PHOTOSYNTHETICA 45 (4): 562-567, 2007

Delayed fluorescence as a direct indicator of diurnal variation in quantum and radiant energy utilization efficiencies of phytoplankton

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Abstract

We compared delayed fluorescence (DF) excitation spectrometry with radiocarbon (^{14}C) technique using a monoalgal culture of *Chlorella vulgaris* grown under natural temperature and irradiance. This was done by monitoring the DF, in parallel to quantum efficiency (QE) and index of radiant energy utilization efficiency (Ψ) as calculated on the basis of carbon uptake measurements by radiocarbon technique. During the diurnal cycle, temperature, irradiance, and chlorophyll (Chl) contents were monitored in the algal culture that was kept in an open transparent plastic tank submerged at the surface of Lake Kinneret, Israel. The DF signal correlated with both the QE ($r^2 = 0.869$, $p<0.01$) and Ψ ($r^2 = 0.977$, $p<0.01$) during a diurnal cycle. We suggest that, besides the measurement of active Chl and phytoplankton population composition, the DF signal provides additional information on the QE and Ψ in phytoplankton population.

Additional key words: *Chlorella*; chlorophyll; index of radiant energy utilization efficiency (Ψ); photosynthesis.

Introduction

Delayed fluorescence (DF) is a unique characteristic of photosynthetically active cells, first described by Strehler and Arnold (1951). The DF signal is emitted after milliseconds upon the transfer of plant cells from light to dark following a non-exponential decay curve of several minutes until charge equilibrium between the donor and acceptor side of the thylakoid membrane is reached (Gerhardt *et al*. 1981). The excitation spectra of DF depend on pigmentation of the examined cells (Arnold and Davidson 1953, Arnold and Thompson 1955), and as such may be used for discrimination between different algal groups, and following mathematical manipulation may be used for the estimation of the contribution of different colour groups to photosynthetic community (Gerhardt and Bodemer 2000). Comparisons between DF spectrometry and conventional methods such as biomass, chlorophyll (Chl) *a* content, and microscopic enumeration have shown that the DF spectrometry is a reliable method and can be used as a very sensitive, non-destructive effective monitoring tool to determine active Chl contents and phytoplankton composition (Friedrich *et al*. 1998, Wiltshire *et al*. 1998, Yacobi *et al*. 1998, Bodemer *et al*. 2000, Gerhardt *et al*. 2005). The intensity of the DF signal is affected by several factors including the efficiency with which the algae use radiation of the specific wavelength for charge separation at photosystem 2 (PS2) (Gerhardt and Bodemer 2000), the irradiation history the cells experienced (Honti *et al*. 2005, Istvanovics *et al*. 2005), the cellular content and package of Chl (Monti *et al*. 2005), and is also dependent on temperature (Wang *et al*. 2004, Zrimec *et al*. 2005) and nutritional status (Mellvig and Tillberg 1986).

Monitoring pulse amplitude modulated (PAM) fluorescence and DF intensity during several diurnal cycles in a *Chlorella* mono-culture grown under natural irradiation revealed a high correlation between the DF signal and the effective quantum yield of PS2 (Kurzbaum, unpublished). This result suggests DF intensity as a proxy for measurements of electron transport rates and photosynthetic rates. In order to test this hypothesis we compared the diurnal variation of DF intensity with radiocarbon based determination of photosynthetic rate and the derived parameters of quantum efficiency (QE) and index of radiant energy utilization efficiency (Ψ). QE and Ψ have been introduced as ecological and physiological parameters used to determine the

Received 30 January 2007, *accepted* 28 June 2007.
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Acknowledgements: This work was supported by the "Yoachai Ben Nun scholarship" from the Israel Oceanographic and Limnological Research Ltd. Technical assistance by V. Gerhardt and U. Bodemer is gratefully acknowledged. We acknowledge two anonymous reviewers whose remarks helped improving the clarity and quality of the presentation.

energy transfer efficiency in ecosystems (Odum 1971, Falkowski and Raven 1997). QE and Ψ were measured by the ^{14}C technique in micro-algal cultures and in phytoplankton, and indicated dependence on the taxonomic affiliation of the examined organism(s), as well as on the environmental conditions, like irradiation history and nutrient availability (Dubinsky 1980, Falkowski 1981, Kishino *et al*. 1986).

