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AN EQUIPMENT FOR INVESTIGATIONS OF PHOTOSYNTHETIC OXYGEN PRODUCTION REACTIONS

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Summary: An equipment for investigation of the photosynthetic oxygen production reaction mechanisms is presented. A very convenient and fast oxygen rate electrode for polarographic application with a 100 µl sample volume, equipped with a system with flash, modulated and continuous illumination is described, allowing quantitative estimation of photosynthetic oxygen production. A special four impulse generator for ignition of groups of four different photoflash tubes with variable dark spacing between the groups and between the flashes in the groups is used. The equipment is destined for investigation of the forward and deactivation (back) reactions of the oxygen evolving centers (S_i states) and for studying the transient effects (induction phenomena) as well the enhancement effects at photosynthesis.

Key words: Emerson transient effect, oxygen evolution, oxygen flash yields, photosynthesis

Introduction

The most important results revealing the nature of the photosynthesis are obtained during the investigation of the photosynthetic oxygen production beginning with the discovery of photosynthesis by Joseph Priestly. Using the polarographic oxygen electrode, especially the rate electrode (Blink's type), it was shown that the first intensive microsecond flash given several minutes after dark adaptation of isolated chloroplasts does not lead to the photosynthetic oxygen production (Allen and Frank, 1955; Joliot, 1965). Lately Joliot et Joliot (1968) demonstrated extremely interesting results con-

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nected with the oscillation patterns of the successive oxygen flash yields obtained after dark adaptation of chloroplast suspensions. These results were used by Kok et al. (1970) for the creation of the so called "four step linear model" of the photosynthetic oxygen evolving reactions.

In this article an equipment for investigation of the photosynthetic oxygen production reaction mechanisms with a polarographic oxygen rate electrode and a system for flash and modulated illumination is described. It will be useful for investigation of the forward and deactivation (backward) reaction rates of the S_i states, the enhancement effect or Emerson's second effect (Emerson, 1957) and the transient phenomena (Blink's effects) (Blinks, 1959).

Equipment description

Polarographic oxygen rate electrode

The main part of the equipment is the oxygen polarographic rate electrode. Detailed description of this part is presented in Fig. 1. The electrode consists of two compartments separated by cellophane (dialysis) membrane. The silver anode is a ring with 3 cm diameter and 3 mm in height situated in the top compartment. The platinum cathode is a disk with 8 mm diameter upon which chloroplast or algae suspension is deposited.

The thickness of the suspension layer is 2 mm, so that the total volume is 100 µl ($3.14 \times 4^2.2$). The cathode is fixed to the piston shown at the right bottom of the same figure. After deposition of the investigated sample, the cellophane membrane is fixed

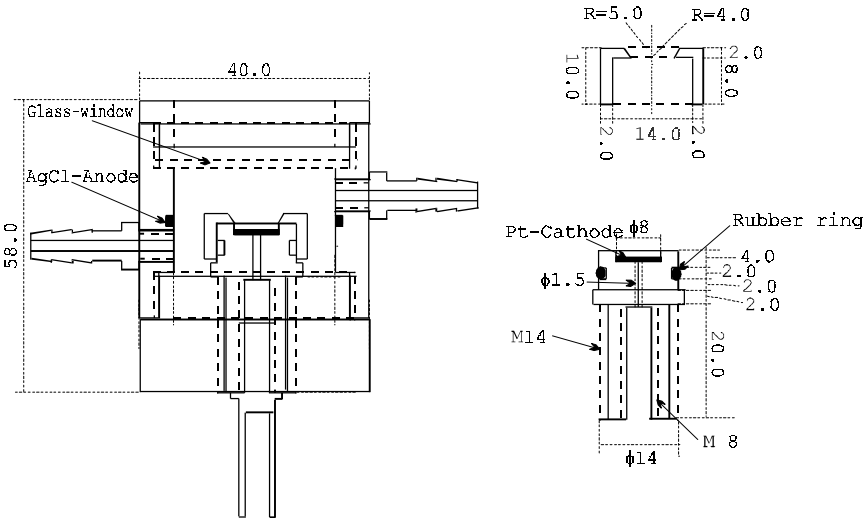

Fig.1. Construction of the polarographic oxygen rate electrode. For details see the text.

by a small cap shown at the right top in Fig. 1. All parts of the electrode system except the anode and cathode and their connections are made from organic glass (Plexiglas). The top compartment of the electrode system could be filled with nutrition solution when the algae suspensions are investigated or with isolating buffer in the case of choroplast suspensions. In both cases 100 mM KCl is added.

