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A method to determine photosynthetic activity from oxygen microsensor data in biofilms subjected to evaporation

Tong Li [a,*], Björn Podola [a], Dirk de Beer [b,1], Michael Melkonian [a,1]

[a] University of Cologne, Botanical Institute, Biocenter Cologne, Germany
[b] Max-Planck-Institute for Marine Microbiology, Bremen, Germany

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ABSTRACT

Phototrophic biofilms are widely distributed in nature and their ecological importance is well recognized. More recently, there has been a growing interest in using artificial phototrophic biofilms in innovative photobioreactors for production of microalgal biomass in biotechnological applications. To study physiological processes within these biofilms, microsensors have been applied in several studies. Here, the 'light–dark shift method' relies on measurement of photosynthetic activity in terms of light-induced oxygen production. However, when applied to non-submerged biofilms that can be found in numerous locations in nature, as well as in some types of photobioreactors, limitations of this approach are obvious due to rapid removal of gaseous species at the biofilm surface. Here, we introduce a mathematical correction to recover the distribution of the actual photosynthetic activity along the depth gradient in the biofilm, based on a numerical solution of the inversed diffusion equation of oxygen. This method considers changes in mass transport during the measurement period as can found on biofilms possessing a thin flow/mass transfer boundary layer (e. g., non-submerged biofilms). Using both simulated and real microsensor data, the proposed method was shown to be much more accurate than the classical method, which leads to underestimations of rates near the biofilm surface. All test profiles could be recovered with a high fit. According to our simulated microsensor measurements, a depth resolution of ≤20 μm is recommended near the surface. We conclude that our method strongly improves the quality of data acquired from light–dark measurements of photosynthetic activity in biofilms.

1. Introduction

Phototrophic biofilms are attached microbial communities driven by photosynthesis, and their importance in nature has long been recognized (Roeselers et al., 2008; Seckbach and Oren, 2010). In recent years, phototrophic biofilms have been applied to biotechnology and environmental biotechnology of microalgae, as well. Here, most applications rely on submerged biofilms, where the light exposed biofilm surface is covered by a layer of water (Berner et al., 2014; Roeselers et al., 2008). Some more recent developments make use of the advantages of biofilms exposed to ambient air (Berner et al., 2014; Schultze et al., 2015; Shi et al., 2007), referred to as porous substrate bioreactors (PSBRs) (Murphy et al., 2014). In view of the technical development of photobioreactors, detailed micro-scale (microsensor) analyses of parameters along the axis (gradient) perpendicular to the biofilm surface have been proposed (Roeselers et al., 2008; Schultze et al., 2015). Such studies might also be of interest to analyze non-submerged (aerial) phototrophic biofilms in the natural environment (Berner et al., 2014).

A number of approaches have been developed to investigate structures and processes inside phototrophic biofilms at the micro-scale level, such as confocal microscopy, microsensor studies, fluorometry, and mathematical modeling (Bachar et al., 2008; Beutler et al., 2009; Kühl and Polerecky, 2008; Ramanan et al., 2013; Revsbech et al., 1981; Wolf et al., 2007). Among these methods, the microsensor-based "light–dark shift method" is a well-established technique to estimate gross photosynthetic activity in phototrophic biofilms since its introduction in the 1980s (Revsbech and Jorgensen, 1983). The method determines the rate of decrease in the dissolved oxygen concentration by an oxygen microsensor, which is induced by applying a short period of darkness to a steady state phototrophic biofilm (Revsbech and Jorgensen, 1983). However, since the diffusion rate of oxygen changes during this dark period, the gross productivity estimated using the acquired data directly might be inaccurate. This limitation was soon recognized and solutions were suggested in several previous studies, which established a solid basis for evaluating data acquired by light–dark shift method. However, these solutions require either a hypothetical distribution of photosynthetic activity or rely solely on the regression of measured data (Glud et al., 1992; Lassen et al., 1998; Revsbech

* Corresponding author.
E-mail address: lit2@uni-koeln.de (T. Li).
[1] Dirk de Beer and Michael Melkonian contributed equally to this work.

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and Jorgensen, 1983; Revsbech et al., 1986). For non-submerged biofilms, besides the limitation referred to above, other processes have to be taken into account: the dynamics of oxygen in a non-submerged biofilm can be described by Eq. (1), assuming that the biofilm is homogenous in planes parallel to its surface (Bergman et al., 2011).

$$\frac{\partial C}{\partial t} = P_g - R_l + D_e \frac{\partial^2 C}{\partial x^2} + u \frac{\partial C}{\partial x} - R_s. \quad (1)$$

The terms on the right hand side represent gross photosynthesis P_g, respiration during light R_l, diffusion and advection caused by evaporation on the surface, and the removal of dissolved gaseous species due to air flow above the surface R_s. D_e is the effective diffusion coefficient, C is the concentration of dissolved oxygen, u is the convective flow rate inside the biofilm,

$$u = q_e/(A_e \cdot \theta) \quad (2)$$

and can be calculated with the rate of liquid volume lost q_e, the exposed surface area of the biofilm A_e, and the areal porosity of the biofilm θ (Eq. (2)). t is time and x is the depth. Without light, no oxygen evolution can be expected (Pessarakli, 2005). Thus after darkening oxygen decreases with a rate:

$$\frac{\partial C}{\partial t} = 0 - R_l + D_e \frac{\partial^2 C}{\partial x^2} + u \frac{\partial C}{\partial x} + R_s. \quad (3)$$

Values measured by the light–dark shift method are the rate of change for the term on the left hand side of Eq. (3) during the measurement period. Immediately after the removal of light (at 0 s of the measurement), $\partial C / \partial t$ does represent accurately the gross photosynthetic productivity. However, its value changes gradually with time, as the removal of light leads to change of the values on the right hand side (Glud et al., 1992; Revsbech et al., 1986; Revsbech and Jorgensen, 1983). In consequence, an accurate representation of the photosynthetic rate is provided only if all terms on the right hand side of Eq. (3) remain constant with time (as the measurement time has to be >0 s), which cannot be assumed: Previous work (Glud et al., 1992) concluded that the respiration rate can be considered stable during the measurement period, but the change in rate of diffusion can have a significant effect on the measured results (Glud et al., 1992; Revsbech and Jorgensen, 1983; Revsbech et al., 1986).

