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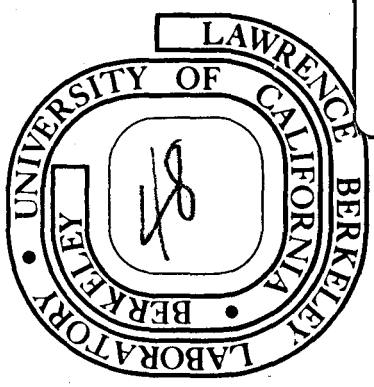

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DISCLAIMER

FLASH KINETICS AND LIGHT INTENSITY DEPENDENCE OF OXYGEN EVOLUTION IN THE BLUE-GREEN ALGA ANACYSTIS NIDULANS

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(Received

SUMMARY

Patterns of oxygen evolution in flashing light for the blue-green alga Anacystis nidulans are compared with those for broken spinach chloroplasts and whole cells of the green alga Chlorella pyrenoidosa. The oscillations of O_2 yield with flash number that occur in both Anacystis and Chlorella display a greater degree of damping than do those of isolated spinach chloroplasts. This increase in damping results from a two- to threefold increase in the fraction (α) of reaction centers "missed" by a flash. The increase in α cannot be explained by non-saturating flash intensities or by the dark reduction of the oxidized intermediates formed by the flash. Anaerobic conditions markedly increase α in Anacystis and Chlorella but have no effect on α in broken spinach chloroplasts. The

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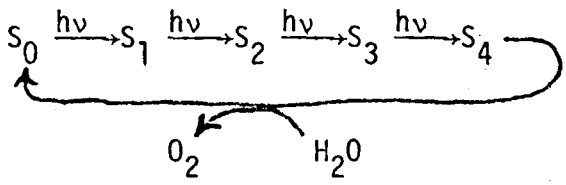
results signify that the mechanism of charge separation and water oxidation involved in all three organisms is the same, but that the pool of secondary electron acceptors between Photosystem II and Photosystem I is more reduced in the dark in the algal cells than in the isolated spinach chloroplasts.

Oxygen evolution in flashing light for Anacystis and Chlorella show light saturation curves for the oxygen yield of the third flash (Y_3) that differ markedly from those of the steady-state flashes (Y_s). In experiments in which all flashes are uniformly attenuated, Y_3 requires nearly twice as much light as Y_s to reach half-saturation. Under these conditions Y_3 has a sigmoidal dependence on intensity, while that of Y_s is hyperbolic. These differences depend on the number of flashes attenuated. When any one of the first three flashes is attenuated, the variation of Y_3 with intensity resembles that of Y_s. When two of the first three flashes are attenuated, Y_3 is intermediate in shape between the two extremes. A quantitative interpretation of these results based on the model of Kok et al. [5,6] fits the experimental data.

INTRODUCTION

Short saturating flashes of light have been an important tool in the investigation of photosynthetic oxygen evolution [1-4]. As a result of such studies, Kok, Forbush and McGloin [5,6] proposed a model for photosynthetic oxygen evolution involving a linear, four-quantum process. Each reaction center acts independently to accumulate oxidizing equivalents with successive photons absorbed until a total of four are stored. Oxygen then appears in a rapid (<1 msec [7]) dark reaction between water and the four-electron oxidized state, S_4:



During an interval of several minutes in the dark, S_2 and S_3 become reduced to S_1, which is stable in the dark. S_0 is only produced in the reaction between S_4 and water. A certain small (ca. 10%) fraction of the reaction centers, α, is not affected even by the saturating flashes (misses). Another small fraction (β) is activated twice by a single flash (double hits).

Some modifications of this model have been suggested, most notably by Joliot and Joliot [7], to correlate fluorescence and oxygen observations, and by Radmer and Kok [8] to include effects of the oxidation state of the acceptor pool on oxygen evolution. Joliot et al. [9] and Bouges-Bocquet [10] presented evidence for a dark reduction of S_1 to S_0. Weiss et al. [11] concluded that the observation that the oxygen yield of the third flash in a series required more light for saturation than did later flash yields could not be explained by the model by Kok et al. [5,6].

We find that the patterns of oxygen evolution in broken spinach chloroplasts and in whole cells of Anacystis nidulans or Chlorella pyrenoidosa are essentially the same. We have confirmed that the oxygen yield of the third flash requires more light to reach saturation than do steady-state flash yields and provide an explanation for this behavior in terms of the model of Kok et al. [5,6].

MATERIALS AND METHODS

The blue-green alga Anacystis nidulans and the green alga Chlorella pyrenoidosa were grown at 22°C in one liter batch cultures in shakers

exposed to continuous illumination. The cultures were aerated by bubbling with a mixture of 4% CO_2 in air. Three times a week about 90% of the culture volume was replaced by sterile growth medium (Kratz and Myers [12] medium C for Anacystis and a modified Myers [13] medium lacking sodium citrate but containing 10^{-5} M $Ca(NO_3)_2$ for Chlorella). Algae from the batch cultures were pelleted by low speed centrifugation (10 min at 3000 x g) and resuspended in a buffered electrolyte solution consisting of 0.1 M KCl, 0.01 M K_2HPO_4, pH = 7.6. The concentration of the algae used for experiments was such that when 1.0 ml of reaction suspension was diluted with 19.0 ml of electrolyte, the absorbance at 625 nm was 0.97 cm^{-1} for Anacystis and 1.15 cm^{-1} at 680 for Chlorella. In repetitive flash experiments the typical dark time between experiments was 3 min for Anacystis and 5 min for Chlorella. No change in the pattern of oxygen evolution in flashing light was observed when the dark time between experiments was increased to 30 min.

Chloroplasts were prepared from the leaves of spinach plants (Spinacia oleracea var. early hybrid #7) grown from seed in a growth chamber as described by Sun and Sauer [14]. The chlorophyll concentration used in oxygen evolution experiments was 300 μg ml^{-1}. NADP (1.5×10^{-4} M) and ferredoxin (0.5 μg ml^{-1}) (Sigma Corp. 66% pure) were added as the electron acceptor system. All experiments were carried out in a buffer which contained 0.4 M sucrose, 0.01 M NaCl, and 0.05 M Tris buffer, pH = 7.6.

In the experiments reported here, the bare platinum electrode, xenon flash lamp and attendant amplification and recording devices previously described by Weiss and Sauer [4] and Babcock and Sauer [15] were used. The circulation electrolyte was 0.1 M KCl, 0.01 M phosphate buffer,

pH = 7.6. For aerobic experiments, the electrolyte was continuously aerated by bubbling with 4% CO_2 in air. For anaerobic experiments, the electrolyte was bubbled with nitrogen. Except where indicated, the interval between each of the 10 μsec xenon flashes was 0.5 sec. The amount of oxygen evolved by a flash was determined to be proportional to the maximum amplitude of the electrode current following the flash, as described by Duysens [16]. Flashes were attenuated by the use of calibrated metal-film neutral density filters (Balzars) used singly or in combination to give the desired transmittance.