Polarographic amplifier

The scheme of the polarographic amplifier is shown in Fig. 2. With the help of potentiometer P2 and the digital voltmeter V a potential of 650 mV could be applied to the anode and cathode electrodes of the polarographic cuvette so the cathode obtains a

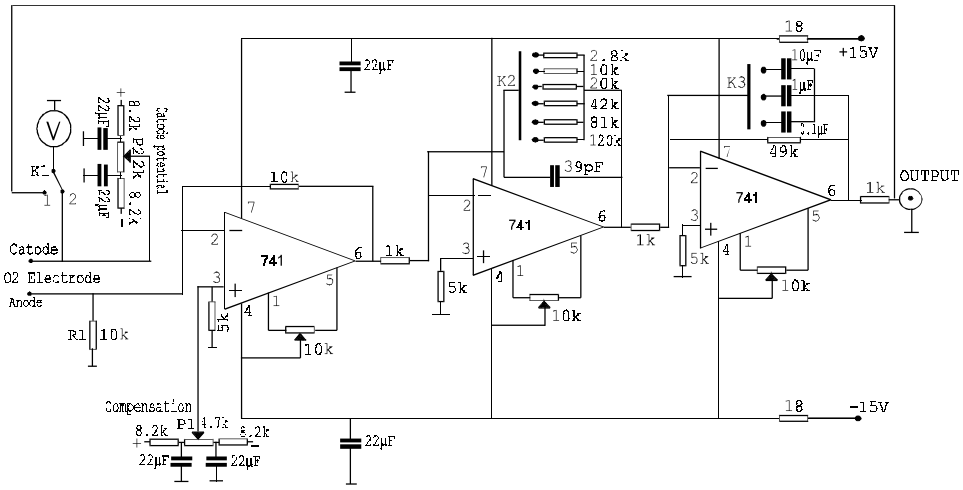

Fig. 2. The electric diagram of the oxygen amplifier. For details see the text.

negative polarity and the anode become positive. The electric current trough the electrode and the resistance of 10 kOhm (R1) is proportional to the concentration of the oxygen in the sample. Because of the very small volume and thickness of the suspension layer the current is proportional to the oxygen production rate. The electric potential obtained over the resistance R1 is amplified from 0.2 V/µA to 7 V/µA depending on the position of the switch K2. The time constant of the amplifier could be changed with the help of the switch K3 and the capacitance of 0.1 mF, 1 mF and 10 mF. When the position of the switch K1 is 1 the voltmeter is connected to the output voltage of the amplifier and in the position 2 – to the cathode. The potentiometer P1 is designated for the compensation of the diffusion currents of the electrode. The output of the amplifier could be connected to the XT recorder or to the computer using an analog digital converter as in our case. It should be noted that the amplifier is extremely stable over time and no changes in its parameters could be observed. Obviously, the amplifier

could be used with concentration type (Clark type) of oxygen electrode or with an oxygen electrode described in our preceding paper (Zeinalov and Maslenkova, 1999).

The systems of irradiation

1. The irradiation with short saturating flashes

The other part of the equipment is the system of illumination with short ($t_{1/2}$=10 μs) saturating (4J) periodic flash sequences or with group of 2, 3 and 4 flashes with variable time between the flashes (100 μs –1.6 s and between the groups (1 s –16 s). The scheme of feeding and ignition of the small photographic flash tubes is shown in Fig. 3. Four similar schemes are used for the 4 flash tubes. The electric diagram of the energy supplier is presented in the next Fig. 4. It allows the transformation of 9 V to 600 V. The periodicity and the generation time of the flashes are determined by an equipment based on a special electronic scheme (Fig. 5) consisting of four waiting multivibrators (two 74123 microchips). The obtained signals (after differentiation and transformation) are directed to the gate electrodes of the thyristors, shown in Fig. 3. The period

