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Quantum yields for oxygenic and anoxygenic photosynthesis in the cyanobacterium *Oscillatoria limnetica*

(blue-green algae/photosystem I growth/phototrophic evolution/H_2S photooxidation)

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ABSTRACT **A comparison of the quantum yield spectra of the oxygenic (H_2O as the electron donor) with the anoxygenic (H_2S as the electron donor) photosynthesis of the cyanobacterium, *Oscillatoria limnetica* reveals that anoxygenic photosynthesis is driven by photosystem I only. The highest quantum yields of the latter (maximum; 0.059 CO_2 molecules/quantum of absorbed light) were obtained with wavelengths which preferentially excite photosystem I (<550, >650) in which chlorophyll *a* and carotenoids are the major pigments. The addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea had no effect on anoxygenic photosynthesis, and no enhancement in quantum efficiency was observed by a superimposition of light preferentially exciting photosystem II.**

Oxygenic photosynthesis efficiently utilizes only a narrow range of the absorption spectrum (550–650 nm) where light is provided in excess to photosystem II via phycocyanin. The quantum yield (0.033 CO_2 molecules/quantum of absorbed light) is lower than the theoretical yield by a factor of 3, possibly due to inefficient light transfer from photosystem II to I. Thus, 3-fold enhancement of oxygenic photosynthesis by superimposition of photosystem I light, and low quantum yields for anoxygenic photosynthesis, were obtained in this region. These results are consonant with the suggestion that such a cyanobacterium represents an intermediate stage in phototrophic evolution.

The photosynthetic apparatus of the cyanobacteria ("blue-green algae") resembles that of eukaryotic algae and higher plants; it includes two photosystems, both utilizing water as the electron donor with the evolution of oxygen. Nevertheless, a number of cyanobacteria have been recently found which, in addition, display bacterial-type anoxygenic photosynthesis, driven by photosystem I with sulfide as the electron donor (1, 2). Thus, when the electron flow between photosystems II and I is inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea ($Cl_2Ph_2Me_2U$), or when only photosystem I is excited by illumination with 703-nm light, anoxygenic photosynthesis proceeds with sulfide as the electron donor. Furthermore, two strains, *Oscillatoria limnetica* and *Aphanothece halophytica* have been shown to readily shift from aerobic growth with oxygenic photosynthesis to anaerobic growth and anoxygenic photosynthesis. In the anoxygenic photosynthesis of these strains, two sulfide molecules are oxidized to elemental sulfur for each CO_2 molecule photoassimilated. The sulfur is then excreted from the cells (3).

The ancient age since cyanobacteria appeared on earth (4) as well as a combination of prokaryotic cellular organization and oxygenic photosynthesis, underly the suggestion that cyanobacteria represent a primitive group of organisms from which the oxygenic plant-type photosynthesis evolved (5). The fact that various cyanobacteria (1, 2) display both anoxygenic and oxygenic photosynthesis and can readily shift from one to the other, points to the possibility that this faculty represents the missing link between bacterial type and plant type phototrophic metabolic patterns.

In the present work with *O. limnetica*, the quantum yield spectra of the oxygenic and anoxygenic photoassimilation of CO_2 were analyzed in order to unravel the pattern by which the absorbed spectrum is used in the two types of photosynthesis and to deduce the interrelationship between photosystem I and II in such an organism.

Abbreviation: $Cl_2Ph_2Me_2U$, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

MATERIALS AND METHODS

Cyanobacterial Strain and Culture Conditions. The *O. limnetica* strain used was described (1). Suspension for all media was in Turks Island salt solution prepared in double strength (6). Growth of *O. limnetica* in the absence of Na_2S was carried out in 250 ml flasks partially filled (30%) with Chu 11 medium (7). The growth medium used in the presence of Na_2S was also described (1). The cultures were incubated at 35° and continuously illuminated by white fluorescent lamps (4300 K, 20 W; incident intensity, 5 W/m^2·sec). Growth was determined by cell protein assay (8) after washing the cells collected on glass filters (Whatman, GF/C) with 5 ml of absolute ethanol to remove elemental sulfur. The logarithmic phase of growth lasted 6–7 days, when the initial cell concentration was 2 μg of cell protein per ml (9).

