ESTIMATION OF PERIPHYTIC PHOTOSYNTHESIS EFFICIENCY USING THE FLUORESCENCE MONITORING SYSTEM : RESPONSE OF FMS PARAMETERS ON LIGHT INTENSITY, TEMPERATURE, AND CHLOROPHYLL CONCENTRATIONS

Nofdianto[a]

[a] ***Staf Peneliti Puslit Limnologi-LIPI***

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ABSTRACT

The development of methods in measuring the photosynthesis process is now increasingly widespread, especially to get a more efficient method and fast. Photosynthetic efficiency of micro-algae periphytic has been estimated under the influence of light intensity and temperature by using the fluorescence monitoring system. The measurement on a colonized substrate by the saturation pulse method has been conducted using a FMS1 (Fluorescence Monitoring Systems, Hansatech). Measurement of the fluorescence parameters was conducted every week on algal periphyton which was cultivated on the artificial substrate during for 5 weeks under light and temperature conditions. The result show that fluorescence maximal value (Fm) increase linearly with chlorophyll a concentrations. For 20°C (experiments where the biomass reached higher values), up to 100 mg chlorophyll a.m^{-2} the change in Fm is approximately linear. After, the response of Fm is hyperbolic, suggesting a saturation law. Our results clearly show that the FMS may be satisfactorily used to measure in vivo chlorophyll a fluorescence of periphyton in laboratory conditions. The instrument is simple to use, with convenient software control, especially when used in PC mode.

Key words : Photosynthesis, micro-algae periphytic, chlorophyll fluorescence, fluorescence monitoring systems,

ABSTRAK

Estimasi efisiensi fotosintesis perifiton dengan menggunakan Monitoring System Fluorescence. Perkembangan metode dalam mengukur proses fotosintesis kini semakin meluas, terutama untuk mendapatkan metode yang lebih efisien dan cepat. Efisiensi fotosintesis perifiton diperkirakan di bawah pengaruh intensitas cahaya dan suhu dengan menggunakan sistem pemantauan fluoresensi. Pengukuran pada substrat dengan metode pulsa jenuh telah dilakukan menggunakan FMS1 (Sistem Monitoring Fluoresensi, Hansatech). *Pengukuran parameter fluoresensi dilakukan setiap minggu pada alga perifiton yang dibudidayakan pada substrat buatan selama 5 minggu dalam kondisi cahaya dan suhu terkontrol. Hasil penelitian menunjukkan bahwa fluoresensi nilai maksimal (Fm) meningkat secara linear dengan konsentrasi klorofil a. Untuk 20 ° C (percobaan di mana biomassa mencapai nilai yang lebih tinggi), hingga 100 mg klorofil per meter persegi perubahan Fm adalah cenderung linier. Setelah itu, respon Fm adalah hiperbolik, menunjukkan hukum kejenuhan. Hasil kami jelas menunjukkan bahwa FMS dapat digunakan efektif dan cepat untuk mengukur fluoresensi klorofil in vivo perifiton dalam kondisi laboratorium. Instrumen ini lebih mudah digunakan, dengan perangkat lunak kontrol yang nyaman, terutama bila digunakan dalam mode PC.*

Kata kunci : Fotosintesis, mikro-alga perifiton, fluoresens klorofil, sistem monitoring fluoresensi

INTRODUCTION

The development of methods in measuring the photosynthesis efficiency is now increasingly widespread, especially to get a more efficient method and fast. Especially for low-level plants such as microalgae periphytic, estimating the efficiency of photosynthesis under the influence of light intensity and temperature by using the fluorescence monitoring system is still very little its information. In this study focused on the influence of photosynthetic ornaments such as chlorophyll a, light intensity, and temperature on the parameters fluoresces.

When a light energy is absorbed by a chlorophyll molecule, the electronic configuration of the molecule is temporarily altered. This "excited" configuration is inherently unstable and of short duration (generally less than 10^{-8} seconds) because several processes compete to dissipate the absorbed energy. In the photosynthetic systems, these processes can be categorized in two groups: a). The photochemical processes which use the energy absorptive for photochemistry during which the electrons are transferred from the pigments to a molecule. These processes direct energy intended for photosynthetic chemical "work". b). The non-photochemical processes dissipate the energy of the photosynthetic apparatus so that it does not lead to photosynthesis: the energy, usually re-emitted as infra red (heat) and red radiation, is called chlorophyllian fluorescence. The FMS (Fluorescence Monitoring System) which allows measurement of the Fo, Fm, and Fv/Fm parameters concerning photosynthesis was used to measure the activity photosynthetic of the microalgae *in situ* (Falkowski & Kiefer, 1985; Topliss & Platt, 1986; Kiefer *et al*., 1989).

