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# Optimization of growth and fatty acid composition of a unicellular marine picoplankton, *Nannochloropsis* sp., with enriched carbon sources

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**Abstract**

A unicellular marine picoplankton, *Nannochloropsis* sp., was grown under $CO_2$-enriched photoautotrophic or/and acetate-added mixotrophic conditions. Photoautotrophic conditions with enriched $CO_2$ of 2800 $\mu$l $CO_2$ $l^{-1}$ and aeration gave the highest biomass yield (634 mg dry wt $l^{-1}$), the highest total lipid content (9% of dry wt), total fatty acids (64 mg $g^{-1}$ dry wt), polyunsaturated fatty acids (35% total fatty acids) and eicosapentaenoic acid (EPA, 20:5$\omega$3) (16 mg $g^{-1}$ dry wt or 25% of total fatty acids). Mixotrophic cultures gave a greater protein content but less carbohydrates. Adding sodium acetate (2 mM) decreased the amounts of the total fatty acids and EPA. Elevation of $CO_2$ in photoautotrophic culture thus enhances growth and raises the production of EPA in *Nannochloropsis* sp.

## Introduction

Increasing attention is being paid to *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs) in microalgae due to their nutritive values for economic marine animals and human health (Wen & Chen 2000). The effects of carbon sources on growth and biochemical composition have been studied for several species of microalgae (Chu *et al*. 1995, Gordillo *et al*. 1998, Wen & Chen 2000). The biomass of *Ankistrodesmus convolutus* increased almost 5-fold when grown with 0.1% (w/v) glucose (Chu *et al*. 1995), while that of *Chlamydomonas humicola* increased 20-fold on acetate (Laliberte & de la Noue 1993). Growth of *Dunaliella viridis* was enhanced when $CO_2$ (1%, v/v) was included in the aeration (Gordillo *et al*. 1998). Cultures of *Nitzschia inconspicua* supplemented with glucose (0.1 w/v), acetate (0.1 w/v) or 5% (v/v) $CO_2$ attained higher biomasses (Chu *et al*. 1996). Acetate was used by *Chlamydomonas humicola* (Laliberte & de la Noue 1993). Lipid content increased at the expense of proteins in *Nitzschia inconspicua* aerated with 5% (v/v) $CO_2$, and gave the highest yield of eicosapentaenoic acid (EPA, 20:5$\omega$3) (0.34 mg $l^{-1}$) (Chu *et al*. 1996). Addition of acetate was supposed to be important for EPA production (Kitano *et al*. 1998). On the other hand, cultures aerated with 5% (v/v) $CO_2$ had a significant increase in carbohydrate content but no in lipids of *Phaeodactylum tricornutum* (Chrismadha & Borowitzka 1994). To optimize the production of the desired chemicals from microalgae, supply of appropriate carbon sources is important and has to be investigated.

*Nannochloropsis* sp., a marine yellow picoplankton, produces polyunsaturated fatty acids, especially EPA. To optimize its EPA production, the present study aims to investigate the effects of $CO_2$ enrichment and acetate addition on the growth and biochemical composition of *Nannochloropsis* sp.

## Materials and methods

### *Organism and growth conditions*

*Nannochloropsis* sp. (PP983), isolated from the East Sea of China, was obtained from the First Institute of Oceanography of the State Oceanic Administration,

Qingdao. It was grown in artificial sea water enriched with Guillard's 'f' solution (main components: 0.88 mM $NaNO_3$, 36.3 $\mu$M $NaH_2PO_4$; micronutrients: 0.08 $\mu$M $ZnSO_4$, 0.9 $\mu$M $MnCl_2$, 0.03 $\mu$M $Na_2MoO_4$, 0.05 $\mu$M $CoCl_2$, 0.04 $\mu$M $\mu$M $CuSO_4$, 11.7 $\mu$M $FeCl_3$, 11.7 $\mu$M EDTA; vitamin: 0.5 $\mu$g cyanocobalamin $l^{-1}$, 0.5 $\mu$g biotin $l^{-1}$, 100 $\mu$g thiamine · HCl $l^{-1}$).

