The Origin of Spectral Effects in Photosynthesis

Yuzeir Zeinalov

Institute of Biophysics, Bulgarian Academy of Sciences, Academic G. Bonchev str., bl. 21, Sofia 1113, Bulgaria. E-mail: zeinalov@bio21.bas.bg

Z. Naturforsch. **64c**, 680–686 (2009); received May 11/June 29, 2009

The non-linearity of photosynthetic light curves and the real reasons leading to the appearance of the "red drop of quantum efficiency" and "enhancement" (Emerson's second) effects are presented. On the contrary to the generally accepted interpretation that these effects are induced from the participation of the two different photochemical systems, it is shown that they are simply a direct consequence of the initial non-linearity of the photosynthetic light curves.

Key words: Photosynthetic Light Curves, Action Spectra of Photosynthesis, Red Drop Effect, Enhancement Effect

The Spectral Dependence of Photosynthesis with Non-Linear Light Curves and the Origin of the "Red Drop" Effect

Emerson and Lewis (1943) demonstrated for the first time that the action spectrum of photosynthesis, after the main absorption maximum (680 nm) in the red region of the spectrum of green algae, was situated noticeably below the absorption spectrum and, as a consequence, the quantum efficiency (yield) spectrum of oxygen evolution decreased to zero very sharply (effect of "red drop"). Later Emerson (1957) discovered that the photosynthetic process in this spectral region might be raised with the addition of short-wave irradiation to the non-effective far red light. The explanation of this phenomenon, known as the effect of "enhancement", was given by the idea of the functioning of two photochemical (pigment) systems in the light-dependent reactions of photosynthesis in green plants. Hill and Bendall (1960) realized this idea by means of the so-called Z-scheme. Almost five decades followed during which this hypothesis was developed. Individual electron carriers as well as their sequence along the complex electron-transport chain were established. Nevertheless, as we have pointed out (Zeinalov, 1977a, b), the "red drop" and the "enhancement" effect could be ensued from the non-linearity of the light curves of photosynthesis at low light intensities and the so-called principle of non-additiveness of the action of light in this process (see Zeinalov, 2009, Fig. 3). We shall now demonstrate a corollary from this non-linearity of the light curves on the action spectra of photosynthesis and particularly on the reduction of the quantum efficiency in the spectrum regions where pigment absorption is lower.

Despite the fact that in the experiments of many founder investigators the non-linearity of the registered light curves of photosynthesis is significantly more pronounced in comparison with the non-linearity arising as a consequence of the operation of Kok's model, we will use the expression (3) presented in Zeinalov (2009) for convenience. Therefore, as was noted there, the reader should agree with the statement that the results obtained in the following part show the minimum effect, which could be observed in the real experiments.

Let us look at Fig. 1, presenting the theoretically calculated data using the model of Kok *et al.* (1970) for a suspension with low (0.1) absorbance. Curve a represents the shape of the irradiance dependence of photosynthesis (oxygen evolution) under low irradiances, inducing at maximum intensity (1.0 a.u.) a light reaction with a rate constant of $1.0\ s^{-1}$, while the slowest forward rate limiting dark reactions has a rate constant of about $20\ s^{-1}$. Curve b represents the absorbance spectrum, while curves c and d are the calculated action (oxygen evolution rate vs. the incident quanta number) and quantum efficiency (oxygen evolution rate vs. the absorbed quanta number) spectra, respectively. All spectral curves are normalized at 680 nm. It could be seen that the action spectrum (curve c) is situated noticeably below the absorption spectrum in both slopes of the

