Journal of Ocean University of China (Oceanic and Coastal Sea Research)
ISSN 1672-5182, April 30, 2005, Vol.4, No.2, pp.157-162
http://www.ouc.edu.cn/xbywb/
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Effect of Growth Phase on the Fatty Acid Compositions of Four Species of Marine Diatoms

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(Received June 17, 2004; accepted March 7, 2005)

Abstract The fatty acid compositions of four species of marine diatoms (*Chaetoceros gracilis* MACC/B13, *Cylindrotheca fusiformis* MACC/B211, *Phaeodactylum tricornutum* MACC/B221 and *Nitzschia closterium* MACC/B222), cultivated at 22 ℃ ± 1 ℃ with the salinity of 28 in f/2 medium and harvested in the exponential growth phase, the early stationary phase and the late stationary phase, were determined. The results showed that growth phase has significant effect on most fatty acid contents in the four species of marine diatoms. The proportions of 16:0 and 16:1n-7 fatty acids increased while those of 16:3n-4 and eicosapentaenoic acid (EPA) decreased with increasing culture age in all species studied. The subtotal of saturated fatty acids (SFA) increased with the increasing culture age in all species with the exception of B13. The subtotal of monounsaturated fatty acids (MUFA) increased while that of polyunsaturated fatty acids (PUFA) decreased with culture age in the four species of marine diatoms. MUFA reached their lowest value in the exponential growth phase, whereas PUFA reached their highest value in the same phase.

Key Words *Chaetoceros gracilis*; *Cylindrotheca fusiformis*; *Phaeodactylum tricornutum*; *Nitzschia closterium*; growth phase; fatty acid

Number ISSN 1672-5182(2005)02-157-06

1 Introduction

In recent years, there is a rapidly increasing interest in polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) due to their involvement in human health and nutrition, including the prevention and treatment of chronic diseases such as coronary heart disease, hypertension, ocular diseases, arthritis and cystic fibrosis (Alonso *et al.*, 1992; Tan and Johns, 1996; Liang and Mai, 2000; Tonon *et al.*, 2002). Microalgal fatty acids also play an important role in animal nutrition as energy sources, membrane constituents and metabolic intermediates (Brown *et al.*, 1996; Fidalgo *et al.*, 1998). Many marine animals appear to have limited ability to synthesize some PUFAs that are important for maintaining good growth and survival. They must be obtained from food. Although EPA and DHA are not essential to some animals, the growth and survival rates will increase if these fatty acids are present in their food (Reitan *et al.*, 1994; Fidalgo *et al.*, 1998; Liang and Mai, 2000).

The microalgal fatty acid composition depends not only on the species and strains used in experiments, but also on factors related to culture conditions including composition of the medium (Yongmanitchai and Ward, 1991; Fidalgo *et al.*, 1998; Lourenco *et al.*, 2002), light intensity (Brown *et al.*, 1996), salinity (Xu and Beardall, 1997) and temperature (Zhu *et al.*, 1997; Li *et al.*, 2003). Several studies have reported that growth phase also has a dramatic effect on the fatty acid compositions of some microalgae (Fernandez-Reiriz *et al.*, 1989; Hallegraeff *et al.*, 1991; Brown *et al.*, 1996; Fidalgo *et al.*, 1998; Belkoura *et al.*, 2000; Wei *et al.*, 2000; Zhukova and Aizdaicher, 2001; Liang *et al.*, 2002; Tonon *et al.*, 2002; Mansour *et al.*, 2003). However, to our knowledge, no one has examined the effect of growth phase on the fatty acid compositions of the four species of marine diatoms used in our experiments.

In this work, we analyzed the fatty acid compositions of four species of marine diatoms, *Chaetoceros gracilis*, *Cylindrotheca fusiformis*, *Phaeodactylum tricornutum* and *Nitzschia closterium*, harvested in three different growth phases (the exponential growth phase, the early stationary phase and the late stationary phase), in order to see if growth phase is a factor which could be used to optimize the microalgal fatty acid composition from the viewpoint of nutritional value for aquaculture and a source of EPA- rich products.

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2 Materials and Methods

2.1 Culture Conditions

Microalgal cultures *C. gracilis* MACC/B13, *C. fusiformis* MACC/B211, *P. tricornutum* MACC/B221 and *N. closterium* MACC/B222 were obtained from the Microalgae Culture Center (MACC), Ocean University of China. Duplicate cultures for each species were carried out in f/2 medium (Guillard and Ryther, 1962) at 22 ℃ ± 1 ℃ using 5-L flasks with continuous aeration and 5000 lx light intensity were supplied by fluorescent tubes. Cultures were monitored daily for cell counts. Duplicate 1 mL aliquots were removed from each microalgal culture and were microscopically determined with a haemocytometer.

