Algal Research 9 (2015) 254–262

Contents lists available at ScienceDirect

Algal Research

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

Photosynthetic efficiency and carbon partitioning in nitrogen-starved *Scenedesmus obliquus*

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ARTICLE INFO

Article history:
Received 2 December 2014
Received in revised form 17 February 2015
Accepted 19 March 2015
Available online xxxx

Keywords:
Carbon partitioning
Nitrogen starvation
Light intensity
Starch
Fatty acid
Triacylglycerol (TAG)

ABSTRACT

This work investigates how the photoacclimated state at the onset of nitrogen starvation and the light intensity during nitrogen starvation influence carbon partitioning and photosynthetic efficiency in nitrogen-starved *Scenedesmus obliquus*. Nitrogen-depleted batch cultivations were performed at an incident light intensity of 200 and 1000 μmol m^{-2} s^{-1}. These nitrogen-depleted batch cultivations were started with biomass that was photoacclimated to an incident light intensity of either 200 or 1000 μmol m^{-2} s^{-1} under nitrogen replete conditions. During initial nitrogen starvation, fatty acids and starch were produced in a 1:4 ratio. This ratio progressively increased towards only fatty acid synthesis. Hereafter, the initially accumulated starch was degraded and likely used as a substrate for fatty acid synthesis. The ratio between starch and fatty acid synthesis correlated strongly to the biomass nitrogen content. Slightly more carbon was partitioned to starch synthesis at high light intensities. Carbon partitioning was not affected by the photoacclimated state at the onset of nitrogen starvation. The photosynthetic efficiency decreased 2-fold at high versus low light intensities but was not affected by photoacclimation. Likely, the observed carbon partitioning is caused by competition between fatty acid and starch synthesis for a common carbon pre-cursor.

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1. Introduction

Oleaginous microalgae can produce large amounts of fatty acids in the form triacylglycerol (TAG). These TAGs are of commercial interest as a resource for food, feed, and biofuels [1,2]. Microalgae can be seen as 'microplants' and have several advantages over agricultural crops that are currently used to produce these commodities. Microalgae have the potential to reach much higher productivities than terrestrial plants, can be cultivated on non-arable land, and have lower freshwater and fertilizer requirements than agricultural crops [1–3].

Microalgal TAGs are only produced under conditions of physiological stress [3]. The most common approach to induce TAG production in microalgae is using nitrogen starvation [3]. During nitrogen starvation, not only TAG accumulation is induced, but also the photosynthetic efficiency and carbon assimilation rate decrease progressively in time, leading to progressively decreasing TAG accumulation rates [4,5]. Furthermore, even in the best TAG producing microalgae species, TAG only accounts for approximately half of the biomass produced after the depletion of nitrogen [5]. Other biomass constituents, such as starch, are produced simultaneously [6]. The overall TAG productivity during nitrogen starvation is thus determined by the product of the photosynthetic rate during nitrogen starvation and the fraction thereof that is partitioned towards TAG synthesis.

Both the light intensity during the nitrogen replete growth phase and the light intensity during the nitrogen starvation phase can affect the photosynthetic efficiency and carbon partitioning during nitrogen starvation, as discussed below.

1.1. Light intensity during nitrogen starvation

It is generally accepted that the photosynthetic efficiency is strongly dependent on the light intensity [7,8], in which the photosynthetic efficiency is here defined as the efficiency of converting light energy into chemical energy (J/J). At low light intensities, photosynthesis is limited by light absorption but at high light intensities photosynthesis is limited by the capacity of the photosynthetic machinery. The excess absorbed light is then thermally dissipated, resulting in a decreased photosynthetic efficiency, and thus also lower biomass yield (g dry weight/mol photon), at high light intensities [7,8]. A high light intensity during nitrogen starvation will thus also reduce the biomass yield during nitrogen starvation [4,9].

Also carbon partitioning may be affected by the light intensity during nitrogen starvation. During nitrogen starvation, photosynthetic products can no longer be used for the synthesis of proteins and nucleic acids. These photosynthetic products are then used for the synthesis of

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http://dx.doi.org/10.1016/j.algal.2015.03.012
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nitrogen-free biomass constituents. Many nitrogen-free biomass constituents could be produced simultaneously. The ratio in which these constituents are made is from now on referred to as carbon partitioning. Many studies report the impact of the light intensity on carbon partitioning towards TAG. The conclusions of these studies are contradicting however. Some studies hypothesize that TAG production is the result of an 'overflow-metabolism'-like response, where TAG is only made when the production rate of excess photosynthetic products exceeds a critical value and that the partitioning ratio of carbon towards TAG increases with increasing light intensity. These studies suggest that optimal TAG production will be achieved at high light intensities [10–12]. Other studies report that the light intensity does not affect the carbon partitioning ratio or maximum TAG content, but does influence TAG productivities and yields, because the light intensity affects the photosynthetic rate and photosynthetic efficiency [9,13,14]. Yet different studies claim that starch is the primary accumulation product, and that low light intensities result in increased partitioning towards TAG [15,16]. Note that the carbon partitioning response to light intensity might very well be species specific, making comparison of aforementioned studies difficult. In conclusion, it is clear that no consensus exists on how the light intensity during nitrogen starvation influences carbon partitioning.

