Algal Research 19 (2016) 69–76

Contents lists available at ScienceDirect

Algal Research

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

Novel micro-photobioreactor design and monitoring method for assessing microalgae response to light intensity

Giorgio Perin [a,1], Elisa Cimetta [b,1], Federico Monetti [b], Tomas Morosinotto [a], Fabrizio Bezzo [b,c,*]

[a] PAR-Lab (Padua Algae Research Laboratory), Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy
[b] CAPE-Lab (Computer-Aided Process Engineering Laboratory) and PAR-Lab (Padua Algae Research Laboratory), Department of Industrial Engineering, University of Padova, via Marzolo 9, 35131 Padova, Italy
[c] Interdepartmental Centre "Giorgio Levi Cases" for Energy Economics and Technology, University of Padova, via Marzolo 9, 35131 Padova, Italy

ARTICLE INFO

Article history:
Received 21 April 2016
Received in revised form 10 June 2016
Accepted 21 July 2016
Available online xxxx

Keywords:
Nannochloropsis
Microscale
Lab-on-a-chip
Photosynthesis
Light response
Microalgae growth

ABSTRACT

Microalgae represent a promising feedstock for the sustainable production of biofuels and many other bio-commodities. However, the optimization of their productivity by assessing the impact that several environmental factors have on microalgae growth still requires intense investigation, as well as numerous and time consuming experiments. In this scenario, microscale technologies are emerging as a valuable tool to improve data production and accordingly to speed up the optimization process, while maintaining a high experimental reliability. Using these approaches for microalgae present additional challenges because of the necessity of dealing with light as a source of energy to support cell metabolism. Currently developed microscale platforms are complex, often requiring a deep experience in the microfluidic field to monitor the device functionality. To overcome this drawback while keeping microscale benefits, here we describe the use of a simple and flexible micro-photobioreactor (micro-PBR) coupled with a quick and reliable growth evaluation method. The system is demonstrated to sustain optimal growth of *Nannochloropsis gaditana* cells, allowing to assess the impact of different light intensities and to monitor the cells photosynthetic functionality by measuring chlorophyll (Chl) fluorescence *in vivo*, while providing for a significant time reduction when compared to traditional experimental approaches.

1. Introduction

The increase in world population and in the energy demand to support modern life have led to a rise in fossil fuels exploitation as primary energy source [1,2]. This phenomenon finds direct correlation with the net greenhouse gases increase in the atmosphere, which is posing serious threats for the future well-being of the society [3,4]. The development of renewable feedstock for the sustainable production of fuels and other oil-derived commodities is thus a strong priority. In this scenario, microalgae are emerging as a promising candidate thanks to their ability to accumulate molecules such as sugars and lipids that can be easily converted into biofuels. Furthermore, they use sunlight and CO_2 as energy and carbon source, and they can thus be at the base of environment-friendly processes [5–7].

However, the realization of such potential still requires large research efforts to improve the process yield and economic performance, from the selection of the most suitable candidate species up to the design of plants and the definition of operation policies. For instance, with concern to the identification of the best species (*e.g.*, the ones exhibiting the highest biomass or lipid productivity), it is a fact that, although the estimated number of microalgae species in nature is between 40,000 and 70,000 [8], the number of those extensively characterized and exploited for industrial purposes to date is still extremely low [9] and an intense bioprospecting effort would be highly advocated [10,11].

The characterization of new or mutant species and most generally the quantification of algae response to external outputs require the assessment and optimization of all growth-affecting parameters, such as CO_2, O_2 and nutrients availability (NH_4^+, NO_3, PO_4) and, obviously, light intensity and dynamics, with the presence of a photosynthetic metabolism further increasing the complexity with respect to other microorganisms [12–18]. Unfortunately, notwithstanding its key importance for the design and optimization of any industrial application, the detailed evaluation of the impact of all factors influencing microalgae

Abbreviations: Chl, chlorophyll; $^3Chl^*$, triplet state chlorophyll; Count, cells count; F_0, chlorophyll fluorescence of dark-adapted cells; Fluo, fluorescence; F_m, maximum chlorophyll fluorescence; HL, high light; LL, low light; ML, medium light; micro-PBR, micro-photobioreactor; NPQ, Non-Photochemical Quenching; PDMS, poly(dimethylsiloxane); ROS, reactive oxygen species.

* Corresponding author at: CAPE-Lab (Computer-Aided Process Engineering Laboratory) and PAR-Lab (Padua Algae Research Laboratory), Department of Industrial Engineering, University of Padova, via Marzolo 9, 35131 Padova, Italy.
E-mail address: fabrizio.bezzo@unipd.it (F. Bezzo).

[1] Equal contributors.

http://dx.doi.org/10.1016/j.algal.2016.07.015
2211-9264/

growth is extremely costly and time consuming [7,19,20]. In this context, mathematical models can represent a value-added to predict and quantify the impact of key inputs (*e.g.*, light, nutrients) and design variables on microalgae growth, and to suggest effective optimization strategies [21,22]. However, complex multiscale effects and the tight interactions between different variables again require numerous and time-consuming experiments (possibly adopting complex design of experiments approaches [23]) to discriminate among different modelling assumptions and identify the model parameters in a reliable way.

In this scenario, microscale technologies have the potential to provide a major step forward in our ability to describe the influence of multiple parameters. By scaling down the cultivation system it is possible to reduce the time and costs required to evaluate the effect of multiple parameters on these microorganisms growth. Despite being mainly developed for human cells biological investigations [24–26], the application of microscale technology recently emerged also in other fields, from eukaryotic microorganisms [27] to plant cells cultivation [28–30].

