Folia Microbiol. **55** (3), 265–269 (2010) **http://www.biomed.cas.cz/mbu/folia/**

Trachydiscus minutus, a New Biotechnological Source of Eicosapentaenoic acid

T. ŘEZANKA[a], M. PETRÁNKOVÁ[a], V. CEPÁK[b], P. PŘIBYL[b], K. SIGLER[a], T. CAJTHAML[a]

[a]*Institute of Microbiology v.v.i., Academy of Sciences of the Czech Republic, 142 20 Prague, Czech Republic*
fax +420 241 062 347
e-mail rezanka@biomed.cas.cz

[b]*Centre for Bioindication and Revitalization, Institute of Botany v.v.i., Academy of Sciences of the Czech Republic, 379 82 Třeboň, Czech Republic*

Received 25 March 2010
Revised version 14 April 2010

ABSTRACT. The yellow-green alga *Trachydiscus minutus* (class Xanthophyta) was cultivated in a standard medium and in media without sulfur and nitrogen. Its yield after a 16-d cultivation reached 13 g dry mass per 1 L medium. The content of oligoenoic ('polyenoic') fatty acid (PUFA), *i.e.* eicosapentaenoic (EPA), was in excess of 35 % of total fatty acids; the productivity was thus 88 mg/L per d. This result makes the alga a very prospective organism that may serve as a new biotechnological source of single cell oil.

Abbreviations

EPA	eicosapentaenoic acid	FAME	fatty acid methyl ester
FA	fatty acid	PUFA	oligoenoic ('polyenoic') fatty acid

Xanthophyceae is a group of photosynthetic organisms found in fresh water, marine waters and moist soils (Maistro *et al.* 2009). Their specific yellow-green color is caused by the presence of pigments chlorophyll *c*, chlorophyll *a* and β-carotene and they completely lack the brown pigment fucoxanthin (Andreoli *et al.* 1999; Adl *et al*. 2005). The Xanthophyta include more than 600 species.

The various members of this group, *e.g*., genera *Botrydium*, *Tribonema* or *Monodus* (Mercer *et al*. 1974; Patil *et al*. 2007) are used for production of PUFAs, especially eicosapentaenoic acid (20:5*ω*–3), docosapentaenoic acid, 22:5*ω*–6) and docosahexaenoic acid (22:6*ω*–3) which belong to essential fatty acids and cannot be produced by humans (Zhou *et al*. 1999; Tonon *et al*. 2002). These acids have been shown to be effective in preventing or treating several diseases, including coronary heart disease, asthma, type 2 diabetes mellitus, and multiple sclerosis, arthritis, kidney and skin disorders, depression and schizophrenia, *etc*. (Andrich *et al*. 2005; Simopoulos 2008).

Attempts at optimization of EPA production by microalgae have shown that the productivity depends on the nutrient status, in particular on nitrogen supply (Hu and Gao 2006; Cohen 1994).

Of all the nutrients evaluated, nitrogen limitation is the single most critical nutrient affecting lipid metabolism in algae. A general trend towards accumulation of lipids, particularly triacylglycerols, in response to nitrogen deficiency has been observed in numerous species or strains of various algal taxa (Hu *et al*. 2008).

The lack of another biogenic element – sulfur – especially in relation to lipid production has so far not been examined in detail (*cf*., *e.g*., Setlik *et al*. 1988).

This work is focused on a little explored alga *Trachydiscus minutus* isolated from the water inside cooling towers of Temelin nuclear power station, which is a rich source of PUFAs, especially EPA.

MATERIALS AND METHODS

Experimental organism. Xanthophycean alga *Trachydiscus minutus* strain Lukavsky et Pribyl 2005/1 was obtained from culture collection of the *Institute of Botany ASCR*, Třeboň, Czech Republic). It is maintained in Zehnder liquid medium (Table I) under irradiance of 25 μmol m^{-2} s^{-1} and at 12–15 °C.

