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ON THE ACTION SPECTRA OF PHOTOSYNTHESIS AND SPECTRAL DEPENDENCE OF THE QUANTUM EFFICIENCY

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Summary. Analysis of the results obtained during the investigation of the photosynthetic oxygen evolution upon irradiation with short saturating flashes and of the widely accepted Kok's model for the reactions leading to the oxygen production showed that the photosynthetic irradiance should be non-linear (quadratic or higher degree of dependence) under low irradiance. This non-linearity of the irradiance curves leads to several consequences on the action spectra of photosynthesis and particularly on the decrease of the quantum efficiency in the spectrum regions where pigment absorption is lower, as we demonstrated in our earlier investigations (Zeinalov, 1982; Maslenkova et al., 1994).

An attempt for estimating the action spectra and spectral dependence of the quantum efficiency of photosynthesis in *Scenedesmus acutus* is presented using a polarographic oxygen rate electrode and irradiation with two light beams, one of them being continuous and the other one modulated. The obtained results show that after compensation of the initial non-linear part of the irradiance curves in the estimating of the action spectra and quantum efficiency of photosynthesis the values for the investigated parameters in the spectral regions with low absorbances increase significantly.

Key words: action spectra of photosynthesis, "enhancement" effect, irradiance dependence of photosynthesis, quantum efficiency, "red drop" effect

Introduction

Investigations on the spectral dependence of photosynthesis have been initiated as early as at the end of the 19th century (cf. Rabinovich, 1956). Notwithstanding their number, we do not as yet have a generally accepted explanation for the deviations

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observed between the absorption and the action spectra of photosynthesis in the various photosynthesising systems. The complex system of pigments and their native forms, whose absorption bands are considerably expanded as a result of the dispersion of light in the biological structure and are mutually overlapping, cause considerable difficulties in the interpretation of the obtained results.

Emerson and Lewis (1943) have demonstrated for the first time that in green algae the action spectrum of photosynthesis after the main absorption maximum (680 nm) in the red region of the spectrum was situated noticeably below the absorption spectrum (effect of "red drop"). Later on, Emerson (1957) discovered that the photosynthetic process in this spectral region might be enhanced when short-waves irradiation was added to the non-effective far red light. An adequate explanation of this phenomenon, known as the effect of "enhancement", was the idea of functioning of two photochemical systems in the light reactions of photosynthesis in green plants. Hill and Bendal (1960), "materialized" this idea by means of the so-called Z-scheme. The four successive decades were a period during which this hypothesis was developed. The individual electron carriers as well as their sequence along the complex electron-transport chain were established. Conversely, we have pointed out (Zeinalov, 1977a, b), that the "red drop" and the effect of "enhancement" could be explained ensuing from the non-linearity of the light curves of photosynthesis at the low light intensities and from the so-called principle of non-additiveness in the action of light in this process. This non-linearity of the irradiance curves leads to several consequences on the action spectra of photosynthesis and particularly on the decrease of the quantum efficiency in the spectrum regions where pigment absorption is lower, as we have demonstrated in our earlier investigations (Zeinalov, 1982; Maslenkova et al., 1994).

All preceding investigations on the action spectra and the quantum efficiency of photosynthesis assumed that under very low irradiance conditions the irradiance (light) dependence of the photosynthesis was a linear function and under the same conditions the quantum efficiency reached its maximum value.

Analysis of the results obtained during the investigation of the photosynthetic oxygen evolution upon irradiation with short saturating flashes and of the widely accepted Kok's model for the reactions leading to the oxygen production, showed that the photosynthetic irradiance should be non-linear (quadratic or higher degree of dependence) under low irradiance. Obviously, this non-linearity of the irradiance curves is a consequence of the oxygen evolving mechanisms. It is well established: i. That for the evolution of one oxygen molecule 4 photons should be absorbed from the given oxygen evolving centre; ii. The obtained oxygen precursors (or S_i-states) after the absorption of the photons are unstable and are submitted to the back deactivating reactions. As a consequence under low irradiances, when the intervals between the arriving of the photons are sufficiently prolonged, photon action is non-effective and the light curves have lower slopes. This conclusion has been experimentally confirmed in our preceding investigations (Zeinalov, 1977a, b).