RESULTS

Oxygen evolution by Anacystis, Chlorella and spinach chloroplasts in flashing light

The pattern of oxygen evolution in flashing light following a dark period is shown in Fig. 1 for Anacystis nidulans, Chlorella pyrenoidosa, and broken spinach chloroplasts. The response of the chloroplasts to the flash series is similar to that described by Kok et al. [5]. The first two flashes result in little or no oxygen evolution. A maximum yield of oxygen results from the third flash. The yield of the fourth flash is considerably smaller than that of the third. Overall, the pattern of oxygen evolution shows large, period-four oscillations which subsequently damp out.

While Anacystis and Chlorella cells exhibit the same basic response, they differ from spinach chloroplasts in one important respect: both of the algae show a greater degree of damping. This can be seen in several ways. Relative to the chloroplasts, the oxygen yield of the third flash (Y_3) in the whole cells is diminished while that of the fourth flash (Y_4)

is increased. In Anacystis Y_4 is actually larger than Y_3. The number and magnitude of oscillations before achieving a steady-state (constant oxygen yield per flash, Y_s) are smaller in the algal cells. Finally, the sum of the oxygen yields of the first four flashes is from 16 to 36% lower in the algae than in the isolated chloroplasts. All three of these effects are expected consequences of increased damping, which causes reaction centers to lose synchrony more rapidly.

Using the model of Kok et al. [5], with $S_0 = 1.0$, $S_1 = 3.0$, and $S_2 = S_3 = 0$ as starting conditions, moderately good fits ($\pm$ 15% maximum) to the experimental data were obtained for Chlorella with $\alpha = 0.22$ and $\beta = 0.014$ and for Anacystis with $\alpha = 0.32$ and $\beta = 0.033$. The values for Chlorella are comparable with those reported previously by Joliot et al. [9] and by Weiss et al. [11]. A good fit to the data for the chloroplasts was obtained with $\alpha = 0.10$ and $\beta = 0.015$. Thus, using the model of Kok et al. and the same starting conditions, we could generate curves which resemble the oxygen evolution flash response of whole cells of Anacystis, Chlorella or isolated spinach chloroplasts primarily by varying the damping parameters. The variations in β are relatively small and contribute little to the differences seen in Fig. 1.

We could also obtain a good fit to the experimental data for Anacystis with $S_0 = S_1 = 2.0$ and $S_2 = S_3 = 0$ as initial conditions and setting $\alpha = 0.20$ and $\beta = 0.06$. This choice of parameters corresponds to a situation in which there is a significant reduction of S_1 in the dark. Results indicating such a dark reduction of S_1 have been reported in Chlorella by Joliot et al. [9] and by Bouges-Bocquet [10]. However, even with these parameters, the value for α is twice that observed for isolated

chloroplasts. Good fits could not be obtained by further increasing S_0 at the expense of S_1.

The flashes used for the experiments shown in Fig. 1 were saturating, so the differences seen in Fig. 1 cannot be due to reaction centers which are simply not excited by the flash. (Flashes were called saturating if the oxygen yield of the steady state was decreased by no more than 50% when the flash intensity was reduced to 20% of its full value. Typical flash saturation curves for Anacystis are shown in Fig. 4.)

The results shown in Fig. 2 indicate that the increased values of α seen in Anacystis and Chlorella are not due to a partial reduction of the S states in the dark between flashes. In these experiments dark-adapted Anacystis cells received three saturating flashes. The time between the first two flashes was 0.5 sec. The time between the second and third flash (Δt_{2-3}) varied from 10^{-4} to 100 sec. The oxygen yield of the third flash (Y_3) is shown plotted as a function of Δt_{2-3}. As Δt_{2-3} increases, Y_3 increases, goes through a "plateau" region of maximum oxygen yield and then decreases. The initial rise of Y_3 is a measure of the turnover time of photosynthesis [17] while the decrease corresponds to the reduction of S_3 [5,9]. The halftime for the increase for Anacystis is 1.2 msec; for the decrease, it is 9.2 sec. Similar curves were obtained for both chloroplasts and Chlorella, although chloroplasts exhibited a shorter rise time (halftime = 0.7 msec) and a longer time for S_3 reduction (halftime = 25 sec). The rise and decay times for Chlorella were similar to those reported by Bouges-Bocquet [10] and Joliot et al. [9]. The flashes used for the experiments shown in Fig. 1 were given at 0.5 sec intervals. When Δt_{2-3} was 0.5 sec, Y_3 had not yet begun to decrease for

any of the three organisms. Thus, the dark reduction of S_3 is not responsible for the differences seen in the experiments shown in Fig. 1.

Radmer and Kok [8] recently suggested that the misses seen at saturating flash intensities are due to the reduction of the primary electron acceptor (Q) of Photosystem II in a small fraction of the reaction centers via equilibration with electron acceptors between Photosystem II and Photosystem I (the "A-pool"). Diner and Mauzerall [18] showed that in both green and blue-green algae the A-pool is more reduced under anaerobic conditions than under aerobic conditions. Fig. 3 shows the pattern of oxygen evolution in flashing light for Anacystis under aerobic (closed circles) and anaerobic (open circles) conditions. The same sample was used for both experiments, and the anaerobic curve was normalized by the same constant used to normalize the aerobic curve. The anaerobic response shows a much greater degree of damping than does the aerobic response. The flash yields are depressed by anaerobiosis, and oscillations are almost absent. This effect is completely reversible upon restoration of aerobic conditions. Similar results were obtained for Chlorella. Isolated chloroplasts, however, showed no important differences in their responses to flashing light between anaerobic and aerobic conditions. Diner and Mauzerall [18] presented evidence that the decrease in oxygen yields in algae under anaerobic conditions is not due to an acceleration of the rate of S_3 reduction. The experiments shown in Fig. 3, then, suggest that the redox state of the A-pool can markedly influence the value of α in Anacystis and Chlorella.

The effects of non-saturating flashes on oxygen evolution

Weiss et al. [11] observed that in Chlorella cells subjected to short flashes of light from either a xenon flash lamp or a Q-switched dye laser,

considerably more light was required to saturate the oxygen yield of the third flash than was needed to saturate the steady-state yield.

We confirmed these findings for Chlorella and observed a similar effect in Anacystis. Fig. 4 shows light saturation curves for the steady-state flash yields (solid circles) and the yield of the third flash (open circles) in Anacystis nidulans. In these experiments all flashes of a series were attenuated to the indicated intensity. The flash yields at infinite intensity were obtained by extrapolation using a double reciprocal plot. These extrapolated values were then used to calculate the flash intensity which half-saturated the flash yields. The small vertical lines in Fig. 4 indicate half-saturation values. The half-saturation intensity for steady-state flashes (Y_s) is only 0.56 of that required to half-saturate the O_2 yield of the third flash (Y_3). A further difference is seen in the shape of the curves. At low light intensities, Y_3 is a sigmoidal function of light intensity while Y_s is linear. This sigmoidal behavior of the Y_3 *vs.* I curve was seen in all experiments.

