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A differential photomicrocalorimetric method for investigating the rate of energy storage in plants ☆

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Abstract

This paper describes the equipment and method for the direct measurement of the quantity and rate of light-induced changes in plants. The photomicrocalorimetric method can be used for the investigation of energetics in photosynthesis and other photobiological processes in higher plants, algae and microorganisms. The rate of energy storage of *Chlorella* cells and a number of agricultural plants under optimal and extreme conditions have been determined.

Keywords: Alga; *Chlorella*; Energy storage; Photomicrocalorimetry

1. Introduction

When investigating photoenergetics it is necessary to use appropriate equipment and methods for the direct measurement of the absorbed light energy. In much of the relevant research [1,2], the energy supply of the plant cell was calculated from the O_2 evolution rate of the CO_2 absorption. The question arises whether this calculation is correct.

According to our data [3–5] it has been found that as much as 30% of the energy can be stored in processes which are not connected with gas exchange. The energy

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measured by the calorimetric method is considerably larger than the storage energy measured by the gasometric method (for difference may be up to 60%), when the plants are under extreme conditions.

The photomicrocalorimetric investigations of plant cell energy were initiated at the Kazan University by Prof. V.Ye. Petrov. These investigations have been developed further at the Kazan Institute of Biology.

The aim of the present work is the description of a photomicrocalorimetric method which allows the direct determination of the rate of light energy storage by, for example, *Chlorella* and higher plants. This permits the direct measurement of the rate of energy storage in plants, and leads to the determination of the intracellular energetic balance of photosynthesizing plants.

2. Experimental

The photomicrocalorimetric appartus was constructed from the differential microcalorimeters DAK-1-1A (type Tian-Calvet) and LKB-2277 (Bio Activity Monitor).

A detailed description of the microcalorimeter has been published by Calvet and Prat [6] Wadsö [7] and Suurkuusk and Wadsö [8].

Particular emphasis is placed on the use of the light appartus (Fig. 1). The precision and reproducibility of the measurement in the operation of the instrument were determined by using a neutral dye-stuff to change the intensity of light flow. The investigations were performed according to the following scheme (Fig. 2):

(1) After loading the calorimetric vessel with distilled water (1 cm^3) or another neutral liquid and setting the dark-zero [$D''O''_{at}$], its level is registered.

(2) Light is delivered to the measuring cell, where it is fully absorbed, and the new steady-state level is set [$L''O''_{at}$].

(3) The rate of the thermal effects (A) is compensated by hand [L_{com}] using (a) the Peltier effect in the measuring vessel, (b) the Joule effect in the comparative vessel. (This type of compenation is more exact.)

When complete compensation is achieved (when $L_{com} = D''O''_{at}$), the following equation is used

$$A = (L''O''_{at}) - (L_{com}) \tag{1a}$$

or

$$A = (L''O''_{at}) - (D''O''_{at}) \tag{1b}$$

The rate A, the measured value I (mA), and the value R (compensative heater, 100 Ω) give the calculated rate of light intensity ($W = I^2R$).

(4) The measuring vessel water is replaced by the same volume of the *Chlorella* suspension and a new steady-state level is established [$D''O''_{res}$]. The observed difference (Δ_1) is caused by dark respiration and other biochemical processes

$$\Delta_1 = (D''O''_{at}) - (D''O''_{res}) = \sum Q_{acz} \tag{2}$$

where $\Sigma\, Q_{acz}$ is the thermogenesis of *Chlorella* cells in the dark.