Fo is defined as the output of fluorescence following dark adaptation of the sample when all the reactive centers of the PS-II and all the molecules accepting electrons are oxidized and completely opened to photo-chemistry. The maximum production of fluorescence (Fm) is reached when algae periphytic adapted to the darkness, are exposed to an intense peak of saturating light. It temporarily reduces all the electrons acceptors of the PS-II. The temporary absence of competition to photochemistry for absorptive energy stimulates a maximal emission of fluorescence of the sample. The difference between Fo and Fm is defined as a variable fluorescence (Fv) corresponding to the maximum capacity of photochemical capture of energy by the sample.

MATERIAL AND METHODS

Periphyton was grown on plexi-glass rectangular pilot with a total volume of about 3.6 liter. Pilots equipped with a pump to maintain water flow and ceramics as a substrate periphyton. Water media using ground water enriched with inorganic compounds (0115 $mg.l^{-1}$ NH4Cl, 0.022 $mg.l^{-1}$ KH_2PO_4, 0.0115 $mg.l^{-1}$ $NaNO_3$ and 0.018 $mg.l^{-1}$ O_3Na_2Si, $5H_2O$) and final pH 7. A three days acclimatization under a light-dark cycle (15/9 hours, ±50 µmol $m^{-2}s^{-1}$) and ±20 °C allowed an homogeneously culture on the tiles before starting the experiments. Then, the pilots were placed in a large temperature-regulated chamber, allowing simultaneous different light intensities from halogen lamp (PhytoClaude day-light, 400 watt). The pilots were arranged from light source to obtain irradiances of 15, 30, 100, 300, and 800 µmol $m^{-2}s^{-1}$ respectively. The experiment duration was 28 days long with a 15/9 light/dark cycle. At least three replications were done for each temperature (10, 20, and 35 °C). The light irradiance was measured with LI 1000 LI-COR sensor. During the experiments, water medium was periodically removed and replaced by new medium in the way to avoid nutrient limitation.

Measurement of chlorophyll fluorescence

The measurement on a colonized substrate by the saturation pulse method has been conducted using a FMS1 (Fluorescence Monitoring Systems, Hansatech). This instrument consists of a portable control unit containing a battery, electronics, hardware, optics and light sources, an optional PAR/temperature leaf clip for whole leaf measurement, and a fiber-optic probe that both delivers light, and collects fluorescence signal from the sample. Data acquisition and recording software allow two different operating modes ("PC", personal computer, or "local"). In this study, the PC mode was used.

Chlorophyll fluorescence was measured at room temperature with a FMS 1 control unit after the sample had been dark-adapted for 30 minutes. The FMS 1 was connected to a leaf-clip holder (modified in dark box) and to a computer (data acquisition software with Modfluor Windows® software package). The probe was placed close to the surface of the periphyton (10 millimeters) and measurements were automatically made by using the automatic data acquisition, where the data points corresponding to the fluorescence signal value (bits) are stored every 110 ms. After conversion from analogical to digital format, the fluorescence signal is plotted versus time and presented graphically immediately on the computer screen (Fig.1). All of the calculated parameters (Fo - Fluorescence level when sample dark-adapted and fully oxidized, Fv - variable fluorescence level (Fm - Fo), Fm - maximum fluorescence yield, and Fv/Fm - maximum quantum efficiency of PSII can be displayed on the "parameters window" and transferred into EXCEL format. Each measurement was repeated three times.

Measurement of the fluorescence parameters was conducted every week on algal periphyton which was cultivated on the artificial substrate during for 5 weeks under light and temperature conditions.

Results and Discussion

Figure 2 shows the correlations between chlorophyll *a* concentration and fluorescence parameters (Fv/Fm and Fm). The Fv/Fm ratio is not significantly correlated with the values of chlorophyll *a* at 10 and 20 °C but there is a negative relationship for 35 °C.