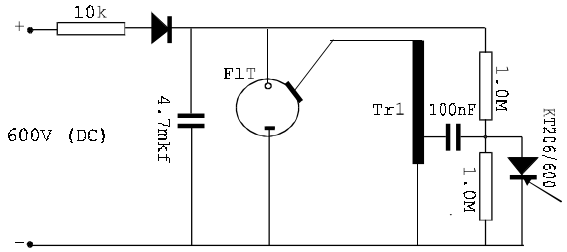

Fig. 3. The electric diagram of the photo-flash tubes

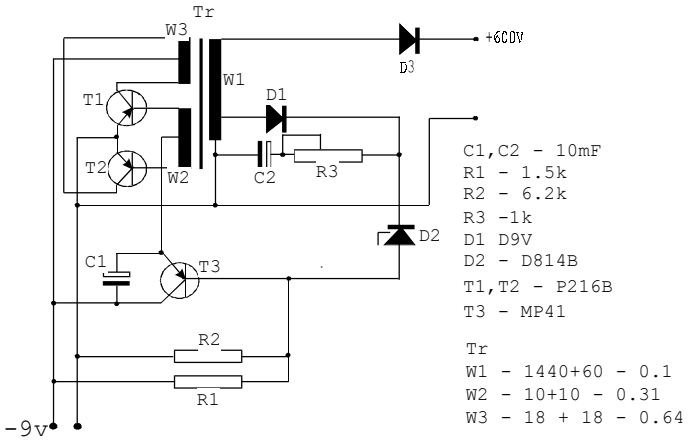

Fig. 4. The scheme of the energy supplier for flash tubes.

