

## Oxygen evolution rate in Antarctic filamentous alga *Stigeoclonium* sp. evaluated by optodes relates to chlorophyll fluorescence parameters

(Short Communication)

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### Abstract

Photosynthetic reactions of algal communities, the essential component of primary production in polar regions, are strongly dependent on environmental factors. Among them, availability and amount of light in particular parts of growing season are of major importance. In this paper, the response of the photosynthetic processes of a filamentous freshwater alga to photosynthetically active radiation (PAR) was studied by two approaches. The simultaneous measurements of the effective quantum yield ( $\Phi_{\text{PSII}}$ ) and oxygen evolution rate (OER) at stepwise increasing photosynthetically active radiation provided data for beneficial correlation analysis of the  $\Phi_{\text{PSII}}$  to OER relationship in a wide range of PAR. In this study, the culture of filamentous alga *Stigeoclonium* sp. was analyzed. The linear relationship between  $\Phi_{\text{PSII}}$  and OER was found for the low PAR (the range of 0 – 200  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ). At high PAR levels (200 – 1000  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) another linear relationship with different slope was found. The approach combining the fluorometric and oxymetric method might be used for calibration of data in follow up studies and, consequently for evaluation of photosynthetic rates ( $\text{O}_2$  evolution) from chlorophyll fluorescence data.

**Key words:** effective quantum yield, oxygen evolution rate, Antarctic phototroph photosynthesis

**DOI:** 10.5817/CPR2018-1-11

### Introduction

Algal communities are an essential part of microbiota. They contribute to the primary production of lakes, streams and rivers in polar regions. Majority of polar algae may survive in harsh and even extreme polar environments. Representatives from almost all algal divisions have been recorded for such extreme environments (Lizotte 2008). Kirk (2011) gives an overview of the studies analysing physiological proper-

Received May 1, 2018, accepted September 6, 2018.

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**Acknowledgements:** The author is grateful to the Czech Polar Research Infrastructure (project CzechPolar2, LM2015078) that enabled sample collection and handling. The experimental part of work has been done in the EEL laboratory (Masaryk University, Brno) and supported by the ECOPOLARIS project (CZ.02.1.01/0.0/0.0/16\_013/0001708).

ties of polar algae by a variety of methodological approaches. These comprise direct and indirect methods. The group of direct methods is based on a measurement of the photosynthetic rate, CO<sub>2</sub> fixation and O<sub>2</sub> evolution, methods of electrochemical analysis and determination of <sup>14</sup>CO<sub>2</sub> and [<sup>14</sup>C]-bicarbonate. Indirect methods involve the measurements of various parameters of chlorophyll fluorescence (e.g. Bates et Cota 1986, Ryan et al. 2011). Among them, the effective quantum yield of photosynthetic processes in photosystem II ( $\Phi_{\text{PSII}}$ ) is widely accepted since it is an evaluator of actual photosynthetic rate. For evaluation of the response of the photosynthetic rate of algae as dependent on incident light, measurements of light response curves (some authors refer as P-I curves) of different photosynthetic parameters are used (e.g. Litchman et al. 2003, Necchi et Zucchi 2001).

During the measurements of light response curves at stepwise increasing light intensity, photosynthetic characteristics (*i.e.* several parameters evaluating the rate of photosynthesis) of the light-adapted sample are evaluated by direct or indirect methods. The results can be used in ecological interpretation of photosynthetic productivity of freshwater algae in polar regions. If the light-response curves are measured on naturally-occurring rather than laboratory grown material (Kirk 2011) the estimates of photosynthetic production are close to reality.

*Stigeoclonium* sp. is an algal species found in several well-hydrated terrestrial ecosystems in Antarctica (e.g. Pushparaj et al. 2004), and James Ross Island (Caisová et al. 2009). This study is focused on the evaluation of the photosynthetic activity of filamentous green alga *Stigeoclonium* sp. at different light intensities. Simultaneous measurements of oxygen evolution and photochemical activity of photosystem II were used. The photosynthetic study is a part of the long-term research activities of Czech Antarctic programme that has been per-

formed at James Ross Island since 2007. Former studies focused on a variety of problems comprising mainly biodiversity and ecophysiological features of terrestrial algae at James Ross Island. Biodiversity of the algae and cyanobacteria have been studied in the Northern part of James Ross Island (Komárek 2014, Nedbalová et al. 2017, Skácelová et al. 2015, 2013). Ecophysiological studies addressed several aspects of algae-environment interaction. Nedbalová et al. (2017) studied temperature and light growth requirements, the fatty acid composition and the phylogenetic relationship in species of the genus *Monoraphidium*. More detailed laboratory-based photosynthetic studies focused on lichen symbiotic algae or cyanobacteria (Bayer et Alba 2017, Marečková et Barták 2017, Sehnal et al. 2014), and filamentous green alga *Zygnema* (Thangaraj 2015).

