

Temperature effect on growth, and selected parameters of *Phaeodactylum tricornutum* in batch cultures*

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The effect of optimal and stress temperatures on the growth kinetics of the *Phaeodactylum tricornutum* CCAP/1055/1 strain (a model diatom with a known genome sequence) in batch cultures was examined. The analysis of the obtained results showed two phases of culture growth. There were significant positive correlations between OD increase of chlorophyll *a* and chlorophyll *c* and protein concentration at different temperatures. The F_v/F_m parameter achieved a maximum level on the 6th or 7th day and then decreased to the values registered on the first day of observation. Genetic material undergoes gradual degradation 10 days after inoculation. The size of the cells was invariable.

Key words: *Phaeodactylum tricornutum*, diatoms, chlorophylls, cell size, photosynthesis, F_v/F_m

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INTRODUCTION

The *Phaeodactylum tricornutum* has been used as a model over several decades to study diatom cell biology, physiology and recently molecular biology and genetics (Martino *et al.*, 2011). Diatoms are the dominant phytoplankton species in the marine environment under nutrient-rich conditions. In freshwater ecosystems diatoms play a major role when high concentrations of nutrients are accompanied with low temperatures. Marine diatoms fix 20 billion tons of carbon per year which corresponds to 40% of the marine and 20% of the global net primary production. This immense carbon fixation is even higher than that of the most productive terrestrial ecosystem, the tropical rainforests (Geider *et al.*, 2001). It is known that environmental changes or experimental conditions significantly influence the morphology, physiology and molecular biology of the diatoms. Morphological transformation of the cells was observed as a result of salinity and temperature stress. The composition of the medium, temperature, inoculum size, UV radiation effected fatty acid production and some physiological parameters of photosynthesis. The temperature and light intensity also influenced the level of carbon fixation, the rate of cell division, cell length and vacuole size (Fawley, 1984; Yongmanitchai & Ward, 1991; Kudo *et al.*, 2000; Liang *et al.*, 2006; Martino *et al.*, 2011). It was observed that size of the cells at 23 to 25°C were larger than those which were grown at lower temperature (Fawley, 1984). Temperature stress effected on proportion between morphotype and transformation of cells into the oval type (Mar-

tino *et al.*, 2011). Chloroplasts were the largest at temperature above 21°C. Increase of the growth temperature resulted in increase of carbon fixation by diatoms (Fawley, 1984), eicosa-pentaenoic acid, and optimum culture temperature for fatty acid production were estimated as 21.5°C to 23°C (Yongmanitchai & Ward, 1991). Low temperature showed predominance of genes typical for stress pathway (Martino *et al.*, 2011).

Despite wide variety of experimental work on the diatoms and the enormous ecological importance of these organisms, so far no correlation between parameters of diatoms *in vitro* culture and their biological condition, manifested by, i. a. the concentration of photosynthetic pigments, proteins, cell size, RNA and DNA content, the F_v/F_m ratio and the optical density (OD_{405} , OD_{600}) was found.

The estimation of the culture conditions, such as its duration and temperature, allowing achievement of diatoms in their optimal stage of development was the goal of our work.

The experiments were performed using the *Pb. tricornutum* CCAP/1055/1 strain as a model diatom with a known genome sequence.

METHODS

The *Pb. tricornutum* CCAP 1055/1 strain was obtained from the Culture Collection of Algae and Protozoa at the Dunstaffnage Marine Laboratory, UK. The diatoms were grown in standard culture conditions in an f/2 medium (Guillard & Ryther 1962), supplemented with sodium metasilicate (3%), f/2 vitamins (filter sterilized and added after autoclaving) 1.6% sea salt (Tropic Marin). *Pb. tricornutum* cells were previously acclimated 5 to 8 days to the experimental temperature. Approximately 500 ml of acclimated inoculum with an optical density (OD_{600}) of 0.3–0.4 was used at the start of 1500 ml the culture. Cells were grown in batch cultures under photoperiod 10:14h D:L with a white light intensity of 40 $\mu\text{Em}^{-2}\text{s}^{-1}$ at optimal temperatures of 15°C and 20°C (according to CCAP 1055/1 strain data, Scottish Marine Institute, Oban, UK) as well as under stress conditions of 12°C and 23°C. Cultures were shaken several times a week during the light phase to keep cells in suspension and maintain an optimal exchange of gas and nutrients. The

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Abbreviations: Chl, chlorophyll; F_v/F_m , maximum photochemical efficiency of PS2; OD, optical density

observations were individually for each culture at different temperatures. Cultures were analysed in duplicate for each temperature and all experiments were performed in quadruplicate.