0939–5075/2009/0900–0680 $ 06.00

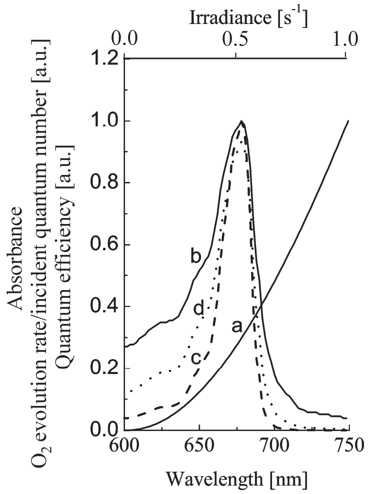

Fig. 1. Theoretically calculated curves according to the model of Kok *et al.* (1970), using the following values for the parameters included in the model: forward rate constant, 20 s^{-1}; back (deactivation) reaction constants: for S_1 state – $k_1 = 0\ s^{-1}$, for S_2 state – $k_2 = 0.05\ s^{-1}$, for S_3 state – $k_3 = 0.5\ s^{-1}$. The values of double hits and misses are neglected. Curve a, irradiance dependence of photosynthesis under low irradiances (0–1 s^{-1}). Curve b, normalized absorption spectrum in a suspension of *Scenedesmus acutus* with low (0.1) absorbance. Curve c, calculated action spectrum of oxygen evolution. Curve d, theoretically calculated quantum efficiency spectrum. The values of curve c (action spectrum) are calculated using the value of absorbance at any given wavelength proportional to the "absorbed light energy". All calculations are performed using relevant computer programs.

maximum, which leads to a dramatic decrease in the values of the quantum efficiency spectrum in this regions. This supports the statement that the non-linearity of the irradiance curves leads to a decrease of the quantum efficiency as well as the appearance not only of the "red drop", but also of a "blue drop" effect on the short wavelength sides of the absorption maxima. Obviously, if all absorbed quanta are used effectively in the photochemical process and the irradiance dependence of photosynthesis is a linear function, the action spectra should coincide with the absorption spectra and the quantum efficiency spectra should be a straight horizontal line without any declines.

The presented, theoretically obtained results in Fig. 1 show the appearance of Emerson's red drop effect (the drop of quantum efficiency after the red absorption maximum at 680 nm) as a logical consequence of the non-linear dependence of photosynthesis on the irradiance intensity under very low irradiances. Thus, the explanation of this effect does not need the assumption that there exist two photochemical systems and that for effective functioning of the entire photosynthetic machinery the "balanced" excitement of these different photosystems is required.

It could be shown that, even if the number of quanta absorbed is the same at different wavelengths, for which the extinction coefficients differ substantially, the quantum efficiency for light with a lower extinction coefficient is lower compared with light with a higher extinction coefficient. We should add that, if the action spectrum is estimated by equalizing the intensity of photosynthesis (the rate of oxygen evolution) at different wavelengths, and if it is calculated as a reciprocal value from the light intensity, the above conclusion remains valid.

Figs. 2a and b illustrate the importance of the decomposition of the suspension layer into sublayers with low optical densities in the process of theoretical calculation of the action spectra and the spectral dependence of the quantum efficiency. Regardless of the fact that the absorbance of the layer considered in this two figures is significantly low (1.0), it is evident that the calculations performed without decomposition (Fig. 2a) are incorrect. No decrease in the quantum efficiency in the middle part of the spectrum (between 500 and 600 nm) was observed and the decrease in the far-red region was obviously not well expressed. The results with decomposition of the entire suspension layer into 20 sublayers presented in Fig. 2b reflect the real situation more adequately. The data in this figure show not only the decrease in the values of quantum efficiency after the maximum at 680 nm, the so-called Emerson's red drop effect, but also the decrease in the middle part of the spectrum.

In Fig. 2c the irradiance intensity was increased up to 20 s^{-1} (a.u.). Even under such high irradiance, the decrease in the quantum efficiency both in the middle part of the spectrum and in the far-red region was well expressed. This means that using suspensions with low absorbance (1.0) we could not estimate the correct values for action spectra and quantum efficiency spectra without compensation of the initial non-linear part of the light curves.

