Algal Research 20 (2016) 153–163

Contents lists available at ScienceDirect

Algal Research

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

Model-based optimization of microalgae areal productivity in flat-plate gas-lift photobioreactors

Christina Evi Pfaffinger [a], Dennis Schöne [a], Sascha Trunz [a], Hannes Löwe [b], Dirk Weuster-Botz [a,*]

[a] Institute of Biochemical Engineering, Technical University of Munich, Boltzmannstr. 15, 85748 Garching, Germany
[b] Systems Biotechnology, Technical University of Munich, Boltzmannstr. 15, 85748 Garching, Germany

ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form 8 September 2016
Accepted 1 October 2016
Available online xxxx

Keywords:
Growth kinetics
Nannochloropsis salina
Nannochloropsis gaditana
Flat-plate photobioreactor
Optimized areal productivity
Mean integral photon flux density

ABSTRACT

The productivity of microalgae used for the production of biofuels is primarily influenced by the availability of exploitable light. To overcome the effects of limitation and photoinhibition, an optimized radiation profile was implemented for the cultivation of *Nannochloropsis salina* 40.85 and *Nannochloropsis gaditana* 2.99 in LED-illuminated flat-plate gas-lift photobioreactors with a depth of 2 cm. For this purpose the light-dependent growth kinetics were characterized for both strains with variable incident photon flux densities up to 2750 μmol m^{-2} s^{-1}. The mean integral photon flux density $\bar{I}^{**}$ was established to describe the light every single microalgal cell is exposed to on average during exponential growth. By implementing a phototrophic growth model based on the light attenuation described by Lambert and Beer, the kinetic growth parameters have been determined including the effects of photoinhibition. Based on the defined optimal range of $\bar{I}^{**}$ for both *Nannochloropsis* strains optimum radiation profiles were developed: Up to an identified upper limit of light, the incident irradiation is increased with rising biomass concentration while the mean integral photon flux density is kept constant at the chosen optimum. Thus, the biomass areal productivity could be increased significantly compared to constant irradiation by 113% to 10.4 g m^{-2} d^{-1} with *Nannochloropsis salina* and by 107% to 14.8 g m^{-2} d^{-1} with *Nannochloropsis gaditana*, respectively. The areal lipid productivity was likewise distinctly improved by the implementation of the radiation profiles up to 11.0 g m^{-2} d^{-1} (+59%) with *Nannochloropsis salina* and up to 6.5 g m^{-2} d^{-1} (+83%) with *Nannochloropsis gaditana*. The concept of optimized radiation profiles may be easily transferred to other microalgal strains as well as other reactor types due to the system-independence of the mean integral photon flux density.

1. Introduction

The world's industry is incapable of providing affordable, sustainable, and reliable energy for the world's population. Moreover, fossil fuels will be running short and oil prices are likely to increase because of the rising demand for energy [1]. One promising alternative for the production of renewable fuels is the utilization of microalgae, which harness sunlight and carbon dioxide for the production of energy-rich lipids. Carbon dioxide can be fixed from different sources including CO_2 from the atmosphere, exhaust gases and soluble carbonates [2]. Beside CO_2 and light, microalgae require nutrients like nitrogen and phosphorus for their growth, which can for example be received from wastewater [3]. Remineralization of microalgae biomass by anaerobic digestion recycles these nutrients and additionally provides energy by the production of methane [4–6]. Microalgal lipids consist mainly of esterified glycerol and saturated or unsaturated fatty acids with a chain length of primarily C_{16} and C_{18} [7,8]. Under unfavorable conditions like nitrogen deprivation, microalgae are able to increase their total lipid content two- to three-fold up to 80% of cell dry weight [8–10]. Compared to other oil crops like soybean, corn or oil palm, microalgae require only a mere fraction of cropping area to produce the same amount of oil [10,11], can be cultivated on non-arable areas and will consequently not compete with food production [12]. Furthermore, microalgae cultivation is less dependent on seasonal weather variations and does not require fresh water as a medium source [12–14]. Microalgae can be cultivated either in open or enclosed photobioreactors depending on the utilized strain: enclosed systems are advantageous for algae which are prone to contaminations while open photobioreactors are suitable for robust microalgae, which can resist high pH or salinity [15,16].