Measurement of Quantum Yield for CO_2 Photoassimilation. The quantum yield for CO_2 photoassimilation is the ratio between the number of CO_2 molecules photoassimilated and the number of light quanta absorbed. Cells in logarithmic growth phase were washed and resuspended (20–30 μg of cell protein per ml) in the growth medium (1) modified as follows: the Na_2CO_3 concentration was 7.1 mM; for the aerobic system Na_2S was omitted; for the anaerobic systems $Cl_2Ph_2Me_2U$ (5μM) and/or $Na_2S·9H_2O$ (3.5 μM) was added to cell suspensions prepared in sealed flasks. Both the aerobic and anaerobic systems were preincubated for 2 hr at 35° in light provided by tungsten lamps (60 W, incident light intensity of 20 W/m^2·sec). Then, 4-ml cell samples in 4-ml stoppered cuvettes were illuminated by specific actinic wavelengths for different periods (up to 6 min) and stirred at 35 ° in the presence of 1.4 μCi/μmol of $NaH^{14}CO_3$ (Radiochemical Centre, Amersham). The total radioactivity introduced into the reaction mixture was determined as described (10), and the value of total inorganic carbon was verified by a Technicon autoanalyzer (model SMA 12/60). The cell suspensions were then filtered through glass filter paper (Whatman GF/C), and the collected cells were washed with the medium suspension solution (4°). Three drops of acetic acid (10% vol/vol) were spread on each filter, the samples were dried overnight at room temperature and cell radioactivity was counted in a gas flow counter (Nuclear Chicago, model C-110 B). The number of CO_2 molecules photoassimilated was determined to be linear with time of illumination.

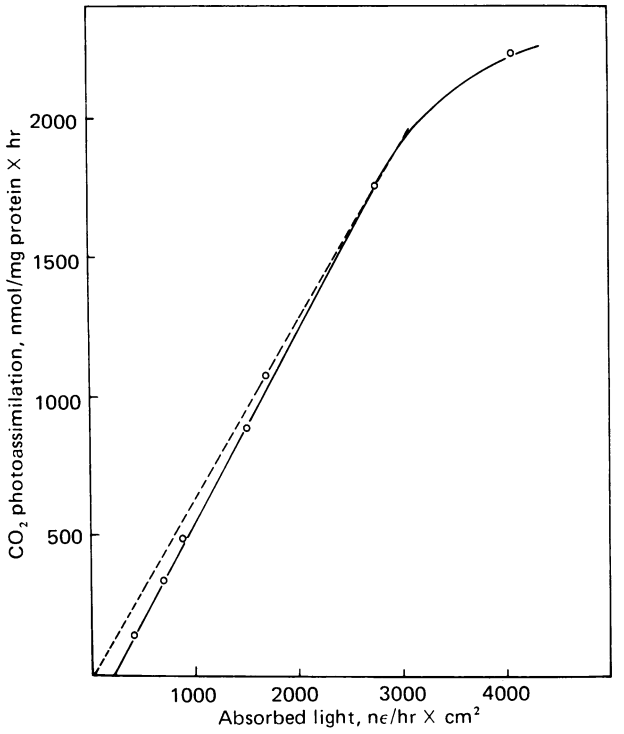

FIG. 1. Dependence of the CO_2-photoassimilation rate on the absorbed light quanta. The aerobic reaction mixture (30 μg of cell protein per ml) was illuminated with 580 nm light: (—) observed; (- - - -) extrapolation to zero light intensity; the slope of the latter line was used to calculate the quantum yield.

