Bioresource Technology 265 (2018) 407–414

Contents lists available at ScienceDirect

Bioresource Technology

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

# Effects of temperature and its combination with high light intensity on lipid production of *Monoraphidium dybowskii* Y2 from semi-arid desert areas

Qiaoning He[a], Haijian Yang[b], Chunxiang Hu[b,*]

[a] Hubei Collaborative Innovation Center for Green Transformation of Bio-resources, Environmental Microbial Technology Center of Hubei Province, Hubei Key Laboratory of Industrial Biotechnology, College of Life Sciences, Hubei University, Wuhan 430062, China
[b] Key Laboratory of Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China

ARTICLE INFO

*Keywords:*
Temperature
Lipid
Microalgae
Carbon allocation
High light intensity

ABSTRACT

Temperature and light intensity are important environmental factors influencing microalgae for biodiesel production. The aim of present work was to study the effects of temperature (15 °C, 25 °C, and 35 °C) and its combination with high light intensity (HL, 400 μmol photon $m^{-2} s^{-1}$) on lipid production of *Monoraphidium dybowskii* Y2 which was isolated from desert. The results demonstrated that algal growth was only inhibited at 15 °C. Promoted lipid content and decreased Fv/Fm were observed in 15 °C and 35 °C. Cellular carbohydrate, protein conversion and membrane lipid (MGDG, DGDG and SQDG) remodeling contributes for lipid accumulation. Stress combined temperatures with HL are benefit for lipid production, especially desired neutral lipid productivity all exceed 40 mg $L^{-1} d^{-1}$. Fatty acids compositions of C16:0 and C18:1 were further promoted under 15 °C or 35 °C combined with HL. Thus, *M. dybowskii* Y2 will well-adapted to outdoors cultivation for biodiesel production.

## 1. Introduction

In recent years, great enthusiasm have been devoted into the research on biodiesel production from oleaginous microalgae for the advantage of biodegradable, sustainable and non-toxic (Faried et al., 2017; Remmers et al., 2018). Photoautotrophic microalgae can capture solar energy and direct transform atmospheric carbon dioxide by photosynthesis to biomass and useful organic compounds such as carbohydrate or lipids (He et al., 2015a). Despite the accumulation of high lipid content in some microalgal strains, current high cost for the cultivation of photoautotrophic microalgae hampered the further development of industrial application for biodiesel production. Currently, a variety of factors like nutrient, temperature, light intensity and pH were studied for their key roles in lipid accumulation of oleaginous microalgae (Breuer et al., 2013; Faried et al., 2017; He et al., 2015a; Ördög et al., 2016; Remmers et al., 2018).

Among all cultural parameters, temperature is one of the most important factors determining microalgae growth and physiology (Ho et al., 2014) as it directly influences the photosynthesis (Bayro-Kaiser & Nelson, 2016; Sharkey & Zhang, 2010), the rate of chemical reactions and biochemical composition (Converti et al., 2009; Sayegh & Montagnes, 2011), the stability of cellular components and many other physiological processes (Legeret et al., 2016). It also strongly affects the lipid productivity, and lipid composition of microalgae, and the effects of temperature are species dependent (Ma et al., 2017; Sonmez et al., 2016). At a low temperature, some algal strains regulate lipid composition to maintain structural integrity and membrane fluidity by increasing the content of unsaturated fatty acids (Guihéneuf & Stengel, 2017; Ma et al., 2017; Wang et al., 2016a). Higher temperature could efficiently stimulate the lipid accumulation and affect the fatty acid synthesis in some microalgae (Han et al., 2016; Venkata Subhash et al., 2014), while in some other microalgae, the lipid content decreased with the increasing temperature. Usually, the optimum growth temperature of photoautotrophic unicellular algae is between 20 °C and 25 °C (Yang et al., 2013), but no accurate definition of temperature range on lipid accumulation. Most microalgae have the ability to grow over a wide range of temperatures; however, the response and adaptation of algae to different temperature stress depends on their origin (de Boer, 2005). Therefore, it is important to estimate the effects of different temperatures on the desert originate oleaginous microalgae.

Temperature, light, and nutrient are critical parameters for the regulation of autotrophic growth of microalgae, and specifically the synthesis and accumulation of lipid. Researches on a range of temperatures for the influence on microalgae have been conducted (Bohnenberger & Crossetti, 2014; Chokshi et al., 2015; Faried et al., 2017; Han et al., 2016; Legeret et al., 2016; Roleda et al., 2013; Yang

* Corresponding author.
*E-mail address:* cxhu@ihb.ac.cn (C. Hu).

https://doi.org/10.1016/j.biortech.2018.06.044
Received 17 April 2018; Received in revised form 12 June 2018; Accepted 13 June 2018
Available online 15 June 2018
0960-8524/ 

et al., 2013). In practice, light intensity and nutrient were accompanied with temperature, especially in outdoor cultivation with photobioreactor or open pond. Some algae species grown under low temperature (−10 °C) and nutrient-replete condition were able to significantly increase lipid content to 57–113% (Roleda et al., 2013). Light and temperature seems to regulate lipid remodeling in *Pavlova lutheri*, with highest TAG levels observed under 200 μmol photon $m^{-2} s^{-1}$ when cultivated for 7 days, and highest TAG levels and contents obtained by day 15 at 18 °C, regardless of irradiance level (Guihéneuf & Stengel, 2017). Furthermore, combined high temperature (40 °C) with high light intensity (2000 μmol photon $m^{-2} s^{-1}$) led to serious photoinhibition and reduced cell growth in *Dunaliella tertiolecta* (Seepratoomrosh et al., 2016). However, it is not possible to draw conclusions about the exact impact of temperature and light intensity on lipid accumulation due to a great variability in the results, biological models and experimental methods in microalgae. And there is very little research on the combination of high light intensity which is above the light saturation curve of oleaginous microalgae with different temperature range, especially for the study on lipid biosynthesis.

*Monoraphidium dybowskii* have great potential as a feedstock for biodiesel production because they grow fast and can accumulate high levels of triacylgycerides with little need for fresh water or land (He et al., 2016; He et al., 2015a; He et al., 2015b; Yang et al., 2018). In present study, effects of temperature and its combination with high light intensity (HL, 400 μmol photon $m^{-2} s^{-1}$) on biomass and lipid production were investigated in a semi-arid desert isolated *M. dybowskii* Y2 to better understand the biodiesel properties of this potentially useful but poorly studied microalgal species. Fundamental understanding of algal physiology and lipid analysis including growth, photosynthetic activity, biochemical compositions, lipid classes and fatty acids profile analysis, as advanced by this study, can therefore be used to optimize algal lipid production under these temperature and high light regimes. The present study help fill the gap and assesses the independent and interactive responses of *M. dybowskii* Y2. Cost-effective and high lipid productivity strategies will be optimized in this microalgae for future industrial biodiesel production.

## 2. Method and materials

### 2.1. Culture and growth conditions

Strain of *Monoraphidium dybowskii* Y2 was used in this experiment. The modified culture medium was BG-11, in which the concentration of $NaNO_3$ was half of the normal BG-11. Other components remain unchanged. The seed was cultured with 400 mL of BG-11 medium in 500 mL Erlenmeyer flasks with the light intensity of 100 μmol photon $m^{-2} s^{-1}$ and a constant temperature of 25 °C. Filtered air was supplied to flasks by using an air compressor.

The experiment was conducted in temperature controlled illumination incubator with three different operating temperatures of 15 °C, 25 °C, and 35 °C. Combination trials of temperature with high light intensity (HL, 400 μmol photon $m^{-2} s^{-1}$) were also carried out. The incubator was illuminated with an external light source (PHILIPS Tornado 23W E27 fluorescent lamp; Philips Co., China) mounted on both sides of the flasks to increase the light intensity.