Culture conditions. The alga was batch cultured in a medium (+S, +N) (in mg/L): KNO_3 4042, KH_2PO_4 340, $MgSO_4 \cdot 7H_2O$ 988, Fe/Na-EDTA 18, $CaCl_2 \cdot 6H_2O$ 10.96, H_3BO_3 3.09, $MnSO_4 \cdot 4H_2O$ 1.18, $CoSO_4 \cdot 7H_2O$ 1.4, $CuSO_4 \cdot 5H_2O$ 1.43, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.88 (Zachleder and Setlik 1982) under conti-

nuous light of 450 μmol m^{-2} s^{-1} (provided by a panel of light tubes L 36W/830 Lumilux; *Osram*, Germany) and at 28 °C. Cultures were bubbled (30 L/h) with air enriched with 2 % CO_2 (*V*/*V*) from the pressure can.

Table I. Zehnder liquid medium[a] (Staub 1961)

Stock solution No.	Component/composition	g/L	Stock solution No.	Component/composition	mg per 100 mL deionised water
1	$NaNO_3$	467	*7*	Solution of microelements	
2	$Ca(NO_3)_2 \cdot 4H_2O$	59		H_3BO_3	310
3	K_2HPO_4	31		$MnSO_4 \cdot 4H_2O$	223
4	$MgSO_4 \cdot 7H_2O$	25		$Na_2WO_4 \cdot 2H_2O$	3.0
5	Na_2CO_3	21		$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	8.8
				KBr	11.9
6	Fe/Na-EDTA	22 mL 35 % HCl		KI	8.3
		+ 250 mL H_2O		$ZnSO_4 \cdot 7H_2O$	28.7
		+ 45 g $FeCl_3 \cdot 6H_2O$		$Cd(NO_3)_2 \cdot 4H_2O$	15.4
		+ 465 g Na_2EDTA		$Co(NO_3)_2 \cdot 6H_2O$	14.6
				$CuSO_4 \cdot 5H_2O$	12.5
				$Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$	19.8
				$Cr(NO_3)_3 \cdot 7H_2O$	3.7
				$VOSO_4 \cdot 2H_2O$	3.5
				$KAl(SO_4)_2 \cdot 12H_2O$	47.4

[a]Ten mL of solutions *1*–*5* is mixed with 0.2 mL of *6*, and 80 μL of solution *7*; then the volume is made up to 1 L with deionized water.

Samples were collected at regular intervals (once a day) and the growth of cultures was assessed as the dry mass. In the stationary phase the culture was divided into 4 aliquots (60 mL) and the same volume of the following solutions added: (*1*) distilled water, (*2*) medium without sulfur (–S), (*3*) medium without sulfur and nitrogen (–S,–N) and (*4*) full medium. In –S medium, the sulfate anion was replaced with chloride; in –S,–N medium, sulfate and nitrate anions were replaced by chloride. The cells at the end of cultivation were centrifuged and lyophilized. Dry mass was determined by filtration with pre-weighed Synpor filters (pore diameter 0.6 μm) and drying to constant mass at 105 ºC.

Light and epifluorescence microscopy. Living material was observed in transmitted light using an epifluorescence microscope *Olympus* BX 60. Microphotographs were taken with a digital camera *Olympus* Camedia C-5050 ZOOM and processed using Adobe Photoshop 7.0. Intracellular oil droplets were observed in the fluorescence microscope thanks to their autofluorescence or after staining with Nile Red (Eltgroth *et al*. 2005). The microscope was equipped with the filter combination U-MNU2 (360–370 nm excitation and >515 nm barrier filter).

