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Impact of temperature and light intensity on triacylglycerol accumulation in marine microalgae

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ABSTRACT

Triacylglycerol (TAG) productivity of *Isochrysis galbana*, *Nannochloropsis oceanica* and *Phaeodactylum tricornutum* was compared to study their suitability for biotechnological applications. Photoautotrophic batch cultures grown at 20 °C and 50 μmol photons m^{-2} s^{-1} showed that *N. oceanica* had the least TAG content and TAG productivity of the three microalgae. Hence, effects of temperature and light intensity on growth rate and accumulation of TAG were subsequently assessed only in *I. galbana* and *P. tricornutum* by cultivation at 20 and 30 °C under 50, 300 and 600 μmol photons m^{-2} s^{-1}. Although *P. tricornutum* did not grow at temperatures higher than 20 °C, an increase in both TAG content (from 28.37 to 39.53%) and productivity (from 15.58 to 31.39 mg L^{-1} d^{-1}) was observed at the highest irradiance values. We also found that combined effects of temperature and light intensity enhanced TAG content (from 18.59 to 31.71%) and productivity (from 11.76 to 21.67 mg L^{-1} d^{-1}) in *I. galbana*.

1. Introduction

Microalgae are a large and diverse group of aquatic organisms found in both freshwater and marine environments, with a huge potential for biotechnological applications. Besides their high growth rates, they produce and accumulate useful biomaterials of commercial interest, such as pharmaceutical and specialty chemicals [1,2]. Biofuels or high valuable molecules derived from microalgal cultures can be considered a sustainable resource [3–5] because their production can be coupled with CO_2 mitigation technologies (1 kg of dry algal biomass require about 1.8 kg CO_2).

Many algal species have been found to produce substantial amounts of neutral lipids (20–50% dry cell weight), and therefore have been referred to as oleaginous algae [3,6,7]. Under stress or unfavorable environmental conditions for growth, these algae can alter their lipid content, mainly in the form of triacylglycerols (TAGs), whereas under optimal growth conditions, they produce only small amounts of TAG.

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Unlike lipids found in membranes, TAGs do not perform a structural role. Instead, they serve as a storage form of carbon and energy under stress conditions promoted by chemical or physical environmental stimuli, when large amounts of TAG are accumulated in the cell. Nutrient starvation, salinity and growth medium pH are the major chemical stimuli, whereas temperature and light intensity are the main physical cues [6,8]. Moreover, growth phase and aging of the culture can also affect TAG content and fatty acid composition [6,9]. Batch, fed batch and semi-continuous operations are traditionally used in biotechnological applications since these regimes facilitate the control of stress conditions (for instance, nutrient restriction or intermittency of nutrient supply), and were successfully implemented to increase oil and biomass productivities [10–12].

Consistent efforts are being made worldwide to achieve the ideal combination of algae species and growing conditions. This study examined the influence of temperature and light intensities on cell growth, specific growth rate, oil content and oil productivity in three marine microalgae (*Isochrysis galbana*, *Nannochloropsis oceanica* and *Phaeodactylum tricornutum*) that are potential resources to obtain either high valuable products, such as polyunsaturated fatty acids, or lipids for biodiesel production. Microalgae were initially batch cultured in f/2 growth medium at 20 °C and 50 μmol photons m^{-2} s^{-1}. Subsequently, effect of photon flux density (PFD) increase (from 50 to 300 and 600 μmol photons m^{-2} s^{-1}) on TAG productivity was assessed in *P. tricornutum* and *I. galbana*, as well as the combined effect of PFD and temperature on oil accumulation was verified in *I. galbana*.

