Algal Research 18 (2016) 198–208

Contents lists available at ScienceDirect

Algal Research

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

Turbidostat operation of outdoor pilot-scale photobioreactors

Jeroen H. de Vree [a,*], Rouke Bosma [a], Rick Wieggers [a], Snezana Gegic [a], Marcel Janssen [a], Maria J. Barbosa [a], René H. Wijffels [a,b]

[a] Bioprocess Engineering, AlgaePARC, Wageningen University, P.O. Boxs 16, 6700 AA Wageningen, the Netherlands
[b] Biosciences and Aquaculture, Nord University, P.O. Box N-8049, Bodø, Norway

ARTICLE INFO

Article history:
Received 16 April 2016
Received in revised form 21 May 2016
Accepted 4 June 2016
Available online xxxx

Keywords:
Microalgae
Outdoor pilot-scale photobioreactors
Areal productivity
Photosynthetic efficiency
Nannochloropsis sp.
Biomass concentration

ABSTRACT

The effect of biomass concentration on areal productivity and photosynthetic efficiency of *Nannochloropsis* sp. CCAP211/78 was studied in three outdoor pilot-scale photobioreactors: an open raceway pond (OPR), a horizontal tubular (HT) photobioreactor and a vertically stacked horizontal tubular (VT) photobioreactor. The reactors were operated continuously as turbidostat at different biomass concentrations. For all systems highest areal productivities were obtained on days with a high light intensity, while the highest photosynthetic efficiencies were obtained on days with a low light intensity. Ground areal biomass concentration exceeding 51 g m^{-2} had a negative effect on the areal productivity and photosynthetic efficiency. No significant effect of biomass concentration on the productivity was found for the HT at ground areal biomass concentration lower than 51 g m^{-2}. Also for the VT, no significant effect of biomass concentration was found with the exception of the highest biomass concentration of 2.0 g L^{-1} (68 g m^{-2}) resulting in decreased productivity. For the open raceway pond the highest biomass concentration (0.5 g L^{-1} or 94 g m^{-2}) resulted in significantly lower areal productivity, compared to the lower biomass concentration (0.25 g L 47 g m^{-2}). Highest areal productivities were obtained for OPR and VT, most likely due to more efficient light interception. In this study we observed that night biomass loss was coupled to net growth. At lower biomass concentrations and concomitant higher growth rates the specific biomass loss rate was higher. Microalgal specific light absorption coefficient was correlated to biomass concentration; higher biomass concentrations resulted in higher specific absorption coefficients, resulting in a steeper light gradient in the microalgal cultures.

Symbols and abbreviations

Symbol	Description	Units
$PE_{sunlight}$	Efficiency of sunlight conversion into biomass	%
$P_{x,ground}$	Ground areal biomass productivity	g m^{-2} d^{-1}
$F_{harvest}$	Harvested volume	L/24 h
C_x	Biomass concentration	g L^{-1}
V_r	Volume of photobioreactor	L
A_{ground}	Ground area occupied by photobioreactor	m^2
$P_{x,vol}$	Volumetric biomass productivity	g L^{-1} d^{-1}
$I_{ground,daily}$	Daily ground areal photon flux density	mol m^{-2} d^{-1}
I_{ground}	Ground areal photon flux density	μmol m^{-2} s^{-1}
ΔH_c^o:	Standard enthalpy of combustion	kJ g^{-1}
E_{PAR}	Conversion factor PAR photons to joule	J mol^{-1}-
D	Dilution rate	d^{-1}
OP	Optical path	m
v_{gs}	Superficial gas velocity	m s^{-1}
Abbreviation	**Description**	
PBR	Photobioreactor	
ORP	Open raceway pond	
HT	Horizontal tubular	
VT	Vertically stacked horizontal tubular	
FP	Flat panel	

1. Introduction

High biomass production costs prevent the current implementation of microalgae in bulk applications; production costs should decrease below 1 €/kg DW. The high production costs are a result of low photosynthetic efficiencies and high energy costs for operation of cultivation systems and harvesting [1]. Currently, efforts are being made to achieve higher photosynthetic efficiencies under outdoor conditions.