In the photosynthetic study of *Stigeoclonium* sp., the optodes were used for determination of oxygen concentration and OER. In general, optodes are optical chemical sensors capable of recording chemical compounds, based on their fluorescence/luminescence properties (*see* Espinosa-Calderon et al. 2011 for review). They measure in either gaseous or aqueous (Tengberg et al. 2006) phase. Recently, an emerging number of application of the optodes is witnessed in a great variety of disciplines including plant science. In photosynthetic studies, the approach is used across species and environments such as e.g. in microalgae cultivated in photobioreactors (Tamburic et al. 2014), seagrasses (Ow et al. 2015), hypersaline soil crusts (Woelfel et al. 2009), shallow sea water (Hydes et al. 2009). In autotrophic microorganisms, the optodes were used to estimate photosynthetic O<sub>2</sub> evolution in a snow alga *Chloromonas nivalis* (Remias et al. 2010), benthic diatoms (Woelfel et al. 2014), cyanobacteria-dominated biofilms (Rubol et al. 2018). Since photosynthetic activities of freshwater microalgae have not been investigated by optodes so far to a large ex-

tent, the presented study focused on simultaneous measurements of photosynthesis in *Stigeoclonium* sp. by two different techniques, *i.e.* optodes and chlorophyll fluores-

cence. The aim of the study was to evaluate the relation of OER to  $\Phi_{\text{PSII}}$  in response to light.

## Material and Methods

### *Algal material handling*

The algal culture of *Stigeoclonium* sp. was isolated from a sample collected from the water basin located in the northern part of Ulu peninsula (63° 50' 14" S, 57° 50' 17" W), James Ross Island, Maritime Antarctica. The collection site was a shallow lake located on a flat surface on the eastern slopes of the Lachman Crags mesa. The culture was cultivated in liquid BBM medium (Bischoff et Bold 1963) at 5°C and

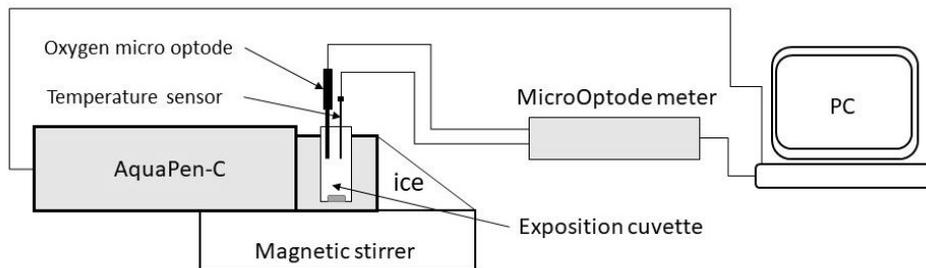
30  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with 16/8h light/dark photoperiod. The algal suspension reaching the optical density of 0.3 at 680 nm was used in the photosynthesis measurements. To analyse the photosynthetic activity in different light intensities of photosynthetically active radiation (PAR), the light response curve approach with simultaneous chlorophyll fluorescence and oxygen evolution measurement was used.

### *The light response curve of $\Phi_{\text{PSII}}$ and oxygen evolution rate*

Grown algal suspension of 2.5ml was transferred to an exposition cuvette. The cuvette with the suspension was coupled to a fluorometric system AquaPen-C AP 100-C (Photon Systems Instruments, Czech Republic) and the oximetric (Unisense, Denmark). The setup was equipped with a magnetic stirrer (Topolino mobil, IKA, Germany) to ensure homogeneity of the suspension during measurements. The measuring protocol consisted of the exposition of the suspension to 8 different PAR levels. Within the protocol applied by the fluorometric system, the suspension was exposed to stepwise PAR of 0, 10, 20, 50, 100, 300, 500, and 1000  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  with each step lasting 300 s. Exposition part of the fluorometric system with integral cuvette was cooled by ice to maintain the temperature of the sample in the temperature range 2 - 7°C. The temperature of the sample was monitored by the thermocouple connected to the oximetric system (Fig. 1.).

During the exposition, steady-state fluorescence ( $F_s$ ) was measured and at the end of each level of PAR. Then, saturation pulse was applied to determine the maximal fluorescence of the light-adapted sample ( $F_M$ ) and the effective quantum yield of the photosystem II photochemistry ( $\Phi_{\text{PSII}}$ ; van Kooten et Snel 1990).

