei HU [b], Qin ZHU [b], Zhiming KONG [a] and Zhili LIU [b]*

[a] National Key Laboratory of Pollution Control and Resource Reuse of China, School of the Environment, Nanjing University, Nanjing, China

[b] School of Life Sciences, Nanjing University, Nanjing, China

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Abstract – There is increasing interest in the use of autotrophic microalgae as alternative sources of eicosapentaenoic acid (EPA), a nutritionally important polyunsaturated fatty acid that plays a key role in the prevention and treatment of several human diseases and disorders, for incorporation in either aquaculture feeds or human diets. In this study, we have demonstrated the feasibility of outdoor cultivation of the prymnesiophyte, *Pavlova viridis*, one of the most promising photoautotrophic producers of EPA, in tubular photobioreactors (PBR). *P. viridis* was cultivated in photobioreactors of two sizes (six-tube and eight-tube) in autumn under the climatic conditions of Nanjing, China. In both photobioreactors the microalgal cells quickly adapted to the new environments outdoors when cultivated with agitation, and had a very short lag growth phase. Chlorophyll *a* contents ranged from 0.037 to 0.066 μg 10^6 cells^{-1} and from 0.041 to 0.063 μg 10^6 cells^{-1} for the 6-tube and 8-tube PBR, respectively. Decreasing temperature and light intensity increased the proportion of polyunsaturated fatty acids in cultures of *P. viridis* to 29.9% and 35.9% in the six- and eight-tube PBR, respectively. Cultivation of *P. viridis* in our closed tubular photobioreactor represents a suitable approach for the production of a high-quality microalgal biomass enriched in EPA.

Eicosapentaenoic acid / *Pavlova viridis* / Microalgae mass cultivation / Temperature / Light intensity / Bioreactor

Résumé – Culture de masse de *Pavloa viridis* (Prymnesiophyceae), algue marine pour la production d'acide eicosapentaenoïque (EPA). Il y a un intérêt grandissant pour l'utilisation de microalgues autotrophiques comme sources alternatives d'acide eicosapentaenoïque (EPA), un acide gras polyinsaturé important nutritionnellement qui joue un rôle dans la prévention et le traitement de plusieurs maladies humaines, pour l'incorporation dans des nourritures en aquaculture ou des aliments pour l'homme. Dans cette étude, nous avons démontré la faisabilité de culture externe de la Prymnesiophyte, *Pavlova viridis*, un des producteurs photoautrophiques d'Epa les plus prometteurs, dans des photobioréacteurs tubulaires (PBR). *P. viridis* a été cultivé dans des photobioréacteurs de deux tailles (six tubes ou huit tubes) pendant l'automne dans les conditions climatiques du Nanjing (Chine). Dans les deux photobioréacteurs, les cellules microalgales s'adaptent rapidement aux

* Correspondence and reprints: liuzl@nju.edu.cn
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nouveaux environnements externes quand la culture est agitée et ont un très court retard de phase de croissance. Les contenus en chlorophylle *a* vont de 0,037 à 0,066 µg 10^6 par cellule et de 0,041 à 0,063 µg 10^6 par cellule respectivement pour les PBR 6-tubes et 8-tubes. La baisse de température et de l'intensité lumineuse augmentent la proportion d'acides gras polyinsaturés dans les cultures de *P. viridis* de 29,9% et de 35,9%, dans les PBR 6-tubes et 8-tube respectivement. La culture de *P. viridis* dans nos photobioréacteurs tubulaires clos représentent une méthode utilisable pour la production de biomasse microalgale de haute qualité enrichie en EPA.

Acide eicosapentaenoïque / *Pavlova viridis* / culture de masse de microalgues / température / intensité lumineuse / bioréacteur

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) of the n-3 series play a key role in the prevention and treatment of a wide range of human diseases and disorders and have been recognized as important dietary compounds. Eicosapentaenoic acid (EPA, 20:5), one of the major PUFAs, is essential for the development of marine organisms and is important for a balanced human diet (Sukenik, 1999). EPA has also been shown to have several highly beneficial effects such as preventing atherosclerosis and cardiovascular diseases, lowering plasma cholesterol and triacylglycerol levels, alleviating inflammatory conditions, and possesses antitumor activities (Gill & Valivety, 1997; Tapiero *et al.*, 2002; Judé *et al.*, 2006).