Cultures were divided into four groups, two photoautotrophic and two mixotrophic cultures aerated with air with either 350 or 2800 $\mu$l $CO_2$ $l^{-1}$, respectively. Duplicate cultures were prepared for each group. For mixotrophic cultures, preliminary experiments showed that the alga could not use glucose. Acetate was chosen as the only usable organic carbon supplement (Sukenik & Carnneli 1990). Sterilized sodium acetate stock solution (500 mM) was added to give 2 mM in the mixotrophic cultures. All cultures were maintained in plant growth chambers (E7 Conviron) at 22 °C under continuous illumination of 50 $\mu$mol photons $m^{-2}$ $s^{-1}$. Aeration was carried out at 200 ml $min^{-1}$. Erlenmeyer flasks 120 ml containing 80 ml and 10 l Schott glass bottles containing 9 l medium were used for growing and harvesting algal cells, respectively. Cells were harvested on 10th day after the inoculation, freeze-dried and analyzed for their biochemical compositions.

*Growth monitoring*

Cell density was measured turbidimetrically at 665 nm and correlated to the dry weight by a standard graph.

*Analytical methods*

The total protein content was measured by the Lowry method. Carbohydrate was determined by the phenol/sulphuric acid method. Cells were counted by using a hemocytometer counting chamber. Total lipids of the cells were extracted with chloroform methanol according to Bligh & Dyer (1959). The extracts were transesterified in 1 M sodium methoxide (60 °C, 20 min) and re-extracted with hexane. After drying under $N_2$ and redissolving in chloroform, the fatty acid esters were analyzed by gas chromatography using a glass column (1.8 m × 2 mm) packed with 5% (w/v) DEGS (Diethylene Glycol Succinate). The injector and detector were maintained at 240 °C, with an injection volume of 1 $\mu$l. The column was initially held at 180 °C for 15 min and then increased to 200 °C at 2 °C $min^{-1}$. $N_2$ was used as the carrier gas. Individual peaks were identified by comparison with retention times of known standards. Quantitative analysis was based on known amount of internal standard (17:0 fatty acid) added to the sample before injection.

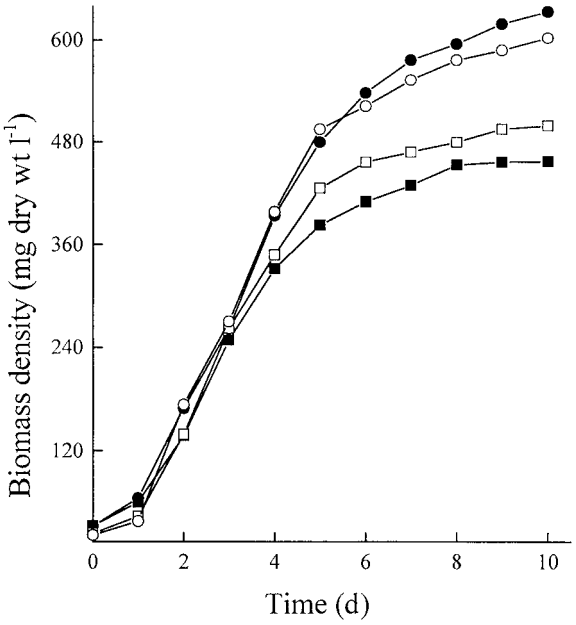


*Fig. 1.* Growth curves of *Nannochloropsis* sp. in photoautotrophic (■, ●) and mixotrophic (□, ○) cultures aerated with 350 $\mu$l $l^{-1}$ $CO_2$ (■, □) or 2800 $\mu$l $l^{-1}$ $CO_2$ (●, ○) at 22 °C and 50 $\mu$mol photons $m^{-2}$ $s^{-1}$. For mixotrophic conditions, acetate concentration = 2 mM.