Fig. 5 shows similar data for Chlorella. When all flashes are attenuated, the Y_3 *vs.* I curve (crosses) differs markedly from the Y_s curve (closed circles) in both half-saturation intensity and shape. Fig. 5 also shows that the extent of the differences between the Y_3 and Y_s saturation curves depends strongly on the number of flashes attenuated. If only one of the first three flashes is attenuated (open circles), the saturation curve for Y_3 is almost identical to that of Y_s. This is true no matter which of the first three flashes is attenuated. When any two of the first three flashes are attenuated (triangles), the resulting saturation curve for Y_3 is intermediate between the two extremes.

DISCUSSION

The responses of Anacystis and Chlorella to non-saturating flashes of light can be understood in terms of the model of Kok et al. [5,6]. According to this model, the average oxidation state of the Photosystem II reaction centers at the start of a series of flashes following a long dark period is significantly different from that during the steady state. At the start of a series of flashes, all of the reaction centers are in the two lowest oxidation states (S_0 and S_1) owing to the reduction of the higher oxidation states in the dark. By the time the steady state is achieved, the four possible oxidation states (S_0, S_1, S_2, S_3) are equally populated. These different conditions result in significant differences in the flash saturation curves.

If α is the average fraction of reaction centers not advanced by a flash, then $(1-\alpha)S_n$ will be the number of reaction centers advanced from oxidation state S_n to state S_{n+1} by a flash. (This ignores double hits, which are small relative to the misses; see Results.) A saturation curve of Y_s is essentially a measurement of how $(1-\alpha)S_3$ changes as the flash intensity changes. At this point it becomes important to distinguish between two kinds of misses. There is that fraction of reaction centers which is missed even at infinite light intensity (α_o) and a variable fraction (α_v) the extent of which depends on light intensity. α_o is the fraction of reaction centers which, at the time of the flash, are closed or are in some way unable to use the light energy supplied by the flash. α_v, on the other hand, is the fraction of reaction centers which do not receive any excitation energy from the flash.

In these terms

$$Y_s = (1-\alpha_v)(1-\alpha_o)S_3 \qquad (1)$$

The saturation curve for Y_3 is slightly more complex. Since the initial state is S_1 rather than S_3, three flashes must be given before oxygen is evolved. Both α_o and α_v occur on each flash. Thus

$$Y_3 = (1-\alpha_v)^3(1-\alpha_o)^3 S_1 \qquad (2)$$

Since $(1-\alpha_v)^3$ decreases more rapidly than $(1-\alpha_v)$ as α_v increases, the apparent half-saturation intensity for Y_3 would be expected to be larger than that for Y_s. Furthermore, the Y_3 saturation curve would be expected to show a non-linear response to light intensity since it has a cubic dependence on α_v. An interpretation of half-saturation values can be made if the relationship between $(1-\alpha_v)$ and the flash intensity, I, is known.

In this model, Y_s is a linear function of $(1-\alpha_v)$. Since α_o is assumed to be a constant in these experiments, in the steady state the term $(1-\alpha_o)S_3$ is also a constant. Because of this, $(1-\alpha_v)$ is proportional to Y_s, and any equation describing Y_s as a fraction of I may also be used to describe $(1-\alpha_v)$ as a function of I.

An equation which we have found empirically to fit the Y_s vs. I curve is

$$Y_s = \frac{I}{i+I} \qquad (3)$$

where I is the flash intensity and i is the intensity which gives half-saturation. We use the proportionality between Y_s and $(1-\alpha_v)$ described in Eq. (1) and write the steady-state saturation curve as

$$Y_s = (1-\alpha_o)S_3\left[\frac{I}{i+I}\right] \qquad (4)$$

The data presented in Fig. 4 have been normalized so that the flash yield at full intensity is unity. The normalized flash yield for the

steady state ($\overline{Y}_s$) can be calculated by dividing Eq. (4) by the constant $(1-\alpha_o)S_3$ to give

$$\overline{Y}_s = \frac{I}{i+I} \tag{5}$$

In a similar fashion, Y_3 and $\overline{Y}_3$ are determined to be

$$Y_3 = (1-\alpha_o)^3 S_1 \left[\frac{I}{i+I}\right]^3 \tag{6}$$

and

$$\overline{Y}_3 = \left[\frac{I}{i+I}\right]^3 \tag{7}$$

This is true when all of the first three flashes are attenuated. In general, when n of the first three flashes are attenuated, the yield of the third flash will be

$$Y_3 = (1-\alpha_o)^3 S_1 \left[\frac{I}{i+I}\right]^n \tag{8}$$

The variation of the shape of the Y_3 saturation curve with the number of attenuated flashes is easily understood in terms of Eq. (8). When only one flash is attenuated, the equation reduces to Eq. (4) with the resulting hyperbolic shape.

Fig. 6 shows a family of curves derived from Eq. (7) using different values of i, as indicated. The solid circles are the data for the saturation curve for Y_3 from Fig. 4. The theoretical curves have been normalized to a value of 1.0 at I = 100 to correspond to experimental data. A good fit is obtained when i = 0.085. A realtively good fit to the Y_s saturation curve was obtained by use of Eq. (5) with i = 0.145.

Fig. 7 shows fits obtained for the data shown in Fig. 5. Good fits were obtained using Eq. (8). The values of i giving the best fits were: $i = 0.070 \pm 0.002$ for the steady state and single flash attenuation curves

(top), i = 0.050 ± 0.002 for the case in which two of the first three flashes were attenuated (middle), and i = 0.045 ± 0.002 when all of the first three flashes were attenuated (bottom).

Sigmoidal behavior in flash light-saturation curves has been described by Diner and Mauzerall [18]. However, their curves were obtained for the steady-state flash yield at low oxygen tensions. Under these conditions, the observed S-shape at low light intensities is a result of the increased reduction of the A-pool and is distinct from the effect described above. Indeed, the sigmoidal shape of the curve disappears at higher oxygen tensions and the curve resembles the saturation curve for Y_s shown in Fig. 4. It is of further interest to note that the model predicts a sigmoidal behavior for the light saturation curve of the third flash which is independent of the redox state of the A-pool and involves the use of only one photon per reaction center per flash.

From the results presented in Fig. 1, it is clear that the method of charge storage in Photosystem II in isolated spinach chloroplasts, and whole cells of Chlorella and Anacystis is basically the same. Sofrová et al. [20] have recently reported that the effects of Tris-washing and heat treatment on protoplasts and cell-free preparations of the blue-green alga Plectonema boryanum are similar to the effects seen in higher plants. Their observations support the contention that the mechanism of charge storage and oxygen evolution is the same for blue-green algae and higher plants. Chlorella, Anacystis and isolated spinach chloroplasts differ primarily in the value of the miss parameter, α. The difference in α probably relates more to the functioning of the primary photochemistry of Photosystem II than to events between the reaction center and the site

of oxygen evolution. (Anacystis may also show a different S_0:S_1 ratio after a dark period, which would indicate different redox conditions on the oxidizing side of System II.) Anacystis and Chlorella show from two to three times more reaction centers missed by a flash than do chloroplasts. The increase in α is not due to insufficient flash intensity nor is it due to the reduction of oxidized intermediates in the dark time between flashes.