Photosynthetic efficiencies obtained under outdoor conditions are still lower than the values obtained under laboratory conditions [2].

* Corresponding author at: Wageningen University, Bioprocess Engineering, AlgaePARC, P.O. Boxs 16, 6700 AA Wageningen, the Netherlands.
E-mail address: jeroen.devree@wur.nl (J.H. de Vree).
URL: http://www.AlgaePARC.com (R.H. Wijffels).

http://dx.doi.org/10.1016/j.algal.2016.06.006
2211-9264/

Under outdoor conditions lower photosynthetic efficiencies are obtained because of photo saturation [3] and because essential parameters for growth, such as temperature, cannot be controlled to the same extend as under laboratory conditions. Photo saturation could be reduced by reactor design [4,5] but is practically complicated because light intensity and light direction vary over the day and over the seasons. The light regime within microalgal cultures can be manipulated by means of controlling the biomass concentration and culture mixing. Too high or too low biomass concentrations can result in suboptimal operation [3]. Too low biomass concentration will result in an incomplete light absorption and possibly photo inhibition, and too high biomass concentrations will result in dark zones where biomass is lost due to cellular maintenance (i.e. endogenous respiration).

In most studies done at outdoor conditions, a fixed daily dilution rate is used as operational strategy [6–9]. However, the application of a fixed dilution rate results in varying biomass concentrations during the day and year, because of day/night cycles and seasonal variations. If the dilution rate is too high the biomass concentration is low and photo inhibition could occur. If the dilution rate is too low the biomass concentration can become so high that the dark zone in the reactor is so large that a significant amount of biomass production is lost due to endogenous respiration [2,10,11]. Operation of photobioreactors with a fixed biomass concentration (turbidostat mode) can prevent culture washouts as dilution of the culture only takes place when growth occurs. Operation at the optimal biomass concentration should theoretically result in the highest productivity as light interception is maximized, photo inhibition is minimized, and at the same time excessive dark zones are prevented [1,6,12].

In laboratory experiments the use of a fixed biomass concentration or a constant light intensity (a fixed amount of light per cell) was shown to positively influence productivity of *Neochloris oleoabundans* [13]. Cuaresma et al., showed that a fixed light absorption by the culture could result in high biomass yields on light [14]. The effect of biomass concentrations on the productivity of different outdoor photobioreactors was shown in model simulations by different authors [15–17]. However, applying a fixed biomass concentration under outdoor production conditions has only been investigated by a limited number of authors [18,19]. Michels et al., investigated the effect of biomass concentration on the productivity of *Tetraselmis suecica*; a biomass concentration of 0.7 g L^{-1} resulted in the highest productivity and biomass yield. Grima et al., investigated the effect of biomass concentration on productivity in tubular photobioreactors with different optical paths for *Tetraselmis suecica* and showed that smaller optical path systems resulted in higher volumetric productivity while higher areal productivities were found for longer optical path systems.

In order to maximize productivity and photosynthetic efficiency in outdoor photobioreactors, the optimal biomass concentration needs to be determined for each type of photobioreactor. Therefore, we compared the performance of three different pilot-scale outdoor photobioreactors under identical climatological conditions. These reactors were operated in turbidostat mode with different biomass concentrations of *Nannochloropsis* sp. The photobioreactors investigated in this study were an open raceway pond (OPR), a horizontal tubular photobioreactor (HT), and a vertically stacked horizontal tubular photobioreactor (VT).