The results presented in Figs. 2b and c give some idea about the variations in the quantum efficien-

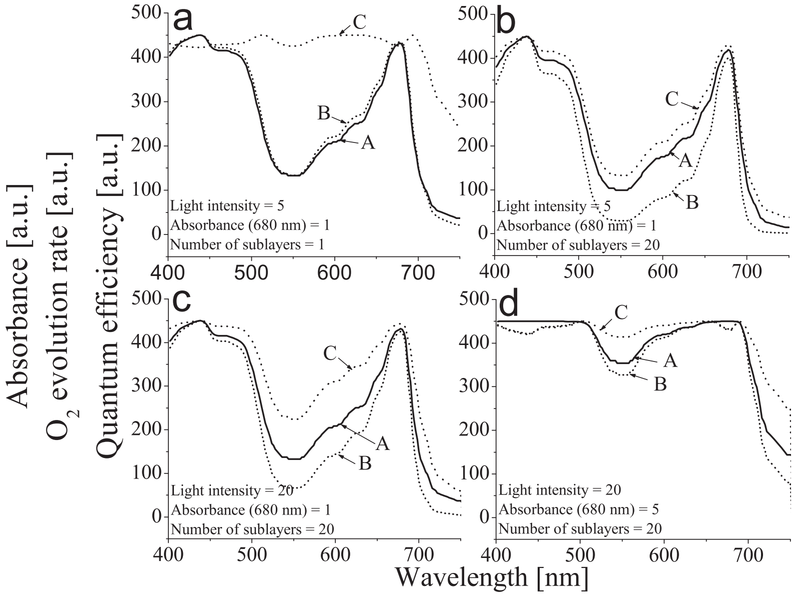

Fig. 2. (a) Theoretically calculated curves according to the model of Kok *et al*. (1970) without decomposition of the suspension layer: Curve A, normalized absorption spectrum in a suspension of *Scenedesmus acutus* with 1.0 absorbance at 680 nm; curve B, calculated action spectrum of oxygen evolution at 5.0 s^{-1} (a.u.) irradiance intensity; curve C, theoretically calculated quantum efficiency spectrum. (b) All parameters are as in (a) except for the procedure of calculation. The entire layer is divided into 20 sublayers, each one with 0.05 absorbance. The oxygen evolution and quantum efficiency spectra are calculated as a sum of the effects of light produced separately in different sublayers. (c) All parameters are as in (b) except that the action spectrum is calculated at 20.0 s^{-1} (a.u.) irradiance intensity. (d) All parameters are as in (c) except that the absorbance is increased to 5.0 at 680 nm.

cy values at different wavelengths and reflect the theoretically calculated action and quantum efficiency spectra in suspensions with not very high absorbance and under low or moderate irradiances. The calculations presented in Fig. 2d show that in the case of a suspension with high absorbance (5.0) and moderate irradiance [20.0 s^{-1} (a.u.)] the decrease in the action spectrum, as well as in the quantum efficiency spectrum in the middle part of the spectrum were noticeably reduced.

Nevertheless, the results presented in Fig. 2 show that the quantum efficiency decrease was visible not only in the long-wavelength slope of the main absorption maximum in the red region of the spectrum (effect of "red drop", Emerson and Lewis, 1943), but also in the middle spectral region between the two absorption maxima. This fact has not been satisfactorily explained in the literature. It is known from detailed investigations of Emerson and Lewis (1943), Haxo and Blinks (1950), and Litvin and Xe (1966) that between 500 nm and 670 nm the curves of action spectra of photosynthesis fall down considerably below the absorption spectrum curves, *i.e.*, the quantum efficiency in this part of the spectrum decreases. This decrease in the quantum efficiency, which begins at 580 nm in the Emerson and Lewis experiments as a result of the high absorbance of the algal suspension used (compare the results presented in Fig. 2c with d), cannot be explained by the assumed non-efficient radiant energy absorption by carotenoids, because significant absorption by these pigments can be expected only up to 540 nm. In the experiments of Haxo and Blinks and Litvin and Xe (where suspensions with low absorbance are used) the decrease in the photosynthetic quantum efficiency on the short-wavelength side of the main absorption maximum in the red region begins already at 650 to 670 nm. The general explanation of this effect, *i.e.* the imbalance of absorbed radiant energy distribution between the two photosystems, is very problematic. In other words, we must assume that a perfect balance of radiant quanta distribution between the two photosystems exists only in the absorption maxima of suspensions, which is very difficult to consider in terms of the quantum mechanics interpretation of electron transitions in chlorophyll molecules.

