

REVIEW

An Engineer Looks at Photosynthesis

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ABSTRACT

Little engineering information is available on which to base the design and analysis of photosynthetic microbial systems. Frequency-response analysis of algal systems supplements basic data and uncovers fundamental time constants for the rate-controlling steps. An engineering perspective of photosynthesis focuses on these bottleneck steps that most strongly influence process economics.

Index Entries: Photosynthetic microbial systems; biomass as a source of liquid fuels; photosynthesis; time constants; control; algae; systems analysis; dissolved oxygen; Bode diagrams; dynamics; pathways; systems.

INTRODUCTION

Biomass formed by green plants is an important potential source of liquid fuels and chemicals. Although a great deal is known about photosynthesis, much of the information is not organized and developed in a suitable engineering context for design, operation, and control of commercial processes. We will survey photosynthesis and show how systems analysis can focus on rate-limiting steps of most concern to process engineering.

Algae are excellent tools for studying photosynthesis; they are important in food chains, self-purification of lakes and streams, and biological waste treatment. Mitsui outlined a plan for commercialization of several products from algae (1). Algae can be converted to food by aquaculture of fish, mollusks, and crustacea. Furthermore, anaerobic di-

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gestion of photosynthetic organisms would be a short route from solar irradiation to fuel gas, although fermentation to higher priced products should be much more profitable.

Merely exploring steady states of photosynthesis is not enough for engineers because, in nature, photosynthesis varies as the sun moves across the sky and as clouds block the light. A systems approach, with a dynamic model for analysis and correlation of information, requires a hypothesis of how the system works. Models have interdependent functions that correspond to physical or chemical steps, such as diffusion through a liquid, excitation of a pigment by a photon, membrane transport, or an enzymatic reaction. These are shown as blocks with connections by arrows to indicate flow of information. There may be feedback loops, alternate paths, cross connections, and other complications.

The relationship between input and output for a given block is called its transfer function, e.g., $G(s)$ in Fig. 1. As an example, concentration of an intermediate biochemical is sensed somehow and used as an information signal for biochemical processes control. The mathematics relating the output signal (reaction rate) to input signal (concentration) form the transfer function. Actually, one block may represent several steps because certain details may presently be unknown. As more information becomes available, several blocks will substitute for the single block, and each should have a relatively straightforward transfer function when there is but one process per block.

A block diagram with dozens of operations would appear to defy rational analysis, but this is not the case here. Different blocks have different time responses, and those with relatively fast response have negligible effect on the overall timing. Usually a few blocks are much slower than all the others, and thus they are "bottlenecks," which determine the response of the system. A dynamic analysis concentrates on these rate-limiting steps and gives little information on the many other steps. However, these dominant steps are relatively much more important and can be studied in real, living situations.

It is appropriate to discuss sinusoidal forcing because it is the basis of much of the terminology of control engineers. After some initial transients, the steady-state output to sinusoidal forcing will itself become pe-

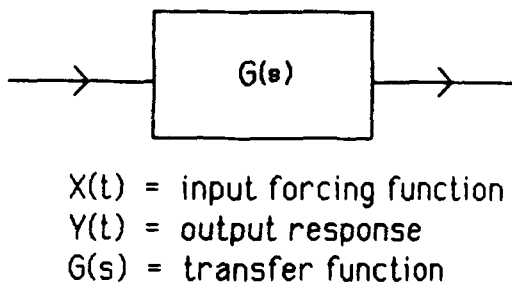


Fig. 1. Block diagram.

riodic. The amplitude ratio of output over input is called the gain, and the displacement in peaks is called the phase shift (Fig. 2). Even when periodic output is not a good sine wave, it is often possible to estimate gain and phase shift. With a modicum of skill and some imagination, a good sine wave can usually be reconstructed from the distorted response.