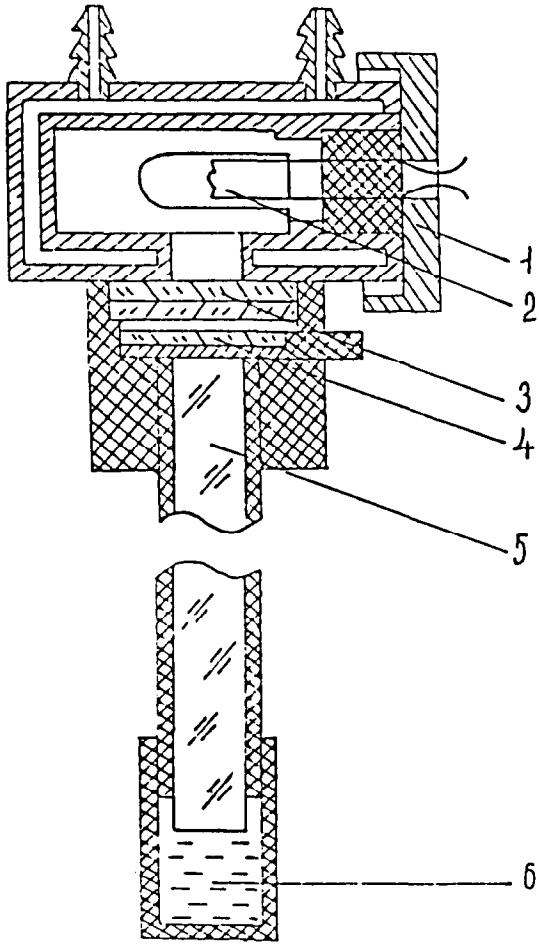

Fig. 1. The construction of the light vessel: 1, lamp holder connected with the water-cooled system; 2, lamp iodine-type KGM-12(V)-100(W); 3, system of constant light-filters for light elimination in the UV and IR spectral ranges; 4, shift-colour filter for changing light flow in the visible spectrum range; 5, quartz light-conductor (10 mm in diameter for DAK-1-1A and 12 mm for LKB-2277); 6, measuring vessel (1 cm^3 for *Chlorella* suspension and 0.5 cm^3 for leaves). The light intensity is regulated by a constant stabilized power supply and can be changed from 0 to 3×10^4 lx.

(5) When the light is turned on, compensation is automatic (at the same value as in (3), above), and a new light steady-state level (L_{es}) is obtained. The rate of energy storage is determined from the equation

$$F = K(L_{es} - L_{com}) = \Delta_2 K \tag{3}$$

where K is the thermal constant of the instrument in the working range.

The first measurement of energy storage by cells of *Chlorella vulgaris* and the non-photosynthesizing microorganism *Serratia marcescens* were carried out using this light vessel (Fig. 1).

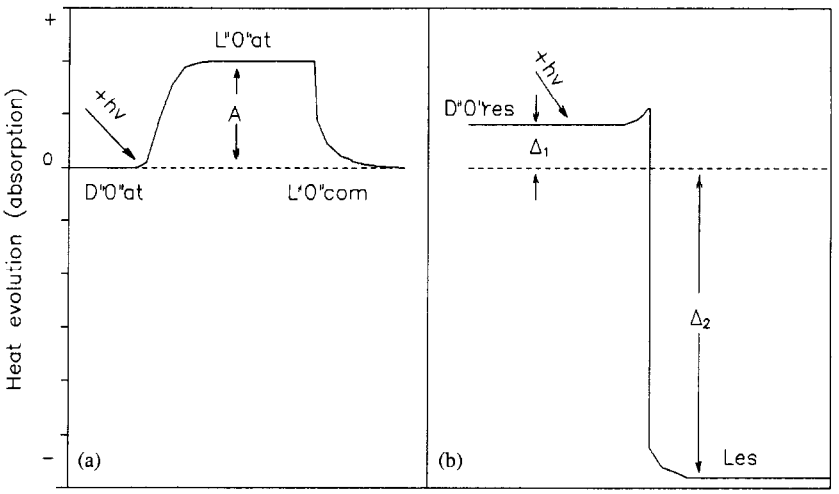

Fig. 2. a. Calorimetric response during illumination. The effect A is compensated by setting the signal from level $L''O''_{at}$ to level $L''O''_{com}$. b. Calorimetric response of a *Chlorella* suspension in the dark (Δ_1) and during illumination (Δ_2).

Improvements to the light vessel and method have made it possible to investigate leaf pieces from high plants. Different types of light vessels have been constructed, with single and multiple light conductors and with a supply of the reaction liquid to the measuring vessel.

Various agricultural plants (wheat, maize, pea and amarant) with different types of metabolism (C_3–C_4) were used for the investigation.

3. Results and discussion

Comparative data for the rate of energy storage by *Chlorella* obtained by gasometric and microcalorimetric methods are shown in Fig. 3. Data obtained by the direct method yield higher values for the stored energy than those obtained using the gas exchange method. Up to 40% of the light energy is utilized by the *Chlorella* cells in optimal conditions. It is stored in reactions which are not directly connected with oxygen evolution, cyclic photophosphorylation being an example of this type of process [9]. The relative magnitude of the effect between energy and gas exchange increases in salt conditions (Fig. 3), indicating its ecological importance.

Fig. 4 shows that in terms of the dry weight (or chlorophylls), the level of stored energy in maize, wheat and pea is between 11.2 and 13.6 J (g dry wt)$^{-1}$ h^{-1}. In buckweat and amarant it is 5.2 and 3.8, respectively. We think that the difference is connected with many reasons, for example the photosynthesis efficiency, metabolic type and morphological peculiarities.