Materials and methods

Algae and culture conditions: We used the unicellular chlorophyte *Chlorella vulgaris* (isolated from Lake Kinneret and kept in the culture collection of the Kinneret Limnological Laboratory, Israel). *Ch. vulgaris* was maintained in the laboratory in small Erlenmeyer flasks and grown on SCM medium (Moss 1972), under irradiance of *ca*. 30 μmol(quantum) m^{-2} s^{-1} and temperature of 17±1 ºC. For the field experiment, a fresh culture was placed in the experimental tank 3 d before the measurements began, thus letting the culture adjust to ambient irradiation and temperature. The algal culture was maintained in a submerged 18 000 cm^3 cylindrical transparent plastic tank with its upper part open to the atmosphere. A small aquarium pump ascertained homogeneity of the culture. The algae were grown in 16 000 cm^3 of SCM mineral medium, and exposed to natural irradiation and temperature that ranged between 20 ºC (early in the morning) and 25 ºC (at noon).

DF signal and spectrum of the *Chlorella* population from the experimental tank was measured over a daily cycle using a custom-made DF spectrometer as described in Gerhardt and Bodemer (2000). The range of the excitation spectrum was set to 400–730 nm, and measurements were done in triplicates. 600 cm^3 of cell suspension were used for each measurement, and after measurement, the sample was recycled to the experiment tank.

The total DF emission intensity of the samples was calculated from the sum of the DF intensity counts as integrated over the excitation wavelength range. Normalised DF intensity was calculated as DF intensity [counts per s] divided by Chl content [kg m^{-3}], and defined in relative units. Normalization was done to compensate for the changes in Chl content of the sample in order to compare the daily DF pattern with Ψ of the phytoplankton population.

Photosynthetic rate, quantum efficiency (QE), and Ψ measurements: The *Chlorella* culture was sampled 7 times from 06:00 to 17:15. Triplicate 50 cm^3 subsamples were taken from the experimental tank and transferred immediately to 60 cm^3 polycarbonate bottles for the measurement of carbon uptake with a modified version of the ^{14}C technique (Steemann-Nielsen 1952, Berman and Pollingher 1974). A spike of ~3×10^5 Bq of ^{14}C-bicarbonate was added to each bottle. The bottles were then placed back in the experimental tank, and after an incubation time of 30 min filtered onto poly-acetate membrane filters (diameter 25 mm, pore size 0.45 μm, *Whatman*) under a mild vacuum (~100 mg Hg), rinsed with filtered lake water, and left overnight in the presence of HCl vapour to eliminate any remaining traces of inorganic ^{14}C. Control samples poisoned by Lugol's solution were run in parallel during each incubation, to compensate for non-biological absorption to filters. The total added ^{14}C was checked for each sampling series by counting 0.1 cm^3 portions withdrawn directly from each of the incubated bottles. Total radioactivity in the particulate fraction retained on the filters was determined by liquid scintillation with quench correction on a *Kontron MR-300* liquid scintillation counter. Photosynthetic rate P [mg(C) m^{-3} h^{-1}] was calculated for the triplicate measurements and averaged for each incubation interval. The average difference between replicates was 3.4 %.

QE was calculated as:

$$QE = P\ PAR^{-1}\ [\mu mol(C)\ \mu mol^{-1}(quantum)] \qquad (1)$$

where PAR = photosynthetically active radiation (400–700 nm) measured during the incubation period [μmol(quantum) m^{-2} s^{-1}] (Dubinsky 1980).

Ψ was calculated following Falkowski (1981):

$$\Psi = P\ (Chl\times PAR)^{-1}\ [mg(C)\ mg^{-1}(Chl)\ mol^{-1}(quantum)\ m^{-2}] \qquad (2)$$

where Chl is the content of Chl *a*.

Chl *a* content was measured by filtration of 5 cm^3 water samples onto glass-fibre filters (*Whatman GF/C*), ground in 90 % acetone, and left overnight at 4 ºC in the dark. Chl content was determined fluorometrically (Holm-Hansen *et al*. 1965), following clarification of the extract by centrifugation for 3 min at 1 100×*g*.