The specific actinic lights employed were obtained by filtering light provided by a Leitz-Prado Universal slide projector through Baird Atomic sharp-cut-off interference filters, blocked to infinity, that peaked at 441 nm, 673 nm, 703 nm, 718 nm (17 nm half band width), 563 (12 nm half band width) 580 nm, 500 nm (15 nm half band width), and 622 nm (27 nm half band width). Light of wavelengths of up to 590 nm was first filtered through 1.5 cm of a saturated solution of $CuSO_4$; longer wavelengths were first filtered by a sharp cut-off filter (Corning 2-60). The incident light intensity was varied with a powerstat and measured with a Yellow Springs Instrument Radiometer, model 65.

The extent of light absorption by the cells (representing 50–300 μg of cell protein per ml) was measured in a cuvette containing the reaction mixture used in the assay. The cuvette was suspended in the center of an integrating sphere apparatus constructed by S. Malkin (Department of Biochemistry, Weizmann Institute of Science, Rehovot, Israel) and illuminated with light filtered through the respective interference filters used in the experiments. Absorbance of cell suspensions was found to be linear with concentration (11). Fig. 2 shows the measured optical density of a reaction mixture containing 300 μg of cell protein per ml at the indicated (⊙) wavelengths.

Fig. 1 shows a sample pattern of the dependence of the CO_2-photoassimilation rate on the absorbed light quanta. This pattern was the same for all wavelengths studied and in all experimental systems in the presence as well as in the absence of Na_2S. At the very low intensity region, below 0.20 μEinstein/hr·cm², no photoassimilation of CO_2 could be measured. The reasons for this "low intensity lag" is unknown. Above the low intensity region, the rate of CO_2 photoassimilation was found to be linearly related to the intensity of the absorbed light. The quantum yield for the photoassimilation of CO_2 was determined from the slope of the straight line extrapolated to zero light intensity (Fig. 1). This calculation led to an underestimation of the quantum yield by no more than 10%.

"Enhancement Experiment". The effect of a combination of two wavelengths, each preferentially exciting photosystem I or II, on the quantum yield, was determined according to Myers (12). When λ_1 is the wavelength region in which quanta are provided in excess to photoreaction II and P_2 is limited by the rate of photoreaction I, enhancement (E) is defined by reference to either wavelength alone as $E_1 = (P_{12} - P_2)/P_1$ and $E_2 = (P_{12} - P_1)/P_2$. The rate of photoassimilation at a combination of different wavelengths of different intensities was determined as in Fig. 1, by avoiding both the "low intensity lag" and saturating intensity regions. Under the conditions employed, the "low intensity lag" in itself accounts for an apparent enhancement of no more than 10%.

Pigment concentration analyses were performed according to Jones and Myers (13), and protein determinations (8) were carried out after washing the cells collected on filters with 5 ml of absolute ethanol to remove elemental sulfur. Concentrations of Na_2S were determined by the methylene blue colorimetric method (14).

$Cl_2Ph_2Me_2U$ was obtained from DuPont, the Na_2S used was analytical grade (BDH, England).

RESULTS AND DISCUSSION

The cyanobacterium, *O. limnetica*, which is capable of both anoxygenic (H_2S-electron donor) and oxygenic (H_2O-electron donor) photosynthesis, contains the photosynthetic pigment complement characteristic of many cyanobacteria (5, 15): chlorophyll *a*, phycocyanin, and carotenoids (Fig. 2*A* and *B*). The cell absorption spectrum was identical for cells grown or incubated in the presence or absence of Na_2S.

Fig. 3 shows the quantum yield for CO_2 photoassimilation in the presence of Na_2S (with or without $Cl_2Ph_2Me_2U$) and for the oxygenic photoassimilation as a function of the wavelength of the exciting light. The quantum yield spectrum for the oxygenic photosynthesis was the same for cells grown in the presence or absence of Na_2S. There was no oxygenic photoassimilation at any wavelength in the presence of $Cl_2Ph_2Me_2U$ (5 μM). In its general pattern, the latter spectrum is similar to that previously shown for other cyanobacteria (16, 17) and eukaryotic algae of similar pigment composition, i.e., *Rhodophyceae* (for reviews see refs. 12, 18, and 19).