2.2 Fatty Acid Analysis

Cultures were harvested at three different points of the growth curve: the exponential growth phase, the early stationary phase and the late stationary phase, respectively (Fig.1). Samples for fatty acid analysis were centrifuged at 4000 r min^{-1}, freeze-dried and preserved at −40 ℃ in test tubes filled with nitrogen for future analysis.

Total fatty acid methyl esters (FAME) were prepared by the modified method of Zhukova and Aizdaicher (1995) and the resulting FAME were analyzed by an HP5890II gas chromatograph fitted with a carbowax capillary column (30 m × Φ0.25 mm). High purity N_2 was used as the carrier gas at a flow rate of 2 mL min^{-1}. Both the injector and detector temperature were 280 ℃. The oven was programmed from 150 ℃ to 200 ℃ at 15 ℃ min^{-1}, then to 250 ℃ at 2 ℃ min^{-1} and held at 250 ℃ until all peaks appeared. Fatty acid methyl esters were identified by comparing the retention times of experimental samples with those of known standards.

2.3 Statistical Analysis

Statistical analyses were performed using SPSS (version 11.5) statistical software with possible differences among groups being tested by one-way ANOVA. Differences are reported as significant when $P < 0.05$.

3 Results

The fatty acid compositions in the four species of marine diatoms are given in Table 1.

The main fatty acids of B13 in all growth phases were 14:0 (17.59% – 21.76%), 16:0 (11.26% – 17.47%), 16:1n-7 (27.76% – 41.16%), 20:4n-6 (3.90%–10.18%) and EPA (4.92%–9.39%) (Table 1).

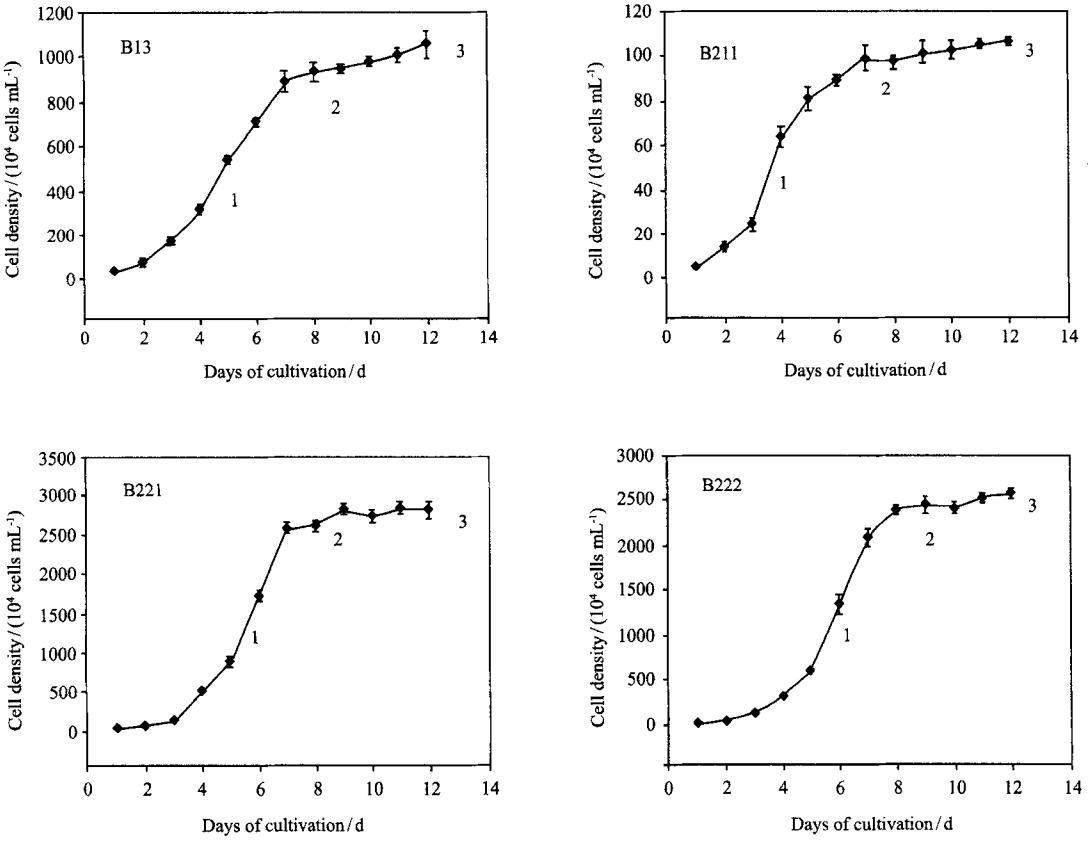

Fig.1 Growth curves of four species of marine diatoms. Data points represent means ± S. D. (n = 4). 1. The exponential growth phase; 2. The early stationary phase; 3. The late stationary phase.