Radmer and Kok [8] proposed that the misses which occur during saturating flashes are due to reaction centers which are unable to respond to a flash because the primary acceptor (Q) is reduced. They also suggested that Q is in equilibrium with the large pool of secondary electron acceptors between Photosystem II and Photosystem I (the A-pool). Diner and Mauzerall [17] and Velthuys and Amesz [18] presented evidence supporting this view. Diner and Mauzerall also showed that anaerobic conditions result in an increased reduction of the A-pool. We find that both Chlorella and Anacystis exhibit a significantly greater degree of misses under conditions of low oxygen tension. This seems to indicate the presence of an equilibrium between Q and the A-pool in these organisms. If this is the case, then the larger value of α seen in Anacystis and Chlorella indicates that the A-pool is more reduced in these organisms than in isolated chloroplasts. Diner and Mauzerall [18] have proposed a model in which the redox poise of the A-pool is controlled by the opposing action of oxygen and some endogenous reductant. The insensitivity of isolated spinach chloroplasts to oxygen tension suggests that they may lack the endogenous reductant.

The similarity in charge storage mechanisms seen in Anacystis, Chlorella, and isolated spinach chloroplasts suggests that the same mechanism is used to oxidize water in all of them. If these organisms are indeed typical representatives of their taxonomic groups, it appears that this mechanism has

remained essentially unchanged through the billions of years of evolution since they shared a presumably common ancestor.

ACKNOWLEDGMENTS

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REFERENCES

1 Allen, F. L. and Franck, J. (1955) Arch. Biochem. Biophys. 58, 124-143

2 deKouchkovsky, Y. and Joliot, P. (1967) Photochem. Photobiol. 6, 567-587

3 Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309-329

4 Weiss, C., Jr. and Sauer, K. (1970) Photochem. Photobiol. 11, 495-501

5 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457-475

6 Forbush, B., Kok, B. and McGloin, M. P. (1971) Photochem. Photobiol. 14, 307-321

7 Joliot, P. and Joliot, A. (1972) in Proc. 2nd Int. Cong. Photosynthesis Res., Stressa, 1971, Vol. 1 (Forti, G., Avron, M. and Melandri, A., eds.), pp. 26-38, Dr. W. Junk N.V. Publishers, The Hague

8 Radmer, R. and Kok, B. (1973) Biochim. Biophys. Acta 314, 28-41

9 Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1971) Photochem. Photobiol. 14, 287-305

10 Bouges-Bocquet, B. (1973) Biochim. Biophys. Acta 292, 772-785

11 Weiss, C., Jr., Solnit, K. T. and Von Gutfeld, R. J. (1971) Biochim. Biophys. Acta 253, 298-301

12 Kratz, W. and Myers, J. (1955) Amer. J. Bot. 42, 282-287

13 Myers, J. (1953) in Algal Culture from Laboratory to Pilot Plant (Burlew, J. S., ed.), pp. 37-62, Carnegie Inst. of Washington Publ. 600

14 Sun, A.S.K. and Sauer, K. (1971) Biochim. Biophys. Acta 234, 399-414

15 Babcock, G. T. and Sauer, K. (1973) Biochim. Biophys. Acta 325, 483-503

16 Duysens, L.N.M. (1972) in Proc. 2nd Int. Cong. Photosynthesis Res., Stressa, 1971, Vol. I (Forti, G., Avron, M. and Melandri, A., eds.) pp. 19-25, Dr. W. Junk N.V. Publishers, The Hague

17 Diner, B. and Mauzerall, D. (1973) Biochim. Biophys. Acta 305, 353-363

18 Diner, B. and Mauzerall, D. (1973) Biochim. Biophys. Acta 305, 329-352

19 Velthuys, B. R. and Amesz, J. (1973) Biochim. Biophys. Acta 325, 126-137

20 Sofrová, D., Šlechta, V. and Leblová, S. (1974) Photosynthetica 8, 34-39

FIGURE CAPTIONS

Fig. 1. Oxygen evolution by dark adapted, broken spinach chloroplasts (●——●), Chlorella pyrenoidosa (o— —o), and Anacystis nidulans (x - - - x) in flashing light. The length of the flashes was 10 μsec. Time between flashes was 0.5 sec. (Reaction mixtures described in MATERIALS AND METHODS.) Oxygen yields were normalized with respect to the average of the last 5 flashes. Dark times preceding the flashes were 5 min for the chloroplasts and Chlorella and 3 min for Anacystis.

Fig. 2. The oxygen yield of the third flash as a function of the time between the second and third flashes (Δt_{2-3}) by dark adapted Anacystis cells. The time between the first and second flashes was 0.5 sec. Oxygen yields were normalized to the value of the oxygen yield when Δt_{2-3} was 0.5 sec.

Fig. 3. Oxygen evolution by dark adapted Anacystis cells in flashing light under aerobic (●——●) and anaerobic (o——o) conditions. Anaerobic conditions were maintained by bubbling the electrolyte flowing past the cells with nitrogen. Both experiments shown were performed on the same sample. The aerobic experiment was performed first and was separated from the anaerobic experiment by 30 min of darkness. Both curves are normalized by division by the average of the final 5 flash yields of the aerobic experiment.

