Algal Research 14 (2016) 72–78

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Concomitant effects of light and temperature diel variations on the growth rate and lipid production of *Dunaliella salina*

H. Bonnefond [a,*], N. Moelants [a], A. Talec [a], O. Bernard [b], A. Sciandra [a,b]

[a] Sorbonne Universités, UPMC Univ Paris 06, INSU-CNRS, Laboratoire d'Océanographie de Villefranche, 181 Chemin du Lazaret, 06230 Villefranche-sur-mer, France
[b] INRIA BIOCORE, 06902 Sophia Antipolis, France

ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form 30 September 2015
Accepted 30 December 2015
Available online xxxx

Keywords:
Dunaliella salina
Triglycerides
Beta-carotene
Light/dark cycle
Temperature variations
Respiration

ABSTRACT

The microalgae *Dunaliella salina* has the capacity to grow in salterns at high salinity. In this particular shallow environment, *D. salina* is exposed to strong light and temperature variations and has developed various strategies such as cell cycle adaptation and storage of dedicated metabolites. The effects of light/dark cycles have already been studied, but few works focused on the concomitant effects of light and temperature variations characterizing salterns and outdoor conditions. In this study, growth, carbon and nitrogen storage, pigments and lipid production of *D. Salina* were measured in laboratory conditions mimicking the outdoor light and temperature conditions. A control experiment with constant temperature was carried out with light variations only. During the night, cell respiration was correlated with temperature, following an Arrhenius law. Many differences with the control at constant temperature confirmed that temperature variations are a crucial parameter in outdoor conditions and should be taken into account to predict growth. Triglyceride and pigment production was tightly linked to the light dark cycle.

1. Introduction

Microalgae have developed numerous mechanisms to permanently adapt to a fluctuating environment. Key points for survival in periodic conditions are cell cycle synchronization with light and temperature [1]. The first consequence of this adaptation is the modulation of carbon and nitrogen acquisition during the cell cycle [2]. The second comprises daily dynamics of storage metabolites like lipids, [3,4]. The green microalgae *Dunaliella salina* grows in salt lakes or in shallow salterns and tolerates wide ranges of salinity, between 0.5 M and 5 M [5]. Due to low water depth, this species has adapted its metabolism and cell cycle to high temperature and light variations over a course of a day. To survive within a changing environment, *Dunaliella sp.* produces different compounds. Carotenoids, such as beta-carotene and lutein, are accessory pigments used as photoprotectors. *D. salina* is among the organisms containing the highest concentrations of carotenoids after metabolic stress [6]. This species can also produce triglycerides (TAG) to store energy and carbon to sustain growth during the night. In addition, *Dunaliella sp.* synthesizes glycerol as an osmo-regulator when grown in hypersaline environments [5]. Industrially, this is the third most important microalgae produced in terms of dry weight (1200 t/year) after *Arthrospira* sp. and *Chlorella* sp. [7]. It is grown mainly for its carotenoids which have strong antioxidant properties that are utilized in the cosmetic and nutritional markets. Its ability to accumulate triglycerides as a potential source of biofuel is also gaining interest.

Photosynthesis is impacted by temperature and light fluctuations. The effect on inorganic carbon acquisition is direct for light, and indirect for temperature, which modulate enzymatic activity. The simultaneous impact of these two factors needs to be studied to better understand the daily pattern of carbon acquisition and storage [8]. Some studies have been carried out with *D. salina* to investigate the individual effects of light or temperature on growth and metabolites [9,10,5], but the effects resulting from their concomitant variations have not yet been studied. Thus the aim of this study was to investigate the effects of concomitant realistic evolution of temperature and light on the metabolic response of *D. salina*, mainly in terms of carbon and nitrogen acquisition and pigment and triglyceride content. These experimental results were compared to a control experiment where temperature was kept constant.

One of our key observations is that a periodic temperature evolution, which is rarely experimentally tested, seems to strongly impact cell dynamics. The cyclic effect of temperature also had a positive impact on carbon fixation.

2. Materials and methods

2.1. Culturing system

D. salina (CCAP 18/19) was grown in duplicate 5 L, temperature-controlled water-jacketed vessels previously washed with 10% HCl and rinsed with milli-Q water and sterile medium. The enrichment

* Corresponding author.

http://dx.doi.org/10.1016/j.algal.2015.12.018
2211-9264/

medium was prepared in 20 L tanks (Nalgen) filled with 3 weeks matured natural seawater filtered on 0.1 μm, and autoclaved at 110 °C for 20 min. After cooling, f/2 medium was added [11]. Nitrates were added separately to the end concentration of 400 μM. Fresh medium filtered through a 0.22 μm sterile filter (SpiralCap, Gelman) was introduced into the continuous cultures with peristaltic pumps (Gilson) at a dilution rate equal to daily growth rate (Table 1). After inoculation, the starting cell concentration was about 2×10^4 cell·mL^{-1} and cultures were first grown in batch mode to allow the algal population to increase rapidly. Then, the turbidostat mode was initiated to stabilize the population at 3×10^5 cell·mL^{-1}, a concentration sufficiently high to allow accurate biochemical analyses on small volume samples, and sufficiently low to prevent nutrient limitation and light shading. Each day, the dilution rate (D) was checked by weighting with a precision balance the input flow during 2 min and adjusted, when necessary, to maintain a constant daily cell concentration. The pH was measured every minute and prevented from exceeding pH 8.3 by computer-controlled microaddition of CO_2 in the bubbling air (see [12]). Homogenous cultures were maintained by gentle magnetic stirring.

