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ENHANCEMENT AND DE-ENHANCEMENT EFFECT IN *ANACYSTIS NIDULANS*

OLGA v.H. OWENS AND GEORGE HOCH
RIAS, Baltimore, Md. (U.S.A.)

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SUMMARY

A de-enhancement phenomenon has been reported to be especially noticeable in the blue-green alga *Anacystis nidulans*. The region of the spectrum in which this phenomenon occurs corresponds to chlorophyll *a* absorption, and has been attributed to a a non-linear response of O_2 production to light intensity. A non-linear response of one or both light beams means that when the two beams are superimposed the resulting rate will be different from the sum of the two beams presented separately.

By means of a modified mass spectrometer and O_2 isotopes, it is possible to examine simultaneously the production and uptake of O_2 by the alga. The results showed that O_2 production in the chlorophyll *a* absorbing region is strictly linear with light intensity. The apparent non-linearity of O_2 production arises as a result of the inhibition of O_2 uptake in light of this spectral region giving rise to an apparent de-enhancement.

INTRODUCTION

An important advance in photosynthetic investigations was the discovery by EMERSON *et al.*[1]. of enhancement. In the enhancement effect, far red light which gives a low quantum yield can be shown to give increased yield when it is supplemented by light of shorter wavelength. These investigations led to the concept of a two pigment system for the photosynthetic mechanism.

Further studies showed that the action spectrum for the supplementary light effect follows the absorption spectrum of the accessory pigments[2,3]. When the supplementary light is in the region of chlorophyll *a* absorption, however, the enhancement effect disappears and a "de-enhancement" or "negative enhancement" occurs. The negative effect is especially noticeable in the blue-green alga *Anacystis nidulans*. De-enhancement was investigated by GOVINDJEE AND RABINOWITCH[4], and by R. GOVINDJEE AND RABINOWITCH[5]. They suggested that the de-enhancement was brought about by an early saturation of photosynthesis in light of the 700-mμ region.

In a previous paper[6] we reported that light given to chlorophyll *a* results in a non-linear slope of net O_2 production *versus* light intensity. In this paper we will show that true O_2 production gives a linear response to light intensity, and that the non-linearity of the net O_2 production is brought about by inhibited respiration resulting in an apparent de-enhancement where no actual de-enhancement occurs.

METHODS

A mass spectrometer with a modified leak system was used in these experiments[7]. In some experiments reported here, light was provided by one 750-W lamp equipped with suitable condenser lenses and a 12-in water bath. The other light source was a slide projector. In other experiments two 750-W lamps were used with their beams at an angle of 30°. Neutral-density screens were used to vary the intensity of the light beams. Spectral regions were isolated with B&L interference filters and suitable Schott or Corning cut-off filters.

The alga was *Anacystis nidulans*. The methods for growth and handling were the same as reported in a previous paper[6].

In most cases 3-min periods of alternating dark and light were used. When rates were low it was possible to use a single batch of cells throughout one experiment. This was the case in Fig. 1. In Fig. 2 and Table I three separate batches were used.

RESULTS

700-mμ intensity curve

In a previous paper three light intensity *versus* rate curves, measured with three different wavelengths, were illustrated[6]. The curves showed that in contrast to 630-mμ light, 678-mμ and 700-mμ light did not give a linear response when net O_2 exchange was observed.

Fig. 1 is a light-intensity curve for weak 710-mμ light using isotopes to demonstrate O_2 uptake and O_2 production rates and in addition, the net O_2 rate, a value that would be observed with a manometer or polarograph. It can be seen from the figure that O_2 uptake is inhibited at lower intensities, while the O_2 production curve is a straight line which extrapolates to zero. The inhibition of O_2 uptake and the linear response of O_2 production produced a net O_2 exchange curve with a break in it, repeating the earlier observations[6]. Thus in weak 710-mμ light O_2 production had a lower value than would have been found from manometry data alone. Additional experiments with 700-mμ light have indicated that in higher intensities of light the O_2 production curve remains linear up to the strongest light available, about one-half the saturation rate obtained in white light.

Enhancement and de-enhancement

Table I gives data for an experiment with 700-mμ and 678-mμ light. Both wavelengths produced inhibited O_2 uptake in weak light. When 678-mμ light was presented simultaneously with 700-mμ light, an enhancement of 700-mμ O_2 production occurred. The table gives values for 700-mμ enhancement at three increasing intensities of the two beams. The last column of the table shows enhancement values which were calculated from "manometric" data, *i.e.* net O_2 exchange minus average dark O_2 uptake. Those values which are less than one demonstrate an apparent de-enhancement. It is clear that in these experiments no actual de-enhancement occurred. On the contrary, an enhancement was found. The slight enhancement values shown in the table were not always present. Further studies are necessary to clarify this point.

Shape of the 700-mμ light intensity curve

The possibility that the 700-mμ light intensity curve might be sigmoid was investigated. If the curve is sigmoid then an apparent enhancement would occur

TABLE I

EFFECT OF 678-mμ (+ CORNING 2-73) AND 700-mμ (+ CORNING 2-62) LIGHT, SINGLY AND IN COMBINATION, ON RATES OF O_2 PRODUCTION AND O_2 UPTAKE

Light periods were 3 min alternating with dark 3 min. Enhancement of 700-mμ light equals the ratio of the rate of 700 mμ in the presence of 678 mμ to the rate in 700 mμ alone. The average dark uptake rate equalled −22.5. Net exchange minus dark uptake is the value for O_2 production that would be recorded from the manometric method. Values are arbitrary units.