Table 1 The fatty acid compositions (% of total fatty acids) in the four species of marine diatoms harvested in the exponential, early and late stationary growth phases (means ± S.D.)

Microalgal species	*Chaetoceros gracilis* MACC/B13			*Cylindrotheca fusiformis* MACC/B211		
Growth phases	1	2	3	1	2	3
14:0	21.76 ± 0.08	18.50 ± 1.32	17.59 ± 0.76	12.11 ± 0.06^{a}	8.22 ± 0.36^{b}	6.99 ± 0.37^{b}
16:0	11.26 ± 0.03	15.14 ± 0.25	17.47 ± 5.34	20.92 ± 0.72	32.09 ± 4.10	33.31 ± 1.76
16:1n-7	27.76 ± 0.37^{b}	35.31 ± 2.22^{ab}	41.16 ± 2.00^{a}	17.49 ± 0.39	22.41 ± 1.01	28.29 ± 3.35
16:2n-7	3.84 ± 0.06^{a}	2.52 ± 0.49^{ab}	1.70 ± 0.28^{b}	3.19 ± 0.35^{a}	1.03 ± 0.30^{b}	0.51 ± 0.01^{b}
16:2n-4	1.41 ± 0.04	1.14 ± 0.23	1.94 ± 0.12	3.09 ± 0.40^{a}	0.96 ± 0.44^{b}	0.30 ± 0.01^{b}
16:3n-4	2.77 ± 0.05	1.80 ± 0.49	1.10 ± 0.12	6.04 ± 0.25^{a}	2.74 ± 0.18^{b}	1.49 ± 0.01^{c}
16:4n-1	0.61 ± 0.05	–	–	0.73 ± 0.05^{a}	0.54 ± 0.04^{b}	0.32 ± 0.01^{c}
18:0	2.30 ± 0.07^{a}	0.79 ± 0.19^{b}	0.74 ± 0.02^{b}	0.35 ± 0.03	0.57 ± 0.17	0.37 ± 0.01
18:1n-9	2.38 ± 0.08	1.87 ± 1.03	1.66 ± 0.32	2.51 ± 0.42^{b}	4.93 ± 0.33^{a}	5.56 ± 0.01^{a}
18:1n-7	1.54 ± 0.03	1.51 ± 0.53	1.36 ± 0.08	1.44 ± 0.19^{b}	3.18 ± 0.02^{a}	3.32 ± 0.01^{a}
18:2n-6	–	0.96 ± 0.19	1.26 ± 0.03	1.63 ± 0.35	1.41 ± 0.17	1.21 ± 0.01
18:3n-6	–	0.12 ± 0.04	–	0.42 ± 0.07	0.46 ± 0.11	–
18:3n-3	–	0.51 ± 0.03	0.25 ± 0.02	0.12 ± 0.01	0.15 ± 0.03	–
18:4n-3	1.03 ± 0.02	–	–	0.47 ± 0.04^{b}	0.70 ± 0.03^{a}	0.73 ± 0.02^{a}
20:4n-6	3.90 ± 0.04	10.18 ± 0.09	5.06 ± 1.25	9.95 ± 0.52	7.68 ± 1.36	7.26 ± 0.03
20:4n-3	0.80 ± 0.02	–	–	0.27 ± 0.01	0.23 ± 0.09	–
20:5n-3	9.39 ± 0.01^{a}	6.92 ± 0.15^{b}	4.92 ± 0.18^{b}	11.66 ± 1.34^{a}	5.59 ± 0.18^{b}	4.35 ± 0.02^{b}
22:4n-6	1.01 ± 0.04	–	–	0.60 ± 0.14	0.19 ± 0.09	0.71 ± 0.04
22:5n-3	1.08 ± 0.05	–	–	0.39 ± 0.00	0.18 ± 0.06	0.13 ± 0.01
22:6n-3	0.26 ± 0.01	1.14 ± 0.30	0.54 ± 0.08	0.94 ± 0.07^{a}	0.19 ± 0.03^{b}	0.21 ± 0.01^{b}
SFA	35.32 ± 0.02	34.43 ± 1.55	35.80 ± 6.09	33.38 ± 0.79^{b}	40.88 ± 3.77^{a}	40.67 ± 1.40^{a}
MUFA	31.68 ± 0.27^{b}	38.69 ± 1.72^{ab}	44.18 ± 2.23^{a}	21.44 ± 0.22^{b}	30.52 ± 0.67^{ab}	37.17 ± 3.35^{a}
PUFA	26.10 ± 0.08^{a}	25.29 ± 0.05^{a}	16.77 ± 2.03^{b}	39.50 ± 1.81^{a}	22.05 ± 0.40^{b}	17.22 ± 0.07^{b}
