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Use of chlorophyll fluorescence to estimate the effect of photoinhibition in outdoor cultures of *Spirulina platensis* †

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Abstract

Chlorophyll fluorescence measurements were used to evaluate the effect of temperature on photoinhibition in *Spirulina platensis* cultures grown in tubular reactors outdoors. Cultures grown at 35 °C during the day time showed a lower reduction in the Fv/Fm ratio as compared to cultures grown at 25 °C. It is demonstrated that the lower temperature photoinhibited cells can undergo a complete recovery once transferred to low light and higher temperature. This recovery does not take place when 100 μg ml^{-1} chloramphenicol is added to cells. The recovery is light dependent and cells incubated in the dark at low temperature do not show a recovery in the Fv/Fm ratio. The data presented strongly support the hypothesis that photoinhibition takes place in outdoor *Spirulina* cultures. At the same time it is demonstrated that fluorescence measurements can be used as a fast reliable indication for photoinhibition in outdoor algal cultures.

Introduction

Photoinhibition has been studied intensively in microalgae and higher plants, mainly under laboratory conditions (Powles, 1984; Kyle & Ohad, 1986; Öquist, 1987). Photoadaption has also been studied in algal population, mainly in relation to its ecological impact i.e. appearance and dominance of specific algal strains in a given aquatic habitat (Neale, 1987).

The deleterious effect of photoinhibition on productivity of outdoor algal cultures was pointed out in many studies on a basis of estimation rather than on factual evidence. To the best of our knowledge, the only work demonstrating that photoinhibition does exist in dense outdoor cultures was recently reported by Vonshak and Guy (1992). An attempt was made to demonstrate that a reduction in photosynthetic activity observed in outdoor algal cultures is correlated with the high light intensity. Furthermore it was demonstrated that by protecting the cultures from this light stress one may even improve productivity of outdoor *Spirulina* cultures.

More detailed studies are required in order to better understand the role of photoinhibition in outdoor algal cultures. Quantitative estimation of the reduction in productivity due to light stress is of great importance. The lack of a reliable parameter to be used as an indicator for the degree of photoinhibition in outdoor cultures may be one of the reasons for the very few papers on this topic. Developing a simple and reliable measure of photoinhibition and being able to estimate the degree by which outdoor cultures are photoinhibit ed at a given time during the day will provide a useful information to the algal biomass producers. This information can be used not only for operational decisions,

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but also in order to compare and select strains of algae more adapted to high light stress.

Chlorophyll fluorescence is used in many laboratory studies as a tool to understand the basic process of photosynthesis as well as the effect of different environmental factors on the photosynthetic capacity (Lichtenthaler & Rinderle, 1988). The ratio of variable fluorescence (Fv) over maximal fluorescence (Fm) which is used as an indication of PS II activity was used to estimate photoinhibitory damage in higher plants (Ögren, 1991), as well as in cyanobacteria (Vonshak *et al.*, 1988). In the fast few years portable instruments were developed to be used as field instrument for assessment of plant stress or plant efficiency. Their principal of use and operation with higher plants was evaluated by Bolhár-Nordenkampf *et al.* (1989) In this work we have used two portable instruments to follow daily changes in the Fv/Fm ratio in outdoor *Spirulina* cultures grown in tubular reactors in order to evaluate the use of this parameter as an indicator for light stress and study the interaction of temperature with the light stress effects.

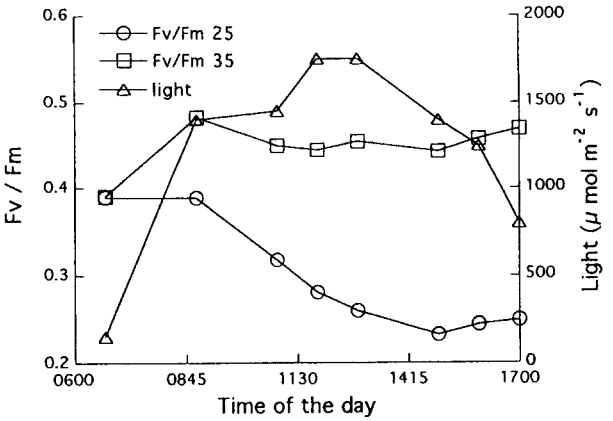

Fig. 1. Changes in Fv/Fm in two *Spirulina* cultures grown outdoors at 25 °C O—O, and 35 °C □ – □. The diurnal changes in light intensity (PAR) are indicated Δ – Δ.

Materials and methods

Organism and culture conditions

Spirulina platensis, strain M-2, of the Culture Collection of the Centro di Studio dei Microrganismi Autotrofi of Florence was used. It was grown in Zarrouk's medium, containing 200 mM sodium bicarbonate (Vonshak *et al.*, 1982). The pH was maintained at 9.4±0.2 by automatic addition of CO_2. The dissolved oxygen concentration was maintained within the range of 8–20 mg L^{-1} by bubbling nitrogen through porous candles in the culture as it flowed into the receiving vessel. The circulation speed of the culture was 0.46 m s^{-1}.

Culturing equipment

The design and description of the photobioreactors used have been described in detail elsewhere (Bocci *et al.*, 1987). The system was built and assembled by Carlo Erba Strumentation, Milan (Italy). Each reactor consists of loops made of ten parallel Pyres tubes (length 2 m, i.d. 4.85 cm) connected to PVC (polyvinylchloride) U-bend with watertight flanges. The reactors are placed in a stainless steel basin containing thermostated demineralized water. The culture is recycled by a PVC pump. The working volume of each reactor is 51 liters.