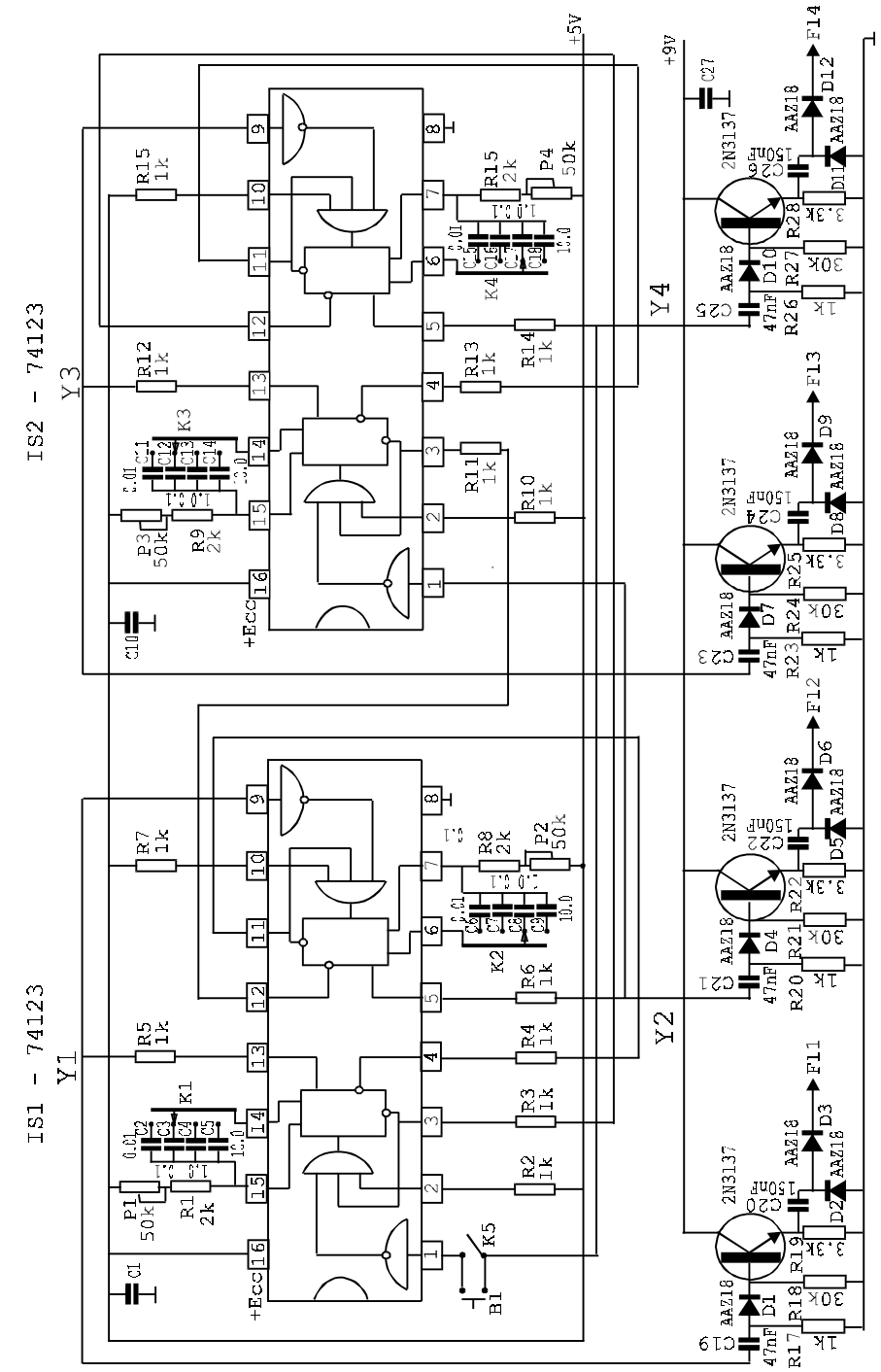

Fig. 5. The electric diagram of the four impulses generator. For details see the text.

of generation of the igniting impulses are determined by the changing the capacitance (C1–C5) stepwise and gradually with the potentiometers R.

2. Irradiation with modulated light

Finally, for illumination with modulated light/dark radiation another device was built (Fig. 6). Two waiting multivibrators (one 74123 microchip) were used, one to determines the dark period and the other one – the period of irradiation. Several types of light emitting diodes were used as light sources. With this scheme the photosynthesiz-

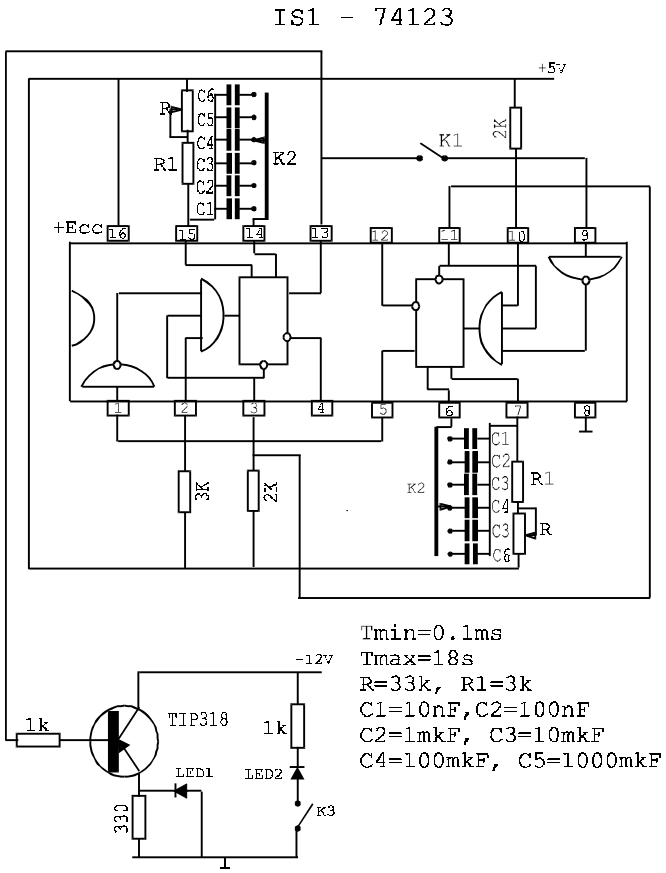

Fig. 6. The electric diagram of the modulated light illumination.

ing samples could be irradiated with different light/dark periods starting from 10 ms up to 16 s. In addition, one of the light emitting diodes from the same model is connected to -12 V and with the help of switch K3 the sample could be irradiated with two light beams with equal spectral distributed energy (equal wavelengths) one of which is modulated and the other one is continuous.