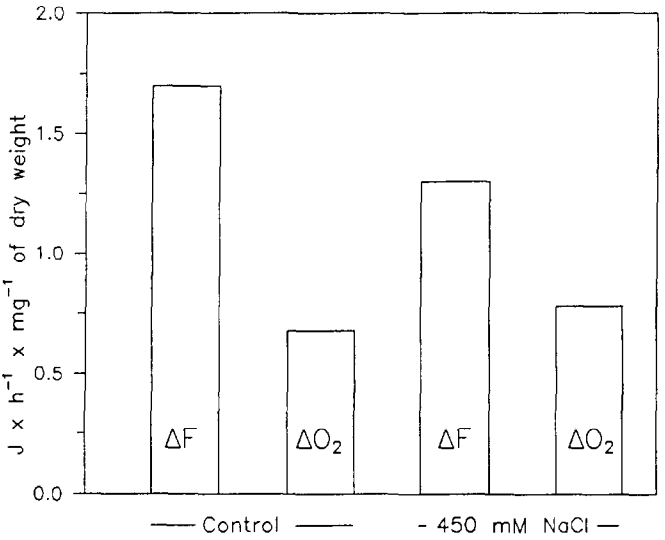

Fig. 3. Comparision of the calorimetrically obtained light absorption ΔF and the energy rate calculated from the O_2 evolution ΔO_2 for a *Chlorella* suspension.

When the specific photosynthesis inhibitor Diuron is inserted in the measuring vessel with a piece of maize leaf, a concentration-dependent effect on the rate of energy storage is observed (Fig. 5). Similar results are obtained with Diuron infiltration at different concentrations. In the dark, Diuron has no observed influence on the thermogenesis [3].

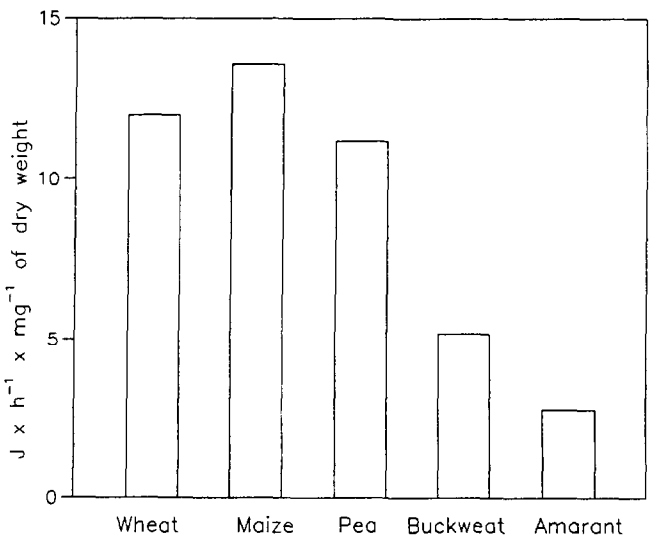

Fig. 4. The rate of energy storage in different agricultural plants.

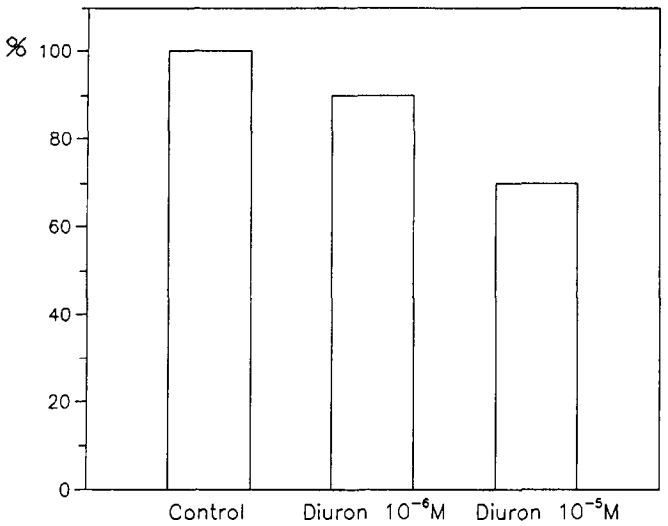

Fig. 5. The effect of the photosynthesis inhibitor Diuron on the rate of energy storage in maize.

4. Conclusion

It has been shown that it is possible to study energy storage in plant leaves, algae and bacteria using a photomicrocalorimeter. The results of this work allow the determination of the adaptive potential of plants under the influence of environmental conditions. This approach can be used in the rapid determination of resistance towards unfavourable environmental conditions.

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