EPA is synthesized by phytoplankton, though other microorganisms such as fungi (especially of the order Mucorales) and bacteria of the genera *Shewanella, Alternomonas, Flexibacter* and *Vibrio* (Gill & Valivety, 1997; Leman, 1997) have long been recognized as potential EPA producers (Radwan, 1991). The marine microalgae, such as the rhodophyte *Porphyridium cruentum* (Servel *et al.*, 1994; Cohen *et al.*, 1988), the diatom *Phaeodactylum tricornutum* (Chrismadha & Borowitzka, 1994; Molina Grima *et al.*, 1994a), the prymnesiophyte *Isochrysis galbana* (Molina Grima *et al.*, 1994b) and the eustigmatophyte *Nannochloropsis oculata* (Tonon *et al.*, 2002) have been proposed for commercial production of EPA under autotrophic conditions.

The marine flagellate microalga *Pavlova viridis* Tseng has been cultivated as an artificial feed for larval fish, rotifers, bivalves and prawns in many mariculture hatcheries in China (Liu, 1995). The nutritional value of *P. viridis* for mariculture is related to its biochemical composition, especially its relatively high lipid (Li *et al.*, 2005) and fatty acid contents including compounds such as EPA and DHA, which are essential nutrients for cultured marine animals (Lu & Lin, 2001). In addition, *P. viridis* has the capacity to adapt well to adverse environments (Zhao & Sun, 2004). *P. viridis* has also been proposed as a potential source of these important polyunsaturated fatty acids for human consumption (Dong *et al.*, 2004). Until now, however, no information has been available on the behavior of *P. viridis* with respect to outdoor mass culture.

The open raceway is presently the major commercial device for outdoor mass production of microalgae. However, algal-based technologies in outdoor ponds are considered commercially not viable for EPA production because of low achievable cell concentrations, low production rates and a reduced degree of control of growth parameters such as growth rate and biochemical composition

(Barclay *et al.*, 1994). These limitations of open ponds could be overcome by use of closed reactors (photobioreactors). In closed systems, protection against contaminating microorganisms is much more easily assured, and much higher cell densities and more efficient control of principal culture parameters can be achieved so as to ensure a sustainable cultivation process (Tredici & Chini Zitelli, 1998). For the cultivation of microalgae, various different reactor designs have been built and tested (Molina Grima *et al.*, 1999; Chini Zitelli *et al.*, 2000).

The aims of the present study were to evaluate the feasibility of outdoor mass culture of *P. viridis* in tubular photobioreactors and to assess the potential of this microalga as an alternative source of EPA.

MATERIALS AND METHODS

Microalgae and culture medium

The marine microalga *Pavlova viridis* (3012) was provided by the Institute of Oceanology, Chinese Academy of Sciences, Qingdao. The inoculum, grown in the laboratory, was cultured in sterilized artificial seawater of the following compositions in g l^{-1}: NaCl, 25; $MgSO_4 \cdot 7H_2O$, 3.5; $MgCl_2 \cdot 6H_2O$, 2.5; $CaCl_2$, 1.2; KCl, 0.8; $NaHCO_3$, 0.2; KNO_3, 0.25 and KH_2PO_4, 0.015 (Yang *et al.*, 2002). The nutrient solution concentrate used to make the growth medium for both the laboratory cultures and mass culture outdoors was enriched with f/2 nutrients (Guillard & Ryther, 1962) in which the vitamin concentrations were doubled (B_1 200 µg l^{-1}, B_2 1 µg l^{-1}, B_{12} 1 µg l^{-1}). The inoculum for mass cultures was grown and maintained at 23 ± 1ºC under a 12 h: 12 h light: dark cycle provided by cool white fluorescence lights at 85-90 µmol photons m^{-2} s^{-1} irradiance (ST-80 illuminometer, Photoelectric Instrument Factory of Beijing Normal University).

For laboratory experiments, artificial seawater was autoclaved at 120ºC for 20 min and the cultures were aerated by gentle bubbling with filtered air, whereas for outdoor experiments the seawater was not sterilized but filtered through 40 µm nylon sieves. Sterile nutrient solution concentrates were used for both outdoor and laboratory cultures.

