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Effect of photoacclimation on microalgae mass culture productivity

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ABSTRACT

Microalgae are capable of adapting their pigmentation to the light regime to which they are exposed. In high density microalgae cultures exposed to sunlight, the high pigment content leads to oversaturation of the photosystems resulting in increased light energy dissipation at the reactor surface, reducing the light use efficiency. In theory, therefore, pigment reduction would maximize biomass productivity. In this study, we have measured the long-term biomass productivity and short-term oxygen production rate of low-pigmented cells of *Chlorella sorokiniana* under mass culture conditions. Reduced pigmentation was obtained through the natural process of photoacclimation under high irradiance. During the time that the pigmentation was reduced, mass culture productivity, light absorption, and light use efficiency were investigated. Photoacclimation kinetics were investigated in a light shift experiment in which the increase in absorption cross section was followed in time upon a shift from high to low light intensity. Improved productivity of low-pigmented cells under mass culture conditions was not observed in any of the experiments. There is no solid explanation based on the experimental data. The most likely explanations are that thermal dissipation mechanisms were still activated and that the photoacclimation process itself consumed a substantial amount of energy at cost of growth processes. It is suggested that photoacclimation can only be exploited in the situation that microalgal cells are grown at a fixed position (e.g. in a biofilm, or multicompartment reactor) without being exposed to rapid light fluctuations.

1. Introduction

The counterproductive photoacclimation behavior of high density microalgae cultures is an inevitable bottleneck in the optimization of the photosynthetic efficiency. Microalgae have the ability to adapt their pigmentation to the light regime to which they are exposed [1]. Under mass culture conditions, microalgae increase their pigmentation, i.e., antenna size, in order to absorb as much light energy as possible. Higher pigmentation is beneficial in the darker zones of the culture but effectuates extensive photosystem (PS) oversaturation of the microalgal cells at the high light exposed side of the reactor. The excess amount of light energy is wastefully dissipated as heat and fluorescence [2]. In theory a decrease instead of an increase in pigmentation would maximize productivity. With a low pigment content, microalgae would be less prone to oversaturation and subsequently show an improved performance at the high light exposed side of the photobioreactor where, normally, most of the light energy is wasted. In our previous study [3], we demonstrated that productivity is, indeed, inversely correlated to the amount of light that is absorbed per cell and that this can be investigated by exploiting light with a spectral composition that minimizes, or maximizes, light absorption.

Photoacclimation may be exploited to improve the productivity of microalgae cultivation systems. The experiment of Grobbelaar et al. indeed demonstrated that, if microalgae are cultivated in a multicompartment reactor, and were thus allowed to acclimate to the light regime in each compartment, productivity is higher compared to a single reactor compartment with a steep light gradient going from full sunlight to complete darkness [4]. This indicates that, if algae are grown in thin compartments or at a fixed location (immobilized in a biofilm), the occurrence of photoacclimation allows microalgae to make more efficient use of the light energy [5].

From a theoretical point of view, the photoacclimation process could also be exploited to study the potential of hypothetical antenna size mutants under mass culture conditions. For example mutants have been created that have a locked-in high light phenotype [6]. In the past, several light shift-down experiments were performed in batch systems to study the dynamics of photoacclimation [7–9]. In the study of Melis [7], it was extrapolated that a two to three times higher productivity could be obtained by employing low-pigmented microalgae if the negative effects that are related to high light damage could be eliminated. However, in batch experiments, biomass productivity cannot be measured in a reliable manner as there is no balanced growth when the light regime is not constant and is dictated by biomass growth itself. Therefore, we developed two continuous experimental setups in which productivity of low-pigmented microalgae was examined

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under well controlled mass culture conditions resulting in balanced growth.

In this work, we have measured the long-term biomass productivity and short-term oxygen production rate of low-pigmented cells of *Chlorella sorokiniana* under mass culture conditions. By using two dedicated experimental setups it was prevented that the high light acclimated algae reverted back to their high pigmented state after being exposed to the darker mass culture conditions. In the first experimental setup, high light acclimated biomass was continuously pumped into a mass culture reactor. The retention time of biomass in the reactor was sufficiently short to maintain a lower pigment content than observed in a normal cultivation with biomass that is allowed to acclimate to the mass culture conditions. In the second experimental setup, cultures with high light acclimated microalgae were analyzed for their short-term oxygen production rate in a biological oxygen monitor (BOM) in which a mass culture was simulated. In both setups, the productivity of the low-pigmented cultures was measured over several days in flat panel photobioreactors operated in turbidostat mode. In an additional experiment, pigment accumulation kinetics were investigated. The increase in absorption cross section was followed in time upon a light shift from high to low light conditions. Model-based calculations served as the theoretical foundation for our hypothesis regarding decreased oversaturation of low-pigmented cells. By comparing the experimental productivity of low-pigmented cells with that of normally pigmented cells, it was investigated whether the use of high light acclimated cells with low pigmentation could indeed result in an increased light use efficiency under mass culture conditions.

