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Keeping the light energy constant — Cultivation of *Chlorella sorokiniana* at different specific light availabilities and different photoperiods

Claudia Holdmann[a], Ulrike Schmid-Staiger[b,*], Helena Hornstein[a], Thomas Hirth[c]

[a] University of Stuttgart, Institute of Interfacial Process Engineering and Plasma Technology, Nobelstrasse 12, 70569 Stuttgart, Germany
[b] Fraunhofer Institute for Interfacial Engineering and Biotechnology, Nobelstrasse 12, 70569 Stuttgart, Germany
[c] Karlsruhe Institute of Technology, Kaiserstr. 12, 76131 Karlsruhe, Germany

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ABSTRACT

Microalgae cultivation is strongly dependent on light availability. Sunlight is the natural energy source for algae cultivation processes, but its fluctuation during the day and the natural day-night-rhythm mean an increasing challenge for an efficient algae production. Additionally, different seasons lead to unequal photoperiods and their influence on the productivity has not been determined in detail yet.

Therefore, we investigated the growth of *Chlorella sorokiniana* at different light intensities and illumination cycles of 24 h/0 h (light/dark), 16/8 h and 12/12 h. The light integral, more exactly the amount of photons per gram biomass and day (specific light availability) was kept constant during 24 h to ensure the comparability of the results of the different photoperiods. During the cultivation in repeated fed-batch mode, the productivity and biomass yield on light of *Chlorella sorokiniana* were determined to characterize the growth.

During the night phase a biomass loss occurred, which was independent of the night temperature and the specific light availability on the day before, but increased with higher biomass concentration. A higher biomass yield on light during the shorter photoperiods led to an improved gross productivity compared to the continuous illumination, especially at a biomass concentration above 4 g L^{-1}. This compensated for the night loss; therefore, the net productivity (during one day) was independent of the photoperiod. In order to determine an optimal process window biomass yield on light and productivity have to be considered. They are diametric with respect to the specific light availability and furthermore dependent on the biomass concentration. The optimal combination of biomass yield on light and productivity could be achieved with a biomass concentration below 4 g L^{-1} during 24/0 illumination and with a biomass concentration below 7 g L^{-1} during shorter photoperiods; the specific light availability should be as high as possible.

1. Introduction

Microalgae are promising sources for renewable resources in the future, as they have a low water demand, a high growth rate and do not need arable land to grow [1]. Although research has been going on for many decades, the scale-up and the development of reliable processes are the biggest hurdles for an efficient microalgae production [2]. Because of that, there are mostly high value products on the market right now: whole cells for human or animal nutrition or extracts, e.g. for cosmetic products or food and feed additives [3]. Nowadays hardly any low value products like biofuels are produced, as it is difficult to remain economically viable during the scale-up from laboratory to large scale [4]. Light intensity is widely considered as the most important parameter for algae cultivation as microalgae are light limited during most cultivation processes [5]. Indoor reactors are usually illuminated with artificial lighting like LEDs, which results in a continuous supply of light energy. In contrast to that, outdoor algae cultures are exposed to fluctuating light conditions during the daytime, but also to the natural day-night-rhythm with changing photoperiods. These parameters can influence the productivity and make it difficult to achieve a reliable process. The photoperiod is the duration of the light phase, thus it defines the time of the day during which algae can photosynthesize. In mid Europe, it is around 12 h in spring/fall, while it is 16 h in midsummer. During outdoor cultivations, the amount of photons is usually considered as the key parameter and the photoperiod is neglected [6–9]. Most outdoor cultivations have a period of several days, thus, the photoperiod does not change a lot during one run of cultivation [10]. During long time cultivations (several months), this parameter might influence the growth of an algae culture. There are several publications about algae cultivations with different illumination cycles, but they all

* Corresponding author.
E-mail address: ulrike.schmid-staiger@igb.fraunhofer.de (U. Schmid-Staiger).

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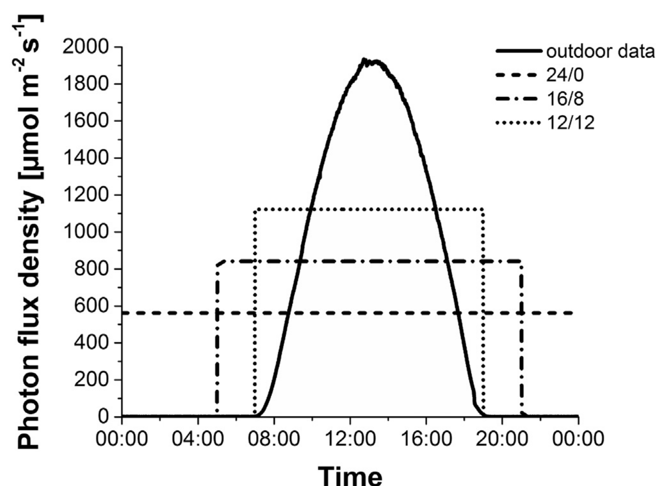

Fig. 1. Photon flux density during one day. Solid line: Data from an outdoor cultivation plant located in Stuttgart, Germany (N48°44′24″ E009°05′56″), recorded at 10/1/2014; sum of two sensors facing north and south. Same light integral with photoperiods of: 1) 24 h illumination (dashed line); 2) 16 h illumination, 8 h darkness (dashed/dotted line); 3) 12 h illumination, 12 h darkness (dotted line).

have in common that the amount of light per day was not constant [11–13]. That means that the algae do not have the same amount of energy available for their growth, which makes it hard to compare the growth between different photoperiods. In this study, the specific light availability per day (quotient of amount of photons on the reactor surface and amount of biomass in the reactor) is kept constant to ensure the comparability of the results. It has been shown before that this parameter is an important factor for the growth of microalgae [14]. An example of the photon flux density during outdoor cultivation and photoperiods of 24/0, 16/8, 12/12 is shown in Fig. 1. As a key parameter, the same amount of photons corresponding to a constant light integral was used.