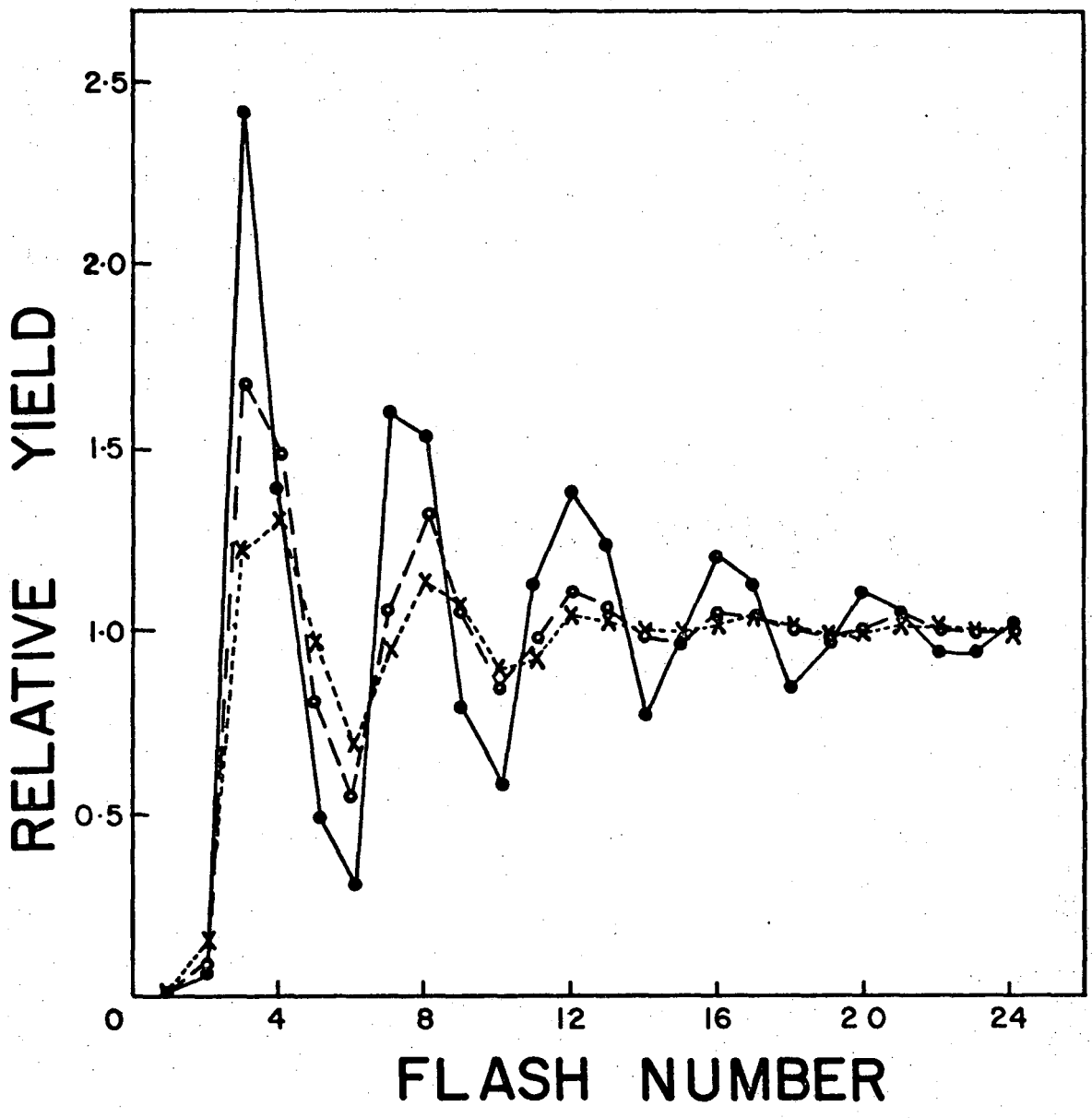
FIGURE CAPTIONS (Cont.)

Fig. 4. Light saturation curves for the steady-state (●——●) flash yields and the yields of the third flash (o——o) in dark adapted Anacystis cells when all flashes are attenuated. After 3 min of darkness, Anacystis cells were subjected to a train of (10 μsec) flashes. The intensity of the flashes was reduced by the use of calibrated Balzars neutral density filters. The oxygen yield of the third flash or of the steady state is shown as a function of the relative flash intensity.

Fig.5. Light saturation curves for various flash yields in dark adapted Chlorella cells. The flashes attenuated were: all flashes, steady-state (●——●), third flash only (o——o), second and third flashes only (Δ——Δ), and all three of the first three flashes (x——x). Samples kept in darkness for 5 min before exposure to the train of flashes.

Fig. 6. Theoretical light saturation curves for the third flash, when all flashes are attenuated. The curves shown are the result of Eq. (7) solved for various values of i. The value of i generating the curve is shown above the appropriate curve. The curves are normalized at I = 100%. The solid circles are the data shown in Fig. 4 for the yield of the third flash in dark-adapted Anacystis cells.

Fig. 7. Fits to experimental data from Chlorella using Eq. (8) (see text). The value of i and n and the flashes attenuated are: (a) i = 0.070, n = 1, ●——● steady-state flashes, o——o third flash only; (b) i = 0.050, n = 2, ●——● second and third flashes only; (c) i = 0.045, n = 3, ●——● all of the first three flashes attenuated. All theoretical curves are normalized at I = 100%.



XBL749-5363

Fig. 1.