Thus, sharp drops in efficiency (decreased quantum yield) of the oxygenic photosynthesis are observed both in the red and blue regions of the spectrum, leaving only a limited segment (550–645 nm) for efficient photosynthesis. The "red drop" region corresponds to the region in which chlorophyll *a* is the main light absorber, and the "blue drop" corresponds to the region in which the carotenoids are the main light absorbers (compare Fig. 2*B* with Fig. 3). "The red drop", also characteristic of all other plant-type phototrophs (for reviews see refs. 12, 18, and 19), is ascribed to absorption of red light via chlorophyll *a* mainly into photosystem I. Photosystem II is thus poorly activated, and the requirement for efficient oxygenic photosynthesis, of both photosystems operating in series, is unfulfilled. The "blue drop" may be similarly explained but, in this case, light is absorbed mainly by carotenoids (12, 16).

If lack of photosystem II light is the cause of the efficiency drops, then superimposition of wavelengths which excite mostly photosystem II both on the red and blue regions should enhance the efficiency. Accordingly, as shown in Fig. 4*c*, very high enhancement (approximately 10-fold) of red light (673 nm) quantum efficiency was obtained by superimposition of light preferentially activating photosystem II (580 nm). In addition,

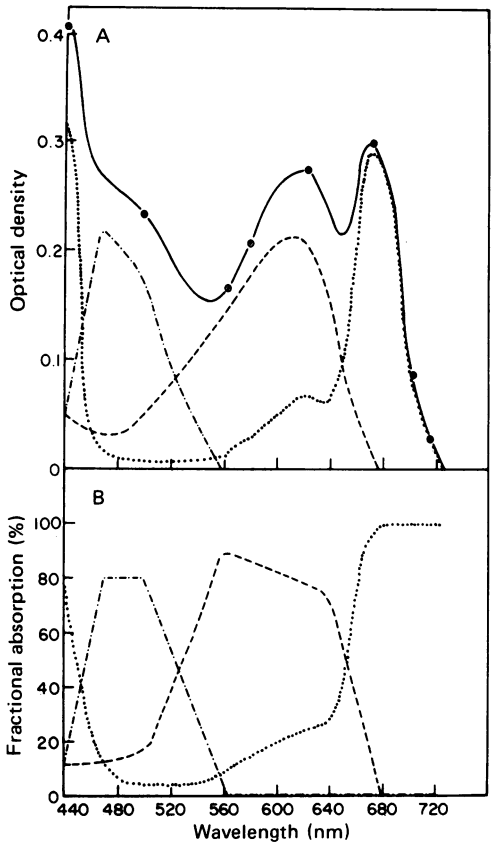

FIG. 2. (*A*) Absorption spectra of *O. limnetica* cells and their pigment components. The absorption spectrum of whole *O. limnetica* cells (300 μg of protein per ml) (—) was measured in the aerobic reaction mixture by the method of Shibata *et al.* (29) and corrected according to the absolute values of absorption measured for the indicated wavelengths (⊙), in the integrating sphere setup. Spectra for pigment components were obtained and adjusted to give best match with the *in vivo* curve according to Jones and Myers (16). Phycocyanin (- - -); chlorophyll *a* (. . .); carotenoids (- . - . -). (*B*) Fraction of the total light absorbed by each of the pigment components at various wavelengths. Values for calculation were taken from Fig. 2*A*. The graphs' connotations are as in Fig. 2*A*.