The "Enhancement" Effect As a Direct Consequence of the Non-Linearity of Photosynthetic Light Curves

In 1957 Emerson, investigating the decrease in the quantum yield (the "red drop" effect) above 680–690 nm in the green unicellular alga *Chlorella*, discovered a new phenomenon with fundamental significance for the future development of the theory of photosynthesis. This was the so-called "enhancement" effect, later known as "Emerson's second" effect. The first effect of Emerson was the CO_2 burst at the beginning of the induction time of photosynthesis. The essence of the enhancement effect is the increase of the low quantum yield in the photosynthetic oxygen evolution in the far-red region of the spectrum (690–720 nm) after simultaneous irradiation with short wavelength light. From the results presented by Zeinalov (2009), it could be expected that the enhancement effect is most likely a consequence of the non-linearity of the light curves. Therefore, it is important to understand that Emerson's enhancement effect is explained entirely by the non-additive action of radiation in photosynthesis or its complete explanation requires the postulation of two consecutively functioning different photochemical systems.

The results presented in Fig. 3 were obtained using the theoretically calculated light curve according to the model of Kok *et al*. (1970) [see Fig. 3 in Zeinalov (2009)]. All calculations were performed after decomposition of the entire suspension layer into 20 sublayers. It was assumed that in the "experiments" two light beams were used – a continuous one with constant intensity (equal numbers of incident quanta) at different wavelengths (400–750 nm) and a modulated background light beam with fixed wavelength (for instance at 700 nm). In other words, the minima of the sinusoid B_1 reflects the action spectrum of photosynthesis without background irradiation while the curve connecting the maxima of the same sinusoid (curve B_2) represents the action spectrum with modulated background light. In the same way, curve C_1 connecting the minima of the sinusoid C shows the quantum efficiency