Obviously, if the irradiance dependence of the photosynthesis is a linear function, the investigations of the action spectra and the quantum efficiency could be carried out under very low irradiances. On the other hand, if this dependence is non-linear, the quantum effectivity of the process will be a complex function of the irradiance intensity. The lowering of this intensity leads to lower rate of photosynthesis, and hence to a quantum efficiency decrease especially in spectral regions where the absorbance is reduced and the rate of the photosynthetic process is lower. This may account for the quantum efficiency decrease 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. The detailed investigations of Emerson and Lewis (1943), Haxo and Blinks (1950), Litvin and I-Tan' (1966), have revealed that between 500 and 670 nm the photosynthesis action spectra curves fall down considerably below the absorption spectrum curves, i.e. the quantum efficiency in this part of the spectrum decreases. This drop, beginning from 580 nm in the Emerson and Lewis' experiments (because of the high absorbance of alga suspension used) cannot be explained by means of the assumed non-efficient radiant energy absorption by carotenoides, because a significant absorption by these pigments can be expected at only up to 540 nm. In the experiments of Haxo and Blinks and Litvin and I-Tan' where suspensions with low absorbances are used, the decrease in photosynthetic quantum efficiency at 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. If this explanation is true, we should assume that the balance restoration occurs at the short-wavelength absorption maximum, i.e. a perfect balance in radiant quanta distribution between the two photosystems exists only at the absorption maxima of suspensions, which is very difficult to explain by the quantum mechanics interpretation of electron transitions in chlorophyll molecules.

The appearance of several new papers considering the action spectra and the maximum value of photosynthetic quantum efficiency (Boichenko, 1998; Govindjee, 1999) indicates that the problems are still unsolved. An attempt for estimation of the action spectra and spectral dependence of the quantum efficiency at *Scenedesmus acutus* is presented using a polarographic oxygen rate electrode and irradiation with two light beams – one of which is continuous and the other one modulated. The obtained results show that after compensation of the initial non-linear part of the irradiance curves the values for the investigated parameters in the spectral regions with low absorbances increase significantly.

Material and Methods

The experiment was carried out using unicellular algae suspensions of *Scenedesmus acutus* (Meyen) Chod.

The oxygen exchange was determined polarographically, using the electrode system of Joliot and Joliot (1968). The amperometric current was recorded with a polarograph OH105 (Radelkis, Hungary) The sample volume, approximately 80 mm^3, was excited with two monochromatic beams, one of which was intermitted by an obturator with a frequency 0.5 Hz (1 s light and 1 s darkness). The intensity of the second beam was adequately selected, so that the initial non-linear part of the irradiance curves could be compensated.

The spectral distribution of halogen lamp ("Ausco", 100 W/12 V) light energy was measured with spectroradiometer "MACAM" SR 3000A – (Scotland) and the obtained results are presented on Fig. 1.

The absorption spectra were recorded with a spectrophotometer SF-14 (USSR) using a suspension with absorbance up to 1.0. The correction index for the scattering was introduced assuming the absorbance at 750 nm for zero. This assumption was conventional and probably scattering at shorter wavelengths was higher, but the recorded absorption spectra of suspensions with different absorbances below 1.0 and their normalization by maxima at 680 nm did not show significant differences in the investigated spectral region.

Results and Discussion

Prior to consideration of the experimentally obtained results let us look at Fig. 2, presenting the theoretically calculated data using the model of Kok et al. (1970) for a suspension with 0.1 absorbancy. Curve *a* represents the shape of the irradiance dependence of photosynthesis (oxygen evolution) under low irradiances, inducing at the maximum intensity (1.0) a light reaction with a 1.0 s^{-1} rate constant, while the slowest forward light-induced reaction 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 maximum which leads to a decrease in the values for quantum efficiency spectrum in this regions. This supports the statement that non-linearity of the irradiance curves leads to decrease of the quantum efficiency on the both slopes of the absorption maximum and to the appearence not only of the "red drop", but also of a "blue drop" effect on the short wave sides of the absorption maxima. Obviously, if all the absorbed quanta are used effectively in the photochemical process and the irradiance dependence of photosynthesis is a linear function, the quantum efficiency spectra should be a straight horizontal line without any declines.

