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Effect of initial biomass-specific photon supply rate on fatty acid accumulation in nitrogen depleted *Nannochloropsis gaditana* under simulated outdoor light conditions

Jorijn H. Janssen[a,*], Jasper L.S.P. Driessen[a], Packo P. Lamers[a], René H. Wijffels[a,b], Maria J. Barbosa[a,c]

[a] Bioprocess Engineering, AlgaePARC, Wageningen University and Research, P.O. Box 16, 6700 AA Wageningen, the Netherlands
[b] Nord University, Faculty of Biosciences and Aquaculture, N-8049 Bodø, Norway
[c] University of Bergen, Department of Biology, PO Box 7803, 5006 Bergen, Norway

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ABSTRACT

Triacylglycerol (TAG) accumulation in the microalgae *Nannochloropsis gaditana* is induced by nitrogen starvation and dependent on the light supplied. We studied under simulated outdoor light conditions the effect of supplied light on the TAG yield by varying the biomass-specific photon supply rate present at the onset of nitrogen starvation. High, intermediate and low average biomass-specific photon supply rates (26, 11 and 6 $\mu mol\, g^{-1}\, s^{-1}$) were achieved by applying equal incident light intensity to different biomass concentrations (1.2, 2.9 and 5.4 $g\, L^{-1}$). The intermediate biomass-specific photon supply rate resulted in the highest time-averaged TAG yield on light; 0.09 $g_{TAG}\, mol_{ph}{}^{-1}$. Sub-optimal yields were attributed to photosaturation, photoinhibition, light falling through reactor without being absorbed and high maintenance requirements. The biomass-specific photon supply rate is important to optimize TAG production by microalgae.

1. Introduction

Due to the increasing world population, the demands for food and energy are rising the need for alternative, sustainable sources for food-commodities and fuels increases [1]. Triacylglycerol (TAG) has a wide range of applications and is used in the food- and petrochemical industry. Microalgae can be used as sustainable alternative for TAG production. For this, the production process should be optimized to be economically feasible and compete with plant-based and fossil oils [2]. Nitrogen starvation is the most applied technique for TAG accumulation in microalgae [3]. *Nannochloropsis* is a marine microalgae species which accumulates large amounts of TAG (up to 54% per dry weight) upon nitrogen starvation and produces the omega-3 fatty acid eicosapentaenoic acid (EPA) [4,5]. Nitrogen starvation is often applied in a two phase batch strategy in which the microalgae are first grown under non-limiting conditions followed by nitrogen starvation to induce TAG accumulation.

Under identical light intensities, low biomass concentration results in a high biomass-specific photon supply rate and a high biomass concentration results in a low biomass-specific photon supply rate. Light supply rate is important as it affects the photosynthetic rate and thereby the biomass and TAG yields. In outdoor cultivation, for a given cultivation system and light intensity, the biomass-specific photon supply rate can be changed by changing the biomass concentration inside the reactor. Biomass concentration dictates the light gradient inside the photobioreactor and, at a fixed light intensity, also the biomass-specific photon supply rate. There are different processes which influence the photosystems and photosynthetic rates: photosaturation, photoinhibition and photoacclimation. When suboptimal biomass-specific photon supply rates are used, these processes can lead to sub-optimal TAG yields (Fig. 1).

One of these processes is photosaturation. At light intensities higher than the photosynthetic processing capacity, photosynthesis is saturated and the excess energy is dissipated as heat, thereby decreasing photosynthetic efficiency [6]. Another process is photoinhibition, which is the process where excess of photons elicit formation of oxygen radicals (ROS) which damage key proteins in the photosynthetic machinery and thereby decrease photosynthetic efficiency and thus increase photosaturation [7]. Microalgae can also adapt their pigmentation dependent on the light intensity received through photoacclimation and thereby adapt their photosynthetic efficiency [7].

In addition to the processes affecting the photosystems and

* Corresponding author.
E-mail address: jorijn.janssen@wur.nl (J.H. Janssen).

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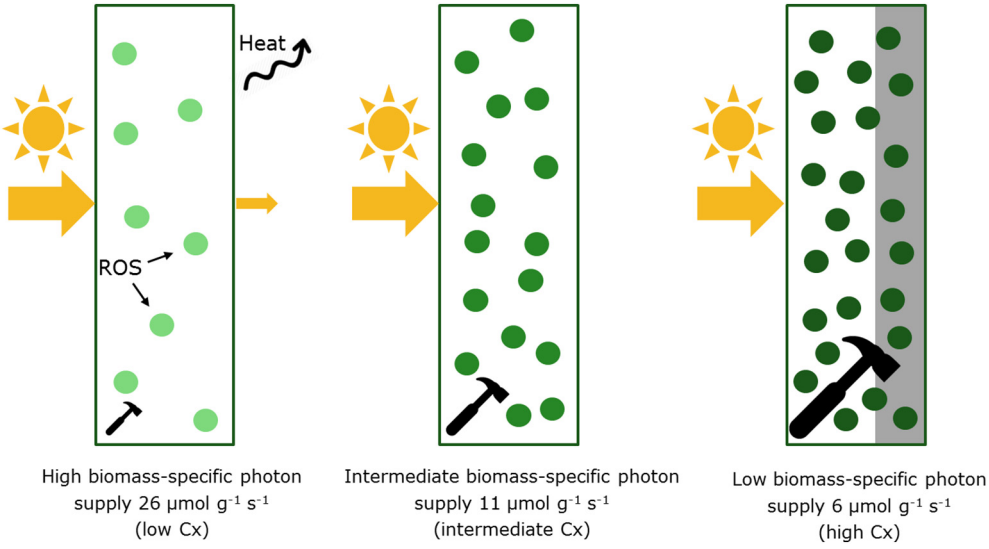

