Algal Research 31 (2018) 387–394

Contents lists available at ScienceDirect

Algal Research

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

The impact of day length on cell division and efficiency of light use in a starchless mutant of *Tetradesmus obliquus*

G. Mitsue León-Saiki[a,*], Tània Cabrero Martí[a], Douwe van der Veen[a], René H. Wijffels[a,b], Dirk E. Martens[a]

[a] Bioprocess Engineering, AlgaePARC, Wageningen University and Research, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[b] Faculty of Biosciences and Aquaculture, Nord University, N-8049 Bodø, Norway

ARTICLE INFO

Keywords:
Microalgae
Scenedesmus obliquus
Day/night cycle
Cell division
Diurnal biochemical changes
Photosynthetic efficiency

ABSTRACT

Large scale microalgal production will be primarily done under natural sunlight conditions, where microalgae will be exposed to diurnal cycles of light and dark (LD) and to differences in the length of both periods (photoperiod). *Tetradesmus obliquus* (formerly known as *Scenedesmus obliquus*), a strain with potential for biofuel production, and the starchless mutant *slm1* were grown under 3 different LD periods: 16:8 h, 14:10 h and 12:12 h. Cell division started a fix number of hours after the light went on (sunrise), independently of the length of the photoperiod. For the wild-type, cell division started approximately 14 h after the beginning of the day and occurred mainly at night. For the starchless mutant *slm1*, timing of cell division was also independent of the photoperiod length (starting 10–12 h after sunrise). However, as opposed to the wild-type, cell division always started during the day. For both strains, growth rate increased with increased length of the light period. The *slm1* mutant is capable of surviving long dark periods (up to 12 h) despite the lack of starch. In general, the *slm1* mutant has a lower photosynthetic efficiency than the wild-type, with the 12:12 h LD resulting into even less efficiency than the other two LD cycles.

1. Introduction

Microalgae can be used as source for commercial products of interest such as biofuels, chemicals, food, and feed [1,2]. Large scale microalgal production will be primarily done under natural sunlight conditions [3,4], where microalgae will be exposed to diurnal cycles of light and dark (LD). Diurnal LD cycles are ubiquitous and many organisms synchronize their metabolism to anticipate the changing environment [5–9]. Environmental cues (known as Zeitgeber, which is German for time indicator) entrain the internal timing to a period of 24 h [8,10]. Cues such as sunrise (dawn), sunset (dusk), changes in light intensity or temperature, as well as light pulses can be used to entrain this diurnal cycle [11]. For photosynthetic organisms, synchronization to the diurnal LD cycle translates into fine-tuning their photosynthetic apparatus to capture sunlight efficiently during the day and to schedule ultraviolet or oxygen sensitive processes (e.g. nitrogen fixation, DNA synthesis or cell division) at night [12–15]. In addition, the length of the light and dark periods under natural sunlight conditions varies depending on the region and the season, which has an impact on biomass productivity and photosynthetic efficiency [16] depending on the species.

The microalga *Tetradesmus obliquus* (formerly known as *Scenedesmus obliquus* [17] and reclassified as *Acutodesmus obliquus* [18]) is an industrially relevant strain whose potential has been demonstrated [19–22]. In addition, de Jaeger et al. [23] developed a starchless mutant, *slm1*, which is incapable of synthesizing starch due to a single nucleotide polymorphism in the small subunit of ADP-glucose pyrophosphorylase, the committed step of starch biosynthesis [24]. This mutant showed a higher maximum triacylglyceride (TAG) yield on light (0.217 $g \cdot mol_{ph}^{-1}$ compared to 0.144 $g \cdot mol_{ph}^{-1}$ for its wild-type) and a higher maximum TAG content (0.57 $g \cdot g_{DW}^{-1}$ compared to 0.45 $g \cdot g_{DW}^{-1}$) in batch cultures under nitrogen starvation [25]. Furthermore, the photosynthetic efficiency of the mutant was comparable to the wild-type under nitrogen starvation.