Photobioreactor and growth conditions

Six-tube and eight-tube photobioreactors installed at the experimental station at Jiangning (Nanjing, China) were used for the outdoor cultivation of *P. viridis*. The photobioreactor consisted of flexible and translucent polyethylene plastic tubes that were connected by two small tanks. The tie-in was connected by PVC U-bends with watertight flanges. The configuration of the two reactors was similar, but the size of the tube was different. The length and diameter were 6 m and 20 cm and 8 m and 31.8 cm for the six-tube and the eight-tube PBRs respectively. The wall thickness of both tubes was 0.2 mm. A schematic drawing of the six-tube photobioreactor is shown in Fig. 1. Microalgae culture medium was cycled through the reactor in the direction of the arrows shown. The culture was lifted using a pump from the lower pond (a) into the upper pond (b) at a rate of 0.2 m^3/min in the six-tube PBR and at 0.4 m^3/min in the eight-tube PBR. PBR tubes were horizontal.

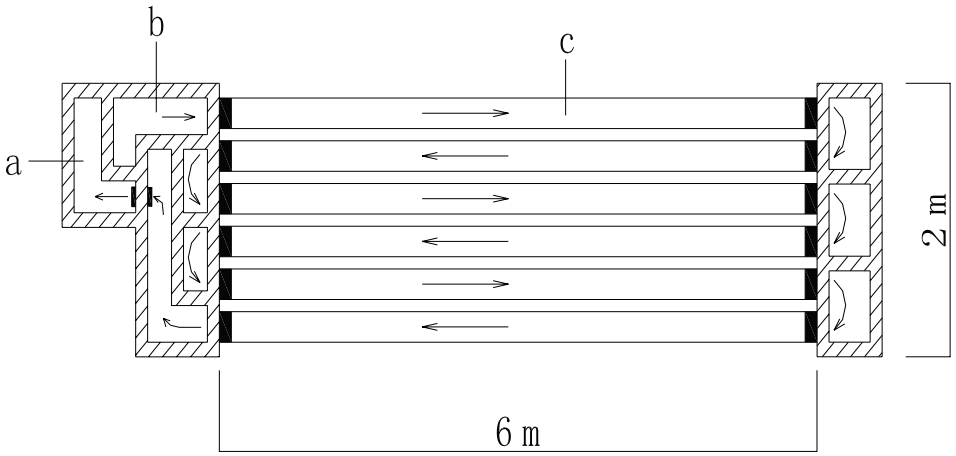

Fig. 1. The schematic drawing of the six-tube PBR. a: lower pond; b: upper pond. c. the tubes. Microalgae culture medium cycled in the reactor following the arrows showing in the drawing.

Maximum daily culture temperature was 31±1ºC in the six-tube PBR and 26±1ºC in the eight-tube PBR. Average minimum night temperature was 16.5±1ºC in both the six- and eight-tube PBRs during the culture periods. The pH of the cultures was not controlled. The basal growth medium of the cultures was not changed throughout the cultivation, nutrient sufficiency being maintained by adding the nutrient formula every 5 days. Samples were collected daily or twice daily for analysis of the optical density (OD), chlorophyll *a* and fatty acid contents of the cultures.

Measurements of growth, chlorophyll *a* and dissolved oxygen content

The growth of the microalga was measured spectrophotometrically (VIS-7220 spectrophotometer, Rayleigh Analytical Instrument Corp., Beijing) at a reference wavelength of 645 nm in a cuvette with a 1 cm light path. OD values were converted to cell counts using a linear regression between optical density and cell counts which was determined in a preliminary experiment ($y = 167.59\ OD_{645}nm - 1.32$; $r = 0.999$; cell counts $= y \times 10^5$). Cells were harvested from 5 ml culture by centrifugation at 6000 *g* for 10 min. Pigments were extracted from the cell pellets in 90% acetone in water, until no further color was obtained. The dilute extracts about 10 ml were centrifuged and chlorophyll *a* contents were determined spectrophotometrically according to Jeffrey & Humphrey (1975). Dissolved oxygen was measured with a dissolved oxygen electrode (JPB-607) (Shanghai Precision & Scientific Instrument Co., LTD, China).

Determination of fatty acid composition

Fatty acid analyses of outdoor *P. viridis* cultures were made on samples collected at the end of the dark period. For fatty acid determination, cells were harvested by centrifugation at $6000 \times g$ for 10 min followed by washing twice with distilled water. The biomass was freeze-dried and analyzed immediately.

