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Microplate-based method for high-throughput screening of microalgae growth potential

Jon Van Wagenen [1], Susan Løvstad Holdt, Davide De Francisci, Borja Valverde-Pérez, Benedek Gy Plósz, Irini Angelidaki *

Technical University of Denmark, Department of Environmental Engineering, DTU Environment, Building 113, DK-2800 Kgs. Lyngby, Denmark

HIGHLIGHTS

- Microalgae specific growth rates can be observed in low-density microplate cultures.
- Microplates may be used determine algae strain specific growth–light (μ–I) curves.
- The μ–I curve can be used to model volumetric productivity of larger algae cultures.
- Replacing photobioreactors with microplates should increase screening throughput.

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ABSTRACT

Microalgae cultivation conditions in microplates will differ from large-scale photobioreactors in crucial parameters such as light profile, mixing and gas transfer. Hence volumetric productivity (P_v) measurements made in microplates cannot be directly scaled up. Here we demonstrate that it is possible to use microplates to measure characteristic exponential growth rates and determine the specific growth rate light intensity dependency (μ–I curve), which is useful as the key input for several models that predict P_v. *Nannochloropsis salina* and *Chlorella sorokiniana* specific growth rates were measured by repeated batch culture in microplates supplied with continuous light at different intensities. Exponential growth unlimited by gas transfer or self-shading was observable for a period of several days using fluorescence, which is an order of magnitude more sensitive than optical density. The microplate datasets were comparable to similar datasets obtained in photobioreactors and were used an input for the Huesemann model to accurately predict P_v.

1. Introduction

Cultivation of microalgae is receiving increasing interest for applications including municipal or industrial wastewater treatment biofuels, bulk chemicals, food, feed or high-value products (Wijffels and Barbosa, 2010). Determination of the feasibility of any cultivation system depends to a large degree on the volumetric productivity achievable by the algal culture. The volumetric productivity achieved in literature shows extreme variation dependent on species and cultivation conditions (Chen et al., 2011). Clearly much of the variation is due to operating conditions, as *Chlorella sorokiniana* strains have been documented to grow at 0.005, 0.23 or 12.2 $g^{-1}\,L^{-1}\,day^{-1}$ (Chen et al., 2011; Cuaresma et al., 2009). With such obvious disagreement of reported values researchers or companies still need to rely on their own measurements to verify that they can achieve required productivity targets.

Screening strains of microalgae for their productivity will continue to be a challenge throughout applied phycology, with over 40,000 species of algae identified and orders of magnitude more yet to be described (Guiry, 2012). While biomass composition is also species-dependent, downstream processing, harvesting and valuable component extraction depends on the type of algae used (Pragya et al., 2013). The need for a high-throughput screening system becomes evident, especially considering the multiplication of variables arising given that e.g., each species (or strain) may have unique optimum responses to medium composition or wastes potentially used as medium nutrients; mutant strains are routinely generated; and combinations of species in mixed cultures may also offer production advantages (Johnson and Admassu, 2013).

* Corresponding author. Tel.: +45 45 25 14 29; fax: +45 45 93 28 50.
E-mail addresses: jovw@env.dtu.dk (J. Van Wagenen), iria@env.dtu.dk (I. Angelidaki).
[1] Tel.: +45 45 25 14 71.

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Microplates, which are fast, low-cost and not requiring intensive labor, would seem an obvious choice for high-throughput studies.

Microplates have been used as a tool for determination of kinetic characteristics of microalgae, but as yet they have not been proven capable of measuring the strain-specific trait maximum specific growth rate μ_{max} as accurately as can be done in larger culture volumes. There are several studies effectively showing that microplates can be used for toxicity tests determining inhibition of growth relative to a positive control, however these studies are not concerned with quantifying algae growth, only its reduction by putative toxins (Blaise et al., 2005; St. Laurent 2007). The promise of screening in microplates is recognized by Chen et al. (2012) and Pacheco et al. (2013), but not validated by comparing growth rate values obtained in microplates with larger scales cultivations. In some cases, the rate measured in microplates underestimated the rates observed in larger, well-mixed vessels (Santos et al., 2012; Skjelbred et al., 2012). Another factor (likely gas transfer, as in Borch unpublished 2012) limits growth when experiments are conducted in microplates (Betts and Baganz, 2006; Duetz, 2007). To overcome this limit, Han et al. (2012) constructed a new type of rotating microplate carrousel in order to increase the CO_2 flow into the microplate to a level comparable to flasks (still significantly lower than a sparged photobioreactor). In some cases, conclusions can be made about the relative preference on an algal strain for one condition compared to another in microplates, but the magnitude of the difference does not match what is observed at larger scales (Santos et al., 2012) Furthermore, low specific growth rates obtained in microplates could be merely strain-specific responses to limitations imposed by the microplate format, as in Pachenco et al. where a *Chlorella* species and *Botryococcus* species have essentially the same specific growth rate, despite the well-documented tendencies of the latter to grow extremely slowly, while the former are typically average to fast growing algae.