The fluorescence maximal value (Fm) increase linearly with chlorophyll *a* concentrations. For 20°C (experiments where the biomass reached higher values), up 100 mg chlorophyll $a.m^{-2}$ the change in Fm is approximately linear. After, the response of Fm is hyperbolic, suggesting a saturation law.

The evolution of the maximum quantum yield of PSII (Fv/Fm) varied ranged from 0.2 to 0.7. The average value of the Fv/Fm ratio slightly increased from 10 to 20 °C to drop at 35 °C (Fig. 3).

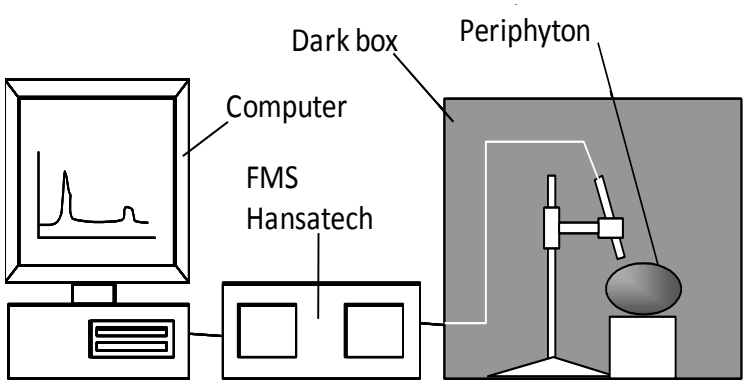

Figure 1. Instrumentation for Measuring the Chlorophyll Fluorescence.

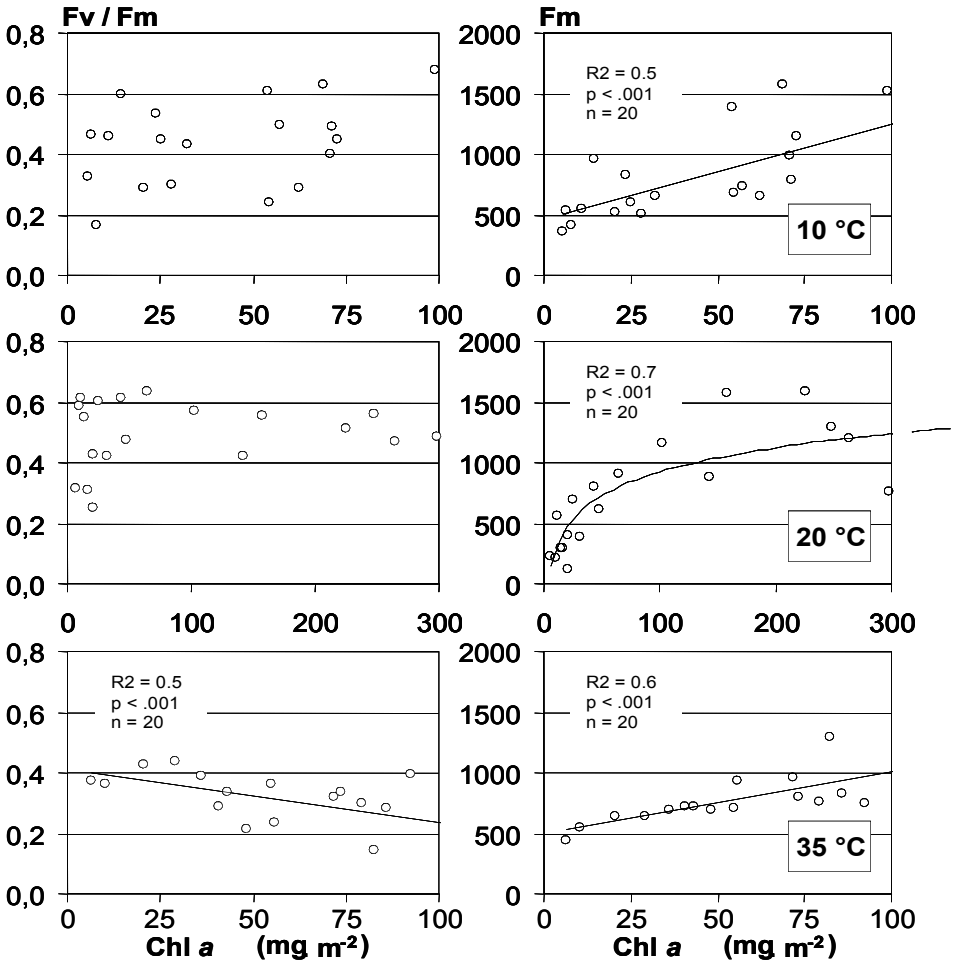

Figure 2. Correlation between Chlorophyll *a* Concentrations and Fluorescence Units (Fo/Fm, Fm) for Different Temperature Conditions of Culture.