PAR measurements and pH control: *In situ* PAR was measured at the surface of the culture's liquid using *192 A Li-Cor* quantum sensor (*Li-Cor*, USA). Measurements of pH were done using a pH electrode (*WTW*, Germany). In order to prevent carbon limitations during the day, the maximal pH of the cultures was adjusted not to exceed 8 by automatic addition of CO_2 using an automatic control system (*Dr. Kuntze*, Germany).

Results

The daily changes in QE (as measured by the radiocarbon technique) and the total (Chl normalised) DF emission intensity followed the same trend with high values during morning and evening and minima at noon (Fig. 1). The two parameters were inversely related with the incident PAR, with high values at 06:00 when DF intensity was 1 481 counts per s, and QE was 0.095 4 μmol(C) μmol^{-1} (quantum). Later on throughout the day, DF and QE declined until noon, when the PAR reached its daily maximum of ~1 900 μmol(quantum) m^{-2} s^{-1}. From noon till dusk the DF intensity and QE increased with the decline in irradiance, reaching their daily maxima when DF intensity was 2 194 counts per s and QE was 0.121 μmol(C) μmol^{-1}(quantum). The apparent similarity of the DF intensity and the QE patterns was confirmed by the high correlation between both parameters (Fig. 2; $r^2 = 0.86$, $n = 7$, $p<0.01$).

The concomitant decline of DF intensity and QE in maximum values between 11:00 and 16:00, and minimum values during morning and evening hours, when PAR was low. Chl content increased rapidly during the day and especially from noon to 15:00. Altogether, Chl contents tripled from morning measurement with 275 mg m^{-3} at 06:00 to evening measurement with 913 mg m^{-3} at 17:15 (Fig. 1).

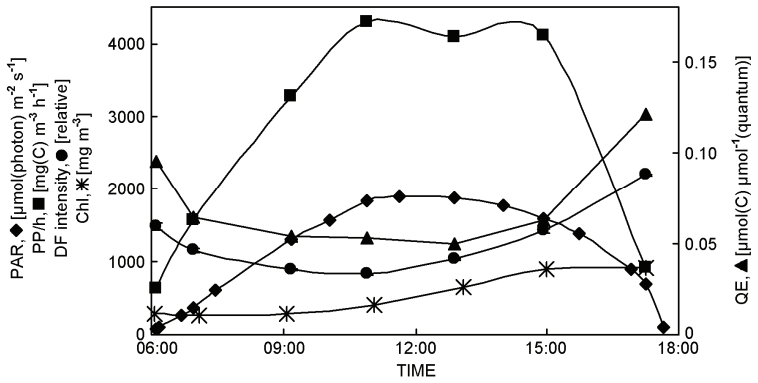

Fig. 1. Diurnal patterns of incident PAR (*diamonds*), primary production (PP/h) (*squares*), quantum efficiency (QE) (*triangles*), DF intensity (*circles*), and chlorophyll (Chl) content (*asterisks*) for *Chlorella vulgaris* growing in the experimental tank.

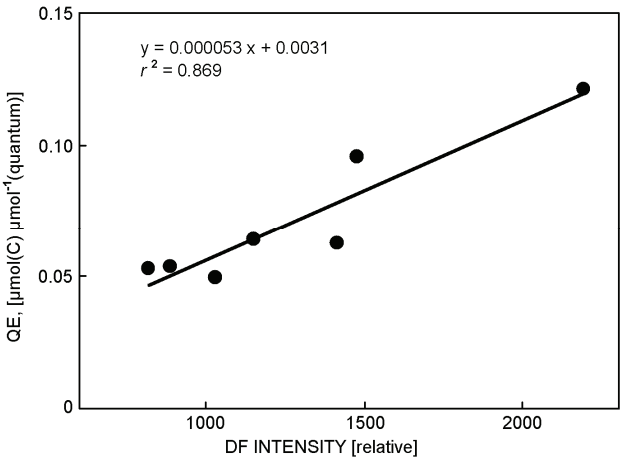

Fig. 2. Relationships between quantum efficiency (QE) and DF intensity ($r^2 = 0.869$, $p<0.01$, $n = 7$).