2.2. Light and temperature

Light was provided by two arrays of six 50 cm fluorescent tubes (Dulux®1, 2G11, 55 W/12–950, lumilux de lux, daylight, OsramSylvania) placed on each side of the vessels. Photosynthetically active radiation (PAR) was measured by a 4π spherical collector (QSL-100, Biospherical Instruments) placed between or in the two turbidostats to assure that no light limitation occurred. Temperature was controlled and monitored using a temperature control unit (Lauda RE 415G). Light and temperature were recorded every minute.

2.3. Culture conditions

A typical meteorological pattern from a meteorological station located in the Laboratory of environmental biotechnology (INRA-LBE Avenue des Etangs F-11,100 Narbonne, south of France), was used as a concrete example of daily natural variability impacting the culture in June. The daily change of temperature in the ponds was calculated by the model of [8], based on this meteorological data. The L/D (14 L:10D) cycle was approached by a truncated sinus square function. These conditions were applied in the duplicate cultures C1-LT and C2-LT (LT, for light and temperature variations), while constant temperature (at the value of 27 °C) was applied in the duplicate cultures C3-L and C4-L (L, for light variation only; Table 1). The light intensity was determined to reproduce the averaged irradiance in the pond. Using the Beer–Lambert law for light attenuation, the average light intensity was calculated from the light at the surface, as detailed in [13].

$$I_{av} = \frac{I_{inc}}{\ln\left(\frac{I_{inc}}{I_{out}}\right)}\left(1 - \frac{I_{out}}{I_{inc}}\right) \quad (1)$$

where I_{inc} is the incident light intensity (μmol quanta·m^{-2}·s^{-1}) impinging the pond, and I_{out} the light at the bottom of the raceway. We assumed here that I_{inc} at noon was such that the photosynthesis rate at the bottom of the pond equaled the respiration rate, corresponding to the (optimal) compensation condition defined by [14]. The compensation condition for *D. salina* was determined for I_{out} = 23 μmol quanta·m^{-2}·s^{-1} [6]. From (1), it followed that, for the maximal incident light intensity in Narbonne, I_{inc} was 1364 μmol quanta·m^{-2}·s^{-1} and the averaged light intensity I_{av} at noon was equal to $I_{inc.} \times 0.22 = 300$ μmol quanta·m^{-2}·s^{-1}. These conditions were obtained by a computer-controlled cultivation device able to maintain long term continuous cultures [12].

Table 1
Experimental conditions applied to the different continuous cultures.

	C-LT		C-L	
	C1-LT	C2-LT	C3-L	C4-L
Maximum light intensity (μmol·m^{-2}·s^{-1})	278	289	297	273
Light pattern	Sinus square function 14L:10D			
Temperature (°C)	Periodic variations (24.4 °C–32.9 °C)		Constant (27 °C)	
Dilution rate (d^{-1})	0.69	0.70	0.42	0.45
Average cell concentration (cell/L)	2.01 × 10^8	2.24 × 10^8	2.20 × 10^8	2.51 × 10^8

2.4. Cell population

Cell concentration and size distribution were monitored every two hours by an automated optical particle counter (HIAC — Royco; Pacific Scientific Instruments). The variability between triplicate measurements was routinely lower than 5%. The mean cell diameter of the population was calculated from its size distribution. Due to high frequency acquisition, continuous functions could be fitted to cell density data using Stavitzky Golay filter [15]. The division rate μ (d^{-1}) was then derived according to the following equation.

$$\mu = \frac{\mathrm{Ln}\left(\frac{n_2}{n_1}\right)}{t_2 - t_1} + D$$

where n_1 and n_2 are the cell concentrations (cell·mL^{-1}) at time t_1 and t_2, respectively, and D the dilution rate (d^{-1}).

2.5. Nutrient analysis

Sampling for biochemical analyses were started after a culture-preconditioning period of 15 days, necessary for biomass stabilization and physiological adaptation of cultures to the experimental conditions, and were performed consecutively during 48 h. Nitrates (NO_3^-) and nitrites (NO_2^-) concentrations were automatically measured on-line [16] to ensure that the duplicate cultures were never N-limited. For particulate carbon and nitrogen analyses, 13.15 mL of culture were filtered in triplicates every 2 h onto glass-fiber filters (Whatman GF/C) and precombusted at 450 °C for 12 h. Samples were kept at 60 °C until analyses were performed with a CHN analyzer (2400 Series II CHNS/O, Perkin Elmer). The variability between triplicate measurements was routinely lower than 6%. Continuous functions of Stavitzky Golay filter were fitted to discrete data of nitrogen and carbon concentrations (μg·mL^{-1}). This allows the computation of the net carbon specific fixation rates of N and C, respectively ρ_N and ρ_C (μg·μgC^{-1}·d^{-1}) according to the following equation:

$$\rho_N = \frac{1}{[C]_{t_2}}\left(\frac{[N]_{t_2} - [N]_{t_1}}{(t_2 - t_1)} + D.[N]_{t_2}\right)$$

where [N] is the particulate nitrogen concentration (μg·mL^{-1}) at time t_1 and t_2, respectively. The computation of ρ_C was done similarly using particulate carbon [C] (μg·mL^{-1}).

2.6. Cellular content analysis

Lipid analysis protocol was derived from the Bligh and Dyer's method [17]. 200 mL of culture was centrifuged (JOUAN G 412) for 10 min at 2000 rpm, and the pellet was stored at −80 °C before lipid extraction. Two successive extractions were performed in a monophasic mixture of chloroform:methanol:salt water (1:2:0.8 *v*/*v*). Chloroform and water were then added for phase separation (2:2:1.8 *v*/*v*). Chloroform phase was evaporated and total lipids (TL) were stored at −80 °C under nitrogen atmosphere to avoid oxidation. Lipid class