The experimentally obtained results are presented in the figures as described below. Curve *a* on Fig. 3 represents the shape of absorption spectra of *Scenedesmus*

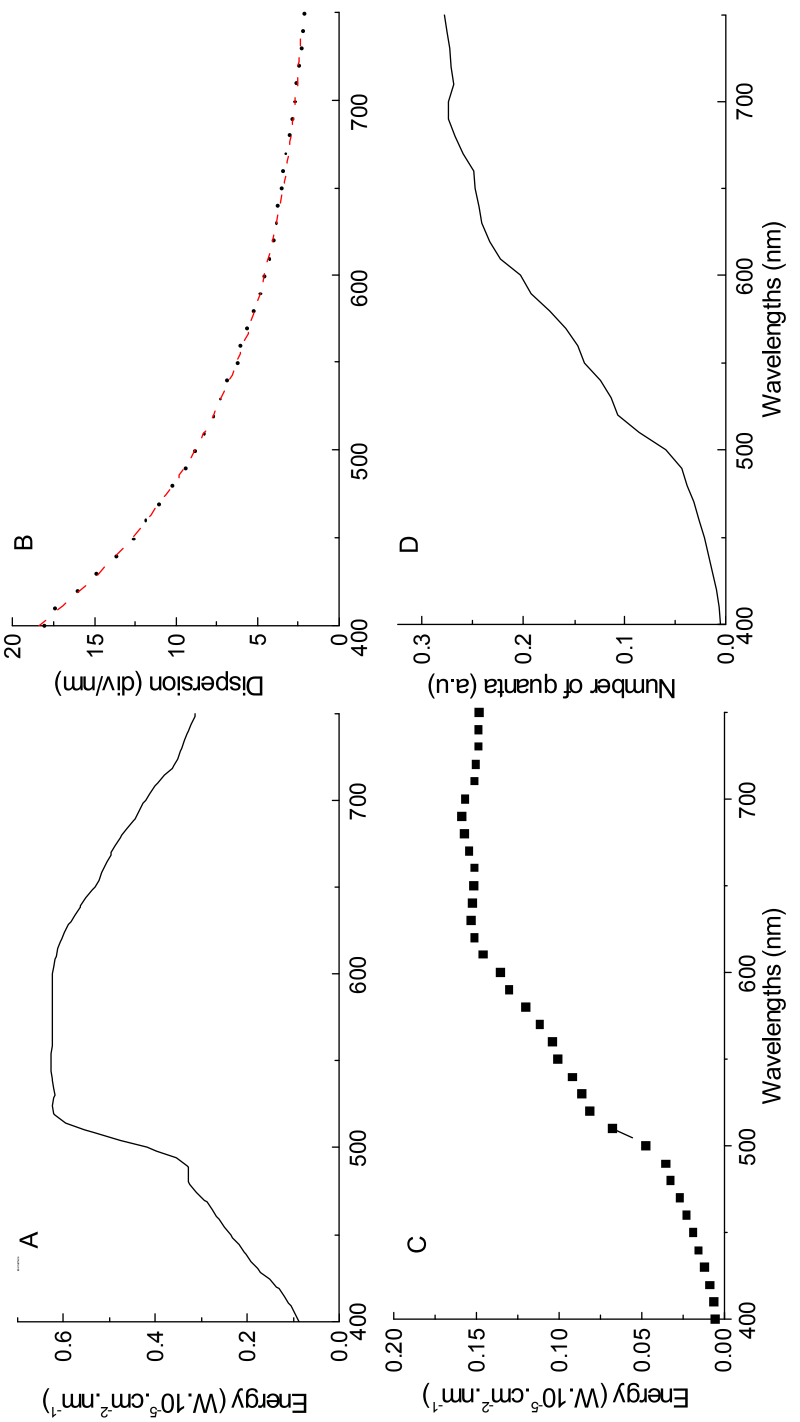

Fig. 1. Spectral distribution of the irradiance energy at halogen lamp (100 W/12 V) – A; Dispersion function of monochromator UM2 – B; energy distribution at the exit slit of the monochromator UM2 – C; quanta distribution at the monochromator UM2 – D.