The daily fluctuations in Ψ paralleled those of the normalised DF signal (Chl normalised) (Fig. 3). Similarly to QE and DF intensity, the Ψ and DF normalised intensity co-varied in a reverse relation with PAR, with high values at 06:00 when DF normalised intensity was 5.385 and Ψ was 4.2 g(C) kg^{-1}(Chl) mol^{-1}(quantum) m^{-2}. Later, the two parameters declined steadily until 15:00. From 15:00 till dusk the normalised intensity and Ψ increased with the decline in irradiance to a value that was approximately half of that measured during early morning [2.400 DF normalised intensity and 1.5 g(C) kg^{-1}(Chl) mol^{-1}(quantum) m^{-2}, respectively]. The apparent similarity of the DF normalised intensity and the Ψ patterns was confirmed by the high correlation between the two variables (Fig. 4; $r^2 = 0.97$, $n = 7$; $p<0.01$).

Discussion

DF intensity displayed a diurnal change that was highly correlated to QE and Ψ in *Chlorella* culture. The DF signal represents QE and Ψ in the culture, and is not a direct indication for carbon uptake rate, but the variable can easily be calculated by measurement of PAR. In addition, an experiment using pulse amplitude modulated (PAM) fluorometry showed that the DF signal was highly correlated to the effective quantum yield of PS2 (Kurzbaum, unpublished). The temporal variability of the DF signal is apparently not related to change of biomass, as measured in this study by Chl content, but reflects short-term variations in the quantum yield of the photosynthetic

apparatus, which in turn reflects the flux of solar energy to which the cell suspension was exposed to. *In situ* variation of quantum yield has been extensively studied with the implementation of prompt fluorescence-based techniques (Schreiber 1986, Kolber and Falkowski 1993, Kolber *et al*. 1998, Torsten *et al*. 2005), and the mechanisms underlying the relationship between irradiance and photosynthetic parameters were elucidated (Kroon 1994, Masojídek *et al*. 1999, Suggett *et al*. 2003). Diurnal changes in biomass-normalized signal intensity of prompt fluorescence are well documented, and attributed to the capacity of the photosynthetic mechanism to channel radiant energy between photochemical and non-photochemical pathways (Kolber and Falkowski 1993, Falkowski and Raven 1997). Kroon (1994) showed that diurnal variation in photosynthetic activity can be explained by the rate of formation of active PS2 units, which in turn are dependent on the formation of a complex between the D1 protein and the electron acceptor Q_B. When excessive radiant energy is provided, the formation of the complex is impaired, and rate of successful electron transfer declines. Thus, considering that DF intensity correlated with the effective quantum yield of PS2 (Kurzbaum., unpublished), although that relationship is statistical, we assume that the rate of D1-Q_B coupling/decoupling is a plausible explanation to account for the relationship of DF and photosynthetic efficiency, since DF intensity is dependent on the rate of electron transfer through PS2 prior the timing of measurement.

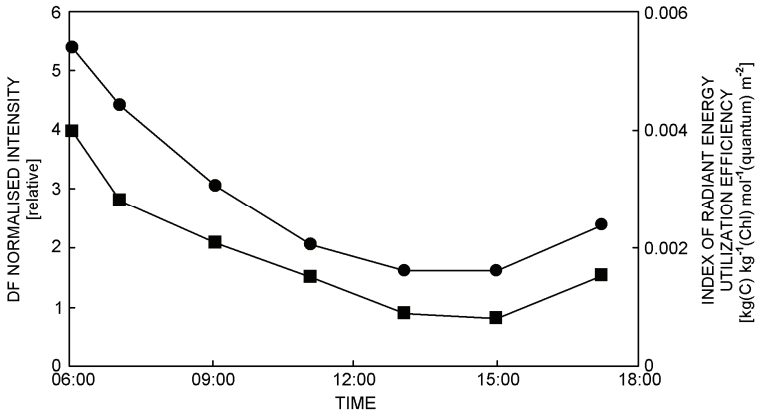

Fig. 3. The diurnal pattern of the index of radiant energy utilization efficiency (Ψ) (*squares*) and DF normalised intensity (*circles*) for *Chlorella vulgaris* growing in the experimental tank. Measurements were done over the course of the same diurnal cycle as in Fig. 1.