Methylation of the fatty acids was performed using a mixture of acetyl chloride-methanol at a proportion of 1:20 (v:v). A known amount of nonadecylic acid (nonadecanoic acid; C19:0) was added as internal standard. All samples were analysed using a HP6890 gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame-ionisation detector and a capillary column (30 m × 0.25 mm × 0.2 μm, INNOWAX, Hewlett-Packard, USA). The flow of carrier gas was helium and a 1 μl methyl ester solution of sample was injected for each analysis. The temperature program was as follows: the initial temperature was 180ºC, and raised to 200ºC at a rate of 2ºC min^{-1}, then from 200ºC to 250ºC at a rate of 5ºC min^{-1}. The final oven temperature of 250ºC was kept for 5 min. Fatty acids were identified from retention times of known standards (Sigma) and mass spectra acquired by gas chromatography-mass spectrometry.

RESULTS

Effects on growth

The mean values of cell counts throughout the incubation time in six- and eight-tube photobioreactors are depicted in Fig. 2. In the six-tube PBR, microalgal cells quickly adapted to the new environment outdoors with agitation and showed no lag stage in the growth curve. In the eight-tube PBR cultures grew for 13 days until the stationary stage was achieved and had only a 1 day lag stage. Cells were observed to be mobile throughout the exponential growth stage in both cultures. *P. viridis* reached its maximum cell density of 1×10^7 ml^{-1} (six-tube PBR) and 6×10^6 ml^{-1} (eight-tube PBR) at the stationary stage.

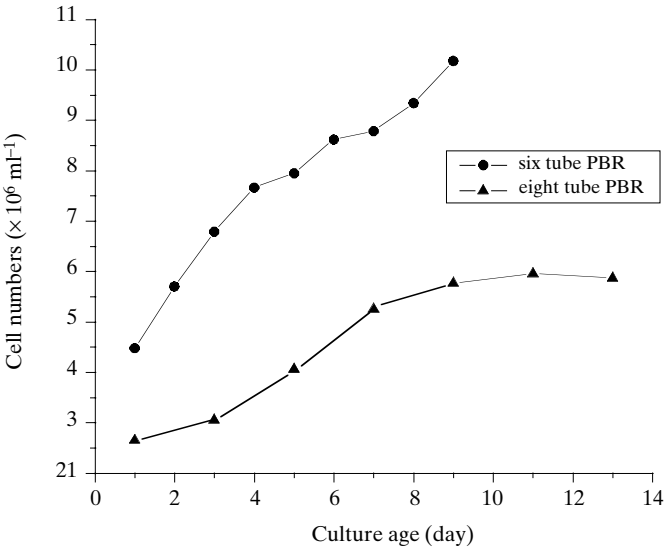

Fig. 2. Growth curves of *P. viridis* cultured in the six-tube PBR and eight-tube PBR. The results are the mean of three measurements of each day.

Effects on chlorophyll *a*

Fig. 3 shows the chlorophyll *a* content of *P. viridis* cultures, calculated from spectrophotometric data. When cultured in the six-tube PBR the chlorophyll *a* contents at the second and third days were lower than that on the first day, but the chlorophyll content significantly increased during subsequent exponential phase of growth. Biomass (as chlorophyll) dropped to 0.051 μg 10^6 cells^{-1} after a maximum at the end of the exponential growth phase of 0.066 μg 10^6 cells^{-1}. The chlorophyll *a* content of the eight-tube PBR increased continuously through the cultivation period and over the growth period rose by 50.8% compared to that on the first day. Results indicated that cellular chlorophyll *a* concentration ranged from 0.037 to 0.066 μg 10^6 cells^{-1} and 0.041 to 0.063 μg 10^6 cells^{-1} for the six-tube and eight-tube PBR, respectively.

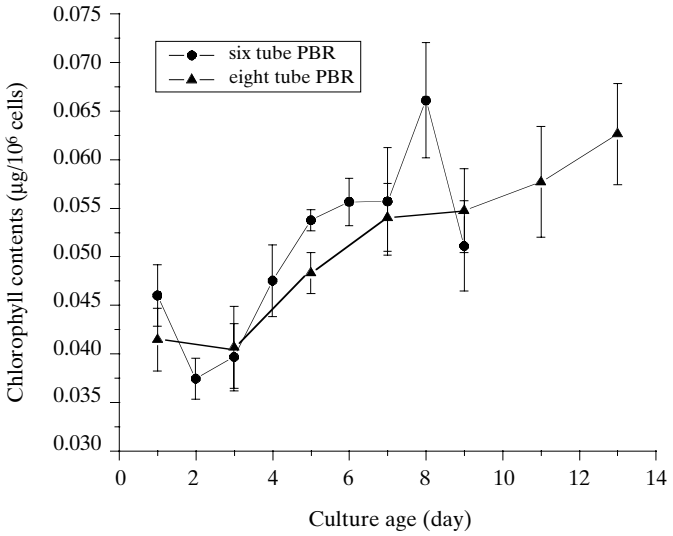

Fig. 3. The chlorophyll *a* content of *P. viridis* cultured in the six-tube PBR and eight-tube PBR. Data are the means ± SD of the three replicates.