Microalgal species	*Phaeodactylum tricornutum* MACC/B221			*Nitzschia closterium* MACC/B222		
Growth phases	1	2	3	1	2	3
14:0	9.35 ± 0.03^{a}	9.38 ± 0.05^{a}	7.57 ± 0.07^{b}	7.93 ± 0.34	6.65 ± 0.42	7.17 ± 0.13
16:0	16.20 ± 0.14^{b}	26.18 ± 0.38^{a}	26.85 ± 0.13^{a}	17.55 ± 1.00^{b}	27.53 ± 0.36^{a}	28.75 ± 0.27^{a}
16:1n-7	25.79 ± 0.47^{c}	36.74 ± 1.02^{b}	42.0 ± 0.02^{a}	26.80 ± 0.07^{b}	38.08 ± 1.45^{a}	37.95 ± 0.10^{a}
16:2n-7	2.45 ± 0.06^{a}	1.09 ± 0.25^{b}	2.87 ± 0.04^{a}	2.90 ± 0.62	1.05 ± 0.05	1.46 ± 0.04
16:2n-4	3.54 ± 0.03^{a}	1.33 ± 0.03^{b}	1.22 ± 0.04^{b}	3.70 ± 0.32^{a}	1.13 ± 0.15^{b}	1.05 ± 0.01^{b}
16:3n-4	6.15 ± 0.04^{a}	2.52 ± 0.24^{b}	1.70 ± 0.02^{c}	5.78 ± 0.22^{a}	1.74 ± 0.20^{b}	1.59 ± 0.01^{b}
16:4n-1	2.00 ± 0.04^{a}	0.52 ± 0.06^{b}	0.52 ± 0.02^{b}	2.03 ± 0.15^{a}	0.53 ± 0.03^{b}	0.49 ± 0.03^{b}
18:0	0.73 ± 0.03^{a}	0.52 ± 0.08^{ab}	0.39 ± 0.02^{b}	10.66 ± 0.05	0.52 ± 0.05	0.41 ± 0.01
18:1n-9	1.85 ± 0.08^{b}	3.38 ± 0.15^{a}	3.70 ± 0.05^{a}	1.97 ± 0.11^{b}	5.85 ± 0.54^{a}	6.52 ± 0.27^{a}
18:1n-7	1.69 ± 0.03	1.18 ± 0.03	1.03 ± 0.02	1.09 ± 0.07^{a}	0.55 ± 0.01^{b}	0.59 ± 0.01^{b}
18:2n-6	0.61 ± 0.01	0.79 ± 0.18	0.86 ± 0.05	0.23 ± 0.02^{b}	0.50 ± 0.04^{a}	0.17 ± 0.01^{b}
18:3n-6	–	0.13 ± 0.03	0.83 ± 0.02	0.35 ± 0.05^{a}	0.36 ± 0.04^{a}	0.11 ± 0.01^{b}
18:3n-3	0.84 ± 0.02^{a}	0.55 ± 0.07^{b}	0.09 ± 0.01^{c}	0.83 ± 0.11^{a}	0.17 ± 0.09^{b}	0.42 ± 0.01^{b}
18:4n-3	–	0.15 ± 0.05	–	0.24 ± 0.02	0.28 ± 0.17	0.18 ± 0.01
20:4n-6	1.06 ± 0.02^{a}	0.47 ± 0.16^{b}	0.83 ± 0.01^{ab}	0.39 ± 0.19	0.27 ± 0.03	0.40 ± 0.02
20:4n-3	1.29 ± 0.01^{a}	0.68 ± 0.02^{b}	0.47 ± 0.01^{c}	0.33 ± 0.00^{b}	1.01 ± 0.03^{a}	0.22 ± 0.01^{c}
20:5n-3	18.33 ± 0.09^{a}	9.58 ± 0.03^{b}	7.27 ± 0.20^{c}	18.02 ± 0.31^{a}	8.24 ± 1.62^{b}	8.79 ± 0.42^{b}
22:5n-3	2.33 ± 0.16	–	0.76 ± 0.05	–	–	0.96 ± 0.03
22:6n-3	0.96 ± 0.01	1.23 ± 0.30	0.30 ± 0.01	2.54 ± 0.15^{a}	0.41 ± 0.11^{b}	0.42 ± 0.02^{b}
SFA	26.28 ± 0.10^{c}	36.08 ± 0.44^{a}	34.81 ± 0.02^{b}	26.14 ± 1.33^{b}	34.7 ± 0.22^{a}	36.33 ± 0.18^{a}
MUFA	29.33 ± 0.58^{c}	41.30 ± 0.60^{b}	46.73 ± 0.02^{a}	29.86 ± 0.25^{b}	44.48 ± 1.98^{a}	45.06 ± 0.18^{a}
PUFA	39.56 ± 0.18^{a}	19.04 ± 0.08^{b}	17.72 ± 0.28^{c}	37.34 ± 0.24^{a}	15.69 ± 2.04^{b}	16.26 ± 0.46^{b}