Experimental results

Following experimental results demonstrate the quality of the above described equipment. In Fig. 7 a serie of oxygen flash yields induced by short (10 μs) and saturating (4 J) flashes after 5 min dark incubation of the isolated pea chloroplasts is presented. The results show well known oscillations in the amplitudes of the oxygen flash yields and also that both after the first and the second flashes a well expressed oxygen consumption reaction occur. This fact has not been interpreted in the literature. It is seen

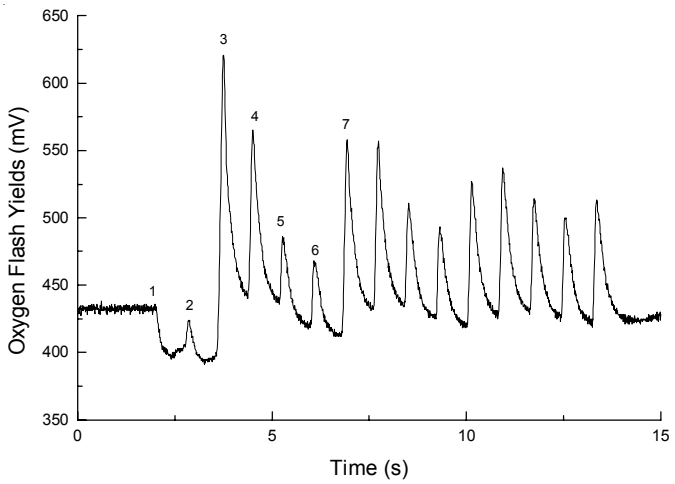

Fig. 7. Oxygen flash yields oscillations induced by a train of short (10 μs) saturating (4 J) flashes at isolated pea chloroplasts.

that the maximum oxygen flash yields (maximum amplitudes) are obtained at the third and the seventh flash.

The results of irradiation of a *Scenedesmus aqutus* cell suspension (100 μmol chlorophyll/ml) with a group of four saturating (4 J) and short ($t_{1/2}$ = 10 μs) flashes with different spacing between the flashes (100 μs, 400 μs, 1 ms, 4 ms, 10 ms, 20 ms, 40 ms, 100 ms, 400 ms and 1 s) and 4 s between the flash groups is shown in Fig. 8. It is seen that the amplitudes of the flash group yields increased with the increase of the dark periods between the flashes in the groups starting at 100 μs. The maximum oxygen yields are obtained at 10–20 ms spacing after which the amplitudes decreased. At 100 ms spacing the effect of the separated flashes in the group yields could be observed which are definitely separated at 400 ms and 1 s. The results presented confirm the general accepted statement that the turnover time of the oxygen evolving centres is about 20 ms. By changing the spacing between the first and second and between the second and third one as well as the spacing between the third and fourth flashes it is possible to investigate the forward rate constants of different S_i states according to

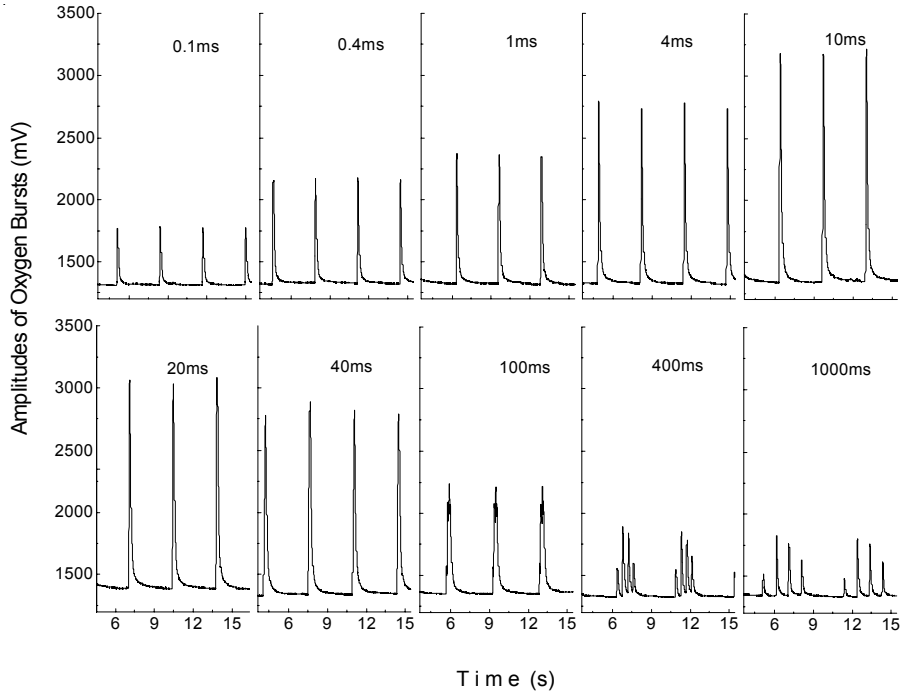

Fig. 8. Oxygen flash yields depending on the spacing (0.1 ms–1 s) between the flashes in the groups of four saturating flashes at *Scenedesmus aqueous.*

the model of Kok et al. (1970). After reaching the steady-state flash yields using only one flash tube with variation of the spacing between the flash sequences it is also possible to investigate the deactivation reactions rate constant of S_3 and S_2 states.