Some microfluidic platforms have been developed in the past years in order to assess the impact of multiple parameters on microalgae growth, too [31–33]. One strategy relies on static microdroplet arrays, produced to develop a finely tuned chemical environment in which microalgae growth takes place [34–36]. However, microdroplet technologies cannot be used for high-throughput studies, given the incompatibility with the application of multiple and simultaneous environmental parameters [34,35]. In order to assess the impact of several environmental parameters on microorganisms growth, microdroplets-based technologies must be replaced with different microscale devices [28,37,38].

The microfluidic platforms that have been developed so far are designed to investigate mainly the impact of nutrients availability, and little consideration was given to the major factor influencing photosynthetic growth, *i.e.* light, with the partial exception of [39], where a microscale platform to assess light intensity and light/dark cycles impact on microalgae growth was indeed provided, although the design was tailored to look at a single colony level. Furthermore, a typical drawback of currently adopted microfluidic platforms is their high level of complexity, and the necessity of highly skilled users in order to be efficiently exploited [40,41], often requiring a deep information technology expertise in order to monitor the device functionality and to produce data output [39,42].

In order to scale-down the growth platform, while maintaining simplicity and flexibility, we developed a simple microfluidic device and an equally important quick and reliable growth evaluation method, based on the *in vivo* Chl fluorescence evaluation. The evaluation approach was also used for investigating the alterations in the photosynthetic performances, directly *in situ*. All experiments were carried out on *Nannochloropsis gaditana*, which was chosen as a model organism for exploitation in industrially relevant applications. Results demonstrate that the microfluidic system was effective in growing microalgae in a batch mode, showing growth rates as high as in lab-scale photobioreactors, but with a dramatic increase in data productivity thus paving the way to more effective microfluidic technologies capable of speeding up the evaluation of how different parameters influence microalgae growth.

2. Materials and methods

2.1. Microfluidic device fabrication

The developed device comprises a matrix of 45 wells, each representing an independent experimental condition, arranged in 9 independent columns with 5 wells each. All the wells are integrated in a microfluidic platform and connected by micro-channels to two lateral flow channels for future microfluidic studies. The platform was designed with the aid of 3D CAD software. The mold was produced *via* stereolithography rapid prototyping, and the final device was replica-molded in poly(dimethylsiloxane) (PDMS). The pre-polymer Sylgard 184 (Dow Corning, USA) was mixed with a cross-linker in a ratio of 10:1 (w/w) and then poured onto the mold and degased in a desiccator. After curing at 65 °C for 3 h, the device is ready for use. We used a thin PDMS membrane to seal the top of the device, reducing evaporation from the wells and maintaining sterility. A simple clamping unit ensured hydraulic sealing. The clamping unit was composed of two perforated metal plates, designed to allow perfect light exposure for the cultured algae, and connected by butterfly screws. The microbioreactor and the clamping system were steam sterilized (20 min at 121 °C) prior to use.

2.2. Preparation of Nannochloropsis gaditana cells

Nannochloropsis gaditana wild type cells (strain 849/5), from the Culture Collection of Algae and Protozoa (CCAP, United Kingdom), were cultured in sterile f/2 media containing sea salts (32 g/L, Sigma-Aldrich, Italy), 40 mM Tris–HCl (pH 8) and Guillard's (f/2) marine water enrichment solution (Sigma-Aldrich, Italy). Cells were routinely kept in pre-culture in Erlenmeyer flasks with 6, 60 or 360 μmol photons/(m^2 s) illumination and 100 rpm agitation at 22 ± 1 °C in a growth chamber.

In the middle of the exponential growth, cells were counted using a cell counter (Cellometer Auto X4, Nexcelom Bioscience, USA) and then centrifuged at 3500*g* for 5 min to remove the exhausted f/2 medium. Cells were used to inoculate the micro-photobioreactor at multiple starting cellular concentrations in fresh f/2 medium. Illumination was always kept constant and intensities ranged from 6 to 360 μmol photons/(m^2 s). The different light intensities were achieved thanks to daylight fluorescent lamps. No external CO_2 supply was provided to the Erlenmeyer flasks and thus carbon dioxide to support growth came directly from the atmosphere.

2.3. Microalgae growth monitoring and photosynthetic parameters estimation in the micro-photobioreactor

Microalgae growth in the micro-photobioreactor was monitored evaluating the *in vivo* chlorophyll (Chl) fluorescence using a FluorCam FC 800 video-imaging apparatus (Photon Systems Instruments, Czech Republic). F_0 was measured after cells were dark adaptated for 20 min, while maximum Chl fluorescence value (F_m) was evaluated after a saturating light pulse of 3000 μmol photons/(m^2 s) with 800 ms duration. The latter value was correlated to the cellular concentration after cells counting using a cell counter (Cellometer Auto X4, Nexcelom Bioscience, USA). The specific growth rate was calculated by the slope of logarithmic phase for the number of cells.

PSII functionality was expressed as PSII maximum quantum efficiency (F_v/F_m) and was calculated as ($F_m - F_0$) / F_m. NPQ activation was monitored by exposing cells to 500 μmol photons/(m^2 s) for 5 min. NPQ values were calculated as previously described in [43].

3. Results and discussion

3.1. Micro-photobioreactor array design

In this work *N. gaditana* cells were grown in a micro-photobioreactor (75 × 50 mm) composed of 45 wells (40 μl of working volume each) linked in columns by a network of microfluidic channels (see Fig. 1 for dimensions details).

This network was also connected to two lateral flow channels allowing for further fluid flow application (Fig. 1). The design of the micro-photobioreactor followed specific criteria such as: (i) support of cultures of microalgae in a no-shear environment, (ii) high-throughput studies with biologically relevant numbers of replicates, (iii) compatibility with on-line imaging, (iv) ease of retrieval of the cell samples for eventual post-processing analyses, and (v), last but not least, capability