1.2. Light intensity during the nitrogen replete growth phase

Microalgae acclimate to the light intensity [8]. The light intensity prior to the onset of nitrogen starvation thus determines the photoacclimated state at the onset of nitrogen starvation. The effects induced by this photoacclimated state are likely to persist during nitrogen starvation because acclimation to a change in light intensity typically occurs with half-times in the order of 10 h, during nitrogen replete conditions [17,18]. This means that the effects of the photoacclimated state will at least persist during initial nitrogen starvation. Because nitrogen starvation can negatively affect protein synthesis, repair and turnover, re-acclimation likely occurs at a much slower rate during nitrogen starvation [17,19]. The effects of the photoacclimated state might therefore also persist during prolonged nitrogen starvation.

The photoacclimation state is reflected in the pigmentation and composition of the photosystem. A change in photoacclimation state can thus have a large impact on the photosynthetic efficiency [8,20,21]. This can also affect carbon partitioning in the case that energetic imbalances are the driving force behind carbon partitioning, as is often proposed [10,12]. It has for example been proposed that microalgae, that are first acclimated to a low light intensity and are subsequently exposed to a high light intensity during nitrogen starvation, can have an enhanced TAG accumulation because of the increased energetic shock [13]. Photoacclimation can also affect the biochemical composition of the biomass, and potentially also the composition of the enzymatic machinery for fatty acid and starch synthesis. When the composition of the enzymatic machinery persists during nitrogen starvation, this can also affect carbon partitioning during nitrogen starvation.

In summary, little is known about the effects of the photoacclimated state at the onset of nitrogen starvation on the photosynthetic efficiency and carbon partitioning during nitrogen starvation, because typically the same light intensity is used during the nitrogen replete phase and nitrogen-depleted phase of a batch cultivation. This makes it impossible to distinguish between the effects of the light intensity during nitrogen starvation and the photoacclimated state. Also no consensus exists on the impact of the light intensity during nitrogen starvation on carbon partitioning. Therefore, in this work, we investigate how the light intensity during nitrogen starvation and the photoacclimated state at the onset of nitrogen starvation influence carbon partitioning and the photosynthetic efficiency during nitrogen starvation in the oleaginous microalga *Scenedesmus obliquus*.

To do so, light-limited, nitrogen replete, chemostat cultivations were performed at a low and a high light intensity to obtain two differently photoacclimated cultures. Subsequently, these differently photoacclimated cultures were used to perform nitrogen-depleted batch cultivations at either a low or a high light intensity (4 experimental conditions in total). The chemostat operation allowed for a high degree of control over the photoacclimated state of the biomass. Furthermore, this setup ensured that the transition from a low to a high, and from a high to a low light intensity was simultaneous with the onset of nitrogen starvation.

2. Materials and methods

2.1. Experimental design

All experiments were started as light-limited, nitrogen replete, chemostat cultivations. These chemostat cultivations were operated at an incident light intensity of 200 or 1000 μmol m^{-2} s^{-1} to obtain two differently photoacclimated cultures. This resulted in an average light intensity during chemostat operation in these cultures of 30 ± 2 and 246 ± 5 μmol m^{-2} s^{-1} for the cultures at an incident light intensity of 200 and 1000 μmol m^{-2} s^{-1}, respectively (calculated as described in Supplementary data 1). In these chemostat cultivations, the dilution rate was chosen such that between 90 and 95% of the NO_3^- present in the feed-medium (10 mM) was consumed (Supplementary data 1). Because the same NO_3^- concentration (10 mM) was used in all cultivations and the biomass nitrogen content was relatively constant under nitrogen replete conditions, the steady-state biomass concentration was approximately 1.5 g/l in all chemostat cultivations (Table 1). Once steady state conditions were achieved (evaluated based on a constant biomass concentration and absorption cross section), chemostat cultivation was continued for more than 4 culture dilutions. During this period, NO_3^- was at no point depleted. Hereafter, chemostat operation was terminated, and batch operation was initiated (Fig. 1). This was accomplished by stopping the medium-inflow and overflow pumps. Because of the near complete NO_3^- consumption during the chemostat operation, all residual NO_3^- was consumed within two hours (Fig. 1). During this period, the consumption of residual NO_3^- was measured every 30 min until all NO_3^- was consumed. Once depletion of NO_3^- was confirmed, the light intensity was set to 200 or 1000 μmol m^{-2} s^{-1} (depending on the experimental condition) and batch operation was followed for at least 21 days (Fig. 1).

There was a short period between the transition from chemostat to batch-operation and the moment of nitrogen starvation/change in light intensity during which nitrogen was not yet depleted. As a consequence, there was a small increase in biomass concentration in this period (Fig. 1). To quantify this difference, a sample was taken when nitrogen was depleted to measure the change in biomass concentration in the period between termination of chemostat operation and complete consumption of NO_3^-. This increase in dry weight (DW) concentration was in all cases less than 13% (Table 1). All experiments were performed in duplicate.

2.2. Reactor design and operation

Cultivations were performed in flat-panel airlift-loop photobioreactors with a working volume of 1.7 l and a light path of 2 cm (Labfors 5 Lux, Infors HT, Switzerland), as described by [6]. The reactors were aerated with air enriched with 2% (v/v) CO_2 at 1 l/min. The condenser was operated at 2 °C to prevent water losses due to evaporation. The reactors were continuously illuminated (24 h/day) using a panel containing 260 LED lamps, with a warm-white spectrum (as specified in the user manual of the Labfors 5 Lux; colour temperature of 4000 K), located on the culture side of the reactor. The temperature was controlled at 27.5 °C and the pH was controlled at pH 7 using automatic addition of a 5% (v/v) H_2SO_4 solution. The O_2 and CO_2 concentration in the off-gas were measured online using a mass spectrometer (Prima dB, Thermo Scientific). Prior to inoculation, the composition of the ingoing gas was

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