Prior to the TAG producing step, which commonly takes place under nitrogen limitation/starvation and LD cycles, biomass must be grown under nitrogen replete conditions. Under nitrogen replete conditions, *T. obliquus* wild-type uses starch as a temporary energy storage compound

Abbreviations: LD, light/dark; mol_{ph}, mol of photons; DW, dry weight; OD_{750}, optical density at 750 nm; TAG, triacylglycerol; D, dilution rate; V_{PBR}, photobioreactor volume; μ_t, time-specific cell division rate

* Corresponding author.
E-mail address: graciela.leonsaiki@wur.nl (G.M. León-Saiki).

https://doi.org/10.1016/j.algal.2018.02.027
Received 31 October 2017; Received in revised form 16 January 2018; Accepted 22 February 2018

during LD cycles [26]. Thus, energy and carbon are stored during the day which are next used at night. When starch synthesis is blocked, as for starchless mutants, different effects on growth under nitrogen replete conditions are observed for different microalgae. However, most of the studies have been done under continuous light [27–30], which is not relevant for outdoor production. Furthermore effects of the absence of starch are expected to be more severe during LD cycles, since the algae use starch during the dark as a source of energy and carbon. To our knowledge, only one report of growth of the starchless mutant of *Chlorella pyrenoidosa* STL-PI was done under 12:12 h LD cycles, showing an increase in growth compared to its wild-type [28]. Additionally, as starchless mutants are made to improve TAG production, most studies on these mutants focus on their performance during the TAG producing step under nitrogen limitation or starvation conditions [22,25,27,29–31], and little is known about their diurnal behavior under nitrogen replete conditions and LD cycles. The diurnal cycles of the starchless mutant of *T. obliquus slm1* were studied under 16:8 h LD cycles, where this mutant showed synchronized growth and cell division even in the absence of starch or any other storage compound, albeit with decreased growth and energy efficiency compared to its wild-type [26]. However, as previously mentioned, production conditions outdoors will include variations in the light and dark periods and it is thus interesting to know how the mutant will react to these variations. Especially, it is interesting to know how the mutant will react to longer dark periods since a temporary energy storage compound is missing.

Therefore, the aim of this paper is to obtain insight into how a starchless mutant of *T. obliquus* copes with different LD periods as compared to its wild-type. For this, scheduling of cell division, energy efficiency and biomass composition were measured under 3 different photoperiods of typical day/night duration throughout the year for both the wild-type and the starchless mutant *slm1*.

2. Materials and methods

2.1. Strains, pre-culture conditions and cultivation medium

Wild-type *Tetradesmus obliquus* UTEX 393 (reclassified from *Scenedesmus obliquus* [17] and *Acutodesmus obliquus* [18]) was obtained from the Culture Collection of Algae, University of Texas. The starchless mutant of *T. obliquus* (*slm1*) was generated as described by de Jaeger et al. [23]. Liquid cultures of 100 mL of filter sterilized (pore size 0.2 μm) defined medium designed by Breuer et al. [19] were maintained in a culture chamber with shaker in 250 mL Erlenmeyer flasks (25 °C, 16:8 h light/dark cycles with 30–40 $\mu mol \cdot m^{-2} \cdot s^{-1}$, 150 rpm, air in headspace). Prior to the start of the experiments, cultures were placed in a shake incubator operating at 25 °C with continuous light (120 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and a headspace enriched with 2.5% CO_2 to reach the desired inoculation cell density.

2.2. Reactor set-up and experimental conditions

T. obliquus was continuously cultivated in a sterile flat panel airlift-loop reactor with a 1.7 L working volume and a 0.02 m light path (Labfors 5 Lux, Infors HT, Switzerland). Reactor set-up, temperature, pH and airflow were set and controlled as described by León-Saiki et al. [26]. Light was provided at an incident photon flux density of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in 3 different light/dark (LD) block cycles: 16:8 h, 14:10 h, and 12:12 h. Cultivations were turbidostat controlled, where the culture was diluted with fresh medium when the light intensity at the back of the reactor dropped below the setpoint (10 $\mu mol \cdot m^{-2} \cdot s^{-1}$). The feeding was stopped during the dark period to prevent washing of the culture.