The initial optical density ($OD_{680}$ nm) was 0.5. All the experiments were conducted in triplicate. For growth evaluation, initially microalgae were inoculated in 300 mL of specified medium in 500 mL flasks and $OD_{680}$ was measured every three days. To further know the effect of temperature with HL on growth and lipid accumulation of the oleaginous microalgae, 400 μmol photon $m^{-2} s^{-1}$ HL was introduced to combine with three temperatures for lipid production.

### 2.2. Biomass analysis and specific growth rate (μ) calculation

Dry weight of microalgae biomass was determined gravimetrically, and the growth was expressed in terms of dry weight (DW). In brief, 20 mL of samples was harvested by centrifugation. Then, the cells was washed twice with distilled water, lyophilized, and weighed. The biomass productivity (VBP, mg $L^{-1} d^{-1}$) was calculated according to the Eq. (1):

$$BP = (B2-B1)/T \quad (1)$$

where B2 and B1 represents the dry weight biomass density at the time T (days) and at the start of the experiment, respectively.

Specific growth rates of microalgal isolates were calculated as previously described (Onay et al., 2014). Specific growth rate (μ):

$$\ln(X1-X2)/(t2-t1) \quad (2)$$

where X1: biomass concentration at the end of the selected time interval in mg $L^{-1}$, X2: biomass concentration at the beginning of the selected time interval in mg $L^{-1}$, t2 − t1: time elapsed between the selected time points in days (d).

### 2.3. Measure of Chl fluorescence

Chl fluorescence was measured using the Handy PEA Chl fluorescence photosynthesis analyzer (Hansatech, U.K.). Microalgal cells were fully adapted for 15 min in darkness and then measured the fluorescence level. The maximum and effective quantum yields of PSII electron transport were calculated as $Fv/Fm = (Fm - F_0)/Fm$.

### 2.4. Determination of protein, carbohydrate and lipid content

Protein and carbohydrate were measured as previously described (He et al., 2015a). Lipid content was estimated as follows: approximately 50 to 100 mg of dried algal sample was used to extract lipid by a Soxhlet apparatus for 6 h at 90 °C, with chloroform-methanol (2:1, v/v) as solvent. Total lipid was dried to constant weight in an oven at 80 °C and weighted. Total lipid content (LC %) and the lipid productivity (LP, mg $L^{-1} d^{-1}$) was recorded.

### 2.5. Lipid analysis

Lipid classes: solid phase extraction (SPE) and thin layer chromatography (TLC) were utilized for analysis. SPE was used to separate lipid extracts into neutral lipid (NL, mostly TAG), glycolipid (GL) and phospholipid (PL). SPE was carried out with a column (20 mm × 150 mm) contains 4 g silica gel cartridges twice with chloroform. Microalgal lipids (approximately 100 mg) were dissolved in 400 μL of chloroform, and were fractionated by using the following solvent systems: NL with chloroform (six column volumes), GL with acetone: methanol (9:1, v/v) (four column volumes) and PL with methanol (four column volumes). Eluted lipid fractions were then dried under nitrogen and the content of each fraction was recorded.

To compare TAG and polar lipid contents of different samples, equivalent quantities (100 mg) of dry algae powder were weighed, and total lipids were extracted, reconstituted in hexane with equivalent volume to make stock solutions. TAGs and polar lipid were separated and quantified by one-dimensional TLC on silica gel plates 60 F254 (Merck KgaA, Darmstadt, Germany). Aliquots of stock solutions (5 mL) along with standards were spotted three times onto silica gel TLC plates developed with a solvent mixture of hexane/diethyl ether/acetic acid (70:30:1, by volume) for TAG and with a solvent mixture of acetone/methylbenzene/$H_2O$ (91:30:7.5, by volume) for polar lipid. Then, TAG and polar lipid were visualized by exposing the plates to iodine vapor.

Fatty acids (FA) composition: Fatty acid methyl esters (FAMEs) were identified and quantified by gas chromatograph mass spectrometry (GC–MS; Thermo Scientific ITQ 700, USA) equipped with a flame ionization detector (FID) and a fused silica capillary column (60 m × 0.25 mm × 0.25 μm; Agilent Technologies, USA). One microliter of each sample were injected. The injector and detector