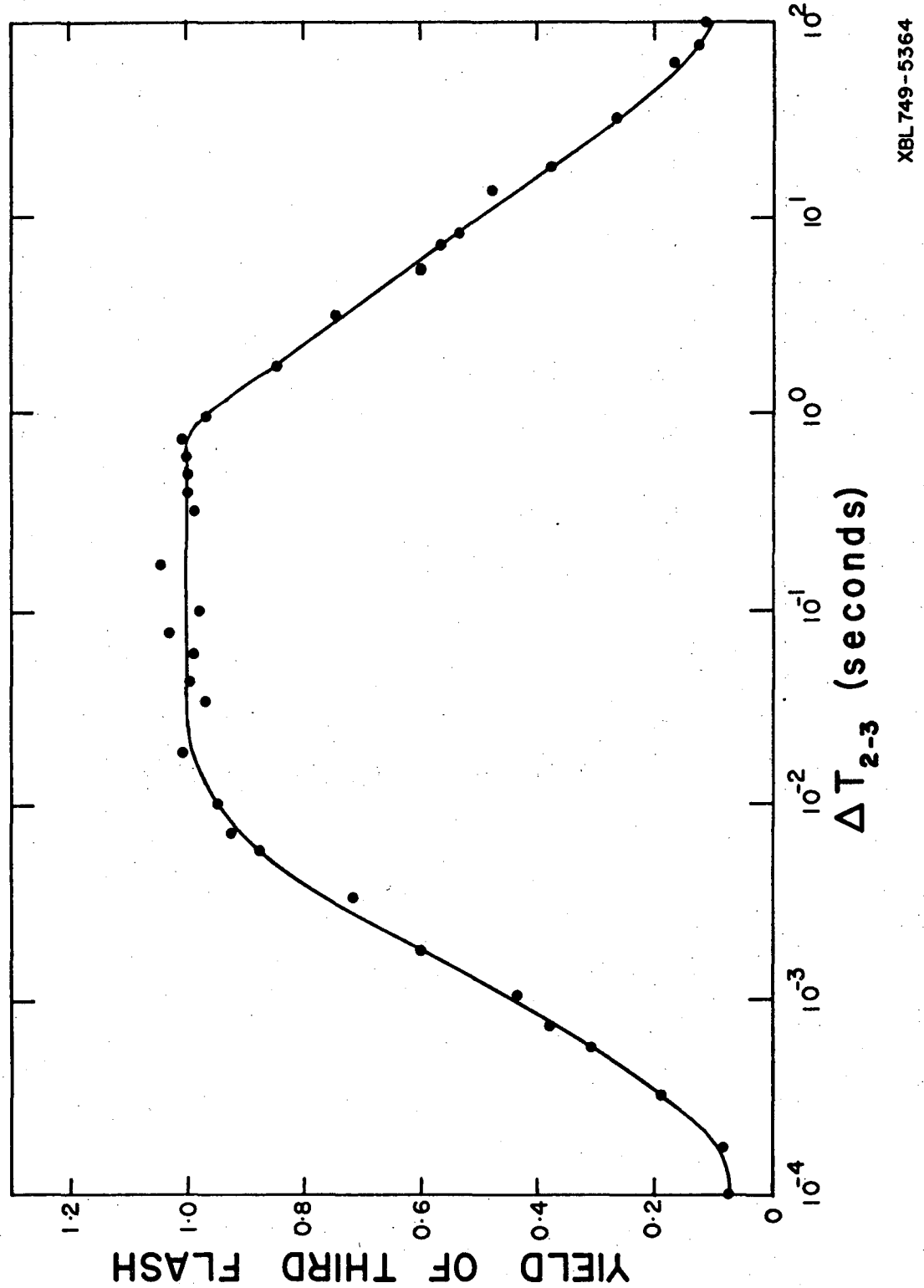
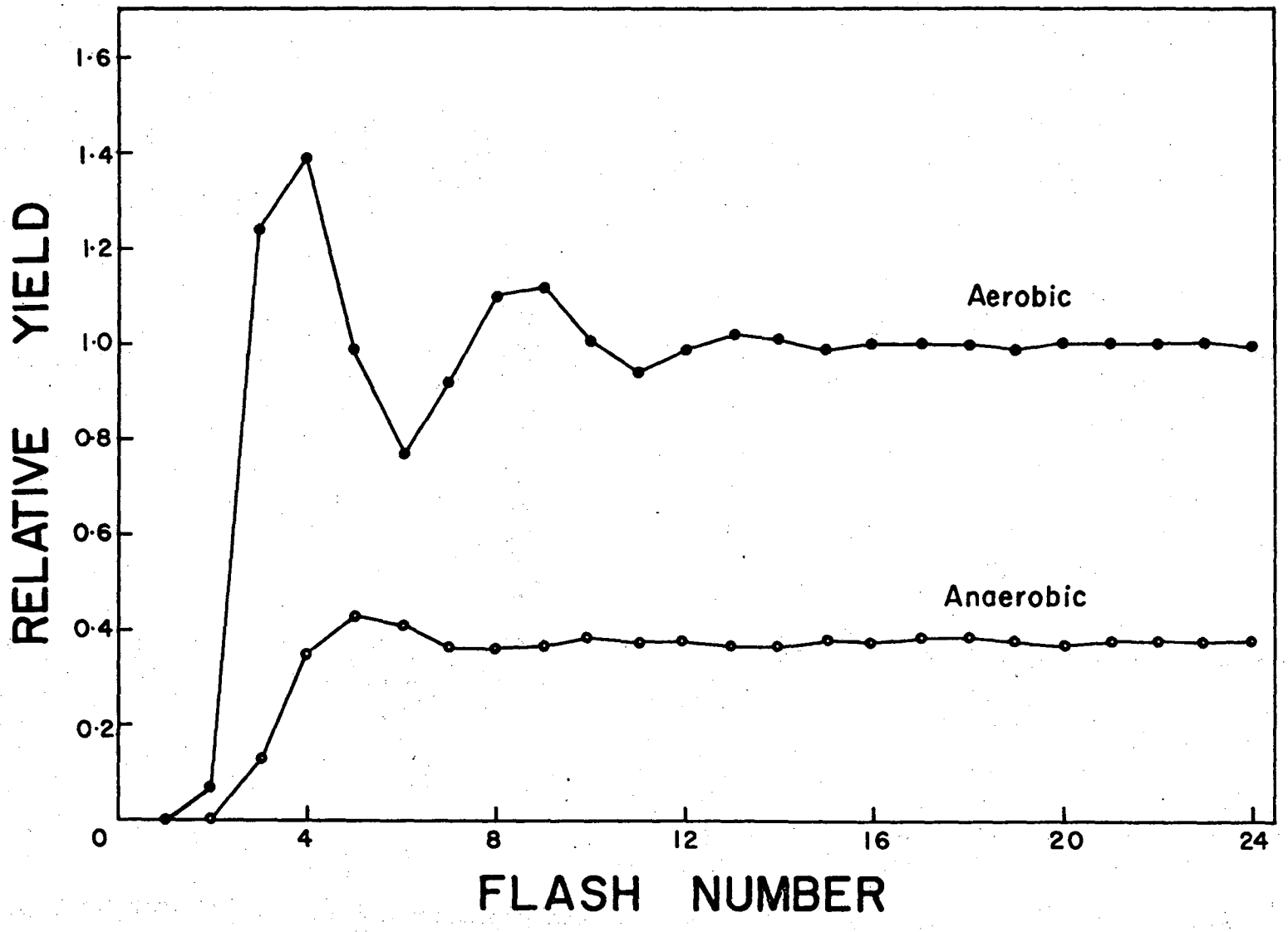
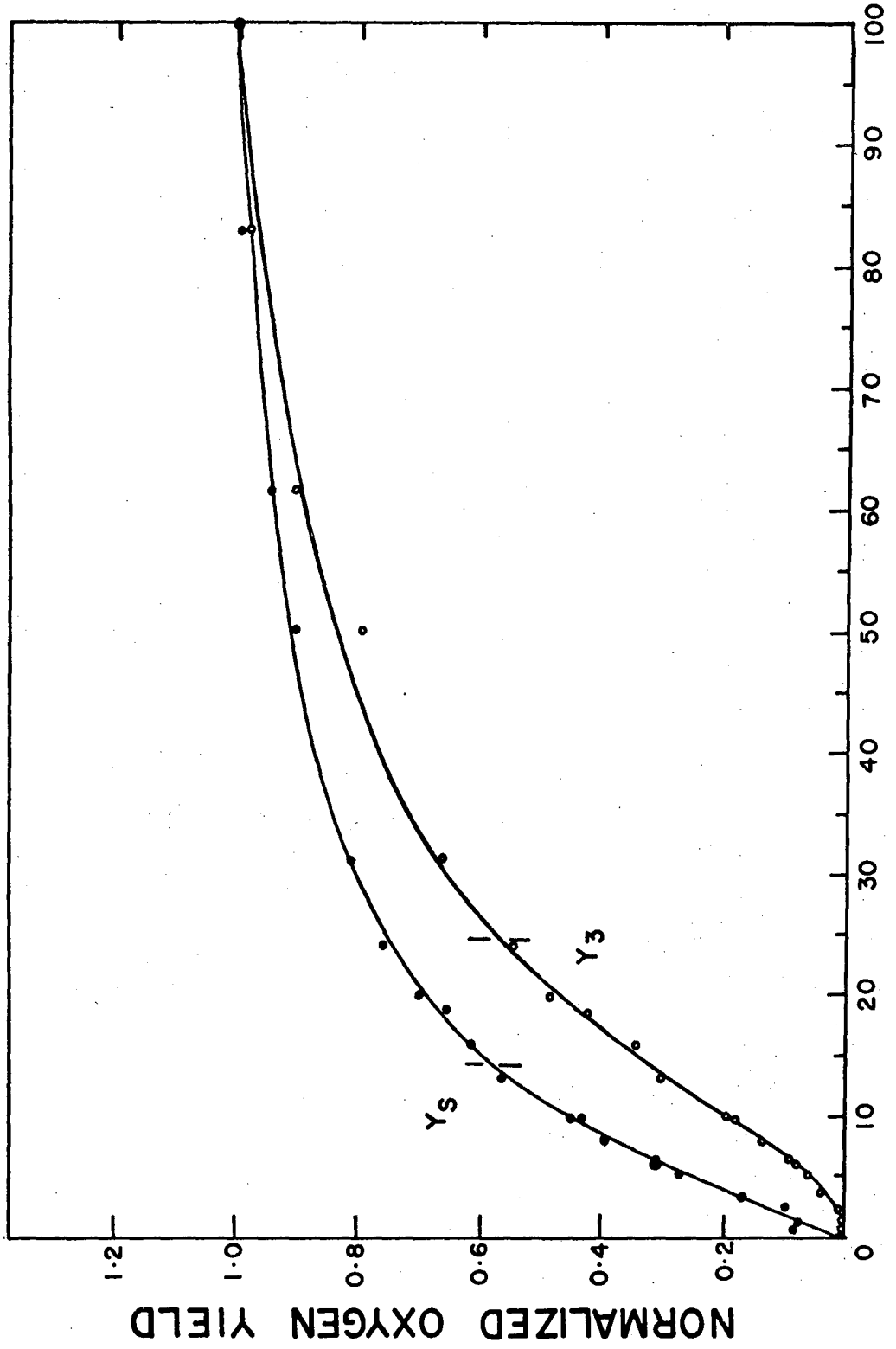