Effects on temperature, light intensity and dissolved oxygen

The water temperature and light intensity during the cultivation of the six- and eight-tube PBRs are presented in Fig. 4 and Fig. 5. The water temperatures of the reactor varied between 13.0 and 31.1, 9.0 and 25.5ºC from 8:00 AM to 14:00 PM in the six- and eight-tube PBRs, respectively. The average light intensities at the reactor surface of the six-tube PBR and eight-tube PBRs were 133 and 165 μmol photons m^{-2} s^{-1} at 8:00 AM and 490 and 292 μmol photons m^{-2} s^{-1}at 14:00 PM, respectively. Changes in the dissolved oxygen concentration of the culture paralleled those in light intensity, with the range between 4.6 to 10.4 mg l^{-1} (Fig. 6). The dissolved oxygen concentration increased rapidly during the morning and attained the highest value when the irradiance peaked at around 1200 μmol photons m^{-2} s^{-1} at 9:00 AM and 1:00 PM with a reduction between 11:00 and 12:00 and a drop later in the afternoon.

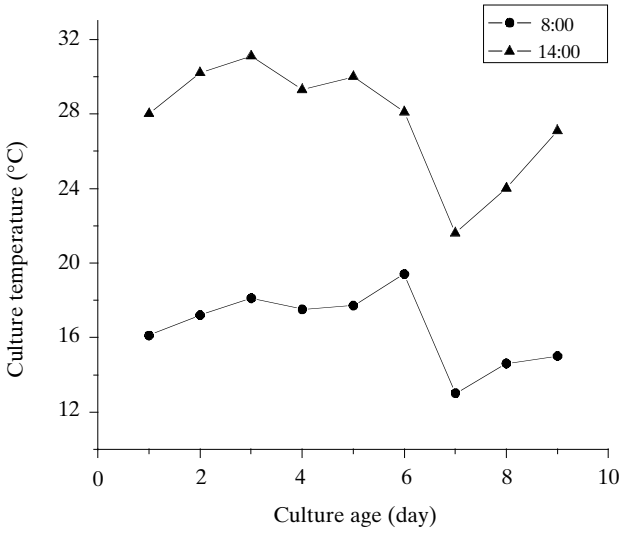

Fig. 4. The temperature during the cultivation times in the six-tube PBR (A) and eight-tube PBR (B) (8:00 AM and 14:00 PM).

Effects on fatty acids profiles and contents

Detail descriptions of the fatty acid compositions determined for the various days during cultivation of *P. viridis* are presented in Table 1 and Table 2. Very similar fatty acid profiles were found at all cultivation times. The major fatty acids were myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6).

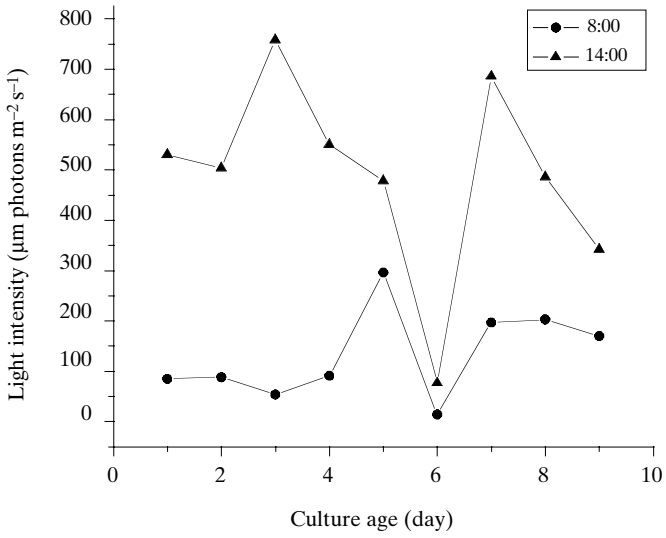

Fig. 5. The light intensity during the cultivation times in the six-tube PBR (A) and eight-tube PBR (B) (8:00 AM and 14:00 PM).