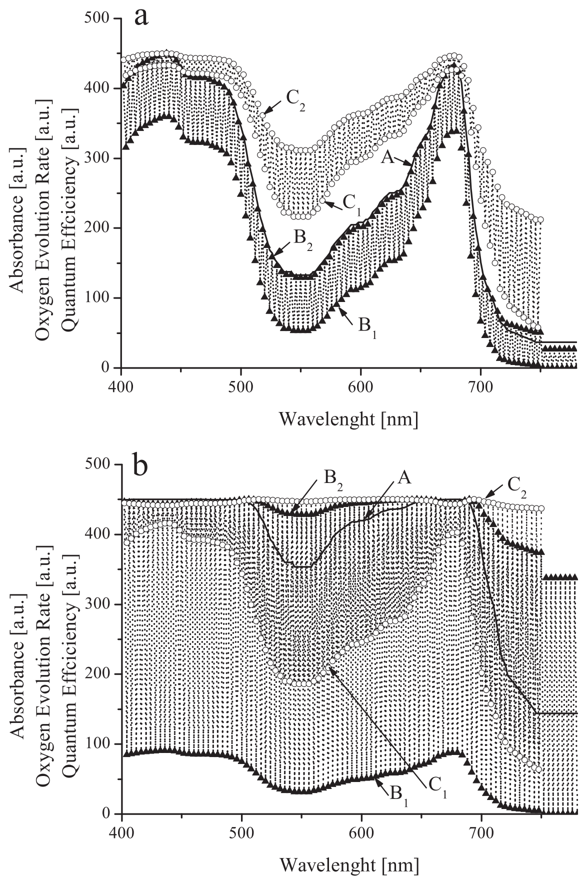

Fig. 3. (a) Curve A, absorbance spectrum of a *Scenedesmus acutus* suspension with 1.0 optical density at 680 nm. Curves B_1 and B_2, theoretically calculated action spectra of photosynthesis produced by continuous irradiation without background irradiation and with supplementary modulated background irradiation, respectively, and the changes in the size of amplitudes – the enhancement spectrum of oxygen evolution. Curves C_1 and C_2, quantum efficiency spectrum without background and with background irradiation, and the changes in the amplitude size (C_2–C_1) – the enhancement spectrum of the quantum efficiency. The continuous (measuring) light beam has $5.0\,s^{-1}$ (a.u.) intensity and the second background (modulated) light beam with $2.0\,s^{-1}$ (a.u.) intensity is supposed to have 700 nm wavelength as it is accepted that the absorbance for this beam is 0.2. Curves B_2 and C_2 are normalized to the absorbance spectrum at 436 nm. The comparison of the amplitudes of oxygen production (curve B) between 750 nm and 780 nm, obtained without continuous irradiation, and the amplitudes of the same curve in the other parts of the spectrum give an impression of the enhancement effect in oxygen evolution. (b) The same calculation as in (a) except that the absorbance is increased to 5.0, the continuous irradiation is $20.0\,s^{-1}$ (a.u.) and the modulated light beam is with $10\,s^{-1}$ (a.u.) intensity.

spectrum of photosynthesis without background irradiation and curve C_2 (connecting the maxima of this sinusoid) coincides with the quantum efficiency spectrum with background irradiation. The data presented in Fig. 3a were obtained considering a suspension with low "optical density" (1.0) and under continuous irradiation with 5.0 s^{-1} (a.u.) and with 2.0 s^{-1} (a.u.) for modulated light beam. The data show a significant decrease in the oxygen evolution rates in the far-red region (after 700 nm) and in the middle part of the spectrum. As a consequence of these drops in the oxygen evolution rates, a very well expressed decrease in the quantum efficiency was observed in these parts of the spectrum (curve C_1). The addition of the second modulated light beam led to a significant increase in the oxygen evolution rate and in the quantum efficiency spectrum in these regions (curve C_2).

Experimental results obtained for the first time by Emerson *et al.* (1957) and repeated later by Govindjee (1963) are presented in Fig. 4a. As it is known, up to now the results presented in this figure are considered to be unexplainable without using the Z-scheme. In 1957, without powerful computing devices, this assumption was easily accepted. However, let us consider the results presented in Fig. 3b obtained by theoretical calculations using an ideal suspension with higher absorbance (5.0), and higher modulated (background) light (20 s^{-1}), as used in all experiments on the enhancement effect. Comparison of the curves of the quantum yield (open circles, C_2) shown in Fig. 4b (which is a part of the calculated Fig. 3b) with the dashed line (closed circles) in Fig. 4a confirmed our main conclusion that the "red drop" and "enhancement" phenomena are a consequence of the non-linearity of the light curves[1].

The postulation of the existence of two different pigment systems participating in the electron transport reactions of photosynthesis, one of which absorbs short-wave light (below 680 nm) and the other long-wave light above 680–690 nm, leads to very clearly expressed contradictions: 1) How can we explain that the light absorbed above