YIELD OF THIRD FLASH
0
0·2
0·4
0·6
0·8
1·0
1·2
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
ΔT_{2-3} (seconds)
XBL749-5364

Fig. 2.



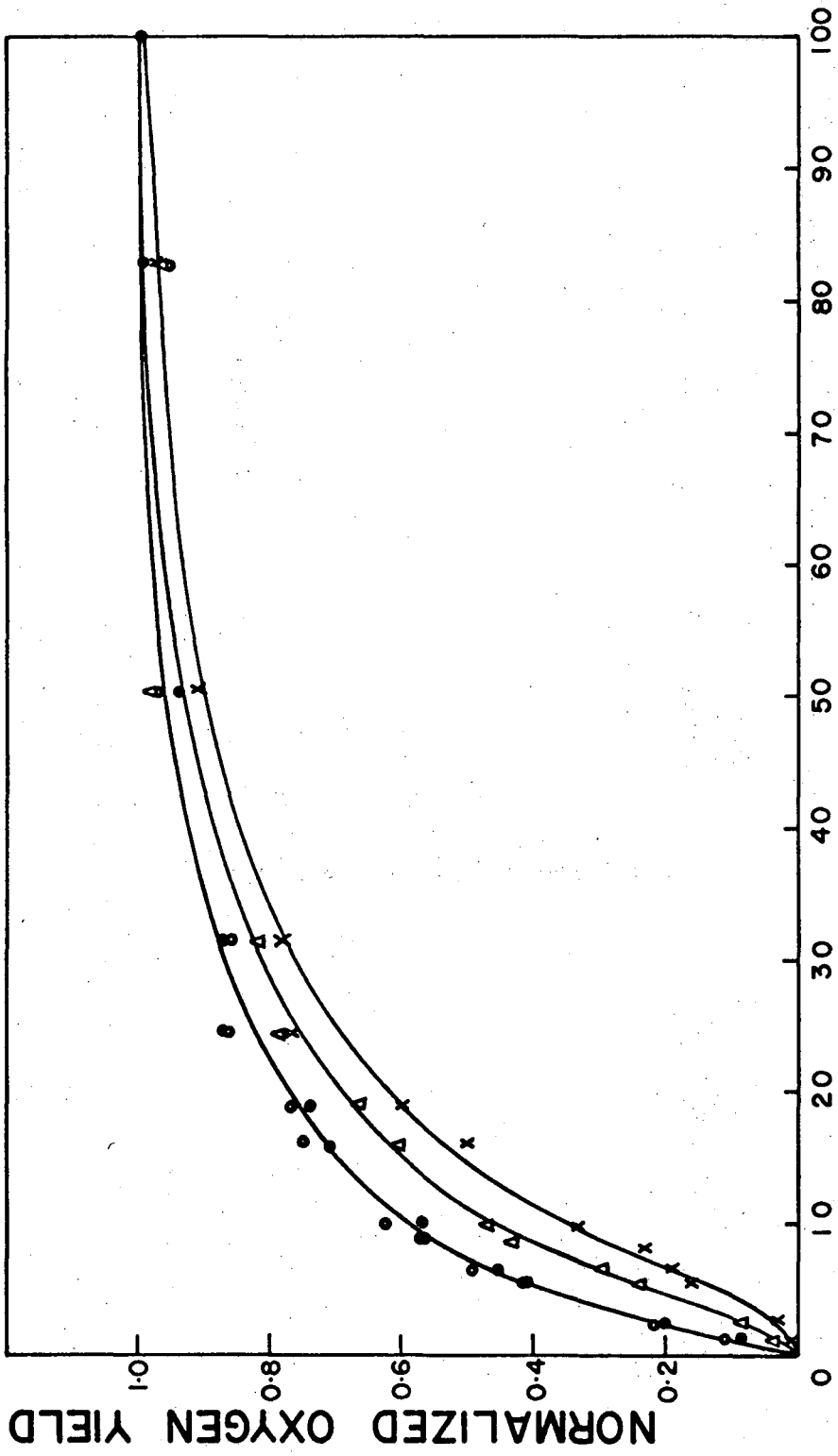
XBL749-5365

Fig. 3.



XBL749-5366

Fig. 4.



XBL749-5367

Fig. 5.