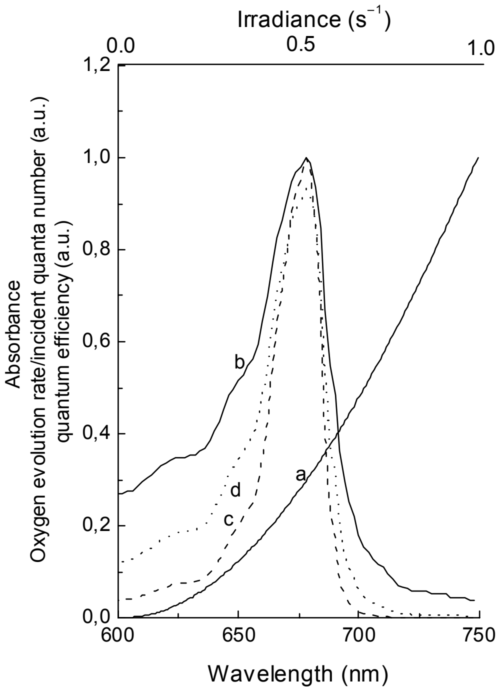

Fig. 2. Theoretically calculated curves according to the model of Kok et al. (1970), using the following values for the parameters included in the model: forward rate constants – 20 s^{-1}; back (deactivation) reaction constants: for S_1-state – $k_1 = 0\ s^{-1}$; for S_2-state – 0.05 s^{-1}; for S_3-state – 0.5 s^{-1}. The values of double hits and misses are neglected. a – irradiance dependence of the photosynthesis under low irradiances (0 – 1 s^{-1}); b – normalized absorption spectrum in suspension of *Scenedesmus acutus* with low (0.1) absorbance; c – calculated action spectrum of oxygen evolution; d – theoretically calculated quantum efficiency spectrum

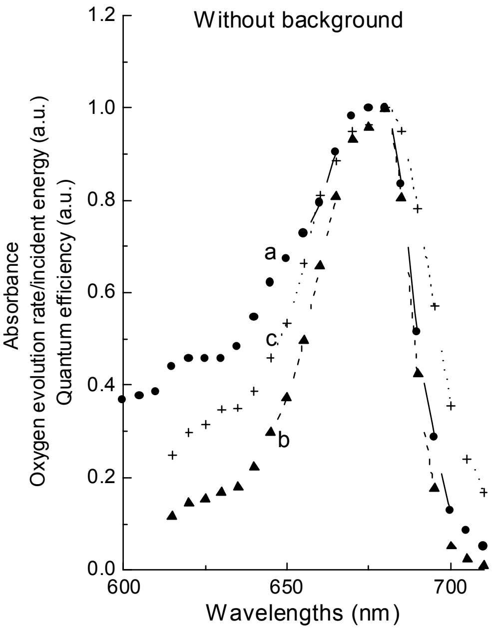

Fig. 3. Normalized absorption spectrum – a; action spectrum – b; and spectral dependence of the quantum efficiency – c in *Scenedesmus acutus* suspension with 0.1 absorbance and without background irradiation

acutus (Meyen) Chod in the spectral region between 600 and 710 nm under low irradiance, representing about 10% of the saturating irradiance intensity and low absorbance (0.1). Curve *b* reflects the action spectrum (oxygen evolution rate vs the incident quanta number at different wavelengths). Curve *c* represents the quantum efficiency spectrum, i.e. the oxygen evolution rate vs the absorbed quanta.

The data showed a well expressed analogy, especially in the region of the shortwave slope of the absorption maximum, between the experimentally obtained and the theoretically calculated results (Fig. 2), i.e. the calculated quantum efficiency spectrum (curve *c*) showed a maximum at 680 nm and a significant decrease both at shorter and at longer wavelengths.

The results presented in the following figures (4 and 5) demonstrate the changes in action spectra and in quantum efficiency spectra obtained using background irradiance at 650 and 680 nm, respectively. The intensity of the background irradiation was chosen to compensate as far as possible, the initial non-linearity of the irradiance curve (Fig. 2, *a*). The action spectra and quantum efficiency spectral curves were slope up on both sides of the absorption maximum after replacing the working point to the more steep slope on irradiance curve with background irradiation.