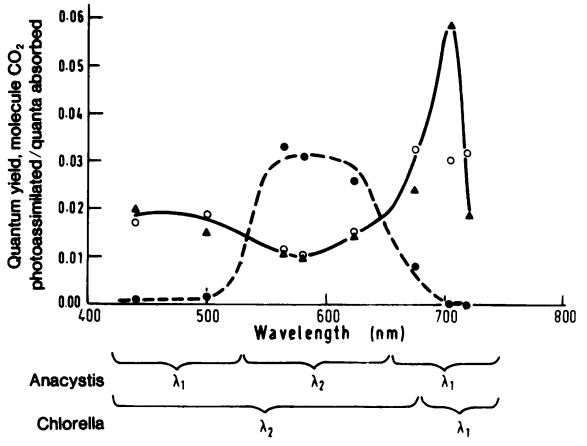

FIG. 3. Quantum yield spectra of oxygenic and anoxygenic photosynthesis. Oxygenic photosynthesis (●) was determined in cells grown and incubated in sulfide-free medium. Anoxygenic photosynthesis was determined in cells grown in the presence of Na_2S and incubated in the presence of 3.5 mM Na_2S (○) or in the presence of 3.5 mM Na_2S and 5 μM $Cl_2Ph_2Me_2U$ (▲). The λ_1 and λ_2 spectral regions (wavelengths preferentially exciting photosystem I and II, respectively), defined by Jones and Myers (16) for *Anacystis* and Govindjee *et al.* (22) for *Chlorella*, are shown.

as expected from a theoretical analysis of two photochemical reactions acting in series (20), the enhancement was linearly dependent on the rate ratio obtained for each wavelength separately. Superimposition of the photosystem II light on blue light (441 nm) also brought about significant enhancement in oxygenic photosynthesis of *O. limnetica* (Fig. 4*d*). However, in the latter case, the maximal enhancement observed was lower than that obtained in the red region (compare Fig. 4*c* to *d*). Therefore, it seems that here as in other photosynthetic systems (18, 19) the carotenoids are pigments that harvest light less efficiently than chlorophyll *a*.

As expected, the efficient segment of oxygenic photosynthesis corresponds to the maximal absorption range of phycocyanin—the main light harvesting pigment of photosystem II (compare Fig. 3 with Fig. 2*B*). However the highest quantum yield observed for the oxygenic photosynthesis of *O. limnetica* is about 0.03, i.e., 66% lower than the theoretical values of 0.10–0.12 molecules of CO_2 per quantum attained by other cyanobacteria and many plant-type phototrophic systems (21, 22). The theoretical values are to be expected only when quanta are absorbed into both pigment systems to give an optimal ratio of rates between the two photoreactions for their operation in series. This situation conforms to the "spillover" model (23) when light quanta are absorbed in excess into photosystem II and the excess quanta spillover into photosystem I thereby poising the rates of the two photoreactions. A control mechanism for the degree of "spillover" has also been suggested (11). As a result of the "spillover" mechanism, the quantum yield is high and constant in the photosystem II spectral region and only small values of enhancement by complementary light are observed. The constancy in quantum yield of the relatively efficient oxygenic photosynthesis in *O. limnetica* indicates that the spillover mechanism does occur because the ratio between the fractions of light absorbed by phycocyanin and chlorophyll *a* in this region changes from three to ten (compare Fig 3 to Fig. 2*B*). However, the finding that the quantum yield is 66% lower than would be expected on a theoretical basis suggests that quanta absorbed mainly by phycocyanin are not spilled over efficiently into photosystem I. Indeed, a 3-fold enhancement was observed by superimposition of photosystem I light (673 nm) on 580 nm light (Fig. 4*a*) that yielded close to theoretical quantum yield values. Two points (at both ends of the oxygenic photosynthesis segment) with theoretical quantum yields are expected. At these points, the optimal ratio between the absorption by the pigments of the photosystems should allow the operation of the oxygenic photosynthesis with the highest quantum yield even without enhancement by complementary light.