The net productivity consists of the gross productivity during the light phase and the night loss (see Fig. 2). The night biomass loss, which is inevitable during a dark phase, is often not considered separately, as only the net productivity is measured. The biomass night loss can be dependent on different experimental conditions: especially on the light intensity during the day [15] and the night temperature, for which different results concerning the dependency exist [16,17].

The effects of both phases (light and dark) must be evaluated separately and then collated, as they result from different metabolic effects. Therefore we analyzed the gross and the net productivity (only light phase and during 24 h) as well as the night loss and the biomass yield on light energy. The net productivity, which is measured after 24 h, is the key parameter for an economic analysis and the biomass yield on light is another important parameter for process optimization [18–20]. One problem of an efficient algae cultivation process is the diametric behavior of the productivity and the biomass yield on light concerning the light intensity: the biomass yield on light improves when the light intensity is lower [21,22], but the productivity increases with an increasing light intensity as long as there is no photoinhibition. Thus, it is important to find the best combination of biomass yield on light and productivity. This is achieved by using a scoring system, which takes into account both, the net productivity and the light yield.

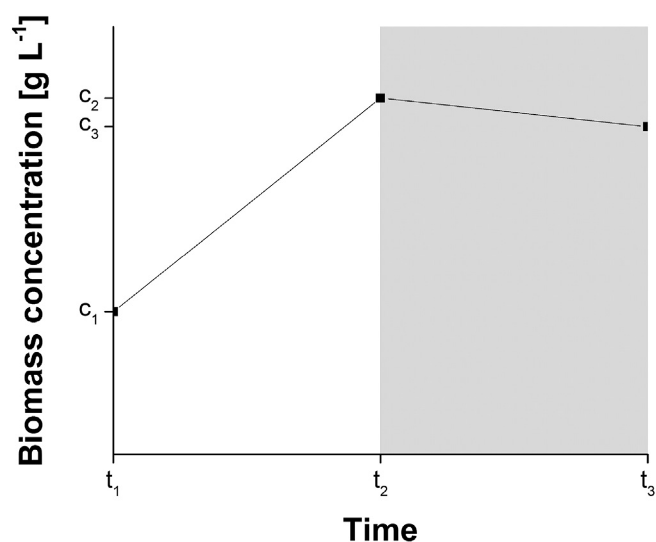

Fig. 2. Schematic representation of the biomass concentration during one day with light and dark phase (grey).

2. Material and methods

2.1. Strain, preculture and medium

The strain *Chlorella sorokiniana* SAG 211-8k was obtained from SAG Culture Collection of Algae (Sammlung von Algenkulturen der Universität Göttingen) from Germany. Stock cultures were kept on modified DSN Medium [23] in Erlenmeyer flasks (50–500 mL). The exact composition of the medium is: 3.5 g L^{-1} sea salt; 1.38 g L^{-1} $MgSO_4{\cdot}H_2O$; 0.56 g L^{-1} $CaCl_2$; 3.2 mg L^{-1} Fe(III) citrate and 40 mL L^{-1} micronutrient solution (20 mg L^{-1} $MnCl_2{\cdot}4H_2O$; 5 mg L^{-1} $ZnSO_4{\cdot}7H_2O$; 5 mg L^{-1} $CoSO_4{\cdot}7H_2O$; 5 mg L^{-1}; $Na_2MoO_4{\cdot}2H_2O$; 0.5 mg L^{-1} $CuSO_4{\cdot}5H_2O$).

After reaching an optical density of about 2, two 500 mL Erlenmeyer flasks (filling volume of 200 mL) were used to inoculate a 5 L glass bottle. In this bottle, the culture was aerated with a sterile air/CO_2 mixture and mixed with a magnetic stirrer. Every 3–4 days 1 L medium was added until a final volume of 5 L was reached. 4 of these 5 L were used to inoculate a lab scale 6 L flat-panel airlift reactor.

2.2. Reactor design

The flat-panel airlift (FPA) reactor was developed at the Fraunhofer IGB and is refined and commercialized by Subitec GmbH. The reactor works on the principle of an airlift reactor and it is mixed solely by aeration with sterile air. The air/CO_2 mixture for aeration is provided by a perforated silicone membrane at the bottom of the reactor. The reactor consists of two PVC half shells equipped with static mixers. These static mixers lead to a circular current in every chamber of the reactor and improve the light distribution on the algal cells. In this study, a 6 L reactor with a culture depth of 3 cm and an illuminated surface of 0.21 m^2 was used. It is well described in previous papers [24–26] especially by Bergmann and Trösch [27] who showed that the static mixers allow an improved productivity compared to other flat panel reactors. All reactors were equipped with silicone tubes and sterile couplings with shut-off function (CPC type HFC35, Infiltec GmbH, Speyer am Rhein, Germany) for medium supply and harvest. These tubes were autoclaved; the reactors were decontaminated with 3% H_2O_2 overnight prior to the cultivation start. Immediately before the cultivation, the reactors were rinsed twice with sterile water to remove remaining H_2O_2.

2.3. Experimental set-up

The reactor was covered with a cloth to avoid scattered light on its surface and illuminated with LEDs (Nichia NSSL157AT-H3) at a photon flux density between 100 and 1700 µmol m^{-2} s^{-1} (see experimental design). A water bath was used for temperature control (day: set point 30 °C; night: set point 10, 20 or 30 °C). Due to the time needed for cooling the reactor the night temperature was reached after about 2 h for 20 °C and 5 h for 10 °C. The heating back to day temperature took

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