In order for a screening protocol to be useful, the investigator should have confidence that the results obtained in laboratory tests will be informative of industrial scale performance. For example in heterotrophic organisms, two strain specific parameters readily measureable at the lab-scale can be used to estimate industrial production rates based on Monod kinetics. These are: maximum specific growth rates μ_{max} and "the value of the limiting substrate concentration at which the specific growth rate is half its maximum value," K_s (Villadsen et al., 2011). There have been several different mathematical approaches to relating the amount of light to the rate of microalgal growth, each requiring different strain specific inputs, but no one formulation has reached a wide acceptance as Monod kinetics (Zonneveld, 1998). Although μ_{max} is often reported when characterizing a strain, it is not sufficient to predict bulk growth at relevant concentrations because light is attenuated by self-shading in algal cultures. This is the principal difference between modeling algal growth and that of other microbes: while with other organisms, one can assume that μ_{max} is essentially obtainable in cultures independent of density, this is not the case in autotrophic cultures. This means that large scale algal cultures are described in a more accurate (and commercially relevant way) by a linear expression, such as volumetric or areal productivity ($g\,L^{-1}\,day^{-1}$ or $g\,m^{-2}\,day^{-1}$, respectively) instead of exponential rates described in the term μ_{max} (day^{-1}). Most algal biomass growth models take light-attenuation into account, but they often require too many other strain-specific input characteristics to be applicable for screening purposes. However, a recently published model (Huesemann et al., 2013) simplifies the process of predicting algal biomass growth modeling by requiring only two measurable strain-specific input parameters: specific growth rate (day^{-1}) as a function of light intensity (known as the μ–I curve) and a light absorbance coefficient, K_a ($m^2\,g^{-1}$).

The dependence of photosynthesis rate on light intensity is a well-known feature of plant biology and in aquatic photosynthesis and the μ–I curve, and has been studied for decades in a similar manner (Sorokin and Krauss, 1958; Sukenik et al., 1989). Known light intensities are applied to nutrient unlimited, well mixed cultures and the increase of biomass is monitored. Only the biomass increase during exponential phase is used for determination of the μ–I curve. The culture is repeatedly (or continuously) diluted with fresh medium to keep concentration low in order to avoid self-shading. Skjelbred et al. (2012) described measurement of a μ–I curve in a microplate for the first time, and confirmed that the maximum observed microplate μ values were comparable to the μ observed in a shake-flask culture. While very illuminating, this result left open the question of whether the entire μ–I curve or only the maximum value of μ was consistent across the different scales of experimentation. This work investigates whether a μ–I curve can be measured at microtiter scale which is equivalent to one obtained in a low cell density, nutrient sufficient batch bioreactor or turbidostat. In addition to direct comparison, μ–I curves obtained in microplates are compared to those from larger reactors in their ability to predict bulk growth characteristics in cultures limited only by light availability using the model described in Huesemann et al. (2013). Many other models of bulk production (Béchet et al., 2013; James and Boriah, 2010; Lee et al., 2014; Slegers et al., 2011; Takache et al., 2012) also require strain-specific inputs that come from empirically derived μ–I curves (Geider et al., 1996). Due to limited amounts of input data available in literature, the bulk models can only be applied to one or two species; by utilizing the methods described here, a larger collection of input data can be readily generated.

2. Methods

2.1. Cell lines

Chlorella vulgaris, *Scenedesmus obliquus*, *Haematococcus pluvialis*, were obtained from SCCAP and maintained in MWC + Se medium. *Chlorella protothecoides* CCAP 211/8D was maintained in MWC medium with yeast extract as N source. *Nannochloropsis salina* (1776) was obtained from CCMP and maintained in L1 medium. *Chlorella sorokiniana* CCAP 211/8 *K* was maintained in modified M8a medium (Cuaresma et al., 2009).

2.2. Instrument sensitivity

Limits of detection and quantification in 24-well plates were calculated according to the IUPAC guidelines (Thomsen et al., 2003). A Coulter Counter enumeration of cells mL^{-1} was compared to signals of in vivo fluorescence (IVF) at 440 nm excitation, 690 nm emission 100 nm bandwidth and optical density (OD) measurements at 750 nm read in a BIOTEK Synergy microplate reader. Instrument detection limits were determined from the standard deviation of the blank ($n = 8$). A calibration curve with four samples each at four concentrations was used to determine the sensitivity of *C. vulgaris* in clear (Costar) and black-walled, clear bottomed (Perkin Elmer) microplates covered with sterile BREATHE EASY gas permeable membranes (Diversified Biotech) after 2 h of incubation. Microsoft Excel was used for a Student's paired *t*-test on calculated detection and quantification limits. The limits of detection and quantification for OD and IVF were determined for *C. protothecoides*, *S. obliquus* and *H. pluvialis* in a black plate with four wells per species and eight for blank. Excel was used to calculate the 95% confidence interval for the detection and quantification limits.