In comparison to the data presented in Fig. 3 (without background irradiation), an increase of the action spectrum on both slopes of the absorption maximum was observed using suspensions with higher absorbances (0.5) (Fig. 6, *b*). The quantum efficiency showed a decrease after 695 nm ("red drop") and before 650 nm. The curves of the quantum efficiency spectral dependence, recorded with background irradiation at 650 nm and 680 nm (Fig. 7 and 8 respectively) did not show a significant drop on the two slopes of the absorption maxima. On the contrary, a decrease at 680–690 nm was obtained independently of the background light wavelength as a result of reaching the saturation region of the irradiance curve. The curve showed also a higher

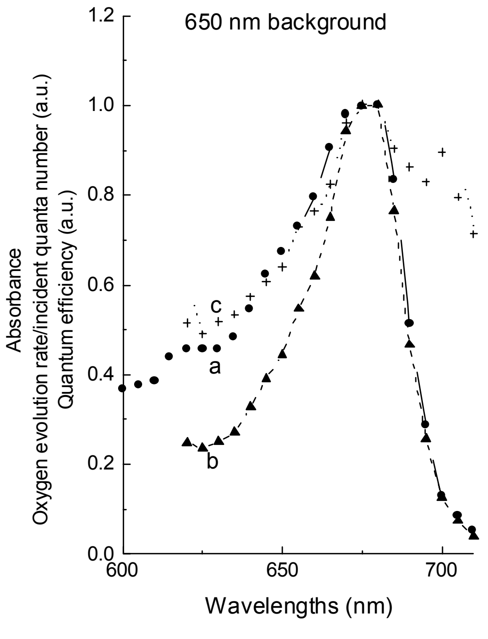

Fig. 4. Normalized absorption spectrum – a; action spectrum – b; and spectral dependence of the quantum efficiency – c in *Scenedesmus acutus* with 0.1 absorbance and with 650 nm background irradiation

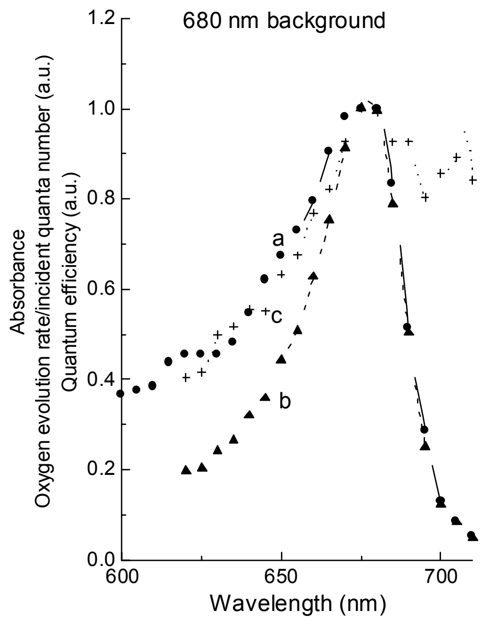

Fig. 5. Normalized absorption spectrum – a; action spectrum – b; and spectral dependence of the quantum efficiency – c in *Scenedesmus acutus* with 0.1 absorbance and with 680 nm background irradiation

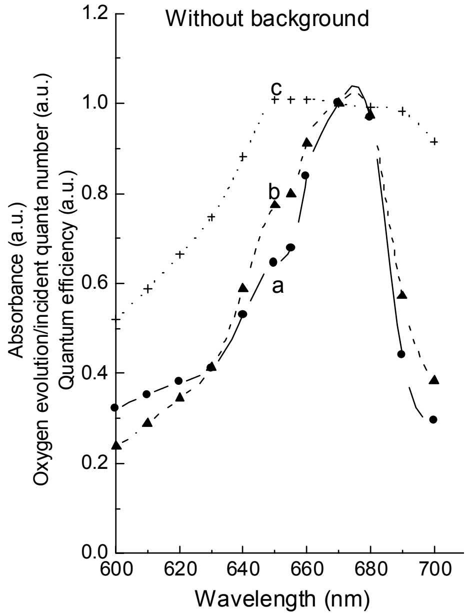

Fig. 6. Absorption spectrum – a; normalized action spectrum – b; and spectral dependence of the quantum efficiency – c in *Scenedesmus acutus* with 0.5 absorbance and without background irradiation.