It has been previously shown (1, 3) that anoxygenic photosynthesis in *O. limnetica* does not require the operation of photosystem II. It proceeds when photosystem II is either inhibited by $Cl_2Ph_2Me_2U$ and/or not excited in the presence of 700 nm light. Fig. 3 reveals that the quantum yield spectra of the photoassimilation reaction are identical in the presence of Na_2S with or without $Cl_2Ph_2Me_2U$. Hence, anoxygenic photosynthesis is driven only by photosystem I at all wavelengths, even under conditions favorable for photosystem II excitation. To further exclude participation of photosystem II in the anoxygenic photosynthesis, we conducted an "enhancement experiment" with the anoxygenic system (in the absence of $Cl_2Ph_2Me_2U$). Enhancement in quantum efficiency of a photoreaction by superimposition of wavelengths, each preferentially activating only one photosystem, should theoretically be observed only if the two photosystems participated in the

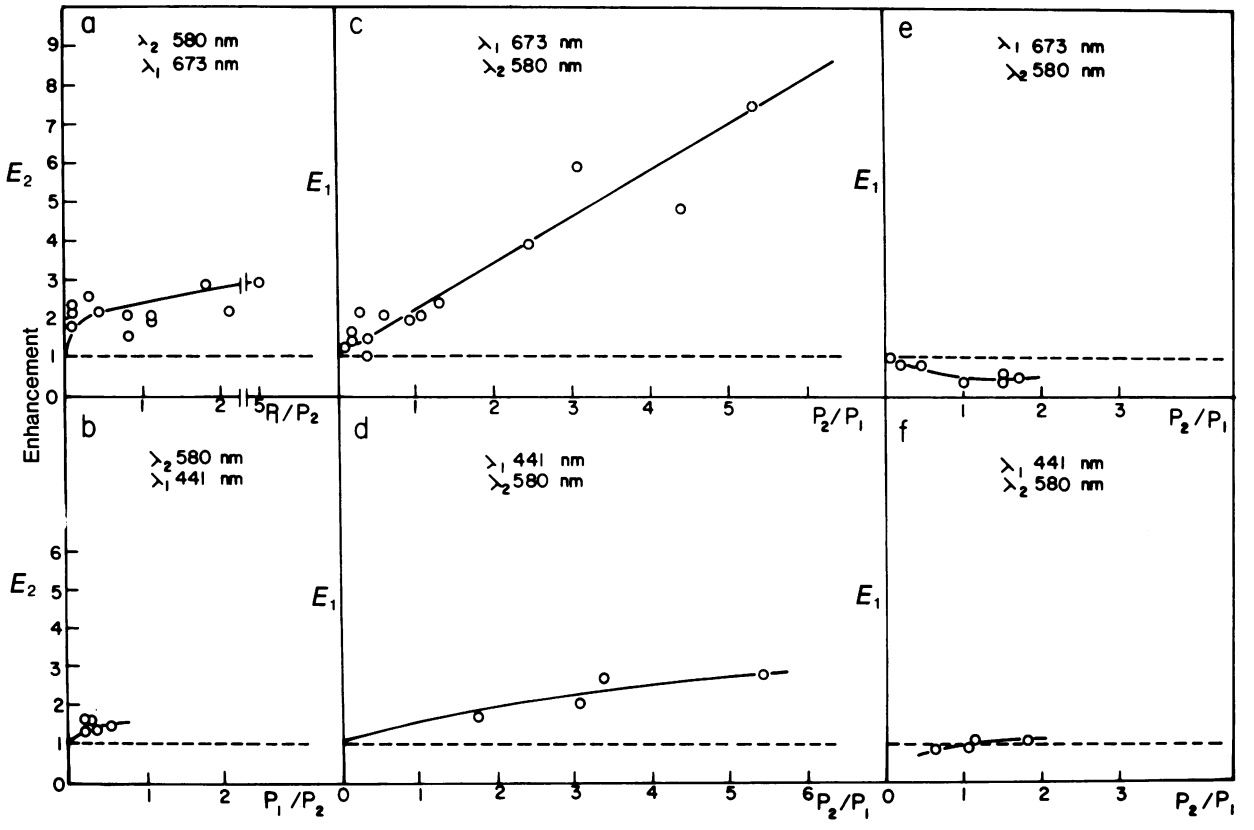

FIG. 4. Enhancement by a combination of wavelengths versus the ratio of rates induced by each wavelength alone. Wavelength λ_1 and λ_2 preferentially exciting photosystem I and II, respectively, and P_1 and P_2 the respective CO_2 photoassimilation rates are indicated in the graphs. Enhancement (E) is defined (12) by reference to either wavelength alone as $E_1 = (P_{12} - P_2)/P_1$ and $E_2 = (P_{12} - P_1)/P_2$; a, b, c, d, aerobic reaction mixture; e, f, anaerobic reaction mixture without the addition of $Cl_2Ph_2Me_2U$.