By using the setup for modulated and continuous irradiation certain results concerning the enhancement phenomena at photosynthesis are shown in Fig. 9. The oxygen evolving amplitudes of the left part in Fig. 9 are obtained using modulated (1 s light/1 s darkness) 650 nm light beam with background irradiation with the same wavelength (650 nm). Both light beams (modulated and continuous) are obtained using light emitting diodes (LEDS VQE13-1B (Germany) I = 20 mA, V=1.8 V, 2.5–4 mcandels). After switching off the continuous (background) irradiation the amplitudes of the modulated oxygen evolution decreased almost 10 times. The onset of the background irradiation again lead to an increase of the modulated amplitudes of the oxygen evolution. The observed enhancement effect (10 time) is obviously different from that discovered by Emerson (1957) (Emerson second effect) firstly with its value (the value of enhancement effect in Emerson's experiments and in the experiments described in the literature is in the range of 1.1–2.0 time) and secondly with the fact that in our case two light beams with equal wavelengths are used instead of two light beams with different wavelengths in the case of Emerson enhancement type of experiments. The observed

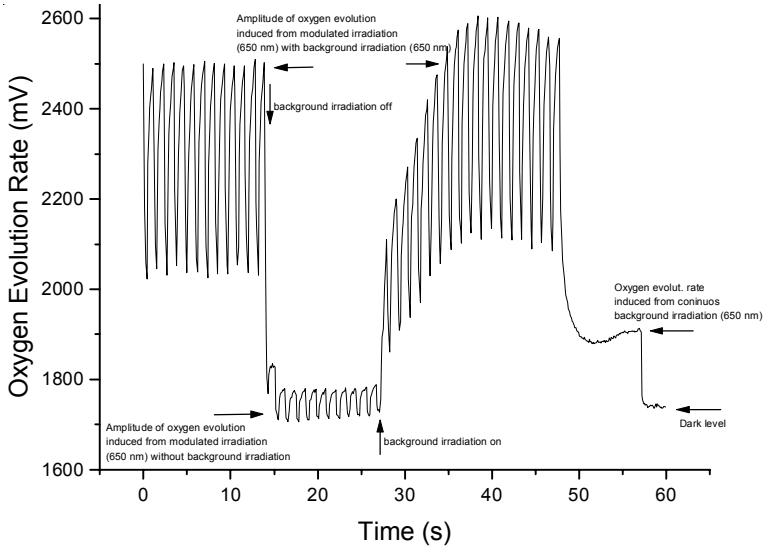

Fig. 9. Oxygen evolution at *Scenedesmus obliquus* induced by two light beams with 650 nm wavelength. One of the beams (12 µmol.m^{-2}.s^{-1}) is modulated 1 s light/1 s darkness and the other one (6 µmol.m^{-2}.s^{-1}) is continuous.

enhancement effect could be explained with the interference of dark respiration (under low irradiations a significant part of the photosynthetically evolved oxygen is used for the respiration) or with the non-linearity of the photosynthetic "light curves" under very low irradiation, which is explained by the fact that the photosynthetic oxygen evolution is a multiquantum process (at least 4 photons are needed for one oxygen molecule) and the obtained intermediate (S_i) states are unstable in the dark.

The same type of results are presented in Fig. 10 using two continuous light beams with equal wavelengths (650 nm). Obviously, the sum of the effects of the two light beams acting separately ($V_{I1} + V_{I2}$) is significantly lower than the effect obtained under their simultaneous action (V_{I1+I2}).

Apparently, the demonstrated observation is raising the question about the nature of the Emerson's enhancement effect and its explanation as a result of the participation of the two different photosynthetic systems in the light reactions of photosynthesis.