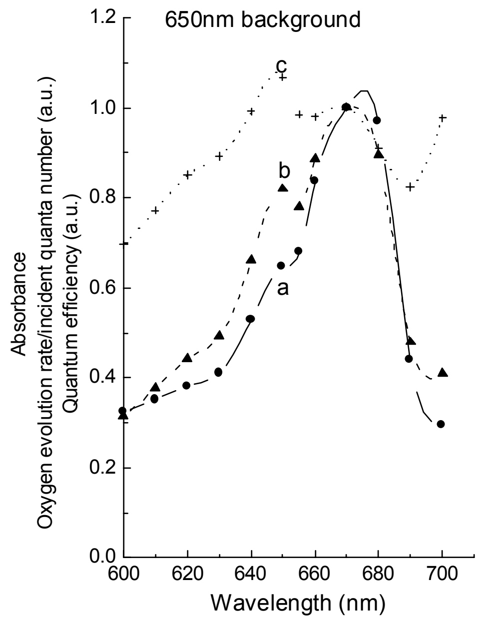

Fig. 7. Absorption spectrum – a; normalized action spectrum – b; and spectral dependence of the quantum efficiency – c in *Scenedesmus acutus* with 0.5 absorbance and with 650 nm background irradiation

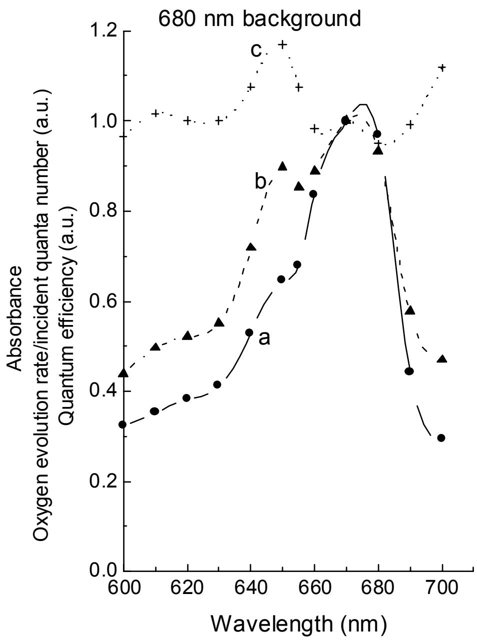

Fig. 8. Absorption spectrum – a; normalized action spectrum – b; and spectral dependence of the quantum efficiency – c in *Scenedesmus acutus* with 0.5 absorbance and with 680 nm background irradiation