photoreaction (12, 20). Fig. 4*e* and *f* reveal that there was no enhancement by any combination of 580 nm, and 441 nm or 673 nm lights.

By being a purely photosystem I-driven reaction, the quantum yield spectrum of the anoxygenic photosynthesis reveals the action spectrum of photosystem I. It therefore affords another means of deducing the efficiency of spillover from photosystem II to I when the utilized light is preferentially absorbed by photosystem II. In the region absorbed mainly by phycocyanin, there were low quantum yields (0.02 CO_2 molecules photoassimilated per quantum) for the anoxygenic photosynthesis. This observation is consonant with the low efficiency of spillover from photosystem II to I in *O. limnetica*. The high quantum yield observed in the blue region, compared to that in the yellow region though lower than that in the red region, agrees with the suggested involvement of carotenoids in photosystem I though with less efficiency than chlorophyll *a*.

While H_2S is a very inefficient electron donor in eukaryotic algae (24, 25), if at all, Gaffron has shown (26) that certain strains of green and red algae as well as cyanobacteria can carry out the anoxygenic photoassimilation of CO_2 by using H_2. This reaction is driven by photosystem I, although possible involvement of photosystem II has been indicated (24). In analogy to the present work, a comparison of the action spectrum of photoreduction with H_2 to that of the oxygenic photosynthesis and enhancement experiments has been conducted in algal cells (24, 27, 28). These studies have revealed that the minimal amount of quanta required for photosynthesis with H_2 by using photosystem I light is similar to that necessary for the anoxygenic photosynthesis with H_2S in *O. limnetica*. However, in both red (28) and green algae (27), and possibly also in other cyanobacteria (16), photosystem II light seems to spillover efficiently to photosystem I. In further contrast with the anoxygenic photosynthesis of cyanobacteria, photoreduction with hydrogen in eukaryotic algae occurs only under special conditions (weak light preceeded by dark anaerobic adaptation) and does not support growth (24). This has been ascribed to the inability of eukaryotic algae cells to shift to anaerobic metabolism.

A comparison of *O. limnetica* anoxygenic versus oxygenic quantum yield spectrum (Fig. 3), reveals that while the entire spectrum absorbed is utilized in anoxygenic photosynthesis, only 33% of it is used for oxygenic photosynthesis. Even the inefficient region of the anoxygenic photosynthesis is far better utilized than the inefficient region of the oxygenic photosynthesis. It appears that photosystem I dominates the absorption spectrum of *O. limnetica* and, similarly to the single photosystem of photosynthetic bacteria, permits anoxygenic photosynthesis to take place along the entire absorbed spectrum. Thus, *O. limnetica* thrives in the H_2S-rich bottom layers of the Solar Lake during winter stratification when light penetrating the two upper plates of photosynthetic bacteria is of low intensity and peaks at about 560 nm (9). A comparison of the *O. limnetica* pattern of quantum utilization in the oxygenic photosynthesis to that previously shown for green algae and higher plants (chlorophyll *b* and carotenoids, the main photosystem II light harvesting systems) reveals that, in contrast to *O. limnetica*, almost all their absorbance spectrum is efficiently utilized in oxygenic photosynthesis (Fig. 3). The patterns of photosynthesis in *O. limnetica* would thus seem more oriented towards anoxygenic rather than oxygenic photosynthesis. It is suggested that this phototrophic pattern of *O. limnetica* possibly

further represents metabolic relicts of cyanobacterial evolutionary history.

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