The changes of the oxygen evolving amplitudes obtained after irradiation with the modulated light beams before switching on the background irradiation, during the induction time, (after switching on the background irradiation (arrow "a")) and in the darkness (after switching off the continuous (background) irradiation (arrow "d")) are presented in Fig. 11. The wavelengths of the two light beams are also 650 nm. The arrows "b" and "c" indicate the switching off and switching on of the modulated irradiation.

In conclusion it should be emphasized that the presented equipment will be very helpful not only for investigations of the kinetic parameters of the oxygen production

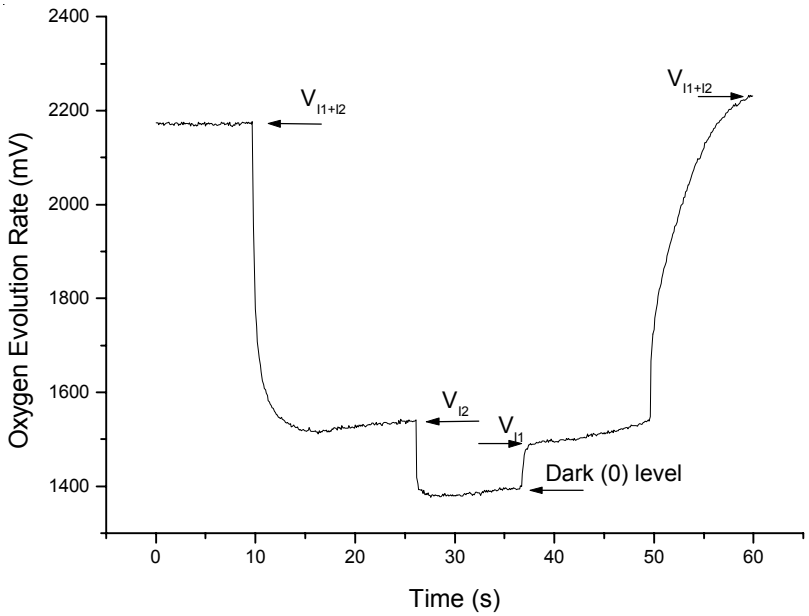

Fig. 10. Oxygen evolution at *Scenedesmus obliquus* induced by two continuous light beams (I_1 – 10 μmol.m^{-2}.s^{-1} and I_2 – 6 μmol.m^{-2}.s^{-1}) with 650 nm wavelength.

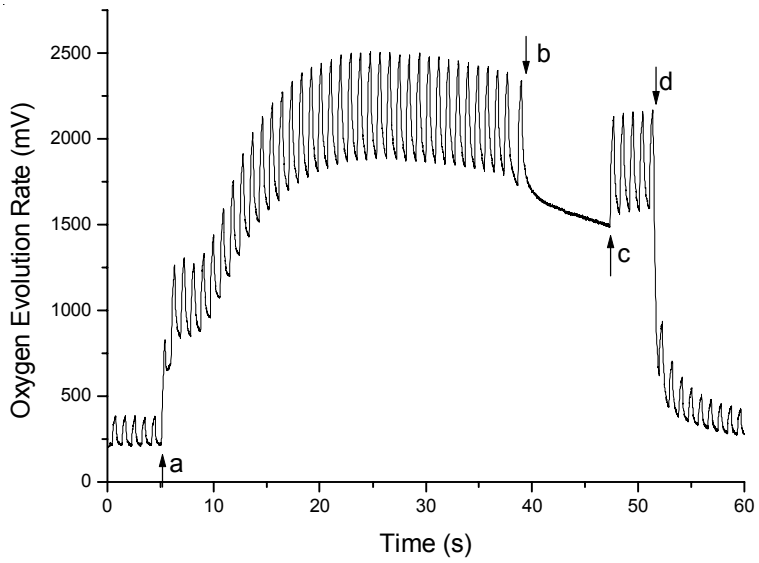

Fig. 11. The dependence of the amplitude of the modulated oxygen evolution at *Scenedesmus obliquus,* during the induction time of photosynthesis. The two light beams are with equal wavelengths (650 nm) and allowing 10 μmol.m^{-2}.s^{-1} and 6 μmol.m^{-2}.s^{-1} irradiances for modulated and continuous beams respectively.

reaction, but also for estimation of the photochemical and photosynthetic activities of different species - isolated chloroplasts and chloroplast fragments as well for establishment of the physiological state of the algae suspensions during the investigation of the maximum value of the quantum efficiency of photosynthesis.

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