Algal Research 2 (2013) 122–126

Contents lists available at SciVerse ScienceDirect

Algal Research

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

Effect of oxygen at low and high light intensities on the growth of *Neochloris oleoabundans*

Claudia Sousa [a,b,*], Ana Compadre [a], Marian H. Vermuë [b], Rene H. Wijffels [b]

[a] Wetsus, P.O. Box 1113, 8900 CC Leeuwarden, The Netherlands
[b] Bioprocess Engineering, Wageningen University, P.O. Box 8129, 6700 EV, Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 30 May 2012
Received in revised form 21 November 2012
Accepted 29 January 2013
Available online 19 February 2013

Keywords:
Neochloris oleoabundans
Oxygen inhibition
Photooxidative damage
Photoacclimation
Photorespiration
Photobioreactor

ABSTRACT

The effect of partial oxygen pressure on growth of *Neochloris oleoabundans* was studied at near-saturating light intensity in a fully-controlled photobioreactor. At the partial oxygen pressures tested (P_{O2} = 0.24; 0.42; 0.63; 0.84 bar), the specific growth rate was 1.36; 1.16; 0.93 and 0.68 day^{-1}, respectively. An increase of the P_{CO2} from 0.007 to 0.02 bar at P_{O2} of 0.84 bar did not show any positive effect on the overall growth of the algae, contrary to what happens at sub-saturating light intensities. These results indicate that at near-saturating light intensity the inhibitory effect of oxygen by photorespiration cannot be overcome. The chlorophyll content of *N. oleoabundans* grown at 200 μmol m^{-2} s^{-1} is about 1.9 times higher than when cultivated at 500 μmol m^{-2} s^{-1}, whereas the carotenoid content was about 1.5 lower, both demonstrating photoacclimation effects. The elevated oxygen concentration in the growth medium does not affect the pigment content both at sub- and near-saturating light conditions. This indicates that elevated oxygen concentrations in the medium do not contribute to photooxidative damage at the light conditions that are predominantly experienced by algae in closed photobioreactors, but only inhibit the growth via photorespiration effects.

1. Introduction

Neochloris oleoabundans is one of the algae which combines high specific growth rate at optimal growth conditions [1–3] with accumulation of lipids with large content of saturated fatty acids during nitrogen starvation conditions [3–5]. These characteristics make this alga species a promising feed stock for biofuel production [6–8]. For large-scale outdoor production of algae, closed photo-bioreactor systems (PBR) have been proposed. To make the production economically feasible, however, bottlenecks still need to be overcome. One of these bottlenecks is the high energy input that is required for mixing to provide the algae with light, carbon dioxide and to remove the photosynthetically produced oxygen [8–10].

In photo-bioreactors oxygen that is produced during photosynthesis, accumulates and induces processes like photorespiration and photoinhibition, both leading to a decrease in biomass yield on light energy of the microalgae [11]. In photorespiration, oxygen binds with the enzyme Rubisco and competes with carbon dioxide needed for photosynthesis. Hence, high oxygen levels lead to lower CO_2 uptake and reduced fixation of light energy into carbohydrates [12].

Photoinhibition occurs mainly at high and over-saturating light intensities. At those conditions an excess of electrons is generated in Photosystem II and these electrons will react with the photosynthetically produced oxygen, leading to the formation of oxygen radicals and other reactive oxygen species (ROS) such as H_2O_2 [13]. In addition, light stimulates the formation of the highly reactive singlet oxygen via photo-activation [14]. The singlet oxygen causes damage of the water-oxidizing center and deactivates the electron transport chain [15,16] and this results in loss of photosynthetic activity and death of cells.

To overcome the photo-oxidative damage caused by photoinhibition at high light, the microalgae have developed several protection mechanisms, generally referred to as photo-acclimation. Photo-acclimation can easily be recognized by changes in the pigmentation of the algae, resulting in lower chlorophyll content and higher carotenoid content of the algae when exposed to higher irradiance. The carotenoid content normally increases to enable the algae to dissipate energy of excited chlorophyll and eliminate ROS and to maintain the photosystem structure [17]. In addition, carotenoids scavenge triplet chlorophyll and quench singlet oxygen [18]. At very high light irradiation, however, the protective mechanisms cannot sufficiently deal with the surplus of electrons and formation of singlet oxygen and the accumulation of ROS occurs, leading to cell damage [14].