Notes: – Not detectable. 1. The exponential growth phase; 2. The early stationary phase; 3. The late stationary phase. For each species, values with different superscript letters (a, b and c) in the same line are significantly different ($P<0.05$).

The most obvious increase was shown by 16:1n-7 (from 27.76% in the exponential growth phase to 41.16% in the late stationary phase) and the obvious decrease by EPA (from 9.39% in the exponential growth phase to 4.92% in the late stationary phase) during the experimental period. The subtotal of saturated fatty acids (SFA) did not change significantly in different growth phases (34.43% – 35.80%). The subtotal of monounsaturated fatty acids (MUFA) increased from 31.68% to 44.18% with increasing cul-

ture age, which was mainly due to the increase in 16:1n-7 (from 27.76% to 41.16%). The subtotal of polyunsaturated fatty acids (PUFA) showed rapid decrease with the culture age (from 26.10% to 16.77%), which was primarily due to the decrease of EPA from the exponential growth phase (9.39%) to the late stationary phase (4.92%) (Table 1).

For B211, the major fatty acids were 14:0 (6.99% -12.11%), 16:0 (20.92% - 33.31%), 16:1n-7 (17.49%-28.29%), 20:4n-6 (7.26%-9.95%) and EPA (4.35%-11.66%) (Table 1). The proportions of the major fatty acids changed markedly during the experimental period. SFA and MUFA increased while PUFA decreased with the growth of B211. Maximal MUFA value (37.17%) was observed in the late stationary phase while maximal PUFA value (39.50%) was observed in the exponential growth phase (Table 1).

In the case of B221, the major fatty acids were 14:0 (7.57%-9.38%), 16:0 (16.20%-26.85%), 16:1n-7 (25.79% - 42.0%) and EPA (7.27% - 18.33%), but their levels changed markedly with the culture age (Table 1). The greatest increase was shown by 16:1n-7 (from 25.79% to 42.00%) and the most significant decrease by EPA (from 18.33% to 7.27%). The SFA maximized in the early stationary phase (36.08%). The MUFA increased with B221 growth and maximized in the late stationary phase (46.73%) whereas the PUFA maximized in the exponential growth phase (39.56%) (Table 1).

The major fatty acids of B222 were 14:0 (6.65%-7.93%), 16:0 (17.55%-28.75%), 16:1n-7 (26.80% -38.08%) and EPA (8.24%-18.02%). The proportions of 16:0 and 16:1n-7 increased while those of 16:3n-4 and EPA decreased with the growth of B222. The SFA and MUFA both maximized in the late stationary phase (36.33% and 45.06%, respectively). The PUFA decreased as the culture grew and maximized in the exponential growth phase (37.34%) (Table 1).

4 Discussion

The results presented in this work showed the differences in the fatty acid compositions among species as well as among the different growth phases in the same species.

In the present study, the major fatty acids in the four species of marine diatoms were 14:0, 16:0, 16:1n-7 and EPA, but the level of each of them changed with the culture age. In addition, B13 and B211 also had high levels of 20:4n-6. Despite the changes in the relative proportions of the main fatty acids, the fatty acid composition remained typical of diatoms at all growth stages. This is in conformity with earlier observations on the fatty acid profiles in these four diatom species (Chrismadha, 1993; Viso and Marty, 1993; Dunstan *et al.*, 1994; Reitan *et al.*, 1994; Zhukova and Aizdaicher, 1995; Tan and Johns, 1996; Liang *et al.*, 1999, 2002; Lourenco *et al.*, 2002).