Optical density of the cultures was measured at 600 (Yongmanitchai & Ward 1991) and 405 nm (Goossens, 2011) with Metertek SP-830 spectrophotometer (1 cm standard cuvette).

Chlorophyll fluorescence was measured by a PAM-210 fluorimeter. Before measurement 1 ml of sample (batch culture) was concentrated, transferred to paper and dark — adapted for 15 min. The software generated F_o (minimum) and F_m (maximum) fluorescence values from which F_v/F_m was calculated (Maxwell & Johnson, 2000).

Protein concentration was measured by Lowry method (1951).

Chlorophyll a and *chlorophyll c* were extracted in 90% acetone, with liquid nitrogen cooling. The concentration of chlorophylls was determined with a spectrophotometer (Jasco V-650) and calculated by Jeffrey & Humphrey method (1975).

Total RNA was isolated in two steps. First, TRI Reagent (Ambion) was used to isolate RNA. Then RNasy Mini Kit (Qiagen) was used to obtain purified RNA from aqueous phase. Electrophoresis were performed in 2% agarose gel in TAE buffer (Tris/acetic acid, 0.5 M EDTA, 8.5 pH) at 80 V.

Measurements of cell length were made on live cells on the 7th and 18th day of culture (culture temperature 12°C or 20°C). A rectangular fragment of Parafilm was cut in order to form a well on the glass slides. One or two drops of the culture were put inside Parafilm well

and covered with a glass cover slip. To calculate precisely the cell length the images were collected using a Nikon Eclipse TE 200 microscope with a Nomarski-DIC prism with a 60× objective (60× LWD 0.52 Nikon, Japan Lens). The microscope was equipped with an Evolution VF Cooled Monochrome Camera (Witkom, Poland). Measurements were performed using Image-Pro Express Version 5.1.0.12. Fifty cells were measured at each temperature both on the 7th and 18th day of culture.

RESULTS AND DISCUSSION

Analysis of the results demonstrated that temperature had significant effects on the growth kinetics of *Ph. tri-cornutum*. Two phases of culture growth were observed (Fig. 1). The faster phase was detected between the 1st and 5th day after inoculation. The second one was slower and was recorded from the 6th till 14th day.

In cultures growing at 20°C and 23°C the highest chlorophylls and proteins concentration as well as OD were detected on the last day of observation. An analysis of the relation between the parameters shows that in the starting 5 days after inoculation, there was a significant positive correlation between OD increase of *chlorophyll a*, *chlorophyll c*, and protein concentration, and F_v/F_m at different temperatures (Fig. 1 and Fig. 2). It is known that the chlorophyll concentration is strongly dependent of temperature growth. 30% lower concentration of *chlorophyll a* per cell volume was detected while temperature decreased from 20 to 10°C (Kudo *et al.*, 2000). In our experiments the pattern and strength of correlations