BODE PLOTS

Slow forcing allows the response of a system to be fully developed, and the gain will be at its maximum, with no phase shift. At higher frequencies of input oscillations, the response cannot track as well, so the gain declines and the output lags to increase the phase shift. The frequency response of a system can be shown in plots of gain and phase shift vs frequency. A logarithm of gain and arithmetic phase shift vs a logarithm of frequency compress the information into a convenient set of two graphs, called the Bode diagram. A simple Bode diagram for a first-order system is shown in Fig. 3. All first-order systems have curves of these same shapes, but there are families of curves for higher-order systems because their equations have additional constants, such as a damping coefficient.

An interactive tutorial about frequency-response analysis will be furnished by the authors on request. It is a program in elementary BASIC

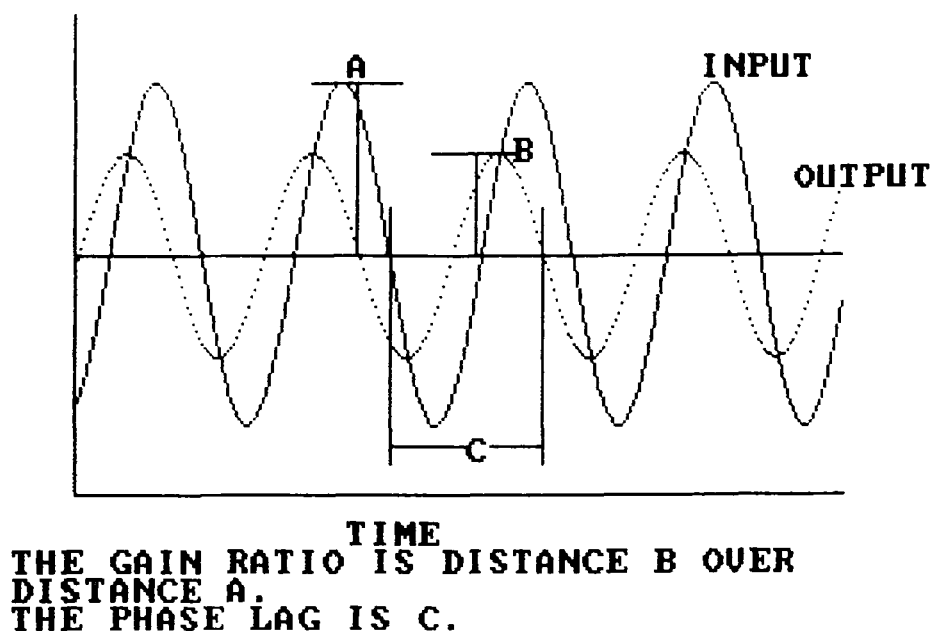


Fig. 2. Gain and phase shift.