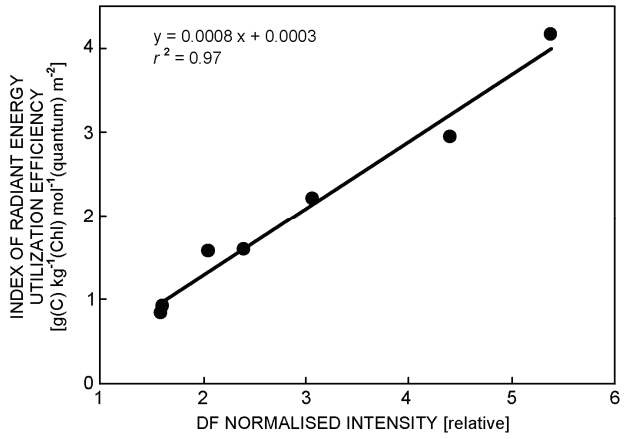

Fig. 4. Relationships between the index of radiant energy utilization efficiency (Ψ) and DF normalised intensity ($r^2 = 0.977$, $p<0.01$, $n = 7$).

Similar diurnal changes of DF intensity signal in *Chlorella* to the current report were described in cultures (Bodemer 2002) and in a natural setting (Istvanovics *et al*. 2005). However, none of mentioned studies with algal cultures and natural populations provided a direct comparison between the DF data with a direct measurement of the fixation of inorganic carbon, and eventually the formation of photosynthetic products, or QE and Ψ parameters. A direct comparison between DF intensity and carbon dioxide uptake was done in higher plants, which showed that the two variables were highly correlated (Wang *et al*. 2004).

Although still based on custom designed instruments, the DF spectrometry has been used so far for diverse assignments. It includes estimation of the contribution of different colour groups of phytoplankton to phytoplankton biomass (Håkanson *et al*. 2003, Istvanovics *et al*. 2005), herbicide toxicology (Katsumata *et al*. 2006), and grazing efficiency (Naddafi *et al*. 2007). Since the intensity of the DF signal depends on the mechanical setup of the detector, the resulting output can not be directly interpreted in terms of electron flow intensity. On top of the matter of the relationship between the intensity of DF and the actual rate of electron flow, an intrinsic handicap of DF spectrometry is the variable, circumstances-dependent relationship between the fluorescence signal and biomass, most often measured as content of Chl. That handicap is common to techniques based on fluorescence record, starting with simple *in vivo* fluorometry (Lorenzen 1967), up to the state-of-the-art technologies fast-repetition-rate-fluorescence (Greene *et al*. 1994) or PAM (Torsten *et al*. 2005). Therefore, fluorescence-based instruments should be calibrated for given compositions of the phytoplankton, under different

environmental conditions. In that respect the DF spectrometer we used in the current study is a convenient instrument, as it excites cells throughout the whole range of 400–750 nm. Thus, it provides an option for calibration for algal groups, which spectrally are fairly similar, but not identical, *e.g*. diatoms *versus* dinoflagellates, or cyanophytes with variable relationships between phycocyanin and phycoerythrin (Bodemer 2004). Conversion of the DF intensity to terms of primary productivity is rather a straightforward issue, once the irradiation climate is determined, and it can be easily achieved by measurement of the solar input and radiation attenuation within the water column. That experimental evidence implies that the DF signal should be cautiously interpreted when applied for estimation of Chl content, as the yield of the signal depends on the environment where the test operates. Istvanovics *et al*. (2005) suggested, therefore, normalizing all measurements to first diurnal readings, in order to monitor phytoplankton population dynamics, to avoid errors originating in the variability of DF/Chl and the attenuation in the daily cycle PAR.

DF is a sensitive fluorometric method that does not require preparatory steps prior application, and is suitable for continuous and on-line monitoring of natural phytoplankton population. In addition, our work suggests that the built-in capacity of the DF spectrometer to identify the contribution of different colour algal groups to the bulk of photosynthetic activity of a given natural sample confers the advantage of indication of photosynthetic efficiency parameters in relation to the composition of phytoplankton. Calibrated for different environmental scenarios, the DF spectrometer is therefore a potential tool for monitoring of QE and Ψ, phytoplankton concentration, and population composition in natural aquatic habitats, and as such joins other Chl-fluorescence-based technologies currently available for *in situ* studies of phytoplankton (Wilhelm *et al*. 2004). Common with other fluorescence-based technologies, DF circumvents the need to use bottle incubations for the estimation of primary productivity, and enables frequent and continuous sampling. The efficient measurement of QE and Ψ by the DF spectrometer at a time resolution of several minutes carries the potential to become an environmental friendly powerful tool for study of phytoplankton populations.

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