FAMEs analysis. The lyophilized biomass (≈100 mg) was saponified in 10 % KOH–MeOH at room temperature overnight. A fatty acid fraction obtained from saponification was partitioned between alkali solution (pH 9) and diethyl ether to remove basic and neutral components. The aqueous phase, containing fatty acids, was acidified to pH 2 and extracted with hexane. The fatty acid fraction was methylated using BF_3–methanol (Rezanka 1990; Rezanka and Mares 1987). Gas chromatography–mass spectrometry of FAME was done on a GC–MS system consisting of *Varian* 450-GC, *Varian* 240-MS ion trap detector with external ionization (EI), and CombiPal autosampler (*CTC*, USA). The sample was injected onto a 25 m × 0.25 mm × 0.1 μm Ultra-1 capillary column (*Supelco*, Czech Republic) under a temperature program: 5 min at 50 °C, increasing at 10 °C per min to 320 °C and 15 min at 320 °C. Helium was the carrier gas at a flow of 0.52 mL/min. All spectra were scanned within the range *m/z* 50–600. The structures of FAMEs were confirmed by comparison of retention times and fragmentation patterns with those of the standard FAMEs (PUFA No. 2 – Animal Source; *Supelco*).

RESULTS AND DISCUSSION

Analysis of lipids from lyophilized biomass (Table II) showed that the major lipids were triacylglycerols, which is also confirmed by two microphotographs (Fig. 1) of the cells of *Trachydiscus minutus* in transmitted light and in fluorescence. Autospores in the stationary phase contain one large central oil droplet (yellow) and several chloroplasts (red). The coloring and fluorescence of neutral lipids, mostly triacylglyce-

rols, stained with Nile Red is caused by specific interactions of the dye with FAs. Glyco- and phospholipids are not stained owing to the presence of polar head groups in their molecule.

Table II. Composition (% of dry mass) of total lipids and fatty acids and dry mass (in g/L) increase[a] in *Trachydiscus minutus* cultivated under different physiological conditions

Component		Distilled water	Medium –S	Medium –S,–N	Medium +S,+N
Total lipids		28	33	39	30
Fatty acid	14:0	26.6	41.8	47.1	53.5
	16:1ω–7	11.6	13.0	10.5	8.6
	16:0	11.7	17.5	17.2	15.0
	18:2ω–6	6.7	6.7	5.4	2.8
	18:3ω–3	5.9	10.7	7.7	3.8
	20:4ω–6	1.7	0.6	0.6	0.9
	20:5ω–3	35.8	9.7	11.5	15.4
Dry mass increase		0.6	1.0	1.0	5.7

[a]Dry mass found at the onset of stationary phase (6.2 g/L) before the samples from mother culture were mixed with the same volume of H_2O, medium (–S), (–S,–N) or full cultivation medium (+S,+N); for medium composition *see Materials and Method*.

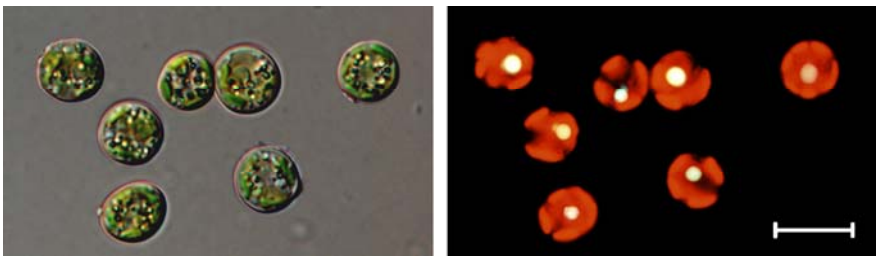

Fig. 1. Microphotographs of the cells *Trachydiscus minutus* in transmitted light (*left*) and in a fluorescence (*right*); *bar* corresponds to 10 μm.

The results of FA analysis of *T. minutus* are in agreement with the published data for other yellow-green algae (Mercer *et al*. 1974; Patil *et al*. 2007) and also with the results of basic experiments on the effect of cultivation conditions, chiefly different temperatures and light intensities, on *Trachydiscus* (Iliev *et al*. 2008).