Analytical procedures

Dry weight was determined in duplicates using 10 ml samples. The procedure has been described elsewhere (Torzillo *et al.*, 1993). Light intensity was measured with a Quantum meter (Li-Cor Inc., model LI-185 B). Chlorophyll concentration was determined according to Bennet and Bogorad (1973).

Fluorescence measurements

Chlorophyll fluorescence was measured on algal samples withdrawn from the tubular reactors and incubated in the dark for 10–15 min, to allow full dark adaptation. Measurements were performed using two fluorometers: For the PEA (Hansatech, U.K.) 3 ml of culture was used and the liquid sample holder was used. For the PAM-2000 (H. Waltz, Germany) a 0.5–ml sample was used with the liquid cuvette type KS-101. Each measurement was done in triplicate.

Results and discussion

Spirulina cultures were grown in a semi-continuous mode by daily dilution to a constant concentration of 1.2 g L^{-1}. The temperature of the cultures was controlled so that one culture was kept at a constant temperature of 25 °C. The heating system in the second

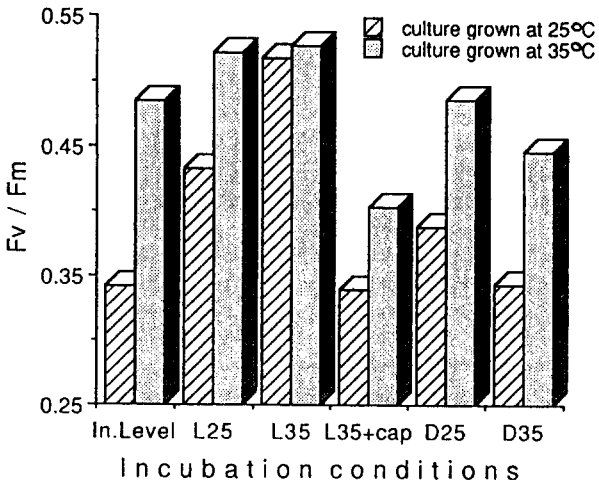

Fig. 2. Effect of different incubation conditions on the recovery of Fv/Fm values in *Spirulina* cultures grown outdoors. In levels-values Fv/Fm at zero time. L = incubation in light (20 μmol photon m^{-2} s^{-1}); D = incubation in dark; cap = 100 μg^{-1} ml of chloramphenicol was added. 25 and 35 indicate the incubation temperature; all samples were incubated for 2.5 h and then dark-adapted for 5 min and measured for Fv/Fm values.

reactor was set to start at sunrise (about 5:30 AM) till reaching 35 °C at about 8:30 AM. The daily changes in chlorophyll fluorescence and light intensity were followed. From Fig. 1 it can be seen that in early morning the Fv/Fm ratio is constant in the 25 °C cultures, while an increase in the ratio may be observed in the heated culture.

This slight increase is most likely due to the increase in temperature, which dropped during the night, when the heating was turned on a faster repair mechanism was enabled as it will be demonstrated later. After 8:30 AM, a further increase in light intensity resulted in a sharp decline in the PS II activity of the 25 °C culture as reflected in the decrease in Fv/Fm ratio. In the early afternoon this ratio was only 50% of that of the 35 °C grown culture. When samples were withdrawn later in the evening (data not shown) a recovery in this decline could be observed.

In order to further evaluate the phenomena of reduction in Fv/Fm as an indicative parameter of a photoinhibitory stress in outdoor grown cultures, early afternoon samples were incubated in the laboratory under different incubation conditions for 2.5 h and the ability to recover, as reflected in the increase in Fv/Fm ratio, was followed. In Fig. 2 it is clearly demonstrated that the PS II activity of the 25 °C grown culture is recovering in dim light.

As it may be expected, this recovery is much higher when cells are incubated at 35 °C. Incubating the cells in the dark or in the light with chloramphenicol prevents the recovery in the PS II activity. The fact that 35 °C grown cells incubated at 25 °C for 2.5 h do not show any significant reduction in the Fv/Fm ratio is providing further support to our assumption that indeed the reduction observed in Fv/Fm is a result of a photoinhibitory stress and not just an effect of temperature. In our *Spirulina* cultures, even when grown at the optimal temperature of 35 °C, the maximum Fv/Fm ratio measured was about 0.6 in full recovered culture. This is a relatively low value compared to the average Fv/Fm ratio of 0.8 found in higher plants. Fv/Fm ratio of 0.6 was also found by Falk *et al.* (1990) with the green alga *Chlamydomonas reinhardtii.*

The two instruments used for this study vary in their cost, measurement device and versatility. Nevertheless in performing the basic measurement of the chlorophyll fluorescence parameters of Fo, Fv and Fm they were very similar. The actual figures were very similar with differences of no more than 5%. Repetitions were very good with variation of less than 5%. The main differences were that the PEA uses a sample holder in which disposable plastic cuvettes are placed. The instrument is provided with a carousel for up to 16 samples. So measurements can be taken while other samples are dark adapted. Volume of samples depends on the chlorophyll concentration and one should not use less then 1 ml. The PAM instrument may provide many more parameters used in fluorescence studies such as quenching analysis yield etc. The sample is placed in a special liquid sample holder that has to be rinsed after each measurement. The fact that there is no barrier between the sample and the sensor may provide a higher sensitivity which is important in diluted cultures. 0.5 ml of culture was enough for the measurement. When used with its light guide and the liquid sample holder, this instrument is less portable for outdoor work.

The growing interest in algal biotechnology requires the application of a modern methodology in this field. Chlorophyll fluorescence measurements using one of the newly developed instruments may be one step forward in further developing this biotechnology.

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