678 mμ			*700 mμ*			*678 mμ and 700 mμ*			*700-mμ enhancement**	
O_2 *production*	O_2 *uptake*	*Net exchange —dark uptake*	O_2 *production*	O_2 *uptake*	*Net exchange —dark uptake*	O_2 *production*	O_2 *uptake*	*Net exchange —dark uptake*	O_2 *production*	*Net exchange —dark uptake*
1.3	−20.1	3.7	3.7	−9.1	17.1	7.4	−9.9	20.0	1.6	1.0
6.0	−13.8	14.7	8.0	−9.3	21.2	18.7	−7.7	34.2	1.6	0.9
23.0	−7.4	38.1	26.5	−7.9	41.1	52.7	−11.2	64.0	1.1	0.6

* Values less than 1.0 represent "de-enhancement."

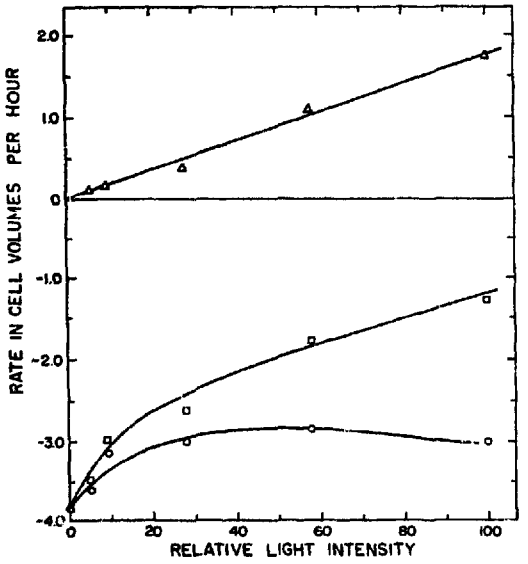
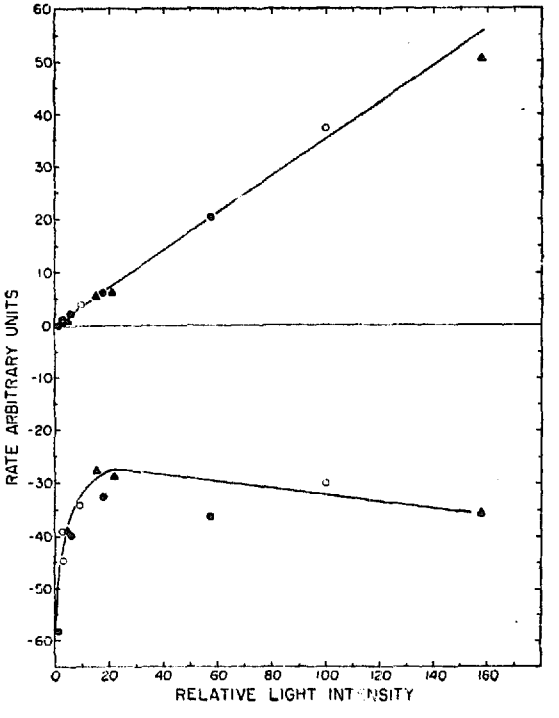


Fig. 1. Relative light intensity of 710-mμ light (B&L 710 interference filter + Schott RG-8) *vs.* rates of O_2 uptake (negative), O—O; net O_2 exchange, □—□; and O_2 production (positive), △—△. Net O_2 exchange was calculated from production plus uptake. The average uptake in the dark was −3.9 cell vol./h.

Fig. 2. Relative light intensity of 700-mμ light *vs.* rates of O_2 uptake and production. Two beams were used, each with a 700-mμ interference filter and a Schott RG-8. Each beam was given separately and then in combination. The light intensity of the combined beams was assumed to be the sum of the two separate beams. O—O, beam A; ●—●, beam B; ▲—▲, combined beams.



when one 700-mμ beam is presented with another 700-mμ beam. Such an experiment is shown in Fig. 2. Several intensities of 700-mμ light were presented separately and in combination. The combination intensity curve does not differ significantly from the individual intensity curves. Neither a sigmoid curve nor a curve with an upward bulge (as suggested by MYERS AND GRAHAM for Chlorella[8]) was found in Anacystis. 678 mμ has also been found to give a strictly linear response. Thus the observed enhancement of 700 mμ (Table I) was due entirely to the presence of 678-mμ light. Fig. 2 also shows the inhibition of respiration in more detail than in Fig. 1. The inhibition reached its greatest extent at a light intensity and O_2 production of about one-fourth compensation.

DISCUSSION

Our observation that respiration is inhibited by weak light in the chlorophyll *a* region of the spectrum helps to explain some results obtained by other investigators.

It has been noted by several investigators[9-11] that the action spectrum in the chlorophyll *a*-absorbing region of the blue-green algae Anabena, Ocillatoria and Anacystis shows a decline, indicating a low efficiency for chlorophyll *a*. This lowered efficiency was not observed by EMERSON AND LEWIS[2] in their quantum-yield studies of Chroococcus. HAXO AND BLINKS[9] using thalli 1–2 cells thick and light bright enough to produce a rate between compensation and saturation, probably obtained values which more nearly resembled the true O_2 production. EMERSON AND LEWIS, on the other hand, used thick suspensions which totally absorbed the light. Their values for O_2 production, always below compensation, may have been as much as three times greater than actually existed resulting in a quantum yield that was too high in the 680–690-mμ region.

Apparent de-enhancement arises from the belief that net O_2 exchange is a true indication of O_2 production. A bend in the net O_2 exchange curve means that the superposition of two beams gives a different rate than the sum of the beams presented separately. Observed de-enhancements can be attributed to the inhibition of respiration which produces the non-linear O_2 exchange curve. True O_2 production, however, is strictly linear. An enhancement occurs when two beams are presented together but no de-enhancement is found.

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