In general, EPA is the major FA (*see* Table II). Its concentration is in the range of 9.7–35.7 % total FA depending on cultivation conditions. Other major FAs in decreasing order are 14:0, 16:0, 16:1, 18:2, 18:3 and 20:4. This content of FAs is highly suitable for further biotechnological processing since C_{14} and C_{16} FA, *i.e*. saturated and unsaturated acids, can easily be separated, producing a concentrate enriched in PUFAs.

To our knowledge, literature reports on the effect of sulfur starvation on the lipid content are scarce (*cf*. Sugimoto *et al*. 2008). Investigations concerned the effect of sulfur starvation on the proportion of sulfoquinovosyl diacylglycerol, a lipid having sulfur in the molecule, and on the content of FAs in this lipid. The lipid was found to contain no PUFAs, 90 % of total FA being due to palmitic acid. Other lipid classes, *e.g*., monogalactosyl diacylglycerol and digalactosyl diacylglycerol, and the acidic lipid phosphatidylglycerol were only negligibly affected by sulfur starvation. For instance, monogalactosyl diacylglycerol was found to contain 20.0 ± 2.2 % of 18:3 in cultivation with sulfur and 32.3 ± 1.0 % in –S cultivation (Sugimoto *et al.* 2008).

Nitrogen starvation has been reported to enhance the FA content in many species of algae (Benamotz *et al*. 1985) but generally it is accompanied by a sharp decrease in proportion of PUFA. Nitrogen starvation was brought about by cultivation in complete medium during the exponential phase, centrifugation and resuspension in nitrogen-free medium and further cultivation for another 8 d. Although the FA content increased to a record high value of 39 %, the proportion of EPA plummeted to just 11.5 %. Consequently, the EPA content was reduced to 4.48 % while the control cultivation contained 10 % EPA.

Nitrogen and sulfur starvation had an adverse effect on EPA productivity. This phenomenon has already been noted, and, as stated above, it reflects mostly a generally lower production of PUFAs under starvation conditions. For instance, increasing $NaNO_3$ concentration from 150 μmol/L to 3000 μmol/L in cultivations of the alga *Nannochloropsis* sp. enhanced the proportion of EPA from 7.9 to 29.9 % total FA while, at the same time, the content of total FA decreased from 62 to 13 % (*W*/*W*) (Hu and Gao 2006). The microalga *Monodus subterraneus* has been found to produce EPA, which is concentrated mainly in the galactolipid fraction as its major FA. Nitrogen starvation increased the FA content but reduced the proportion and content of EPA to 19.5 % of FAs and 1.8 % of dry mass, respectively (Cohen 1994). On the other hand, under nitrogen starvation the FA content constituted over 35 % of dry mass and the proportion of arachidonic acid exceeded 60 % of total FAs. This can be ascribed to the fact that, though structurally similar, the two FAs are biosynthesized by two different mechanisms, *viz*. ω–3 and ω–6 pathway (Khozin-Goldberg *et al*. 2002).

Table III. Comparison of EPA productivity (mg/L per d) of microalgae under various culture conditions