## Results

*Growth*

$CO_2$ enrichment enhanced the growth of *Nannochloropsis* sp. Elevation of $CO_2$ from 350 to 2800 $\mu$l $l^{-1}$ raised the biomass yield by 39% in photoautotrophic culture and by 21% in mixotrophic culture. As sodium acetate was added to the growth medium, biomass yield increased by only 9% at the low $CO_2$ and slightly decreased at the high $CO_2$ level (Figure 1, Table 1).

*Biochemical composition*

Contents of lipids, carbohydrates and proteins ranged 7–9%, 7–13% and 34–41%, respectively, on a basis of dry weight (Table 1). Protein content increased under the high $CO_2$ level in both mixotrophic and photoautotrophic cultures as well as under the low-$CO_2$ mixotrophic condition. The highest carbohydrate content was found with the cells grown in mixotrophic

*Table 1.* Biomass yield and biochemical composition of *Nannochloropsis* sp. grown in photoautotrophic and mixotrophic cultures aerated with 350 $\mu$l $l^{-1}$ $CO_2$ or 2800 $\mu$l $l^{-1}$ $CO_2$ at 22 °C and 50 $\mu$mol photons $m^{-2}$ $s^{-1}$. Acetate concentration = 2 mM in the mixotrophic culture. Data are the means ± SD of three replicates.

| | Photoautotrophic | | Mixotrophic | |
|---|---|---|---|---|
| | 350 | 2800 $\mu$l $CO_2$ $l^{-1}$ | 350 | 2800 $\mu$l $CO_2$ $l^{-1}$ |
| Biomass yield (mg $l^{-1}$)[a] | 457 ± 15.1 | 633 ± 27.1 | 499 ± 11.1 | 603 ± 20 |
| Lipid (% w/w) | 7 ± 0.3 | 9 ± 0.5 | 7 ± 0.8 | 8 ± 0.6 |
| Carbohydrate (% w/w) | 8 ± 0.3 | 11 ± 1 | 7 ± 0.4 | 13 ± 1 |
| Protein (% w/w) | 34 ± 2.6 | 41 ± 2.9 | 39 ± 0.9 | 41 ± 2.6 |

[a]Dry weight.

culture enriched with $CO_2$; and the highest lipid content was recognized in cells grown photoautotrophically under the high $CO_2$ concentration.

*Fatty acid profiles*

The predominant fatty acids of *Nannochloropsis* sp. were palmitic acid (16:0), palmitioleic acid (16:1) and EPA (20:5$\omega$3) (Table 2) irrespective of the carbon source supplied. Both photoautotrophic and mixotrophic cultures had higher percentages of palmitic acid under the low-$CO_2$ than the high $CO_2$ conditions. The photoautotrophic conditions gave higher EPA as a percentage of total fatty acids (TFA) but lower palmitioleic acid than the mixotrophic ones, regardless of the $CO_2$ levels.

Elevation of $CO_2$ from 350 to 2800 $\mu$l $l^{-1}$ increased the amount of TFA by 10% under photoautotrophic and by 29% under mixotrophic conditions. TFA content decreased significantly in cells grown with acetate (2 mM) under either 350 or 2800 $\mu$l $l^{-1}$ $CO_2$. The polyunsaturated fatty acids (PUFAs) increased as percentages of TFA from 29% to 35% in photoautotrophically grown-cells, and from 25% to 30% in mixotrophically grown-ones when the $CO_2$ in aeration was raised to 2800 $\mu$l $l^{-1}$ $CO_2$.

EPA content increased as $CO_2$ concentration was raised from 350 to 2800 $\mu$l $l^{-1}$. The addition of acetate to the medium decreased the EPA amount relative to TFA and dry mass under both high and low concentrations of $CO_2$. The photoautotrophic cultures with $CO_2$ enrichment resulted in the highest EPA percentage of TFA, which is 25%, equivalent to 1.6% as a percentage of dry biomass.