Saturated fatty acids were present at 30-35 % of the total fatty acids for the six-tube PBR and at 29-42 % in the eight-tube PBR. The fatty acids found in the biomass showed a notable increase in polyunsaturated fatty acids (20:5 and 22:6) with culture age, whereas saturated (14:0, 16:0 and 18:0) and monounsaturated fatty acids (16:1, 18:1, 18:2 and 18:3) decreased as culture progressed in the

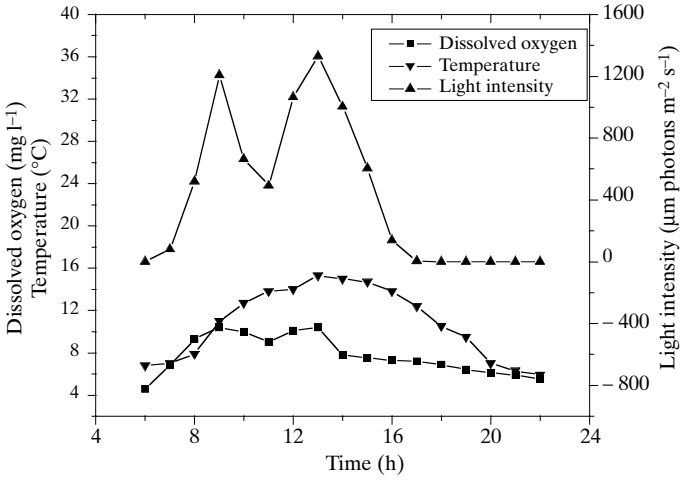

Fig. 6. The temperature, light intensity and dissolved oxygen contents during 6:00 AM to 22:00 PM someday in the eight-tube PBR cultivation.

Table 1. The major fatty acids composition (percent of total fatty acids) of *P. viridis* at different culture days in the six-tube PBR (SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Data are the mean of three replicates.The microalgae were harvested at different times of growth. Fatty acids were extracted and converted to their methyl esters before analysis by GC. Each peak was identified by the retention time compared to known standards. Only the major fatty acids are shown. Minor fatty acids are not reported, thus totals do not add up to 100%.

Fatty acid	*Culture age (days)*								
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
14:0	16.0 ± 0.9	17.4 ± 0.9	15.4 ± 0.9	16.7 ± 1.7	18.7 ± 1.8	18.4 ± 1.7	17.3 ± 1.8	19.2 ± 1.6	19.9 ± 1.5
16:0	15.8 ± 0.3	15.7 ± 0.5	15.2 ± 0.2	14.9 ± 0.3	14.9 ± 0.6	13.8 ± 0.2	11.8 ± 0.3	13.8 ± 0.6	13.9 ± 0.7
16:1(n-9)	19.3 ± 1.7	19.5 ± 2.0	15.4 ± 0.7	14.3 ± 0.5	15.2 ± 0.7	14.8 ± 0.4	13.4 ± 0.4	16.2 ± 0.8	16.1 ± 0.7
18:0	2.5 ± 0.2	1.4 ± 0.3	1.7 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	0.9 ± 0.1	0.9 ± 0.2	1.8 ± 0.4	1.1 ± 0.2
18:1(n-9)	1.5 ± 0.2	1.4 ± 0.3	1.5 ± 0.2	1.4 ± 0.4	2.2 ± 0.3	1.7 ± 0.2	1.7 ± 0.3	1.8 ± 0.3	1.3 ± 0.6
18:2(n-6)	3.5 ± 0.7	2.2 ± 0.5	3.5 ± 0.5	3.2 ± 0.5	2.2 ± 0.2	2.6 ± 0.5	1.6 ± 0.3	1.6 ± 0.4	1.6 ± 0.7
18:3(n-3)	1.9 ± 0.3	1.3 ± 0.2	2.2 ± 0.3	2.3 ± 0.2	2.2 ± 0.3	2.0 ± 0.2	1.4 ± 0.5	1.7 ± 0.1	1.7 ± 0.6
20:5(n-3)	16.3 ± 1.2	18.0 ± 1.3	16.4 ± 1.1	17.6 ± 1.6	17.0 ± 2.0	21.0 ± 1.9	20.3 ± 2.1	20.9 ± 1.9	21.2 ± 2.2
22:6(n-3)	5.8 ± 0.9	6.8 ± 0.7	6.6 ± 0.5	6.9 ± 0.9	6.6 ± 0.3	7.5 ± 0.9	7.2 ± 0.6	7.7 ± 0.5	8.7 ± 0.7
SFA	34.3	34.5	32.3	32.6	34.7	33.1	30.0	34.8	34.9
MUFA	26.2	24.4	22.6	21.2	21.8	20.1	18.1	21.3	20.7
PUFAs	22.1	24.8	23.0	24.5	23.6	28.5	27.5	28.6	29.9

Table 2. The major fatty acid composition (percent of total fatty acids) of *P. viridis* at different days during cultivation in the eight-tube PBR. (SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Data are the mean of three replicates. Only the major fatty acids are shown. Minor fatty acids are not reported, thus totals do not add up to 100%).