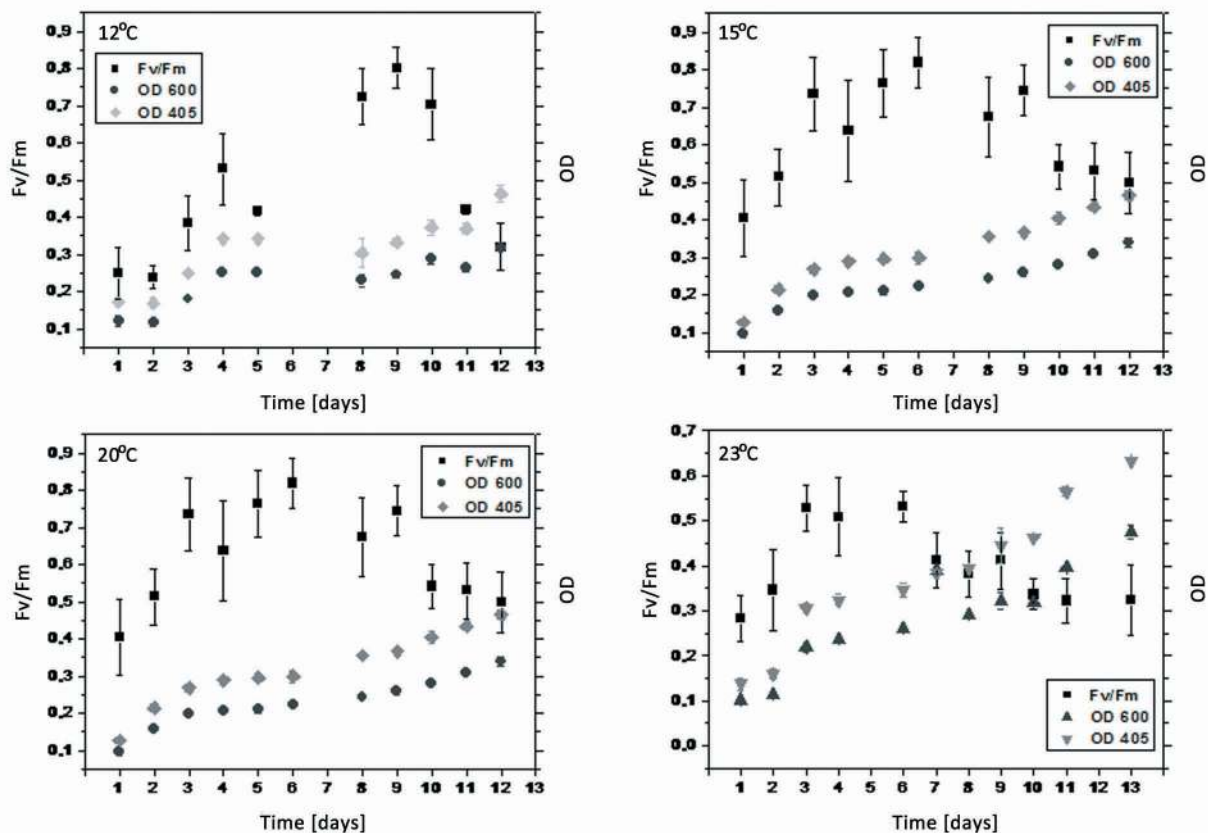


Figure 1. Effect of temperature on OD and Fv/Fm of *Phaeodactylum tricornutum* in batch culture

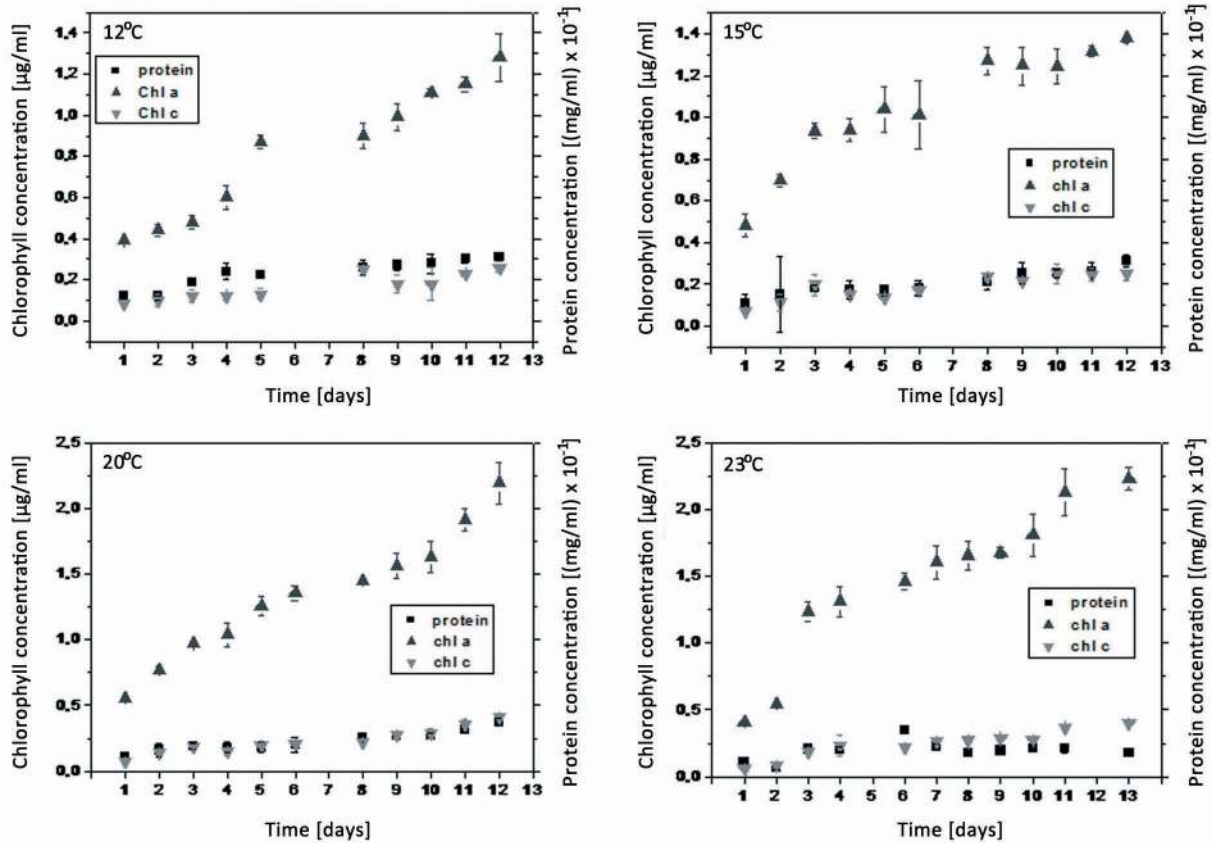


Figure 2. Effect of temperature on chlorophyll *a*, chlorophyll *c*, and protein concentration in *Phaeodactylum tricornutum* batch cultures