During the whole exposition to the PAR levels, the changes in the concentration of dissolved oxygen ( $c_{\text{O}_2}$ , temperature compensated) was measured using the micro optode (MicroOptode OP-430, Unisense, Denmark) connected to MicroOptode meter (Unisense, Denmark). The oxygen evolution rate (OER) was determined according to the equation:  $\delta c_{\text{O}_2} / (\delta t \cdot c_{\text{chl}a})$  and expressed as  $\mu\text{mol}(\text{O}_2)\cdot\text{mg}(\text{chl}a)^{-1}\cdot\text{h}^{-1}$ . From each sample, photosynthetic pigments were extracted in dimethylsulfoxide after the measurements. Concentrations of chlorophyll *a* was determined according to Welburn (1994).



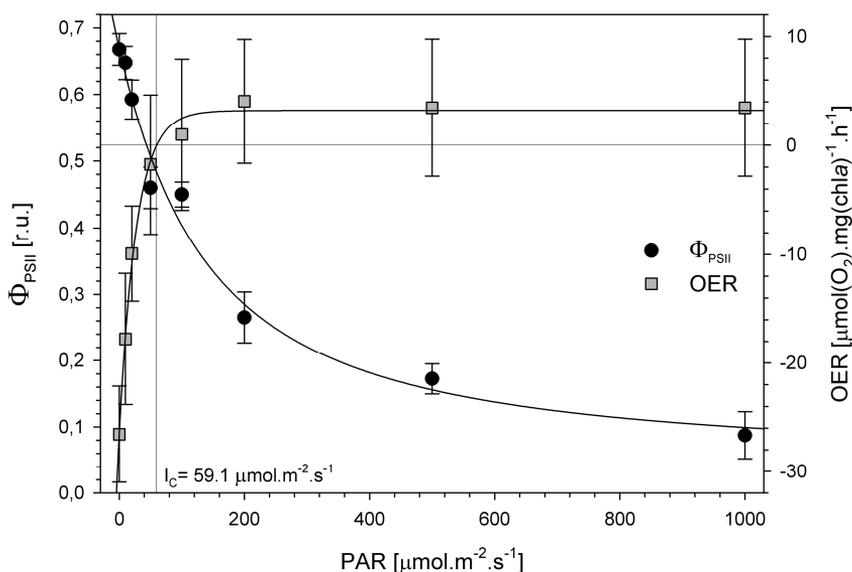
**Fig. 1.** Scheme of the instrumental setup consisting of a fluorometer, microoptodes linked to a PC.

## Results and Discussion

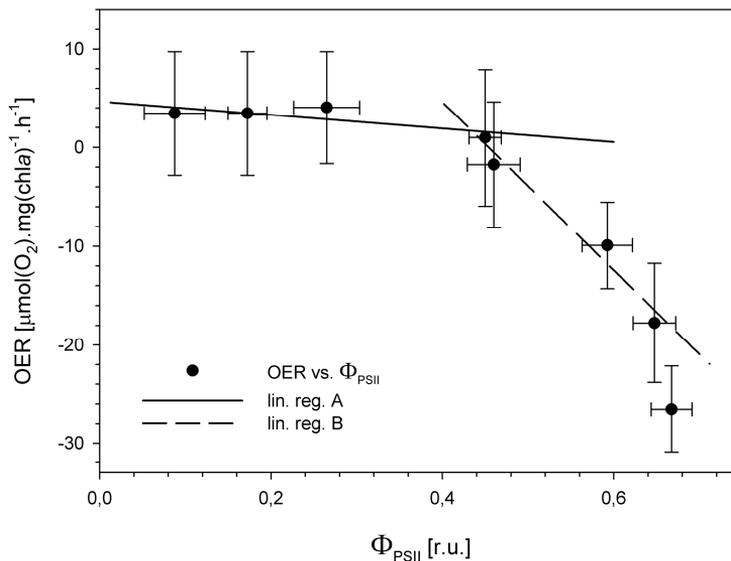
The effective quantum yield of PS II photochemistry –  $\Phi_{\text{PSII}}$  determined at the end of each particular PAR levels (Fig. 2) reached the values from 0.088 ( $\pm 0.036$ ) at maximal PAR level of  $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  to its maximum of 0.668 ( $\pm 0.024$ ) reached in the dark. Within the range of PAR levels,  $\Phi_{\text{PSII}}$  showed an exponential decrease (from its maximum at lowest PAR to a minimum at the highest PAR). The minimal value of oxygen evolution rate (OER; Fig. 2) of  $-26.56 (\pm 4.43) \mu\text{mol}(\text{O}_2)\cdot\text{mg}(\text{chl}a)^{-1}\cdot\text{h}^{-1}$  was reached in the dark and its maximum of  $4.04 (\pm 5.68) \mu\text{mol}(\text{O}_2)\cdot\text{mg}(\text{chl}a)^{-1}\cdot\text{h}^{-1}$  was found at PAR level of  $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The irradiance compensation point derived from the OER data (Fig. 2) was  $59.1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The OER values recorded at higher PAR levels ( $>200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) did not show any additional increase (plateau or limited slight decrease).