Fig. 1. Schematic overview of different biomass-specific photon supply rates and the processes expected to influence the TAG yield. The size of the hammer represents the volumetric maintenance energy requirements.

photosynthetic efficiency, TAG yield on light will also be affected by the metabolic processes downstream of light absorption; TAG yield decreases when absorbed light energy is used for other processes than TAG production. For instance, light energy is necessary for cell maintenance, which are non-growth related processes (*e.g.* turnover of cellular materials or maintain concentration gradients across cell membranes) [8,9]. The volumetric energy requirement for cell maintenance is dependent on the biomass concentration [10]. When there is not enough light energy supplied for maintenance, cells need to degrade storage products such as TAG to generate energy to maintain themselves, leading to a lower TAG yield. Another factor contributing to a low TAG yield on light, but now under high biomass-specific photon supply rates, is the loss of light that goes through the reactor without being absorbed.

Previous outdoor studies showed that the amount of biomass at the start of nitrogen starvation affected TAG yield on light for *Chlorella zofingiensis* [11,12]. Increasing initial biomass concentration showed increased lipid productivity for the biomass concentrations tested (0.02, 0.15, 0.35 and 0.5 g L^{-1}) with a reactor depth of 17 cm at varying outdoor light conditions [11]. In lab-scale experiments, no significant effect of biomass-specific photon supply rate at the start of nitrogen starvation on the overall TAG production for *Chlorella zofingiensis* was found between the tested biomass-specific photon absorption rates 4.7, 3.5 and 2.9 µmol g^{-1} s^{-1} using continuous light conditions [13]. For *Nannochloropsis oculata* a maximum TAG productivity under continuous light conditions was found at 13 µmol g^{-1} s^{-1} [14].

In this research, the effect of the biomass-specific photon supply rate on TAG yield in *Nannochloropsis gaditana* during nitrogen starvation was studied at lab-scale under simulated outdoor light conditions. Simulated outdoor light conditions were used at lab-scale to be more representative of outdoors conditions. The different biomass-specific photon supply rates were set by applying equal light intensities to different biomass concentrations present at the moment of nitrogen starvation. The outdoor light intensity was simulated using a half-sinus incident light intensity curve with a peak at noon of 1500 µmol m^{-2} s^{-1} and a day: night cycle of 16: 8 h. The average initial biomass-specific photon supply rates were; 26, 11 and 6 µmol g_{dw}^{-1} s^{-1} at the start of nitrogen starvation. This required initial biomass concentrations of 1.2, 3.0 and 5.4 g L^{-1}. It was hypothesized that there is an optimal biomass concentration and thus biomass-specific photon supply rate at the start of nitrogen starvation where the TAG yield on light is maximal. Besides TAG, the omega-3 fatty acid eicosapentaenoic acid (EPA) was studied in more depth. EPA is a fatty acid present in the photosynthetic membranes and TAG, and therefore differences are expected at different biomass-specific photon supply rates.

2. Materials and methods

2.1. Strain, cultivation medium and pre-cultivation

The microalgae *Nannochloropsis gaditana* CCFM-01 was obtained from the Microalgae Collection of Fitoplancton Marino S.L. Pre-cultures of *N. gaditana* were kept in 250 mL Erlenmeyer flasks with 100 mL culture, incubated at 25 °C in an orbital shaker incubator (125 rpm). The cultures were maintained at low light conditions (30–40 µmol m^{-2} s^{-1}), in a 16:8 h day:night cycle. A week before inoculation, the microalgae were transferred to continuous high light conditions (118 µmol m^{-2} s^{-1}) with air enriched with 2.5% CO_2 for inoculum production. The growth medium was based on [15] and contained: NaCl 445 mM; KNO_3 33.6 mM; Na_2SO_4 3.5 mM; $MgSO_4{\cdot}7H_2O$ 3 mM; $CaCl_2{\cdot}2H_2O$ 2.5 mM; K_2HPO_4 2.5 mM; NaFeEDTA 28 µM; $Na_2EDTA{\cdot}2H_2O$ 80 µM; $MnCl_2{\cdot}4H_2O$ 19 µM; $ZnSO_4{\cdot}7H_2O$ 4 µM; $CoCl_2{\cdot}6H_2O$ 1.2 µM; $CuSO_4{\cdot}5H_2O$ 1.3 µM; $Na_2MoO_4{\cdot}2H_2O$ 0.1 µM; Biotin 0.1 µM; vitamin B1 3.3 µM; vitamin B12 0.1 µM; and 10 mM $NaHCO_3$. For nitrogen depleted growth medium KNO_3 was replaced with 33.6 mM KCl to keep equal osmolarity. Since the nitrate and phosphate concentration in the medium was high enough for a biomass concentration of approximately 5 g L^{-1} extra nitrate and phosphate were added before it became limited during the growth phase. For the intermediate concentration (2.9 g L^{-1}) 2.8 g KNO_3 was added at day 7 of the growth phase. For the high biomass concentration (5.4 g L^{-1}) 2.6 g KNO_3 was added at day 7 and 4.8 g KNO_3 and 0.6 g K_2HPO_4 were added at day 13 of the growth phase. During pre-cultivation in Erlenmeyer flasks 100 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) was added as pH buffer. The pH of the growth media was adjusted to pH 7.5 and filter sterilized prior to use (pore size, 0.2 µm).

2.2. Photobioreactor and experimental setup

Experiments were performed in an aseptic, heat-sterilized, flat-panel, airlift-loop photobioreactor (Labfors 5 Lux, Infors HT, Switzerland, 2010) with a working volume of 1.8 L and a reactor depth of 20.7 mm. Mixing was provided by aeration of the culture with 1 L min^{-1} filtered air mixed with 2% CO_2. The pH was maintained at