2. Materials and methods

2.1. Inoculum production

Inoculum production was done as described by [9]. *Nannochloropsis* sp. CCAP211/78 was cultivated in 250 mL Erlenmeyer flasks, followed by cultivation in a flat panel photobioreactor and a horizontal tubular photobioreactor located in a greenhouse. The flat panel photobioreactor was a 25 L flat panel photobioreactor having an optical path of 40 mm, pH was controlled at 7.5 ± 0.5 by blending CO_2 in the airflow, and temperature was controlled at 25 °C. The indoor horizontal tubular photobioreactor (280 L) was operated at a liquid velocity in the tubes of 0.3 m s^{-1}, a temperature of 25 °C, and pH was controlled at 7.5 by on demand addition of carbon dioxide.

Nannochloropsis sp. CCAP 211/78 was cultivated in seawater (Eastern Scheldt, the Netherlands) enriched with a stock solution resulting in the following concentrations (in mM); $NaNO_3$, 25; KH_2PO_4, 1.7; Na_2EDTA, 0.56; $Fe_2SO_4 \cdot 7H_2O$, 0.11; $MnCl_2 \cdot 2H_2O$, 0.01; $ZnSO_4 \cdot 7H_2O$, $2.3 \cdot 10^{-3}$; $Co(NO_3)_2 \cdot 6H_2O$, $0.24 \cdot 10^{-3}$; $CuSO_4 \cdot 5H_2O$, $0.1 \cdot 10^{-3}$; $Na_2MoO_4 \cdot 2H_2O$, $1.1 \cdot 10^{-3}$. For pre-cultures (100 mL culture, 250 mL Erlenmeyer flasks) HEPES (20 mM) and Na_2EDTA (5 mM) were added to the seawater, and pH was adjusted to 7.5 followed by heat sterilization (121 °C, 20 min). The nutrient stock solution was added to the heat sterilized seawater through a sterile filter (0.2 μm).

2.2. Outdoor pilot-scale photobioreactors

A short description of each photobioreactor (Fig. 1) is given in this section; a more detailed description of the used outdoor systems is given by Bosma et al. [20]. All outdoor photobioreactors were operated at a pH of 7.5 by on-demand CO_2 addition and culture temperatures were maintained between 20–30 °C. Volumes of the systems were: HT; 560 L, VT; 1060 L and ORP; 4713 L. Optical path length in the tubular systems was 0.046 m and depth of the open raceway pond was 0.2 m. Each system covers a ground area of ± 25m^2. High dissolved oxygen concentrations in the tubular photobioreactors by increasing the airflow in the bubble column, when concentrations of 300% were reached. In the open raceway pond dissolved oxygen concentrations reached a maximum of 160%.

2.3. Turbidostat operation

All cultivation systems were operated in turbidostat mode to study the effect of biomass concentration on the productivity. During turbidostat operation harvesting starts automatically when the biomass concentration set point is reached. Biomass concentration (turbidity) was measured by a single channel NIR absorption probe (AS56-N, Optek, Elscolab, the Netherlands) and harvesting via a pump was initiated via a programmable logic controller (PLC) and supervisory control and data management system (SCADA). Harvesting was done at a flow rate of 12.5 L min^{-1}, water addition took place at a flow rate of 10 L min^{-1}. The stability of turbidostat operation was visualized in supplementary data a previous publication on the construction of the pilot plant where experiments were executed [20]. The biomass concentrations studied under turbidostat operation are given in Table 1. Operational setpoints for turbidostat operation are given in the supplementary data A. The corresponding biomass densities when expressed in gram per m^2 of occupied ground area are included in Table 1. The research started with biomass concentrations comparable to the biomass concentrations encountered during chemostat operation in a previous study [9]: ORP; 0.5 g L^{-1}, HT; 1.5 g L^{-1} and VT; 1.0 g L^{-1}. In a next step biomass concentration was set to lower and higher values in order to determine the effect on photobioreactor productivity.

Natural seawater was added on demand when a lower liquid level in the system was detected as a result of harvesting, or evaporation (in the case of the ORP). Simultaneously, the stock solution used to enrich the natural seawater was automatically added to the culture flow proportional to the addition of seawater. An increase in culture depth as a result of precipitation, in the ORP pond was prevented by automated harvesting.