Assuming that all growth conditions like pH, which can be controlled by the CO_2 influx, as well as macro- and micronutrients are optimized, temperature is constant, and the culture is adequately

Abbreviations: FAME, fatty acid methyl ester; PBR, photobioreactor; PFD, photon flux density.

* Corresponding author.
E-mail addresses: c.pfaffinger@tum.de (C.E. Pfaffinger), dennis.schoene@googlemail.com (D. Schöne), saschatrunz@yahoo.de (S. Trunz), h.loewe@tum.de (H. Löwe), d.weuster-botz@lrz.tum.de (D. Weuster-Botz).

http://dx.doi.org/10.1016/j.algal.2016.10.002
2211-9264/

homogenized, light is the only growth-limiting factor in enclosed photobioreactors [17,18]: Microalgae productivity depends on the growth rate, which is controlled by the light-availability at every point in the photobioreactor [19]. In dense microalgal cultures, rapid light attenuation and self-shading effects lead to a heterogeneous radiation profile in the photobioreactor and consequently to locally varying kinetics [20–23]: While negligible mutual shading causes exponential microalgal growth, completely absorbed light leads to linearly growing cells [24]. Furthermore, very low photon flux densities cause photolimitation and thus reduce growth rates. Rising photon flux densities results in increasing growth rates until light saturation and therefore the maximum growth rate is reached. A further increase in the photon flux density (PFD) can reduce microalgal growth because of the phenomenon of photoinhibition [13,25–27]. Therefore, the crucial point in microalgae cultivation is to identify the optimal photon flux density to increase productivity.

For the efficient production of biofuels *Nannochloropsis* sp. are promising organisms since these lipid-rich strains are extremely robust and tolerate high temperatures as well as high irradiances [28–30]. Additionally, *Nannochloropsis* sp. is suitable for mass cultivation because of the reduced risk of contamination due to the possibility to apply high salinity [30]. Several studies [31–34] estimated the optimal incident PFD for different *Nannochloropsis* species using various methods: Kandilian et al. [31] depicted a model-free optimal search for *Nannochloropsis oculata* in flat-plate photobioreactors by fitting three different incident photon flux densities identifying 236 μmol m^{-2} s^{-1} as optimum average PFD. Sforza et al. [32] carried out seven cultivations of *Nannochloropsis salina* (*N. salina*) in flat-plate photobioreactors under different irradiation up to 1000 μmol m^{-2} s^{-1} experimentally determining 150 μmol m^{-2} s^{-1} as optimum PFD. Furthermore, Huesemann et al. [33] and van Wagenen et al. [34] performed 9 respectively 6 cultivations of *N. salina* in Roux bottles with different incident PFD up to 850 μmol m^{-2} s^{-1}. The optimum incoming PFD was given with 250 μmol m^{-2} s^{-1} using a biomass growth model [33].

However, identifying the optimum incident PFD is only the first step towards the increase of microalgal productivity. The attenuation of incoming light caused by mutual shading and absorption effects of the growing cells will limit productivity because of photolimitation. One possibility to overcome this problem is the establishment of a radiation profile, which increases the incoming PFD with rising biomass concentration to delay photolimitation, maximize productivity, and reduce the overall process time. For this purpose, a deeper understanding of the light-dependent growth kinetics of the applied microalgae strain is essential.

In this work, the growth kinetics depending on photon flux density of the photoautotrophic organisms *Nannochloropsis salina* and *Nannochloropsis gaditana* will be characterized to identify the optimal PFD in flat-plate gas-lift photobioreactors. Based on the identified growth kinetics a radiation profile will be implemented to increase biomass and lipid areal productivity.