Organism	Culture vessels	Culture mode	EPA productivity	Reference
Porphyridium cruentum	pond[a]	winter	0.5	Ratledge 1997
Porphyridium cruentum	flasks	batch	3.6	Akimoto *et al.* 1998
Isochrysis galgana	glass tubes	semi-continuous	4.6	Otero *et al*. 1997*a*
Phaeodactylum tricornutum	glass tubes	semi-continuous	5.2	Otero *et al*. 1997*b*
Nannochloropsis oculata	?	batch	5.5	Sukenik *et al*. 1989
Isochrysis galgana	fermenters	continuous	7.2	Grima *et al*. 1993
Porphyridium cruentum	pond[b]	optimal weather	8.0	Ratledge 1997
Isochrysis galgana	fermenters	continuous	15.3	Grima *et al*. 1994
Phaeodactylum tricornutum	glass tubes	batch	19.0	Yongmanitchai and Ward 1991
Isochrysis galbana	cylindrical fermenters	continuous	23.8	Sevilla *et al*. 1998
Phaeodactylum tricornutum	glass tanks	continuous	25.1	Yongmanitchai and Ward 1992
Monodus subterraneus	Erlenmeyer flasks	semi-continuous	25.7	Cohen 1994
Nannochloropsis sp.	tubular photobioreactors	continuous	32.0	Zittelli *et al*. 1999
Phaeodactylum tricornutum	glass vessels[c]	batch	33.5	Garcia *et al*. 2000
Monodus subterraneus	helical tubular photobioreactor	semi-continuous	56.0	Lu *et al.* 2001
Monodus subterraneus	flat plate reactors	semi-continuous	58.9	Qiang *et al*. 1997
Trachydiscus minutus	light tubes	batch	88.0	*this paper*
Nitzschia laevis	fermenters[d]	perfusion-cell bleeding	175	Wen and Chen 2001

[a]Open pond in winter. [b]Open pond in best-case conditions. [c]Mixotrophic growth. [d]Heterotrophic growth.

Under optimum cultivation conditions, the EPA productivity by *T. minutus* in our experiments was around 88 mg/L per d. EPA productivity is a function of four parameters – the EPA proportion of FAs, the FA content, the specific growth rate, and the biomass concentration at harvest. Among microalgae, *T. minutus* is nearly a top producer of EPA (Table III).

This work was supported by the *Institutional Research Concepts* AV0Z50200510 and AV0Z60050516 and by project no. 1M0571 (*Ministry of Education, Youth and Sports of the Czech Republic*).

REFERENCES

ADL S.M., SIMPSON A.G.B., FARMER M.A., ANDERSEN R.A., ANDERSON O.R., BARTA J.R., BOWSER S.A.S., BRUGEROLLE G., FENSOME R.A., FREDERICQ S., JAMES T.Y., KARPOV S., KUGRENSI P., KRUG J., LANE C.E., LEWIS L.A., LODGE J., LYNN D.H., MANN D.G., MCCOURT R.M., MENDOZA L., MOESTRUP Ø., MOZLEY-STANDRIGE S.E., NERAD T.A., SHEARER C.A., SMIRNOV A.V., SPIEGEL F.W., TAYLOR M.F.J.R.: The new higher level classification of eukaryotes with emphasis on the taxonomy of Protists. *J.Eukaryot.Microbiol.* **52**, 399–451 (2005).

AKIMOTO M., SHIRAI A., OHTAGUCHI K., KOIDE K.: Carbon dioxide fixation and polyunsaturated fatty acid production by the red alga *Porphyridium cruentum*. *Appl.Microbiol.Biotechnol*. **73**, 26–278 (1998).

ANDREOLI C., MORO I., LA ROCCA N., RIGONI F., DALLA VALLE L., BARGELLONI L.: *Pseudopleurochloris antarctica* gen. et sp.nov., a new coccoid xanthophycean from pack-ice of Wood Bay (Ross Sea, Antarctica): ultrastructure, pigments and 18S rRNA gene sequence. *Eur.J.Phycol.* **34**, 149–159 (1999).

ANDRICH G., NESTI U., VENTURI F., ZINNAI A., FIORENTINI R.: Supercritical fluid extraction of bioactive lipids from the microalga *Nannochloropsis* sp. *Eur.J.Lipid Sci.Technol.* **107**, 381–386 (2005).

BENAMOTZ A., TORNABENE T.G., THOMAS W.H.: Chemical profile of selected species of microalgae with emphasis on lipids. *J.Phycol*. **21**, 72–81 (1985).

COHEN Z.: Production potential of eicosapentaenoic acid by *Monodus subterraneus*. *J.Am.Oil Chem.Soc*. **11**, 941–945 (1994).