Fatty acid	*Culture age (days)*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
14:0	20.7 ± 2.6	19.0 ± 1.9	16.1 ± 1.3	16.2 ± 1.6	15.4 ± 1.3	17.2 ± 1.6	16.4 ± 1.5
16:0	19.6 ± 1.6	18.6 ± 0.8	17.4 ± 0.9	15.8 ± 0.4	14.1 ± 0.5	15.2 ± 0.3	11.5 ± 0.4
16:1(n-7)	22.3 ± 2.3	22.4 ± 2.0	20.7 ± 1.7	23.1 ± 1.5	19.7 ± 1.7	18.0 ± 1.4	17.0 ± 1.4
18:0	1.8 ± 0.1	1.7 ± 0.2	1.5 ± 0.2	1.2 ± 0.6	1.2 ± 0.2	1.6 ± 0.2	0.8 ± 0.3
18:1(n-9)	2.4 ± 0.6	2.9 ± 0.9	3.2 ± 0.9	1.5 ± 0.3	1.1 ± 0.4	1.0 ± 0.3	0.8 ± 0.3
18:2(n-6)	1.7 ± 0.2	2.8 ± 0.9	2.6 ± 0.5	1.6 ± 0.3	1.6 ± 0.3	2.5 ± 0.5	1.1 ± 0.3
18:3(n-3)	1.9 ± 0.3	1.8 ± 0.2	2.1 ± 0.4	2.2 ± 0.6	1.7 ± 0.4	1.8 ± 0.2	1.6 ± 0.4
20:5(n-3)	12.2 ± 1.9	12.4 ± 1.5	17.0 ± 1.7	18.7 ± 1.6	19.9 ± 1.6	23.1 ± 1.8	25.2 ± 2.2
22:6	4.0 ± 0.7	6.8 ± 0.9	5.7 ± 0.6	6.3 ± 0.9	7.5 ± 0.6	8.5 ± 0.6	10.7 ± 1.2
SFA	42.1	39.3	35.0	33.2	30.7	34.0	28.8
MUFA	28.3	31.57	28.6	28.4	24.1	23.3	20.5
PUFAs	16.2	19.2	22.7	25.0	27.4	31.6	35.9

eight-tube PBR. An increase of polyunsaturated fatty acids by one fourth was observed in the six-tube PBR, (22.1 to 29.9% of the total fatty acids) whereas it increased from 16.2 to 35.9% in the eight-tube PBR when cultivation ended.

DISCUSSION

Although EPA is found in many species of microalgae, only a few strains, such as *Phaeodactylum tricornutum* (Reis *et al.*, 1996) and *Isochrysis galbana* (Molina Grima *et al.*, 1994b), are suitable for industrial production because of their fast growth rate and high EPA content. The EPA production potential of microalgae depends on the characteristics of the specific algal species such as higher achievable cell densities and more efficient control of the principal culture parameters as well as the cultivation strategies developed. The algae can be grown in the open systems such as ponds, tanks and closed systems, either photoautotrophically, mixotrophically or heterotrophically. Due to its high EPA content, *P. viridis* has been proposed as a potential source of EPA (Lu *et al.*, 2001). Although in China *P. viridis* is currently cultivated in spring in large outdoor tanks as a feed for the aquaculture of fish larvae (Liu, 1995), data from outdoor mass cultivation of these microalgae is scanty.

Microalgal mass cultures have been widely investigated for production of health foods, pharmaceuticals, pigments, vitamins, carbohydrates, and other fine chemicals (Borowitzka, 1999). Microalgae have higher CO_2 fixation rates than terrestrial plants and thus can utilize CO_2 from flue gas to produce biomass (Negoro *et al.*, 1991). However, in order to utilize solar energy for mass algae production, efficient cultivation systems are required. Closed photobioreactors such as tubular photobioreactors have the potential to overcome the main limitations encountered in open ponds and are promising sources for production of algal biomass. Such approaches can bring EPA production by microalgae closer to becoming a reality (Chini Zittelli *et al.*, 1999; Amos & Zhang, 2001). Nevertheless, most of the proposed tubular photobioreactors are still very expensive for microalgal mass cultivation. In order to utilize the potential advantages of tubular photobioreactors, some aspects of the microalgae used in tubular photobioreactors have been studied.