varied with temperatures. Several days after inoculation (from 6th till 14th day) chlorophylls and proteins concentrations as well as their OD increased continuously at all temperature growths (Fig. 2).

On the other hand the F_v/F_m parameter which provides information on the functionality of PS2 (Lichtenthaler *et al.*, 2005) achieved a maximum level at 6th or 7th day and then decreased to the values registered on the first day of observation. The increase of F_v/F_m in the beginning of experiments and stabilization of this parameter after third day after inoculation were observed at 18°C (Liang *et al.*, 2006). At the highest temperature (23°C) the values of this parameter were the lowest. Ge-

netic material (total level of RNA) undergoes a gradual degradation 10 days after inoculation (Fig. 3).

The temperature affects the relative proportions of *Pb. tricornutum* morphotype and cell size. There are three types of diatom cells shape: triradiate, fusiform and oval cell. Pt8_{6F} monoclonal strain (CCAP 1055/1) displayed mainly fusiform cells (80% fusiform, 6% triradiate and 10% oval) in 19°C. The decrease on fusiform cells and increase of the oval ones was observed when temperature was changed to low values. This conversion was

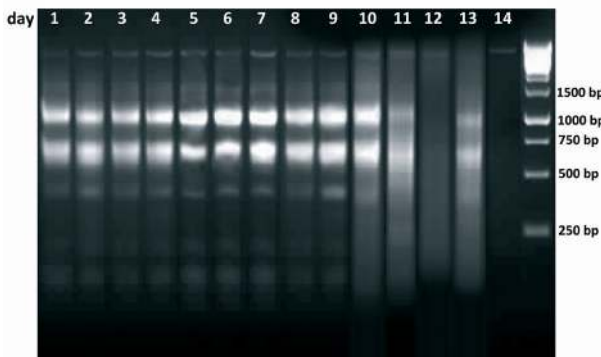


Figure 3. Total RNA isolated over two weeks from *Phaeodactylum tricornutum* grown at 15°C. The right line is the molecular weight marker 1 kb (Fermentas)

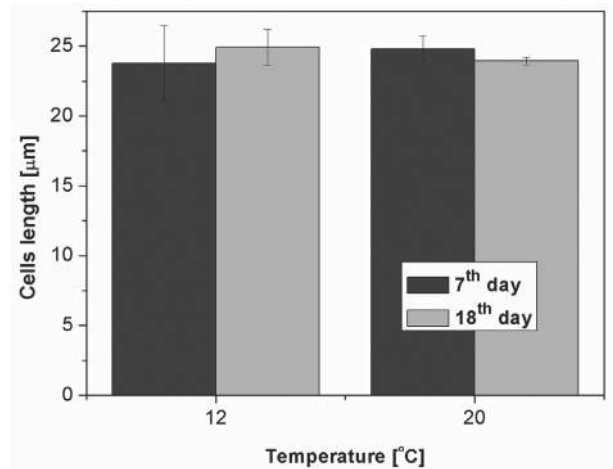


Figure 4. Effect of temperature on *Phaeodactylum tricornutum* cell length on 7th and 18th day after inoculation. Each value is the average of fifty measurements

reversible when cells were transferred to higher temperature (Martino *et al.*, 2011). These results cause that we chose two temperatures (12 and 20°C) for experiment. In our experiment only fusiform of *Ph. triornutum* was observed. The size of cells was invariable (approximately 25 µm) and independent of both observation time and growth temperature (Fig. 4). The same length of fusiform cells was described by Lewin *et al.* (1958).

All tested parameters clearly shown that the biological conditions of *Ph. triornutum* are optimal in the period between 4th and 6th day from inoculation. Both the rate of the culture growth and proteins, RNA or photosynthetic pigments concentration demonstrated the highest values. Similarly, cell size and Fv/Fm ratio indicated the best condition of the tested diatoms. Presented results define the best time for applying of *Ph. triornutum* CCAP/1055/1 strain, which is commonly used as a model organism in studies of physiology and biochemistry of diatoms.

Acknowledgements

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