ELTGROTH M.L., WATWOOD R.L., WOLFE G.V.: Production and cellular localization of neutral long-chain lipids in the haptophyte algae *Isochrysis galbana* and *Emiliania huxleyi*. *J.Phycol.* **41**, 1000–1009 (2005).

GARCIA M.C.C., SEVILLA J.M.F., FERNANDEZ F.G.A., GRIMA E.M., CAMACHO F.G.: Mixotrophic growth of *Phaeodactylum tricornutum* on glycerol: growth rate and fatty acid profile. *J.Appl.Phycol.* **12**, 239–248 (2000).

GRIMA E.M., PEREZ J.A.S., CAMACHO F.G., SANCHEZ J.L.G., ALONSO D.L.: N-3 PUFA productivity in chemostat cultures of microalgae. *Appl.Microbiol.Biotechnol*. **38**, 599–605 (1993).

GRIMA E.M., PEREZ J.A.S., CAMACHO F.G., SEVILLA J.M.F., FERNANDEZ F.G.A.: Effect of growth rate on the eicosapentaenoic acid and docosahenaenoic acid content of *Isochrysis galbana* in chemostat culture. *Appl.Microbiol.Biotechnol*. **41**, 23–27 (1994).

HU H., GAO K.: Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. *Biotechnol.Lett.* **28**, 987–992 (2006).

HU Q., SOMMERFELD M., JARVIS E., GHIRARDI M., POSEWITZ M., SEIBERT M., DARZINS A.: Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *Plant J.* **54**, 621–639 (2008).

ILIEV I., FURNADZHIEVA S., ANDREEVA R., LUKAVSKY J., PETKOV G.: Influence of temperature and light intensity on the growth and composition of *Trachydiscus minutus.* Abstr. D-10, *Conf. Responses of Plants to Environmental Stresse*s, Elena (Bulgaria) 2008.

KHOZIN-GOLDBERG I., BIGOGNO C., SHRESTHA P., COHEN Z.: Nitrogen starvation induces the accumulation of arachidonic acid in the freshwater green alga *Parietochloris incisa* (Trebuxiophyceae). *J.Phycol*. **38**, 991–994 (2002).

LU C., RAO K., HALL D., VONSHAK A.: Production of eicosapentaenoic acid (EPA) in *Monodus subterraneus* grown in a helical tubular photobioreactor as affected by cell density and light intensity. *J.Appl.Phycol*. **13**, 517–522 (2001).

MAISTRO S., BROADY P.A., ANDREOLI C., NEGRISOLO E.: Phylogeny and taxonomy of Xanthophyceae (Stramenopiles, Chromalveolata). *Protist* **160**, 412–426 (2009).

MERCER I.A., LONDON R.A., KENT I.S.A., TAYLOR A.J.: Sterols, sterol esters and fatty acids of *Botrydium granulatum*, *Tribonema aequale* and *Monodus subterraneus*. *Phytochemistry* **13**, 845–852 (1974).

OTERO A., GARCIA D., FABREGAS J.: Factors controlling eicosapentaenoic acid production in semicontinuous culture of marine microalgae. *J.Appl.Phycol*. **9**, 465–469 (1997*a*).

OTERO A., GARCIA D., MORALES E.D., ARAN J., FABREGAS J.: Manipulation of the biochemical composition of the eicosapentaenoic acids-rich microalga *Isochrysis galbana* in semicontinuous cultures. *Biotechnol.Appl.Biochem.* **26**, 171–177 (1997*b*).

PATIL V., KALLQVIST T., OLSEN E., VOGT G., GISLEROD H.R.: Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture Internat.* **15**, 1–9 (2007).

QIANG H., ZHENGYU H., COHEN Z., RICHMOND A.: Enhancement of eicosapentaenoic acid (EPA) and γ-linolenic acid (GLA) production by manipulating algal density of outdoor cultures of *Monodus subterraneus* (Eustigmatophyta) and *Spirulina platensis* (Cyanobacteria). *Eur.J.Phycol*. **32**, 81–86 (1997).