Although the combined effect of oxygen and light has been described in detail, the effect of accumulating oxygen on algal growth is only studied independently at controlled low light conditions [19–21]. Upon an increase in oxygen concentrations in the algal cultures a

* Corresponding author at: Bioprocess Engineering, Wageningen University, P.O. Box 8129, 6700 EV, Wageningen, The Netherlands. Tel.: +31 582843190.
E-mail address: claudia.sousa@wetsus.nl (C. Sousa).
URL: http://www.algae.wur.nl (C. Sousa).

2211-9264/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.algal.2013.01.007

general decrease in specific growth rates has been observed. This inhibition at low light conditions is related to the carboxylation/oxygenation ratio of the enzyme Rubisco and its affinity for oxygen and the oxygen inhibition effects at low light conditions can be compensated by an increase of the carbon dioxide concentration [20]. In outdoor cultivation; however, algae will experience different light conditions. It is thus important to know how the algae respond on accumulated oxygen at controlled culture conditions at higher light intensities and investigate if addition of carbon dioxide could be used to overcome the inhibiting effects of oxygen at higher light conditions as well. In this paper, the effect of oxygen partial pressure on the growth of *N. oleoabundans* exposed to near-saturating conditions was determined in a fully-controlled photo-bioreactor operated in turbidostat mode and compared with the inhibiting effects of oxygen on growth at low light conditions. The magnitude of this effect on the specific growth rate as well as on the biomass yield on light energy and pigment content was determined. Finally, the CO_2/O_2 ratio was increased to see if the inhibiting effect of O_2 in microalgae could be overcome.

2. Material and methods

2.1. Cultures and medium

Adapted f/2 medium [22] was used to grow and maintain *N. oleoabundans* (UTEX 1185) cultures. The medium was composed of artificial seawater (in mM): NaCl, 419; $MgCl_2 \cdot 6H_2O$, 48.2; $CaCl_2 \cdot 2H_2O$, 3.6; Na_2SO_4, 22.5; and K_2SO_4, 4.9. The artificial seawater was enriched with the following nutrients (in mM): $NaH_2PO_4 \cdot 2H_2O$, 2.50; and $NaNO_3$, 32; trace elements (in μM): EDTA–FeNa, 29.3; $CuSO_4 \cdot 5H_2O$, 0.10; $Na_2MoO_4 \cdot 2H_2O$, 0.07; $ZnSO_4 \cdot 7H_2O$, 0.19; $CoCl_2 \cdot 6H_2O$, 0.19; and $MnCl_2 \cdot 4H_2O$, 2.27; and vitamins (μg L^{-1}): thiamine, 200; biotin, 1.00; and cyanocobalamin, 1.00. The pH was adjusted to 7.8 with 0.5 M NaOH. *N. oleoabundans* was pre-cultured in an incubator with orbital shaker (Innova 44R, New Brunswick Scientific, USA) under fluorescent light (40 μmol $m^{-2}s^{-1}$) at 25 °C and 120 rpm. The air inside the incubator was enriched with 2% carbon dioxide. In the reactor experiments the culture media was enriched with 10 mM $NaHCO_3$.

2.2. Photobioreactor

A 3 L jacketed bioreactor (Applikon Biotechnology, The Netherlands) was used to perform continuous turbidostat experiments. All sensors and regulators of the experimental set-up were connected to an Ez-controller equipped with BioExpert© software (Applikon Biotechnology, The Netherlands). The culture was illuminated with two light panels (20 × 20 cm) with red (627 nm) LED lights (SL3500, Photon Systems Instruments, Czech Republic). The incident light intensity was measured with a PAR quantum sensor (model SA-190, LI-COR Biosciences, USA) before the start of each experimental run. The measurements were done at different heights and radial positions to determine the average incident photon flux density (PFD_{avg}). The average value for the different experiments at high-light intensity was always ~500 μmol $m^{-2}s^{-1}$, while at low light intensity the average incident photon flux density was ~200 μmol $m^{-2}s^{-1}$. The measured and controlled process parameters were: pH, temperature, oxygen and carbon dioxide partial pressure in the liquid phase (P_{O2} and P_{CO2}), liquid level, stirrer speed and optical density (OD) [20].