2. Materials and methods

2.1. Strains and seed culture preparation

The unicellular photoautotrophic microalgae *Nannochloropsis gaditana* 2.99 and *Nannochloropsis salina* 40.85 were provided by the Culture Collection of Algae (SAG) at the University of Goettingen. For strain maintenance shaking flasks were used with modified artificial seawater (ASW) medium (27.0 g L^{-1} NaCl, 6.6 g L^{-1} $MgSO_4$ 7 H_2O, 1.5 g L^{-1} $CaCl_2$ 2 H_2O, 5.0 g L^{-1} KNO_3, 70 mg L^{-1} KH_2PO_4, 14 mg L^{-1} $FeCl_3$ 6 H_2O, 19 mg L^{-1} Na_2EDTA, 1.0 mL L^{-1} trace element solution) [35] under laboratory light and ambient temperature conditions with daily manual shaking. Seed cultures were prepared at 25 °C under continuous irradiation with 83 ± 17 μmol m^{-2} s^{-1} in 250 mL bubble columns with ASW medium in a modified Profors incubator system (Infors, Bottmingen, Switzerland) [36]. The seed culture was mixed by applying a gas flow rate of 12 L h^{-1} air to each reactor, adding CO_2 in excess (5%, v/v). For the inoculation of the PBR, seed cultures in the late exponential respectively early stationary phase were used aiming an initial cell dry weight of approximately 0.05 g L^{-1} in the PBR.

2.2. Cultivation in flat-plate gas-lift photobioreactors

For all bioprocesses steam sterilized 1.8 L flat-plate gas-lift photobioreactors (PBR) (Labfors Lux 5, Infors HT, Bottmingen, Switzerland) were used. Continuous irradiation is performed by an LED panel with 260 high performance LEDs with a characteristic spectrum in the visible range of light (400–800 nm) reaching a maximum irradiance of 4200 μmol m^{-2} s^{-1} on the surface area of the PBR (0.09 m^2). The light's path length through the cultivation chamber of the PBR is 2 cm and temperature control (25 °C) is conducted by means of a temperature chamber attached on the light-averted side of the PBR. Furthermore, measurement of pH and dissolved oxygen is performed by optical sensors (Easyferm Plus ARC and VisiFerm DO ECS 120 H0, Hamilton Germany GmbH, Hoechst, Germany). A constant pH was maintained by the controlled addition (0–5%) of CO_2 to the airflow. The gas flow rate was kept constant at 120 L h^{-1} during the overall experiment duration and ensured adequate mixing.

ASW medium with 5 g L^{-1} KNO_3 was used for batch cultivations in the PBRs. To reduce experimental time, batch cultivations for the determination of the light-dependent growth kinetics were terminated when light became the only growth limiting state variable and consequently growth became linear. In contrast, investigations of the optimized radiation profiles consisted of two phases with the biomass production (I) and the lipid production phase (II). The second phase was induced by diluting the culture to approximately 0.8 g L^{-1} cell dry weight (CDW) with nitrogen-free ASW medium. Particular attention was paid to the maintenance of a residual KNO_3 concentration of 0.4 g L^{-1} to prevent cell lysis due to abrupt nitrogen limitation [37,38] (own observations). Loss of culture volume due to sampling was compensated by the addition of sterile ASW-medium (nitrogen-free in the lipid production phase) eliminating possible nutrient limitations (except for nitrogen limitation).

2.3. Determination of cell dry weight, growth rate and cell count

The cell dry weight was determined gravimetrically in triplicate: glass microfiber filters (GF/C, Whatman, GE Healthcare) retaining particles bigger than 1.2 μm were dried for 24 h, weighed, loaded with a defined volume of microalgae culture, dried for 24 h, and weighed again. CDW was estimated by the difference in weight of unloaded and loaded filter divided by the volume applied. The measured triplicates were used to determine the average CDW and the standard deviation.

The specific growth rates with standard deviations were determined by exponential regression of the measured CDW during the exponential growth phase as a function of process time.

For the determination of the cell count, a hemocytometer (Neubauer Improved, LaborOptik Ltd., Lancing, Great Britain) with a height of 0.1 mm and a corner square area of 0,025 mm^2 was used. For each sample, six corner squares have been counted. With the obtained total cell count and the volume of the counted corner squares (0.015 mm^3) the number of cells per mL was calculated.

2.4. Determination of nitrate concentration

To determine the nitrate concentration in the microalgal cultures, 1 mL of the sample was centrifuged at 13,000 rpm for 5 min. The resulting supernatant was analyzed by using a colorimetric enzyme assay with photometric endpoint determination of nitrite as well as nitrate (Nitrite/Nitrate colorimetric method, Roche Diagnostics GmbH, Penzberg, Germany).