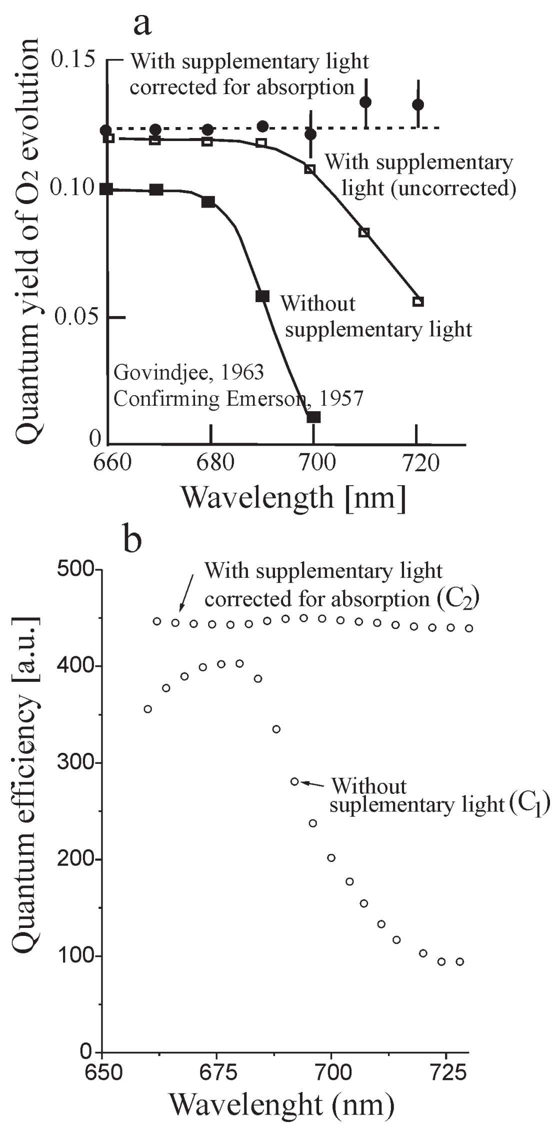

Fig. 4. (a) Experimentally obtained results by Emerson *et al.* (1957) and repeated by Govindjee (1963, 2000) for "red drop" and "enhancement" effects of Emerson. Solid line (with solid squares), quantum yield without complementary light; solid line (open squares), quantum yield with supplementary light (without correction for absorbance); dashed line (solid circles), quantum yield with supplementary light after correction for absorption. (b) Theoretically calculated dots of quantum efficiency without supplementary light (C_1) and with supplementary light after correction for absorption (C_2). The difference between the two curves shows the increase of quantum efficiency (Emerson enhancement effect). Curve C_2 is analogous to the curve with supplementary light corrected for absorption in Fig. 4a. The graph is a part (between 660 and 730 nm) from Fig. 3b.

[1] It should be noted that the theoretically calculated enhancement spectrum of quantum efficiency (Fig. 4b) describing almost exactly the experimentally obtained spectrum (Fig. 4a) is not obtained up to now using the postulate of the two photosystems (Z-scheme).

700 – 750 nm (Pettai, 2005a, b) could evolve oxygen, a process performed only by photosystem II (PSII)? The supporters of the "Z-scheme" and the two photosystems affirm that the short-wave PSII is in a state to absorb light up to 780 nm. Nevertheless, in this spectral region light is absorbed predominantly by PSI and in this way some of the absorbed quanta cannot be used effectively, thus leading to the "red drop". Obviously, all these assertions are not based on any experimental proof. 2) Another interesting experimental result is the increase in the quantum yield with supplementary light in the region of the PSII absorption [between 660 and 680 nm; see Fig. 4a or Govindjee (1963, Fig. 1)]. According to Govindjee and Govindjee (1965), this effect is explained with the so-called "spurious enhancement", arising from the non-linearity of the light curves. However, if the non-linearity of the light curves leads to such an increase in the quantum efficiency in the spectral regions with high absorbance (in the absorbance maximum at 680 nm), the effect will be much more pronounced in the regions with low absorbance, *i.e.* between 690 nm and 750 nm, which is actually registered. 3) On the other hand, why does the use of higher light intensities not lead to the positive enhancement effect? Enhancement effects have been obtained (see Table II in Govindjee and Rabinowitch, 1960) in all experiments, not only in the one which is cited, under low, even very low light intensity conditions. As shown, under such conditions the light curves are non-linear or "S"-shaped, so the non-additive behaviour of the light action cannot be excluded without compensation of the initial non-linear part! The correct explanation of this phenomenon should be based on the non-linearity of the light curves.