RATLEDGE C.: *Biotechnology: Products of Secondary Metabolism, Vol. 7*. VCH Press, Weinheim 1997.

REZANKA T.: Identification of very long polyenoic acids as picolinyl esters by Ag^+ ion-exchange high-performance liquid-chromatography, reversed-phase high-performance liquid-chromatography and gas-chromatography mass-spectrometry. *J.Chromatogr*. **513**, 344–348 (1990).

REZANKA T., MARES P.: Unusual and very long-chain fatty-acids produced by Basidiomycetes. *J.Chromatogr*. **409**, 390–395 (1987).

SETLIK I., BALLIN G., DOUCHA J., ZACHLEDER V.: Macromolecular syntheses and the course of cell cycle events in the chlorococcal alga *Scenedesmus quadricauda* under nutrient starvation: effect of sulfur starvation. *Biol.Plant.* **30**, 161–169 (1988).

SEVILLA J.M.F., GRIMA E.M., CAMACHO F.G., FERNANDEZ F.G.A., PEREZ J.A.S.: Photolimitation and photoinhibition as factors determining optimal dilution rate to produce eicosapentaenoic acid from cultures of the microalga *Isochrysis galbana*. *Appl. Microbiol.Biotechnol.* **50**, 199–205 (1998).

SIMOPOULOS A.P.: The importance of the ω-6/ω-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Exp.Biol.Med.* **233**, 674–688 (2008).

STAUB R.: Ernährungs-physiologisch-ökologische Untersuchungen an der planktonischen Blaualge *Oscillatoria rubescens* DC. *Schweiz. Z.Hydrol*. **23**, 82–198 (1961).

SUGIMOTO K., MIDORIKAWA T., TSUZUKI M., SATO N.: Upregulation of PG synthesis on sulfur-starvation for PS I in *Chlamydomonas*. *Biochem.Biophys.Res.Commun.* **369**, 660–665 (2008).

SUKENIK A., CARMELI Y., BERNER T.: Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloropsis* sp. *J.Phycol.* **25**, 686–692 (1989).

TONON T., HARVEY D., LARSON T.R., GRAHAM I.A.: Long chain polyunsaturated fatty acid production and partitioning to triacylglycerols in four microalgae. *Phytochemistry* **61**, 15–24 (2002).

WEN Z.Y., CHEN F.: A perfusion-cell bleeding culture strategy for enhancing the productivity of eicosapentaenoic acid by *Nitzschia laevis*. *Appl.Microbiol.Biotechnol.* **57**, 316–322 (2001).

YONGMANITCHAI W., WARD O.P.: Growth of and *ω*-3-fatty-acid production by *Phaeodactylum tricornutum* under different culture conditions. *Appl.Environ.Microbiol.* **57**, 419–425 (1991).

YONGMANITCHAI W, WARD O.P.: Growth and eicosapentaenoic acid production by *Phaeodactylum tricornutum* in batch and continuous culture systems. *J.Am.Oil Chem.Soc.* **69**, 584–590 (1992).

ZACHLEDER V., SETLIK I.: Effect of irradiance on the course of RNA synthesis in the cell cycle of *Scenedesmus quadricauda*. *Biol. Plant*. **24**, 341–353 (1982).

ZHOU N., RICHMOND A.: Effect of light-path length in outdoor flat plate reactors on output rate of cell mass and of EPA in *Nannochloropsis* sp. *J.Biotechnol.* **70**, 351–356 (1999).

ZITTELLI G.C., LAVISTA F., BASTIANINI A., RODOLFI L., VINCENZINI M., TREDICI M.R.: Production of eicosapentaenoic acid by *Nannochloropsis* sp. cultures in outdoor tubular photobioreactors. *J.Biotechnol*. **70**, 299–312 (1999).