Even under optimum temperature, polar phytoplankton communities (cyanobacterial particularly) exhibit extremely low photosynthetic rate (Fritsen et Priscu 1998). However, “fast-growing” algal and cyanobacterial species, e.g. *Chlorella*, and *Synechocystis* showed maximal values of OER more than 10-times higher (Schuurmans et al. 2015) than OER in *Stigeoclonium* sp. reported in this study.

In this experiment, the analysis of the relationship between  $\Phi_{\text{PSII}}$  and OER showed non-linearity within the whole PAR range (Fig. 3). However, if the analysis of the relationship is done separately for lower (from 0 to  $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and higher (from 200 to  $1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) PAR levels, then linearity of the relationship in this two particular ranges was found. Genty et al. (1989) showed a linear relationship between fluorescence derived photosynthetic rate and directly measured for higher plants. In cyanobacterial blooms, a linear relationship can be seen between fluorescence-based ETR and photosynthetic  $\text{O}_2$  evolution at PAR up to  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . However, a non-linear relationship of ETR to  $\text{O}_2$  evolution at the PAR levels above  $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (Masojidek et al. 2001). Similarly, Beer et Axelsson (2004) found a linear relationship between  $\text{O}_2$  evolution and fluorescence-based ETR at low irradiances, however, at high irradiances, there was a decrease in ETR while OER remains almost constant. Nonlinearity of ETR and OER relationship in high PAR is generally attributed to the secondary photosynthetic processes, i.e. oxygen-consuming processes (photorespiration, Mehler reaction) and documented in algae (see e.g. Masojidek et al. 2001).



**Fig. 2.** Light response curves of effective quantum yield of photochemistry of PSII ( $\Phi_{\text{PSII}}$ ; black symbols) and oxygen evolution rate (OER; grey squares), measured at a stepwise increasing photosynthetically active radiation (PAR). Data points are means of 5 replicates  $\pm$  standard deviation. The location of light compensation point ( $I_c$ ) is indicated. The best-fit curves equations are: ( $\Phi_{\text{PSII}}$ )  $y = 0.042 x e^{(1237.735/(x+448.132))}$ , (OER)  $y = 29.706 x (1 - e^{-0.037x})$ .



**Fig. 3.** Oxygen evolution rate (OER) of *Stigeoclonium* sp. as dependent on the effective quantum yield of photosynthetic processes in photosystem II ( $\Phi_{\text{PSII}}$ ) recorded for PAR ranging 0-1000  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ . Linear regression A ( $y = -6.877x + 4.657$ ) is for the data recorded for the PAR interval of 0 – 200  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ . Linear regression B ( $y = -85.088x + 38.617$ ) is for the data recorded within the PAR range of 200 – 1000  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ . Data points represent means of 5 replicates  $\pm$  standard deviations.

Non-linearity of the relation between photochemical and biochemical processes measured within a range of PAR is well documented for sea macroalgae (Figueroa et al. 2003). The authors reported non-linear relation between the effective quantum

yield ( $\Phi_{\text{PSII}}$ ) and the quantum yield of photosynthetic  $\text{O}_2$  evolution ( $\Phi_{\text{O}_2}$ ). If the  $\Phi_{\text{O}_2}$  is calculated from the data presented here for *Stigeoclonium* sp., they give non-linear relationship to  $\Phi_{\text{PSII}}$  (not shown) as well.

## Concluding remarks

The method of simultaneous measurements of OER and  $\Phi_{\text{PSII}}$  used in this study proved to be an useful tool for detailed photosynthetic study in algal suspension. Such combination of the two methods allows an analysis of the photosynthetic processes, the relation of photochemical and biochemical processes of photosynthesis in particular. Moreover, the combination of the methods might be used in the field and semi-field conditions, since a battery-operated in-

strument, as used in this study, provides an opportunity for complex measurements performed in the field (Antarctic station). Such approach is helpful to define the differences between the samples collected in the field in the environments with natural variation of physical and chemical factors and those samples cultivated in the lab and/or collection of polar autotrophic microorganisms.

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