When applying the light–dark shift method for measuring photosynthetic activity to a non-submerged biofilm, two additional factors attributed to the absence of a thick liquid phase on the biofilm surface have to be considered (Bergman et al., 2011; Schlichting and Gersten, 2000):

1) For a non-submerged system directly exchanging dissolved gases with the gas phase above the biofilm surface, the flow/mass transfer boundary layer (compared to submerged biofilms) can be considered as non-existent in case of a considerable air flow. With the gas phase above the biofilm being in effect a sink for the oxygen produced inside the biofilm, during the light–dark cycle, the rate of change of oxygen concentration at the biofilm surface will always be 0 (or almost 0), and the change rate near the surface an underestimation of the photosynthetic activity. This effect also occurs in submerged system at the interface between the boundary layer and the bulk liquid, however, the effect in the biofilm itself is dampened due to the relatively thicker flow/mass transfer boundary layer.
2) The evaporation of water at the biofilm surface exposed to air induces a convective flow through the biofilm in the direction perpendicular to the biofilm surface, which in effect adds a mass flow towards the surface.

These two processes have a strong effect throughout a non-submerged biofilm, and, therefore, need to be considered for the interpretation of acquired experimental data to obtain a profile representing a valid photosynthetic activity.

In summary, direct results from the light–dark shift measurement do not accurately represent the actual photosynthetic activity in biofilms, especially for measurements performed in non-submerged biofilm systems. Thus, in the present contribution we propose a mathematical treatment to achieve an adequate recovery of the distribution of actual photosynthetic activity from measured microsensor data.

2. Materials and methods

2.1. Suspension and biofilm cultivation of microalgae

In this work, a Twin-Layer algal biofilm, which is an immobilized biofilm system that separates the bulk medium and biomass by means of a micro-porous membrane (Nowack et al., 2005), was used as a model for a non-submerged biofilm. Stock cultures of the green algae *Halochlorella rubescens* (strain CCAC 0126; Culture Collection of Algae at the University of Cologne; www.ccac.uni-koeln.de) in suspension was kept at 16 °C at a light intensity of about 20 μmol photon m^{-2} s^{-1} photosynthetically active radiation (PAR). To obtain sufficient material for biofilm inoculation, stock cultures were transferred into 2 L Erlenmeyer flask filled with 1.3 L of Bold's basal medium (BBM) (Bischoff and Bold, 1963), and were cultivated at a light intensity of about 50 μmol m^{-2} s^{-1} PAR at 23 °C with 0.5 L min^{-1} compressed air with 1% (v/v) supplementary CO_2. Biomass was harvested after 3 weeks of growth, and was inoculated onto polycarbonate filters (PC40, 0.4 μm pore size, 25 mm diameter, Whatman, Dassel, Germany) with a circular inoculation area of 2.55 cm^2 as described by Naumann et al. (2013). Inoculated filters were then transferred into a bench-scale Twin-Layer system for cultivation according to Schultze et al. (2015). Tubes were aerated with a flow rate of 0.75 L min^{-1} with compressed air. Sodium discharge lamps (SON-T AGRO 400 W, Philips, Hamburg, Germany) were used as light source with a 14–10 hour light–dark cycle at an intensity of 1000 μmol m^{-2} s^{-1} 1 PAR. Temperature in the tubes was 25 ± 2 °C. 1 L BBM culture medium was used at a medium flow rate of 3 mL min^{-1} per tube and the culture medium was replaced every 2 days.

2.2. Microsensor measurements

A schematic representation of the experimental setup for microsensor measurements based on previous works by Gieseke and de Beer (2004) is given in Fig. 1: the biofilm immobilized on a polycarbonate membrane was placed inside the biofilm measurement cell under a controlled atmosphere of compressed air at a flow rate of 1 L min^{-1} (Fig. 1A). The non-inoculated area of the polycarbonate filter and glass fiber were covered with a black plastic foil to exclude light and gas exchange in this area. 50 mL of BBM culture medium were circulated though the measurement cell with a flow rate of 3 mL min^{-1} by means of a peristaltic pump and were exchanged every 1 h during the measurement. The evaporation rate was monitored by recording the change of the volume in the medium container. Biofilm temperature during the experimental period was 23 ± 0.5 °C. Light was supplied at an intensity of 1000 μmol m^{-2} s^{-1} (on biofilm surface) from the front side by a halogen lamp (KL-1500, Schott, Mainz, Germany) equipped with a 3-fold splitter to ensure even distribution. The movement of the sensor was enabled by the computer-controlled micromanipulator (Pollux Drive, PI miCos, Eschbach, Germany). Data were recorded with a depth resolution of 20 μm until 500 μm depth (at which the switch from light to dark has no significant effect on the oxygen concentration measured). Microsensor measurements were carried out on biofilms cultivated for 30 days. Microsensor signals were amplified and converted into digital data (DAQpad 6015 and 6009, National Instruments, Munich, Germany) prior to their further processing on a computer (Fig. 1B). Software used for system control and data acquisition were custom