In the present work, for the first time, an outdoor closed photobioreactor has been assembled for *P. viridis* cultivation. The intention of our study was to evaluate the function and usability of this device. Our work shows that the reactor can be successfully used to cultivate *P. viridis* under natural climatic conditions, and has shown the potential to overcome the limitations encountered in outdoor open ponds and other traditional culture systems. The closed reactors have several advantages such as 'clean' algal culture, better mixing, easy operation and the ability to be used outdoors in natural daylight in autumn. We considered the electricity, water usage and other costs and found the device to be economical for commercial production.

We measured the dissolved oxygen, light intensity and temperature on the last day of the cultivation in the eight-tube PBR. In our study the highest oxygen concentrations did not cause cell death under conditions of this low light irradiance. It has been previously reported (Vonshak, 1997a) that, in large ponds with small water circulation and turbulence, O_2 concentrations may reach as high as 500% of saturation, inhibiting photosynthesis and growth, and eventually leading to culture death (Marquez *et al.*, 1995). This phenomenon was not observed in our reactor.

We studied the fatty acid profile during cultivation. The fatty acid composition of *P. viridis* in our results was similar to those found in other *Pavlova* species except they have much higher abundances of 18:4 (Volkman *et al.*, 1991). The results showed that the content of polyunsaturated fatty acids increased in *Pavlova* with culture age in a six-tube or an eight-tube PBR.

Temperature is a very important factor affecting cell growth, lipid composition and n-3 PUFA formation of microalgae. The effect of temperature on EPA biosynthesis appears to be complex and has not been clarified. An increase in the level of unsaturation in microbial lipids is thought to be one of the responses of the microorganism to maintain fluidity in, and hence function of, membranes during acclimatization to low temperatures (Harwood & Jones, 1989). Seto *et al.* (1992) found that cells grown at 20°C contained 60% more EPA than cells grown at the optimal growth temperature of 25°C. Similarly, Sukenik *et al.* (1993) showed that cultures grown at a low temperature (20°C) were characterized by a relatively high level of EPA, as compared with cells grown at a high temperature (30°C). In contrast with these authors, Teshima *et al.* (1983) found maximal EPA synthesis at the optimal growth temperature of 25°C. Although the effect of temperature is not easy to establish in outdoor cultures, where both temperature and irradiance vary during the day, the present work would suggest that minimum morning temperatures (8:00 am) play a key role in

influencing the fatty acid levels and composition. Our results showed that although the fatty acid profile of the microalga *P. viridis* varied significantly during the cultivation period, the total fatty acid content remained rather stable around a value of 11% of dry biomass.

Among the factors cited above, solar irradiance played a vital role in determining the productivity of the microalgae. In microalgal outdoor culture, and provided that pH and nutrient supply are suitable, the main factors determining the growth rate are temperature and light availability to cells. Renaud *et al.* (1991) showed an increasing saturation of the fatty acids of *N. oculata* with increasing irradiance in large scale outdoor cultures. On the other hand, Seto *et al.* (1992) reported that high light intensity has no effect on the EPA distribution and content. Some species such as *Chaetocerns calcitrans, Isochrysis* sp. or *Pavlova lutherii* also exhibited no significant difference in their 20:5 content (as a percentage of total fatty acids) as a function of irradiance (Thompson *et al.*, 1990). The experiments carried out in our eight-tube outdoor reactor confirm that decreasing light intensity causes an increase of the relative PUFAs content (that accumulate up to 36 % of the total fatty acids) due to the decrease in saturated and monounsaturated fatty acid contents.

Although lower temperature and light intensity bring about higher contents of EPA and other PUFAs per unit mass, microalgal growth rate and biomass accumulation are decreased. Therefore, the highest overall production yields of PUFAs and EPA could not be achieved at low temperature levels. Culturing an alga at its optimal temperature for the highest biomass density and then shifting it to a lower temperature can potentially generate higher yields of EPA and other PUFAs. However, the feasibility of such a practice depends on the balance of the cost and commercial values of the product.

Our results indicate that outdoor mass culture of the marine microalgae *P. viridis* is suitable for the production of fatty acids and closed photobioreactors appear the system of choice.

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