The reactor was inoculated at an optical density (OD_{750}) of 0.1. Cultures were allowed to reach steady state, which was defined as a constant biomass concentration and 24 h-dilution rate for a period of at least 3 residence times. After steady state was reached, liquid samples were freshly taken from the reactor and either immediately used for cell count (1 mL) and dry weight measurements (3 mL, in triplicate) or centrifuged for 5 min at 2360 × *g* for biochemical analysis (12 mL for proteins, 5 mL for starch, 5 mL for triacylglycerides (TAG) and 5 mL for total carbohydrates). For biochemical analysis, the resulting pellet was transferred to bead beating tubes (Lysing Matrix E; MP Biomedicals Europe) or glass tubes (for total carbohydrates analysis) and stored at −20 °C. Pellets were freeze dried and stored again at −20 °C until further analysis. Sampling was done in intervals of 1 h for cell counts. Biomass composition was analyzed in intervals of 3 h for the 14:10 h and 12:12 h LD. For the 16:8 h LD, biomass composition was obtained from a previous publication [26]. In addition, at least 3 daily overflow samples were collected for each photoperiod and strain.

2.3. Analyses

Dry weight (DW) concentration was determined in triplicate as described by Kliphuis et al. [32]. Starch was measured using a total starch kit (Megazyme, Ireland) as described by de Jaeger et al. [23] with the modification that 5 mg of freeze dried biomass was used for the analysis. Protein content was measured using a colorimetric assay (Bio-Rad DC protein assay) as described by Postma et al. [33] with the difference that 10–12 mg of freeze dried biomass was used for analysis. Triacylglycerol (TAG) content was determined as described by Remmers et al. [22]. Total carbohydrates were extracted and quantified according to DuBois et al. [34] and Hebert et al. [35].

2.4. Cell number and size

T. obliquus cells aggregate and form coenobia [36]. To separate the cells, a 1 mL cell suspension was sonicated on ice for 30 s at 30% amplitude using a probe sonicator (Sonics vibra-cell, USA). The absence of coenobia after sonication was verified under the microscope. Cell number and size were determined using a Beckman Coulter Multisizer 3 (Beckman Coulter Inc., USA). The sonicated culture was diluted 200 times with Isoton® II diluent solution. Cells with diameter above 2.5 μm were counted. As some cell counts were done only in duplicate (n = 2), we show the range of values measured by including the maximum and minimum values found.

2.5. Dilution rate, doubling time and time-specific cell division rate

Dilution rate (D_{24h} in day^{-1}) was calculated by logging the medium (feed) and acid consumption over 24 h (V_{24h} in L) and the volume of the photobioreactor (V_{PBR} in L) (Eq. (1)) [37]:

$$\mu_{24h} = D_{24h} = \frac{V_{24h}}{V_{PBR}} \quad (1)$$

Dilution rates over small intervals of time were calculated by logging the medium and acid consumption in intervals of 10 min, followed by a moving average per 60 min. Dilution patterns were repeated daily. Values corresponding to 1 h were averaged and used for the time-specific cell division rate (μ_t), which was calculated based on a cell number balance (C_{cells}) and D_t following:

$$\frac{dC_{cells}}{d_t} = -D_t \cdot C_{Cells} + \mu_t \cdot C_{Cells} \quad (2)$$

$$\mu_t = \frac{\frac{dC_{cells}}{d_t} + D \cdot C_{Cells}}{C_{Cells}} \quad (3)$$

Hourly values were added up to get the cumulative cell division rate. The average dilution rate over the light period was calculated by dividing the daily average dilution rate (D_{24h}) by the amount of hours of light supplied, with the following equation: