Journal of Applied Phycology **1**: 131–135, 1989.

Use of a solid support in the study of photosynthetic activity of the cyanobacterium *Spirulina platensis*

Avigad Vonshak[1], Mirta Sivac[2] & David Walker[2]
[1]*Microalgal Biotechnology Laboratory, The Jacob Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Sede-Boker Campus, Israel 84993*; [2]*Research Institute for Photosynthesis, University of Sheffield, Sheffield S10 2TN, U.K.*

Accepted 6 January 1989

Key words: *Spirulina platensis*, cyanobacterium, photosynthetic activity, solid support system

Abstract

Oxygen evolution activity of *Spirulina platensis* cells ttached to nitro-cellulose filters or glass fiber filters (GF/C) was measured using the leaf disc electrode (LD-2 Hansatech Ltd, Kings Lynn, U.K.), originally designed for its use with leaves of higher plants. Measurements were performed in saturating (CO_2) as described previously for leaf discs and pieces. Photoinhibition could be induced in cells on the solid support as indicated by a significant increase in their quantum requirement (from 11 to 33 after 25 min exposure to a photon flux density of 2500 μE $m^{-2}s^{-1}$ and a smaller effect on the photosynthetic rate at light saturation. Photoinhibited cells showed recovery from the photoinhibitory treatment when illuminated under dim light.

Introduction

The response of photosynthesis to light can supply useful information about the nature of the photosynthetic process. In algae and higher plants the response is usually linear at low photon flux densities and saturates at higher levels, and healthy plants, i.e. those which have not been subjected to stress of any kind, display quantum requirements near the theoretical minimum (Bjorkman & Demmig, 1987). The use of the liquid phase electrode in the study of the photosynthetic activity of algal cultures is common practice today. However, some problems may arise when comparing quantum requirements of different algae or those of the same alga but obtained by different investigators. These problems are a consequence of differences in the light path of the measuring cell used, chlorophyll and cell concentrations, degree of mixing in the cell, total area illuminated, etc. and are inherent in the liquid system.

In the last ten years *Spirulina platensis* has been widely used as a model system for the outdoor cultivation of monoalgal biomass. The culture as a source of protein and chemicals has been growing in importance and has led to the setup of several commercial production sites all over the world, e.g. in Israel, Thailand, U.S.A., Taiwan (Vonshak & Richmond, 1988). This development has stimulated the study of basic aspects of photosynthesis in this cyanobacterium, and the search for early warning probes of stress and for screening techniques to select strains of better productivity (Vonshak, 1988; Vonshak & Guy, 1987).

In this work, algal cells were attached to a solid support in order to obtain a stable system that could then be studied using the leaf–disc electrode, originally designed for use with leaves. Photosynthetic activity, i.e. kinetics of oxygen evolution and the relationship between photosynthesis and light, and chlorophyll fluorescence emmission were studied in *P. platensis* on solid support and the effect of photoinhibitory PFD on that activity was examined.

Materials and methods

Plant material

Spirulina platensis was grown in batch cultures in Zarouk's medium using a rotary shaker. Temperature was 30 °C and PFD was 120 μE $m^{-2}s^{-1}$ (white) provided by incandescent lamp.

Algal cells on solid support

Several types of filters were tried, and the best results were obtained with the glass microfibre GF/C (Whatman) type filter. A volume of 30 to 100 ml culture sample (according to the chlorophyll concentration required) was filtrated through the GF/C filter of 50 mm in diameter placed on a 100-ml capacity filter holder (Sartorius SM16219), the vacuum was provided by a water suction system. The filter was then washed with 20 ml distilled water. A 10-cm^2 disc was cut and when it was not used immediately, it was place on a piece of wet styrofoam, in a covered petri dish. Chlorophyll was determined as described by Bennet and Bogorad (1973).

Measurements of oxygen evolution and chloropyll fluorescence

Photosynthetic oxygen evolution activity of *Spirulina platensis* cells attached to nitro-cellulose filters or glass fiber filters (GF/C) was measured using the leaf disc electrode (LD-2 Hansatech Ltd., Kings Lynn, U.K.) in saturating (CO_2) as described previously for leaf discs and pieces (Delieu & Walker, 1983; Walker *et al.*, 1983).

Light response curves of photosynthetic oxygen evolution were obtained using a high light intensity lamp (Bjorkman & Demmig, 1987). The cells were illuminated at each PFD for 60 s; PFD was varied by inserting neutral density filters between the light source and the cell (Walker & Osmond, 1986; Walker, 1987). Photosynthetic rates, quantum yield and quantum requirement were calculated by a computer (Walker, 1987) assuming that light absorption by the attached cells was 85%.

Chlorophyll fluorescence was measured at 740 nm using a fluorescence sensor (Hansatech Ltd, Kings Lynn, Northfolk, U.K.). Red actinic light (peak output 660 nm) was provided by an array of light emitting diodes.

Results and discussion

Three different types of filters were tested as a possible supports for *Spirulina* cells: nitro-cellulose, Whatman No. 1 and the glass fiber GF/C type. All three of them held the algal cells well and were non-toxic. The samples kept the initial activity for longer periods and were easier to handle when GF/C filters were used, probably because this filter has a high water absorbance. When activity was assayed every 3 h in samples on GF/C filters, which were otherwise kept under dim light, no significant decrease in activity could be observed up to 10 h from the time when cells were first attached to the filter (not illustrated).

Characteristic photosynthetic O_2 evolution and chlorophyll fluorescence kinetics displayed by *P. platensis* immobilized on a GF/C filter are demonstrated in Fig. 1. Respiration in the dark and photosynthetic O_2 evolution in the light can be measured in *Spirulina* in the same way as in leaf discs. It is worth noting that the characteristic oscillations in photosynthetic oxygen evolution and chlorophyll fluorescence displayed by leaves of many species were not observed (see also Sivak and Vonshak, 1988). The fluorescence kinetics displayed upon illumination and darkening, re-

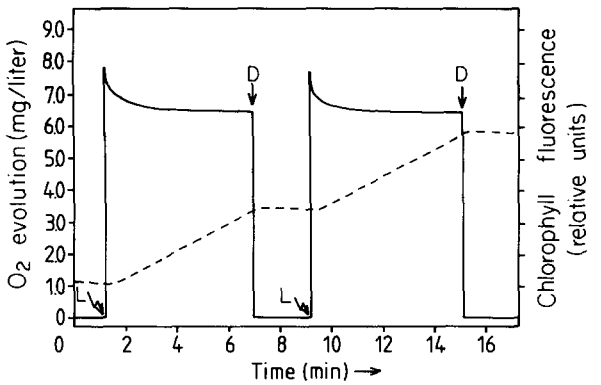

Fig. 1. Photosynthetic oxygen evolution (——) and chlorophyll fluorescence (– – –) emission by *Spirulina platensis* cells attached to glass fiber filters (GF/C), measured using the leaf disc electrode in high light intensity (red, 650 μE $m^{-2}s^{1}$) and saturating (CO_2).
L: Light on; D: Light off.

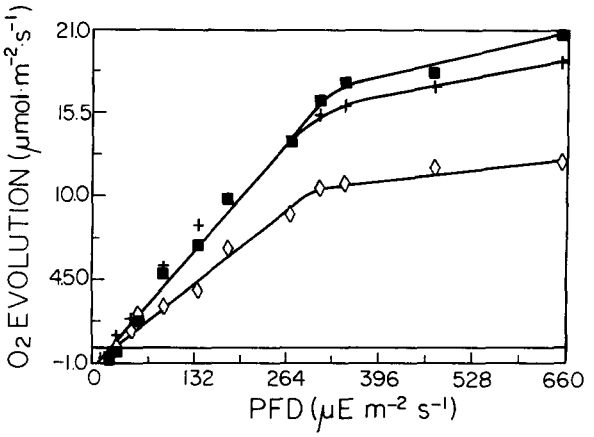

Fig. 2. Effect of cell concentration on the light response curves of Spirulina platensis cells attached to a GF/C filter. The amounts of chlorophyll per filter were 160 μg ◇------◇, 200 μg +------+, 240 μg ■------■.

sponse to light-intensity, kinetics of quenching components and F_v/F_o ratios were similar in cells in suspension and those on solid support, indicating that filtration and assay on filter did not affect to a significant extent the photosynthetic process. In the gas-phase the signal to noise ratio was better than in the stirred suspension, allowing a more precise determination of quenching components and a better definition of the kinetics (Sivak & Vonshak, 1988).

Light response curves were determined by measuring oxygen evolution at various light intensities (Fig. 2). The effect of the amount of cells in the sample (measured in chlorophyll units) was evaluted. The apparent quantum requirement was higher when a small amount of cells was used, probably because a larger proportion of the incident PFD was not absorbed by the cyanobacteria, but was rather reflected and/or transmitted. When the amount of cells (chlorophyll) was increased to 240 μg per filter, the apparent quantum requirement decreased to 13 (Fig. 2). Further increase in the amount of Chl per sample did not affect the quantum requirement, indicating that the amount of light absorbed did not increase any further; however, dark respiration and rate of O_2 evolution at the higher light intensities employed, increased.

The optimum temperature for growth and photosynthesis by *platensis* is around 35 °C (Richmond, 1988). The same seems to apply for photosynthetic oxygen evolution in solid support. In this case, rates at the higher light intensities were higher at 35 °C than at 25 ° or 30 °C quantum requirements were unchanged (not illustrated).

To assess the usefulness of this experimental approach in the investigation of physiology of *P. platensis* (or other blue-green algae), the effect of photoinhibitory light intensities on quantum requirement was examined. The light responce of *Spirulina* attached to a GF/C was measured as described above, before and after illumination at a PFD of 2500 μE $m^{-2}s^{-1}$ for 25 min (Fig. 3). Quantum requirement was greatly increased by this treatment (from 11 to 33), indicating that the response of *Spirulina* to high PFD is similar to that displayed by many higher plants. Recovery from this photoinhibitory treatment, as indicated by a decrease in quantum requirement, was observed in samples left under dim light (not illustrated, but see Vonshak *et al.*, 1988).

Preliminary measurements of reflectance and transmitance by samples prepared as described above indicated that for the amounts of chlorophyll employed here, the amount of light effectively absorbed was around 85%, a value very similar to that reported for leaves of many species of higher plants (Bjorkman & Demmig, 1987). If this

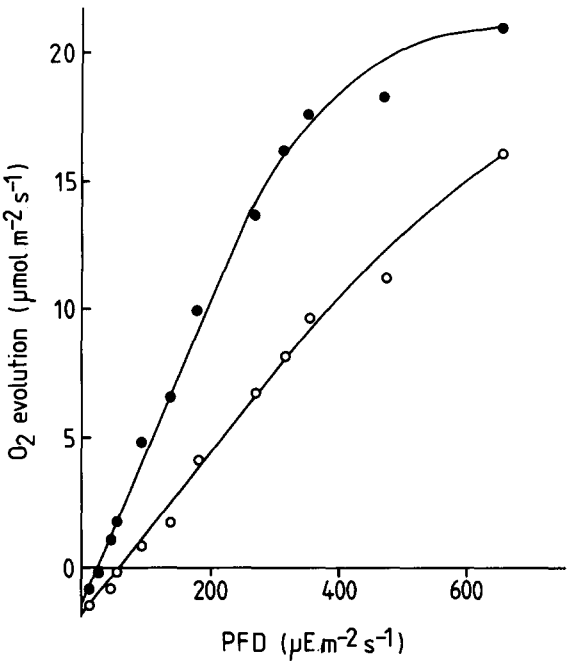

Fig. 3. Photoinhibition in *Spirulina platensis*. Light response curves were determined on a sample on solid support before (control O—O) and after (photoinhibited O—O) illumination for 25 min with a PFD of 2500 μE $m^{-2}s^{-1}$. Relative quantum requirement (not corrected for the fraction of light absorbed) increased from 13 to 39.

factor is employed for the conversion of quantum requirement into 'absolute' quantum requirement, the minimum values determined for *Spirulina* were around 11. This value compares favorably with those obtained by Ogawa and Aiba (1978), who estimated that the quantum requirement for CO_2 uptake in *Spirulina* was around 20. Quantum requirements reported for cyanobacteria are significantly higher than those displayed by higher plants (Bjorkman & Demmig, 1987). In higher plants quantum requirements can be increased by stress e.g. photoinhibition (Bjorkman, 1987), but for unstressed plants they are remarkably constant and near the theoretical minimum (Bjorkman & Demmig, 1987). Although quantum requirement in *Spirulina* increased when cells were illuminated in photoinhibitory conditions (Fig. 3) quantum requirements were high (at least three photons higher than the theoretical minimum) in unsaturated cultures, suggesting that at least in *Spirulina* but probably in other cyanobacteria as well, photosynthesis is running below its theoretical efficiency. The pigment composition of the antennae and/or an intrinsic inefficiency in the way that the absorbed energy is channelled into the reaction centers could be contributory factors. On the other hand, pseudocyclic electron transport, i.e. the reduction of molecular oxygen or Mehler reaction, is a process which utilises light without affecting net oxygen evolution (for a review see Sivak, 1987). If this process were running at high rates, the effect would be decreased quantum efficiency, measured as O_2 evolved per photon absorbed. Pseudocylic electron transport in higher plants is supposed to have a role in ATP synthesis but during photosynthetic induction and at high PFD it could also contribute to the prevention of photoinhibitory damage by dissipating light energy in excess of that used by carbon assimilation. At present there are no data concerning the rate of electron transport to oxygen in cyanobacteria, but the synthesis of extra ATP and the avoidance of photoinhibitory damage could be as relevant in cyanobacteria as they are in higher plants.

Conclusion

The use of cells attached to filters allows the application to cyanobacteria of methods which previously could only be used with leaves, such as the analysis of chlorophyll fluorescence and its quenching components during changes in the composition of the gas-phase and illumination, and the simultaneous measurement of CO_2 uptake and O_2 evolution under controlled CO_2 and O_2 concentrations (Sivak & Vonshak, 1988). Furthermore, the use of a solid conjunction with the leaf–disc electrode provides a convenient means of measuring quantum requirement in *Spirulina* and other algae in which activity is not significantly altered by filtration on a suitable filter. It may also prove useful in the study of the effects of environmental stress. We believe that this experimental approach could provide a convenient screening procedure which could be further extended to the study of the effect of other environ-

mental stresses likely to be encountered in the large scale culture of *Spirulina* and other microalgae of growing economic importance.

Acknowledgements

This work was supported by grants from the Agricultural and Food Research Council of the United Kingdom.

References

Bennet J, Bogorad L (1973) Complementary chromatic adaption in a filamentous blue–green algae. J. Cell Biol. 58: 419–435.

Bjorkman O (1987) High irradiance stress in higher plants and interaction with other stress factors. In: Biggins J (ed), Progress in Photosynthesis, Vol. IV. Martinus Nijhoff, Dordrecht, 1–18.

Bjorkman O, Demmig B (1987) Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170: 489–504.

Delieu T J, Walker D A (1983) Simultaneous measurement of oxygen evolution and chlorophyll a fluorescence from leaf pieces. Plant Physiol. 73: 542–549.

Ogawa T, Aiba S (1978) CO_2 assimilation and growth of a blue–green alga *Spirulina platensis* in continuous culture. J. Apll. Chem. Biotechnol. 28: 5151–521.

Richmond A (1987) *Spirulina*. In: Borowitzka M A, Borowitzka L J (eds), Micro–algal Biotechnology. Cambridge U.P., Cambridge, 85–121.

Sivak M N (1987) The effect of oxygen on photosynthetic carbon assimilation and quenching of chlorophyll emmission *in vivo*: phenomenology and hypotheses concerning the mechanisms involved. Photobiochem. Photobiophy. Suppl.: 141–156.

Sivak M N, Vonshak A (1988) Photosynthetic charcteristics of *Spirulina platensis* on solid support. Chlorophyll fluorescence kinetics. New Phytol. 110: 241–247.

Vonshak A (1987a) Laboratory techniques for the culturing of microalgae. In: Richmond A (ed), Handbook for Microalgal Mass Culture. CRC Press, Boca Raton FL, 117–146.

Vonshak A (1987b) Biological limitations in developing the biotechnology for algal mass cultivation. Sciences de l'Eau 6: 99–103.

Vonshak A, Guy R (1987) Photoinhibition as a limiting factor in outdoor cultivation of *Spirulina platensis*. In: Stadler T *et al.* (eds), Algal Biotechnology. Elsevier Applied Science Publishers, London, 365–370.

Vonshak A, Guy R, Poplowsky R, Ohad I (1987) Photoinhibition and its recovery in two different strains of *Spirulina*. Plant and Cell Physiology, 29(4): 721–726.

Vonshak A, Richmond A (1988) Mass production of the blue–green alga *Spirulina*: An overview. Biomass 15: 233–247.

Walker D A (1987) The use of oxygen electrode and fluorescence probes in simple measurements of photosynthesis. Oxygraphics Pub., Sheffield, 1–145.

Walker D A, Osmond C B (1986) Measurement of photosynthesis *in vivo* using dependence steady-state photosynthetic O_2 evolution and chlorophyll *a* fluorescence transients. Proc. Royal Society London B., 227: 267–280.

Walker D A, Sivak M N, Prinsley R T, Cheesbrough J K (1983) Simultaneous measurement of oscillations in oxygen and chlorophyll *a* fluorescence in leaf pieces. Plant Physiol. 73: 534–541.