## Discussion

Several species in the genus *Nannochloropsis* are commonly used as high-quality food organisms due to their high contents of EPA (Sukenik *et al*. 1993). The *Nannochloropsis* sp. in the present study is a species newly found in the East Sea of China. Its fatty acid composition and content appeared to be equivalent to those reported in other *Nannochloropsis* species, such as *N. salina*, *N. oculata*, *N. gaditana* and *N. limnetica* (Mourente *et al*. 1990, Krienitz *et al*. 2000). EPA content was 18–25% of TFA in *Nannochloropsis* sp. in the present study, and was 12–18% of TFA reported by Mourente *et al*. (1990) in *N. salina*, *N. oculata* and *N. gaditana*. The percentage of EPA in algal biomass varied in a range of 1.6–3.8% (w/w) in *Nannochloropsis* sp. grown under varied conditions by Sukenik *et al*. (1993), and was 1.6% (w/w) in the present study. The highest EPA percentage and cellular content (3.8%, w/w) of *Nannochloropsis* sp. grown in different seasons were obtained during winter with lower irradiance and temperature (Sukenik *et al*. 1993), and the lowest EPA content (1.6%, w/w) was achieved during the summer. By comparison, laboratory-controlled cultures at constant levels of temperature and illumination resulted in a much lower EPA percentage (Sukenik *et al*. 1993). The cellular fatty acid composition was supposed to be dependent on the interactive balance between temperature and light and may vary with algal species and experimental conditions (Sukenik *et al*. 1993).

The enhanced growth of *Nannochloropsis* sp. by addition of $CO_2$ was observed in the late growing phase probably due to carbon-limitation during this period. *Nannochloropsis* sp. grew best in culture aerated with the enriched $CO_2$, while its biomass yield was the lowest with the ambient $CO_2$ level. The effect of acetate on growth was so little that it can be ignored.

*Table 2.* Fatty acid composition (% total fatty acid) of *Nannochloropsis* sp. grown on photoautotrophic and mixotrophic cultures aerated with 350 $\mu$l $l^{-1}$ $CO_2$ or 2800 $\mu$l $l^{-1}$ $CO_2$ at 22 °C and 50 $\mu$mol photons $m^{-2}$ $s^{-1}$. Acetate concentration = 2 mM in the mixotrophic cultures. Data are the means ± SD of three replicates.

| | Photoautotrophic | | Mixotrophic | |
|---|---|---|---|---|
| Fatty acid | 350 | 2800 $\mu$l $CO_2$ $l^{-1}$ | 350 | 2800 $\mu$l $CO_2$ $l^{-1}$ |
| TFA mg $g^{-1}$ DW | 58.2 ± 0.4 | 63.8 ± 1.7 | 33.7 ± 1.1 | 43.6 ± 0.6 |
| 14:0 | 4.3 ± 0.4 | 4.1 ± 0.2 | 4.6 ± 0.7 | 5.7 ± 0.5 |
| 16:0 | 27.5 ± 0.4 | 25.4 ± 0.6 | 31.8 ± 1.1 | 24.4 ± 0.5 |
| 16:1 | 25.1 ± 0.9 | 25.6 ± 0.6 | 27.9 ± 1.6 | 27.1 ± 1 |
| 18:0 | 1.2 ± 0.1 | 0.5 ± 0.3 | 1.1 ± 0.1 | 0.8 ± 0.2 |
| 18:1 | 10 ± 0.2 | 7.1 ± 0.3 | 7.9 ± 0.1 | 9.4 ± 0.3 |
| 18:2 | 4.1 ± 0.9 | 4.8 ± 0.1 | 2.9 ± 0.3 | 4.5 ± 0.7 |
| 20:0 | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 |
| 20:1 | 1.2 ± 0.3 | 1.2 ± 0.1 | 0.6 ± 0.5 | 0.7 ± 0.6 |
| 20:4 | 2.1 ± 0.5 | 3.4 ± 0.7 | 2.4 ± 0.5 | 3.5 ± 0.1 |
| 20:5 | 21.9 ± 1.5 | 25.3 ± 1 | 18 ± 1.5 | 21.2 ± 1 |
| 22:6 | 1.4 ± 0.6 | 1.4 ± 0.2 | 1.3 ± 0.3 | 1.3 ± 0.3 |
| Others | 1.1 ± 0.1 | 1.2 ± 0.2 | 1.1 ± 0.1 | 1.3 ± 0.1 |

DW: dry weight; TFA: total fatty acids.