2. Material and methods

2.1. Organism and medium

Chlorella sorokiniana CCAP 211/8 k [10] was obtained from the American Type Culture Collection (ATCC). The algae were cultivated in filter sterilized (pore size 0.2 μm) M8-a medium with the following composition (in g L^{-1}): urea, 1.80; KH_2PO_4, 1.48; $Na_2HPO_4 \cdot 2H_2O$, 0.52; $MgSO_4 \cdot 7H2O$, 0.40; $CaCl_2 \cdot 2H_2O$, 0.013; EDTA ferric sodium salt, 0.116; $Na_2EDTA \cdot 2H_2O$, 0.0372; H_3BO_3, $6.18 \cdot 10^{-5}$; $MnCl_2 \cdot 4H_2O$, 0.013; $ZnSO_4 \cdot 2H_2O$, $3.2 \cdot 10^{-3}$; $CuSO_4$, $1.83 \cdot 10^{-3}$. The cultures were pre-cultivated in 250 mL shake flasks containing 100 mL of medium. The cultures that were employed to inoculate the short retention time reactor were maintained at pH 6.7, 25 °C and were exposed to a continuous light intensity of 120–150 μmol photons m^{-2} s^{-1}. One or two days prior to inoculation, the cultures were placed in another incubator at 400–500 μmol photons m^{-2} s^{-1} and at 37 °C in order to pre-acclimate the algae to the reactor conditions. The cultures used to inoculate the reactors for the biological oxygen monitor experiments were maintained at pH 7 at 25 °C under a 16/8 h day/night cycle and a light intensity of 30–40 μmol photons m^{-2} s^{-1}.

2.2. Basic photobioreactor setup and operation

Microalgae were continuously cultivated in flat-panel airlift photobioreactors (Algaemist, Technical Development Studio, Wageningen University, the Netherlands) with a working volume of 0.4 L, an optical depth of 14 mm, and an illuminated area of 0.028 m^2 (See Fig. 1A for a schematic overview). The reactors were equipped with a black cover on the backside to prevent exposure to ambient light. Warm white light was provided by 6 Bridgelux LED lamps (BXRA W1200, Bridgelux, Livermore, USA) which are integrated in the Algaemist system. In Fig. 1B, the emission spectra of the used light sources are shown. Reactor temperature was maintained at 37 °C, and the pH was maintained at 6.7 (±0.1) by means of on-demand CO_2 supply. The reactors were operated in turbidostat mode to ensure a constant light regime; A light sensor was used to record the light passing through the reactor and if the outgoing light intensity dropped below the required setpoint (see below), the culture was automatically diluted with fresh medium employing a peristaltic pump. Further details of the photobioreactor setup and its operation are provided in [11], with the exception that the gas stream of di-nitrogen was, at all times, 500 mL min^{-1}. The incident light intensity of the reactors was measured with a LI-COR 190-SA 2π PAR (400–700 nm) quantum sensor (LICOR, USA) at 28 points evenly distributed over the light-exposed surface of the front glass panel of the culture chamber. A dummy reactor, with only a front glass panel installed, was used for the purpose of light measurements.

2.3. Setup and operation of the short hydraulic retention time reactor

The setup consists of three photobioreactors that were operated in turbidostat mode (see Fig. 2). In reactors 1 and 2 (high light reactors, illuminated from two sides) high light acclimated algae with reduced pigmentation were grown. As can be seen in Fig. 2, the overflow of reactors 1 and 2 was connected to a third reactor (reactor 3). This means that if reactor 1 and 2 are diluted, the algae suspension of these reactors will be pumped into reactor 3. In the high light reactors 1 and 2, the

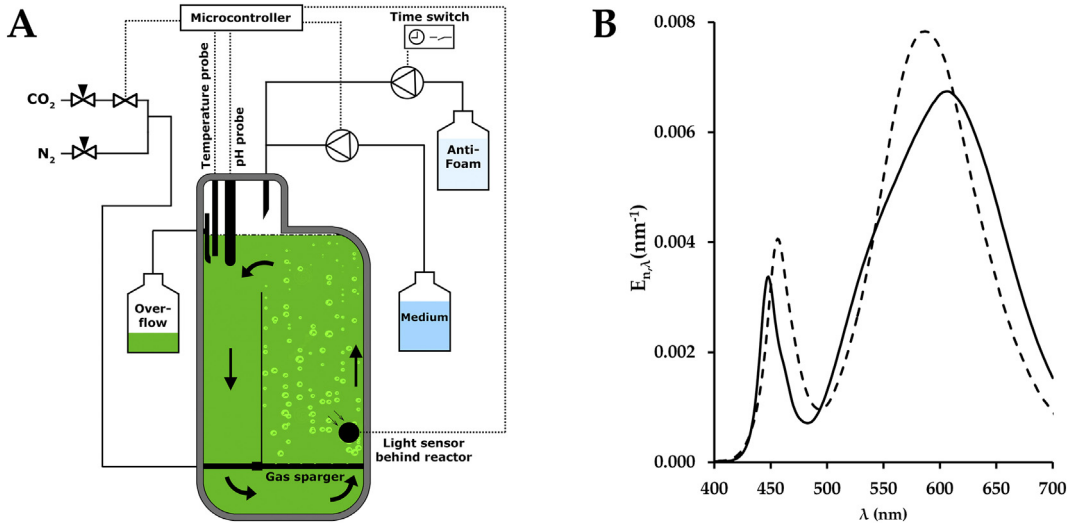

Fig. 1. (A) Schematic overview of the experimental setup. (B) Solid line: emission spectrum of the warm-white LEDs (BXRA W1200, Bridgelux, Livermore, USA) of the Algaemist. Dashed line: emission spectrum of the additional LED light source (warm white 45mil chip, Bridgelux, Livermore, CA). The parameter $E_{n,\lambda}$ represents the relative fraction of PAR photons present within a 1 nm wavelength interval.