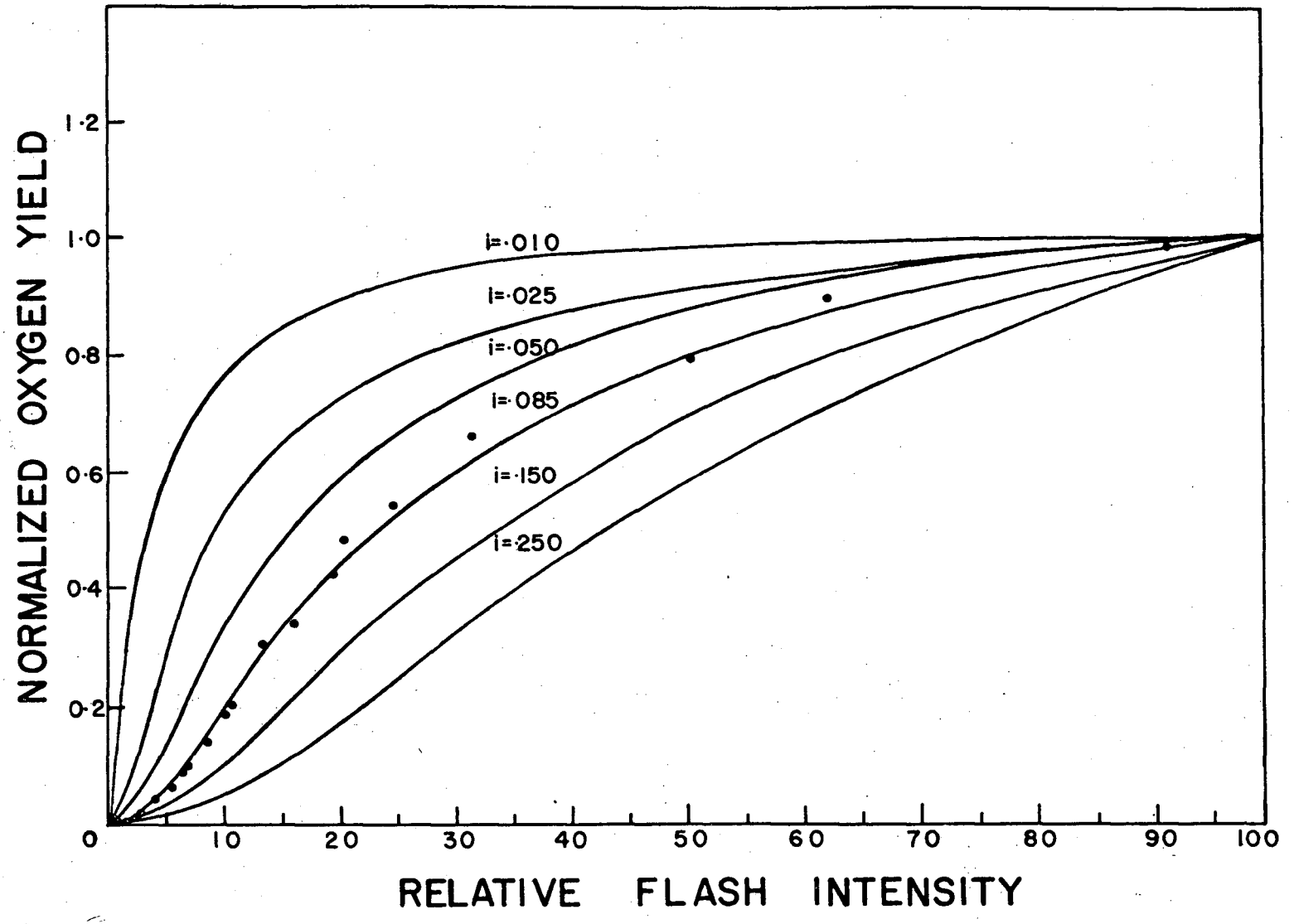

NORMALIZED OXYGEN YIELD
1·2
1·0
0·8
0·6
0·4
0·2
0
i=·010
i=·025
i=·050
i=·085
i=·150
i=·250
10
20
30
40
50
60
70
80
90
100
RELATIVE FLASH INTENSITY
XBL749-5368

Fig. 6.

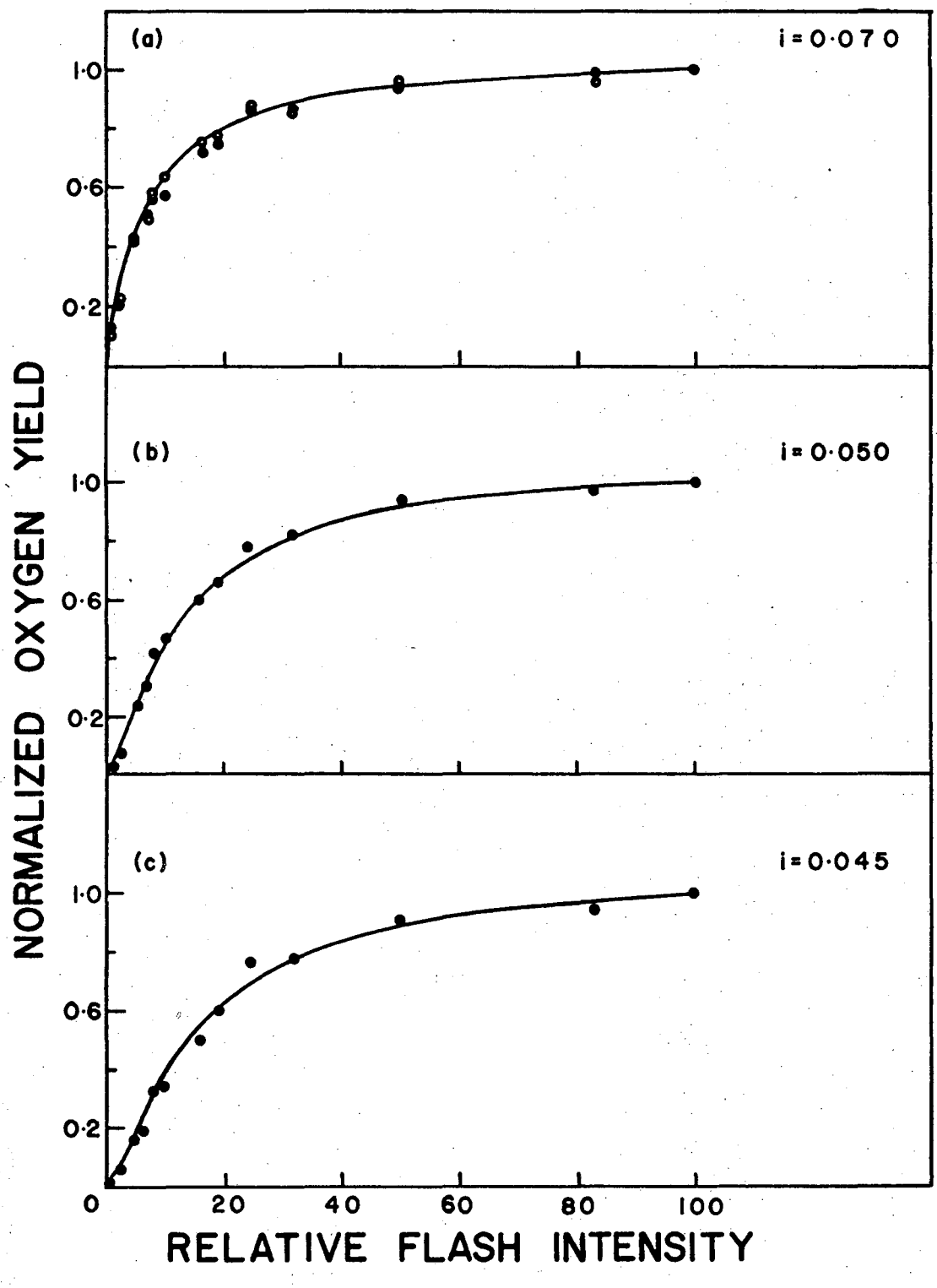

(a)
i = 0·070
(b)
i = 0·050
(c)
i = 0·045
1·0
0·6
0·2
0
20
40
60
80
100
NORMALIZED OXYGEN YIELD
RELATIVE FLASH INTENSITY
XBL749-5369

Fig. 7.

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