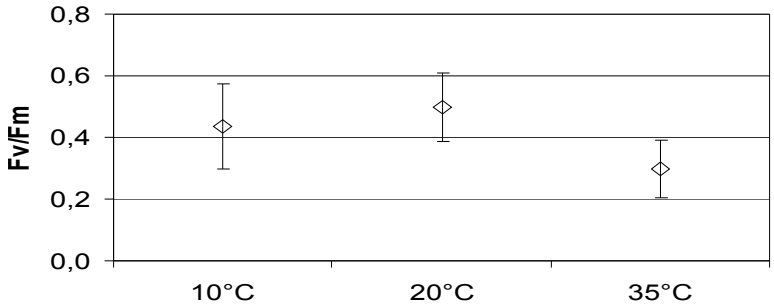

Figure 3. Variation of the Fv/Fm Ration Versus the Temperature Condition of the Culture of Periphyton.

The Fv/Fm ratio is slight correlated with irradiance light conditions of the culture (Fig. 4) : there is a decrease from very low light to higher light levels.

The maximum quantum yield of PSII, usually expressed as Fv/Fm unit is given by the equation: Fv/Fm=(Fm–Fo)/ Fm. A change in Fv/Fm is due to a change in

the efficiency of non-photochemical quenching. Dark adapted values of Fv/Fm reflect the potential quantum efficiency of PSII and are used as a sensitive indicator of plant photosynthesis performance, with optimal values of around 0.8 measured for most plant species (Bjorkman and Demmig, 1987; Johnson *et al*., 1993). The observed values in this study are commonly lower than the theoretical value : here, the Fluorimeter Monitor System, and especially the sensor is not used in the conditions as planned for this kind of device (generally, fluorescence response is obtained from a leaf measurement).

The measure made with Fv/Fm ratio appears to indicate, not a quantitative response of the photosynthesis rate as a function of chlorophyll *a* content (as it is the case for Fm) but the ability for the photosynthetic system to operate.

The results of this experiment showed that the Fluorescence Monitoring System (FMS) as a tool in quantifying the fluorescence of chlorophyll in vivo in algal periphyton can be used in laboratory conditions. The instrument is simple to use, with convenient software control, especially when used in PC mode.

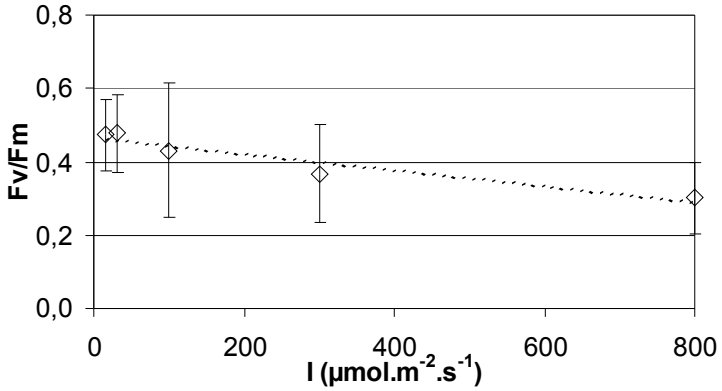

Figure 4. Evolution of the Fv/Fm Unit, which Observed on the Treated Periphyton Under Various Light Intensities.

Fm unit is not commonly used as fluorescence parameter (see Maxwell & Jonson, 2000) : the study showed that the variations of the values were significantly correlated with increasing chlorophyll *a* concentrations per surface unit.

However, the variations of the Fv/Fm ratio appeared to be quite biomass-independent. This means that for Fm and Fv/Fm parameters, the response is complex, related to chlorophyll *a* concentration, but also to light scattering and re-absorption phenomenon (Ting & Owens, (1992; Büchel & Wilhelm, 1993), to temperature and the type of box containing the algae (Mouget & Tremblin, 2002).

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