The cells were adapted to the turbidostat conditions for at least 3 days, before the specific growth rate (μ) was determined from the dilution rate. The optical density at 750 (OD_{750}) and 680 nm (OD_{680}) was measured in a UV–visible spectrophotometer (UV-1650 PC, Shimadzu). The cell dry weight concentration and pigment content were determined off line as well.

2.3. Dry weight concentration

To determine the dry weight concentration, 5 mL samples in triplicate were washed with 10 mL of ammonium formate 0.5 M, filtered through a pre-weighed glass fiber filter (Whatman GF/F), and washed again with 40 mL of ammonium formate 0.5 M. The filters were dried in an oven at 95 °C, for 24 h, in aluminum trays, cooled in a desiccator for at least 2 h, and then weighed on a 5 digit analytical balance (ME235P-SD, Sartorius, Germany).

2.4. Chlorophyll and carotenoids

Chlorophyll and carotenoid contents of the algae were determined in triplicate at the end of each experiment. A 2 mL algal aliquot collected from the reactor was centrifuged at 3760 rpm and 4 °C for 10 min (Allegra™ X-12 R Centrifuge). The pellets were frozen at −80 °C, prior to further analysis. Chlorophyll was extracted by adding 5 mL of methanol (100%) to the biomass pellet. The cells were disrupted by ultrasound (Sonorex Digitec, Bandelin) combined with temperature shock (incubation at 60 °C and 0 °C). The suspension was centrifuged at 3760 rpm and 4 °C for 10 min. The supernatant was collected and the chlorophyll and carotenoid contents were determined at 470, 652 and 665 nm in a UV–visible spectrophotometer (UV-1650 PC, Shimadzu). Modified Arnon's equations [23] were used to calculate chlorophyll and carotenoid concentrations in the extracts [24]. Chlorophyll and carotenoid contents were presented per gram of biomass which was calculated based on the dry weight concentrations in the samples used.

3. Results and discussion

3.1. Controlled cultivation of algae at high and low light intensities

Fig. 1 shows a typical run at 0.21 bar of oxygen partial pressure of *N. oleoabundans* cultivated at high light conditions. In this experiment the algae were cultivated using an average incident light irradiance of 500 μmol m^{-2} s^{-1}. The accompanying average photon flux density experienced by the algae inside the photobioreactor was 230 μmol $m^{-2}s^{-1}$, using an estimated light gradient inside the photobioreactor, as was described by Sousa et al. (2011). The PI (photosynthesis–irradiance) curve for this alga [20] shows that *N. oleoabundans* experiences near-saturation conditions at 230 μmol $m^{-2}s^{-1}$. When growing the algae at an average low incident light intensity of 200 μmol $m^{-2}s^{-1}$, they experience 92 μmol $m^{-2}s^{-1}$

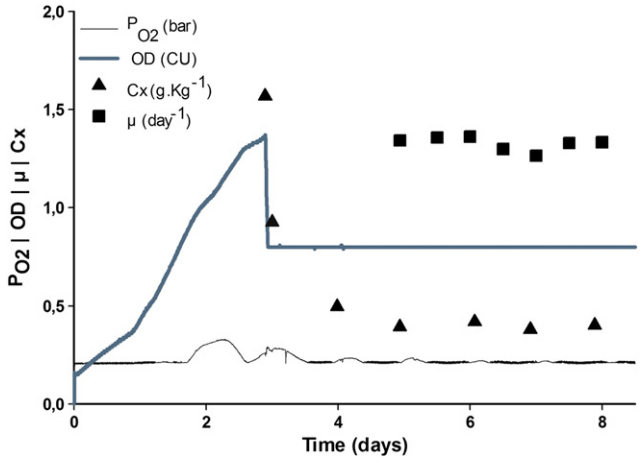

Fig. 1. Graphical representation of the partial oxygen pressure (P_{O2}), optical density (OD), dry weight concentration (C_x), pH and specific growth rate (μ) of *Neochloris oleoabundans* in time at incident light intensity of 500 μmol m^{-2} s^{-1}.