The increase of the amplitudes in oxygen evolution induced by one modulated light beam on the background of the second continuous beam with the same (650 nm) wavelength in *Scenedesmus obliquus* is shown in Fig. 5. The results show that the value of enhancement [the ratio of the amplitude size of modulated oxygen evolution, obtained under the action of the two light beams ($V_{I_1+I_2} - V_{I_1}$) and the amplitude obtained under the action of the modulated beam only (V_{I_2}) was higher than seven.

The following experimentally obtained results give additional confirmation of the statement that the enhancement effect is a consequence of the non-linearity of the photosynthetic light curves. A suspension of *Chlorella pyrenoidosa* was irradiated with two light beams (Fig. 6), one of which was modulated at 700 nm (1 s light/1 s dark), and the second one was continuous background light with different wavelengths between 600 nm and 700 nm. The amplitude of the modulated oxygen evolution rate induced by the 700-nm beam changed after applying background radiation of

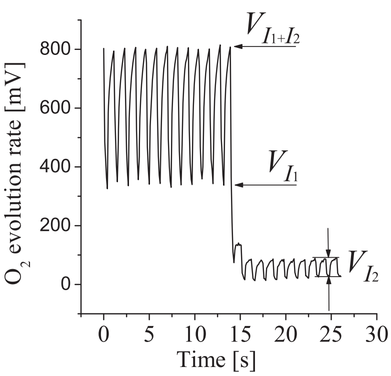

Fig. 5. Oxygen evolution in *Scenedesmus obliquus* induced by two light beams with equal (650 nm) wavelength. One of the beams, I_2, is modulated (1 s light/1 s darkness, 6 μmol m^{-2} s^{-1}) and the other one, I_1, is continuous (12 μmol m^{-2} s^{-1}).

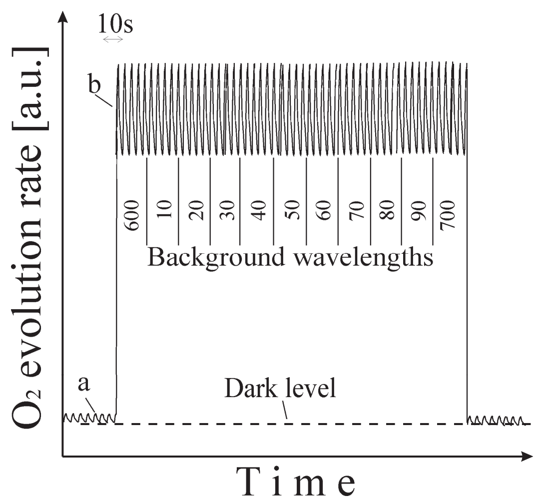

Fig. 6. The amplitudes of the modulated (0.5 Hz) oxygen evolution rate in *Chlorella pyrenoidosa* induced by (a) a 700-nm beam without background radiation and (b) after compensation of the initial non-linear part of the light curve with background radiation of different wavelengths between 600 and 700 nm.

different wavelengths, whose intensity was chosen in such a way to yield an equal oxygen evolution rate in the linear part of the light curve. The intensity of the 700-nm modulated beam was kept constant. The amplitude of the modulated oxygen evolution rate remained constant (in the limit of experimental errors) in all investigated spectral regions (600 to 700 nm). If Emerson's second effect exists as a separate phenomenon, we should not obtain any enhancement in the case of addition of a 700-nm light beam. The equal degree of enhancement at 700 nm and other wavelengths shows that Emerson's second effect is only a particular case of the principle of the non-additive action of radiation in photosynthesis (Zeinalov, 1977a) and that it does not exist even as a second-order effect. Comparison of the enhancement values obtained in our experiments (approx. 5 to 10 times, in some experiments reaching 20!) with those in Emerson's second effect investigations (approx. 1.2 to 2.2) shows that the effect provoked by the non-linearity of the irradiance curves was much stronger than the observed Emerson's enhancement effect. Obviously, the effect of irradiance on photosynthesis is non-additive not only for the beams with different wavelengths but also for the beams with equal wavelengths. This statement was confirmed by Warner and Berry (1987) and by Milin and Sivash (1990).