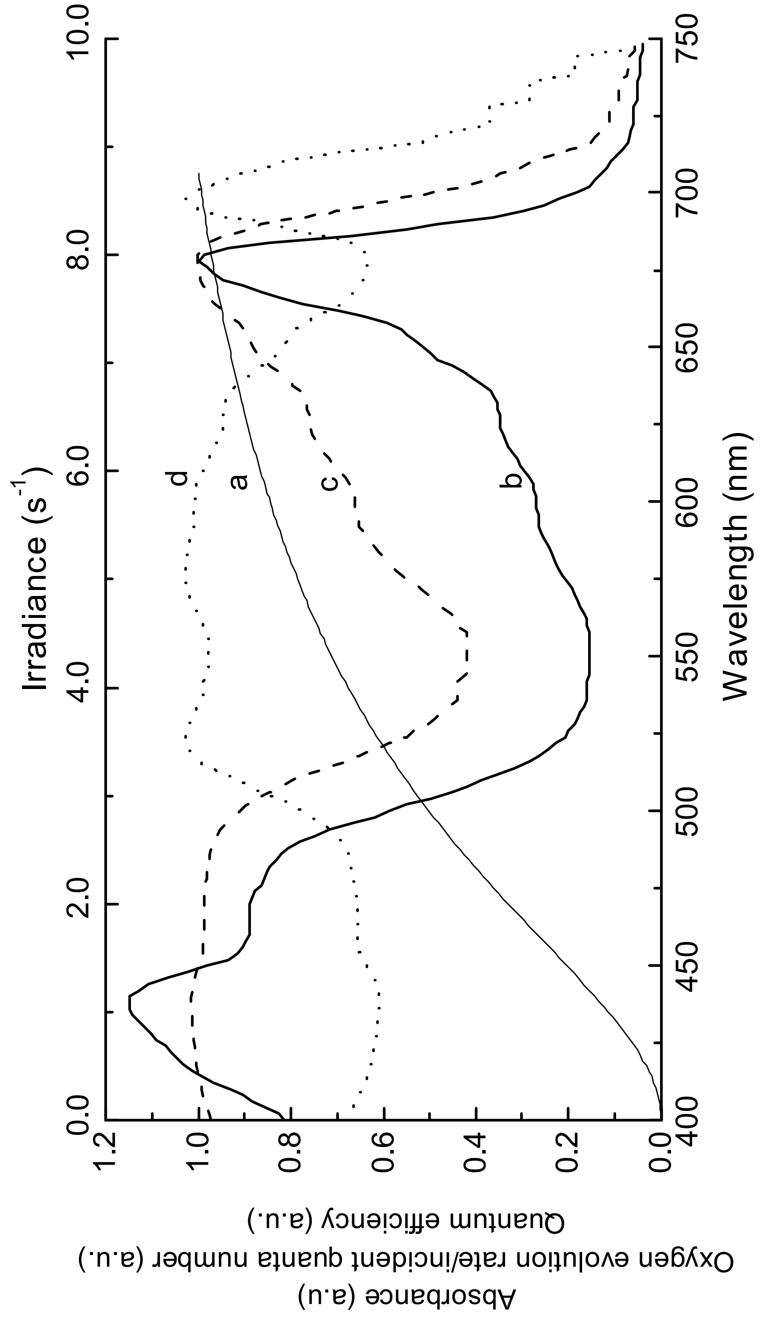

Fig. 9. Theoretically calculated curves according to the model of Kok et al. (1970): a – irradiance dependence of the photosynthesis under high irradiance conditions (10 s^{-1}); b – normalized absorption spectrum in suspension of *Scenedesmus acutus* with 1.0 absorbance; c – calculated action spectrum of oxygen evolution; d - theoretically calculated quantum efficiency spectrum

efficiency of the quanta absorbed by chlorophyll *b* and the formation of a maximum at 650 nm. The results presented in Fig. 9 give an explanation of the changes in the quantum efficiency values at different wavelengths, and reflect the theoretically calculated action and quantum efficiency spectra in suspensions with higher absorbances (1.0) and higher irradiance intensity (10 s^{-1}). Obviously, due to the non-linearity of light curves, the different sublayers of the suspensions could not lead simultaneously to the linear part of the "irradiance curves". We can obtain the linear part of the average "irradiance curve" from the different sublayers, but in this case the surface sublayers will attain the light saturated region of their individual "irradiance curves" especially at the regions of absorbance maxima. Therefore, the effect of irradiance on the surface layers at 670–680 nm will be reduced and this will lead to formation of a minimum. Having in view that the absorbance of some of the separated chloroplasts at this regions could reach values higher than 0.5–0.8, even with suspensions with absorbance (0.5) which are used in the experiments of figures 6, 7 and 8, we could obtain some decrease in quantum efficiency curves at absorbance maxima.

The presented results show that if the "irradiance curves" of photosynthesis have a non-linear part at low irradiances which is a consequence of the nature of process itself, the exact determination of action spectra and the spectral dependence of quantum efficiency is very complicated and the estimated action spectra will be dependent on the intensity of the irradiance and the rate of the oxygen evolution. The results indicate also that even after applying background irradiation, the registered quantum efficiency spectra of a suspension whose absorption is not very high (0.5) show decreases at both slopes of the absorption maximum. In contrast to the results of Emerson and Lewis (1943) and in agreement with the data of Haxo and Blinks (1950), Litvin and I-Tan' (1966), the results presented in this investigation show that the use of suspensions with absorption lower than 0.5 leads to a quantum efficiency reduction at spectral regions, with decreased absorptions, even after "compensation" of the non-linearity of the irradiance curves. Most probably, as a consequence of the higher absorbance of individual algae cells, where the chloroplast structures and chlorophyll distribution is not homogenous by far, the exact compensation of the non-linearity of the separated sublayers is not possible to occur simultaneously. The results show that on the quantum efficiency spectra could be found regions with local maxima (i.e. at 650 nm in Fig. 7 and Fig. 8), or with minima (i.e. at 680 nm), whose origin is also connected with the non-linearity of the irradiance curves. On the other hand, the use of suspensions with higher absorbances in the investigations of the photosynthetic action spectra and especially of the maximum values for quantum efficiency, will lead to smoothing of the action spectra and to a decrease in the absolute value for quantum efficiency. In conclusion, we can point out that most probably, the efficiency of photosynthesis remains almost constant in the investigated spectral region (600 to 700 nm) and the observed deviations are connected with the non-linearity of the irradiance curves.

A serious consequence of the non-linear character of photosynthetic "light curves" in optically non-dense suspensions was that both the quantum efficiency curves and the action spectra showed a clearly expressed structure. Maximal values of oxygen evolution vs incident (absorbed) quanta number were obtained in the regions where the absorption was maximal.

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