Acetate might have been playing a role in increasing the buffering capacity of the system rather than participating in metabolic improvement. Similarly, *Nitzschia inconspicua* has been reported to grow better with 5% (v/v) $CO_2$ than on organic carbon sources (glucose or acetate) (Chu *et al*. 1996), which might be linked to the incapability of taking up the organic carbon under light.

The fate of carbon sources incorporated into microalgal cells varies with species and is related with many factors, such as light-dark cycles and nitrogen levels. More lipids were produced in *Nitzschia inconspicua* with $CO_2$ enrichment (Chu *et al*. 1996). Sukenik & Carnneli (1990) found that the uptake of acetate by *Nannochloropsis* sp. was a light dependent process and its synthesis of lipids was greatly affected by diurnal light-dark cycles. Mixotrophic cells of *Chlamydomonas humicola* grown on acetate accumulated proteins at the expense of carbohydrates (Laliberte & de la Noue 1993). In the present study, the elevated $CO_2$ level could have increased the overall rate of photosynthesis and thus the provision of more acetyl units than previously. Then synthesis rate of fatty acid was enhanced under enriched-$CO_2$ conditions. On the other hand, the higher level of $CO_2$ increased the contents of proteins and carbohydrate to greater extents compared with that of lipids.

It is generally assumed that microalgae have two biosynthetic pathways of production of EPA, that is, desaturation of 18:2$\omega$6 is conducted by either $\Delta$6 or $\Delta$15 ($\omega$3) desaturase trails, resulting in either 18:3$\omega$6 or 18:3$\omega$3, which respectively lead to 20:4$\omega$6 and 20:5$\omega$3. Although the addition of acetate to enhanced the amount of EPA in the total fatty acids in three species of microalgae, *Rhodomonas salina*, *Nitzschia* sp. and *Navicula saprophila* (Kitano *et al*. 1998), it decreased the EPA proportions in *Nannochloropsis* sp. in the present study. Kitano *et al*. (1998) reported that mixotrophically grown *Navicula saprophila* in the presence of acetate had enhanced production of EPA through $\Delta$15 desaturation pathway. Lower EPA amount in mixotrophically grown *Nannochloropsis* sp. with acetate implies the desaturation of 18:2$\omega$6 to formation of EPA could been achieved without the involvement of $\Delta$15 desaturation pathway.

In high-$CO_2$-grown cells, 18:2$\omega$6 was of higher relative amount, and available for $\Delta$6 desaturation, which gave rise to an increased production of 18:3$\omega$6 and subsequent 20:5$\omega$3 (Tsuzuki *et al*. 1990). In *Nannochloropsis* sp. of the present study, 18:3$\omega$6 was not detected, suggesting rapid turnover of 18:3$\omega$6; and higher contents of EPA were found with the higher $CO_2$ level under both photoautotrophic and mixotrophic conditions. By contrast, Tsuzuki *et al*. (1990) found that high $CO_2$ cultures of *Porphyridium cruentum* and *Euglena gracilis* showed lower EPA contents compared with air-grown cultures. On the other hand, $CO_2$-enrichment has been shown to raise

EPA content in *Navicula saprophila* and *Phaeodactylum tricornutum* (Kitano *et al*. 1998). The present study demonstrated that elevation of $CO_2$ level could raise the production of EPA on a dry-mass basis by affecting the desaturation pathways of fatty acids.

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