Based on the analysis of the obtained results, it is evident that the presently accepted interpretation of the "red drop" and "enhancement" effects, with the misbalanced excitation of the two different photosystems at the red region of the spectrum, is wrong. The theoretically obtained results, presented in this work, should be accepted as the only possible and adequate interpretation of these effects.

Emerson R. (1957), Dependence of yield of photosynthesis in long-wave red on wavelength and intensity of supplementary light. Science **125**, 746.

Emerson R. and Lewis C. M. (1943), The dependence of the quantum yield of *Chlorella* photosynthesis on wave length of light. Am. J. Bot. **3**, 165–178.

Emerson R., Chalmers R., and Cedersland C. (1957), Some factors influencing the long-wave limit of photosynthesis. Am. J. Bot. **43**, 133–143.

Govindjee (1963), Emerson enhancement effect and two light reactions in photosynthesis. In: Photosynthetic Mechanisms in Green Plants (Kok B. and Jagendorf A. T., eds.). National Academy of Sciences – National Research Council, Washington, D.C., publication no. 1145, pp. 318–334.

Govindjee (2000), Milestones in photosynthesis research. In: Probing Photosynthesis: Mechanisms, Regulation and Adaptation (Younis M., Pathree U., and Mohanty P., eds.). CRC Press, Taylor and Francis Group, London, UK, pp. 9–39.

Govindjee and Rabinowitch E. (1960), Action spectrum of the "second Emerson effect". Biophys. J. **1**, 73–89.

Govindjee and Govindjee R. (1965), Two different manifestations of enhancement in the photosynthesis of *Porphyridium cruentum* in flashing monochromatic light. Photochem. Photobiol. **4**, 401–415.

Haxo F. T. and Blinks L. R. (1950), Photosynthesis action spectra of marine algae. J. Gen. Physiol. **33**, 389–422.

Hill R. and Bendall F. (1960), Function of the two cytochrome components in chloroplasts: a working hypothesis. Nature **186**, 136–137.

Kok B., Forbush B., and McGloin M. (1970), Co-operation of charges in photosynthetic O_2 evolution. I. A linear four step mechanism. Photochem. Photobiol. **11**, 457–475.

Litvin F. F. and Xe I.-T. (1966), Action spectra of photosynthesis and Emerson effect in higher plants. Dokl. Akad. Nauk SSSR **167**, 1187–1190 (in Russian).

Milin A. B. and Sivash A. (1990), Effect Emersona: Novij podhod (The effect of Emerson: A new approach). Fiziologia i biochimiya kulturnih rastenii **1**, 27–31 (in Russian).

Pettai H., Oja V., Freiberg A., and Laisk A. (2005a), Photosynthetic activity of far-red light in green plants. Biochim. Biophys. Acta **1708**, 311–321.

Pettai H., Oja V., Freiberg A., and Laisk A. (2005b), The long-wavelength limit of plant photosynthesis. FEBS Lett. **579**, 4017–4019.

Warner J. W. and Berry R. S. (1987), Alternative perspective on photosynthetic yield and enhancement. Proc. Natl. Acad. Sci. USA **84**, 4103–4107.

Zeinalov Y. (1977a), Non-additiveness in the action of light at the photosynthesis of green plants. Compt. Rend. Acad. Bulg. Sci. **30**, 1479–1482.

Zeinalov Y. (1977b), The principle of non-additiveness in the action of light and the concept of two photosystems at the photosynthesis in green plants. Compt. Rend. Acad. Bulg. Sci. **30**, 1641–1644.

Zeinalov Y. (2009), On the minimum quantum requirement of photosynthesis. Z. Naturforsch. **64c**, 673–679.