The present study showed a general tendency for increased proportions of SFA and MUFA and reduced proportions of PUFA with increasing culture age over the experimental period. The results thus agree with several previous studies which showed the similar trend in some microalgae (Fernandez-Reiriz *et al.*, 1989; Siron *et al.*, 1989; Belkoura *et al.*, 2000; Mansour *et al.*, 2003). On the other hand, the data differ from other reports, which showed a slight increase with culture age in the relative proportions of EPA and DHA in some microalgae (Hallegraeff *et al.*, 1991; Shamsudin, 1992). In addition, the fatty acid composition of *P. tricornutum* observed are different from that reported by Chrismadha (1993) in the same species, which showed no significant influence of growth phase on chemical composition of *P. tricornutum*. These differences may be due to different microalgal species or strains and growth conditions. For instance, the data presented by Chrismadha (1993) were obtained from a batch culture in a tubular-photobioreactor, while 5-L glass flasks were used in the present study.

Some variations in the microalgal fatty acid composition caused by the growth phase may relate to the relative distribution of the major lipid classes in these microalgae (Brown *et al.*, 1996; Fidalgo *et al.*, 1998; Zhukova and Aizdaicher, 2001; Tonon *et al.*, 2002). Triacylglycerols (neutral lipids) from microalgae have a greater proportion of saturated and monounsaturated fatty acids such as 16:0 and 16:1n-7 while the proportions of the polyunsaturated fatty acids such as EPA and DHA are correlated with the proportions of polar lipid. Neutral lipids are considered to be storage lipids for algae while more polar lipid fractions are likely to be associated with the photosynthetic membranes of the algae (Sukenik and Carmeli, 1990; Brown *et al.*, 1996; Fidalgo *et al.*, 1998; Zhukova and Aizdaicher, 2001; Tonon *et al.*, 2002). The increases in the proportions of 16:0 and 16:1n-7 fatty acids with the increasing culture age in our study support the view that these fatty acids have a role as readily mobilizable energy reserves (Brown *et al.*, 1996). The phenomenon appears to be a complex matter of the dynamics of cellular division, nitrogen metabolism and photosynthetic fixation of carbon (Sukenik and Carmeli, 1990; Brown *et al.*, 1996; Fidalgo *et al.*, 1998).

EPA and DHA are two important fatty acids due to their potential pharmaceutical use in human health promotion and their nutritional value in aquaculture feed. In our experiments, EPA and DHA of B13 represented 4.92%-9.39% and 0.26%-1.14% of the total fatty acids, respectively. This is consistent with those of *C. gracilis* reported by Volkman *et al.* (1989),

but the EPA content is lower than that published by Thompson *et al.* (1990) in the same species. EPA of B211 was 4.35%–11.66% in the present study, which agrees with the findings of Liang *et al.* (1999) and Tan and Johns (1996) in the same species, but differs from the observations of Dunstan *et al.* (1994). EPA and DHA of B221 represented 7.27%–18.33% and 0.30%–1.23% of the total fatty acids, respectively. This agrees with the data analyzed by Viso and Marty (1993) and Lourenco *et al.* (2002), but differs from the results reported by Zhukova and Aizdaicher (1995) and Thompson *et al.* (1990). The proportions of EPA and DHA in B222 were 8.24%–18.02% and 0.41%–2.54%, respectively, differing from the data published by Viso and Marty (1993), Dunstan *et al.* (1994) and Zhou *et al.* (1996). All these differences could be related to different strains, culture media, culture conditions and methods for fatty acid analysis.

The four species of marine diatoms investigated in our study are commonly used in many mariculture systems. They are all rich in PUFA, and grow well in large-scale cultures, either indoors or outdoors (Kawamura and Takami, 1995; Daume *et al.*, 1999; Liang *et al.*, 1999, 2002; Lourenco *et al.*, 2002). The results showed that maximal PUFA values were obtained in the exponential growth phase in B211, B221 and B222. This is useful for the production of PUFA-rich microalgae for aquaculture feeds/supplements, enrichment of live feeds or EPA-rich oil for human nutraceuticals.

Acknowledgements

This work was supported by the National High Tech 863 Project of China (863-819-02-01).

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