2. Materials and methods

2.1. Organism and culture conditions

Two species of microalgae, *P. tricornutum* (Bacillariophyceae) and *I. galbana* (Prymnesiophyceae), were kindly provided by the "Elizabeth Aidar Microalgae Culture Collection" (Department of Marine Biology, Fluminense Federal University, Brazil), while *N. oceanica* (Eustigmatophyceae) was obtained from the Instituto de Estudos do Mar Almirante Paulo Moreira (IEAPM-MM), Arraial do Cabo, Rio de Janeiro, Brazil. The microorganisms are available at the Laboratório de Estudos Aplicados em Fotossíntese (LEAF-Culture Collection, Institute of Chemistry, Federal University of Rio de Janeiro, Brazil). The microalgae were batch-cultured photo-autotrophically in 2.0 L Erlenmeyers flasks containing 1.2 L of enriched seawater (34 psu salinity) of f/2 medium [13]. Cultures were gassed with filtered atmospheric air by using aquarium compressors with a 3.5 L/min air flow, and exposed to lateral illumination (50, 300, and 600 μmol photons m^{-2} s^{-1}, on the outer surface of the vessels) provided by fluorescent lamps (Philips 23W, white light), under 12:12 h photoperiod. Culture flasks were maintained in an incubator (Forma Scientific Inc., Ohio – USA) at temperature calibrated either at 20 ± 2 or at 30 ± 2 °C. The volume of the culture medium was monitored and, when necessary, adjusted by adding sterile seawater in order to minimize evaporation effects.

Cell growth was followed by direct microscopic cell counting according to Silva et al. [14]. In order to avoid the occurrence of a "lag phase", cultures were set at an initial cell density of about 5×10^4 cells/mL for *I. galbana* and *P. tricornutum*; and 5×10^5 for *N. oceanica*. Aliquots of 10 mL algal suspension were filtered through pre-weighted glass fiber filters (Whatman GF/F) and washed with the same volume of an isotonic ammonium bicarbonate solution (0.5M) for dry weight determination [15]. Filters were reweighted after drying in 70 °C until constant weight was reached.

Acclimation to high light intensities was obtained by a gradual increase of light intensity from 50 to 600 μmol photons m^{-2} s^{-1}, with cultures in exponential growth phase were exposed successively to 150, 300 and 600 μmol photons m^{-2} s^{-1}. Cells were kept in the desired condition for about four weeks before starting the experiments.

2.2. Triacylglycerols extraction and quantification

Cells were collected in intervals corresponding to early (from 0 to 72 h), intermediate (from 72 to 144 h) and late (from 144 to 192 h) stationary growth phase. Cells were harvested by centrifugation (8000–13,200g for 10 min at 21 °C) in a Sorvall RC5B centrifuge. Thereafter, biomass corresponding to two culture flasks was pooled and dried at 70 °C until constant weight was reached. The material was macerated in a mortar to obtain powder texture, transferred into a cellulose cartridge and weighted. Neutral lipids were extracted with petroleum ether (distillation range: 30–60 °C) in a Soxhlet extraction system maintaining the solvent condensation reflux at about 120 drops min^{-1}. Extraction was finished when the solvent became colorless in the compartment containing the cartridge. Residual solvent was eliminated in a water bath at 60 °C. The material was heated at 100 °C until constant weight was reached. Lipid content was determined gravimetrically, as follows:

$$\text{Oil content (\% dcw)} = \left[\left(W_{(g)} - W_{o(g)}\right)/DCW_{(g)}\right] \times 100$$

where $W_{o(g)}$ and $W_{(g)}$ are the weights of the Soxhlet extractor flask without sample and after extracting the oil, respectively. DCW corresponds to the dry cell weight, and TAG productivity was calculated as follows:

$$TAG_{prod} = (X \cdot TAG\%)/T$$

where TAG_{prod} is triacylglycerol productivity (mg L^{-1} d^{-1}), X is dry biomass concentration (mg L^{-1}) and T is cultivation time (days).

2.3. Mathematical model

To study the combined effects of temperature and light intensity on the triacylglycerol accumulation in *I. galbana*, experiments were performed according to a mixed full factorial experimental design. Light intensity (x_1) was tested under (+) 50, (0) 300 and (–) 600 μmol photons m^{-2} s^{-1}, while temperature (x_2) was tested in (+) 20 and (–) 30 °C. The limits chosen for the variables were based on literature review and operating conditions. According to Massart et al. [16], the proposed predictive model is:

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