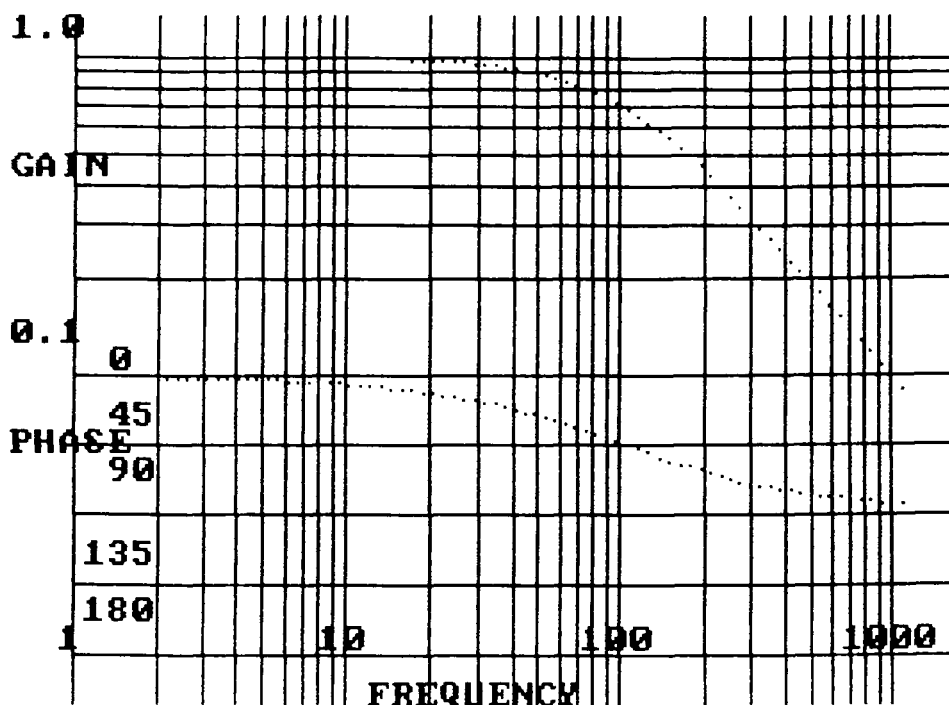


Fig. 3. First-order bode diagram.

for the IBM-PC, but conversion to a different computer system should be easy. Figures 2 and 3 came from this program.

The midpoint in transition of the slope of the gain curve and crossing points of the phase curve relate to fundamental time constants of the system. Experimentally, effects of the fast steps can not be measured. In other words, slow steps mask the effects of fast steps. A biochemist would consider this a serious defect and would not use frequency response analysis to unravel the details of a pathway. On the other hand, an engineer finds it appropriate to employ a technique that focuses on only the controlling steps.

LINEARITY

A linear differential equation has no powers, no products and no log or trig functions of the variables. Many systems are governed by linear differential equations or by equations that do not depart greatly from linearity in a narrow range of interest. When linearity prevails, the effects of several forcing functions or several blocks can be superimposed. The overall response is the product of the responses of individual blocks in the system. This is so important in simplifying the analysis of systems that engineers very often treat highly nonlinear systems as if they were linear. The degree of error introduced by assuming linearity may be tol-

erable, and the systems analysis or the design of a controller may be adequate for a given task.

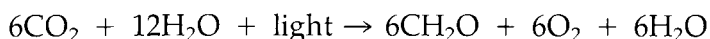
Photosynthesis is definitely nonlinear. For example, rate vs intensity of illumination is not a straight line, and at high intensities, the rate plunges as chlorophyll is bleached and damaged. Nevertheless, the operating range can be restricted so that linearization gives valid results for research on photosynthesis.

Bode diagram analysis, although rigorous only for linear systems, allows the effects of different blocks to be handled graphically. For example, if the effect of a first- and a second-order block are each plotted, the effect of both in series is additive on these log scales. A ruler or dividers can be used to sum the gains and the departures of each phase shift from 0° at a selected frequency.

PHOTOSYNTHESIS

The photosynthetic apparatus of all plants is composed of stacks called thylakoids (2). In the cyanobacteria, the thylakoids lie free in the cytoplasm. In other algae, they are separated from the cytoplasm by pairs of membranes to form chloroplasts (Fig. 4). Photosynthetic pigments and the enzymes required for the primary light-dependent reactions are associated in the thylakoids.

Only the primary events in photosynthesis require light, and subsequent reactions can take place in the dark. Light energy is converted to the chemical energy of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP), and oxygen is evolved. The secondary reactions form glucose and other reduced products from carbon dioxide. The overall reaction is:



Despite intensive research, some partial reactions of photosynthesis are still largely unknown. Whereas the path of carbon has been elucidated, the movement of oxygen through intermediate biochemicals is not thoroughly understood. Oxygen reactions are topics of current research, so progress should be rapid.

Emerson and Arnold (3,4) measured oxygen evolution by *Chlorella*, a green alga, using short flashes of light. On the average, a maximum of 1 O₂ molecule was evolved/2480 chlorophyll molecules/flash. These early experiments led to the concept of the photosynthetic unit, a group of chlorophyll and carotenoid molecules capable of acting cooperatively to reduce carbon dioxide.

Photosynthetic pigments resonate when energized by photons of light. The process may be viewed as photons being absorbed by an antenna of pigments that transfer energy through an array of pigments to a reaction center. This center or energy trap consists of several hundred

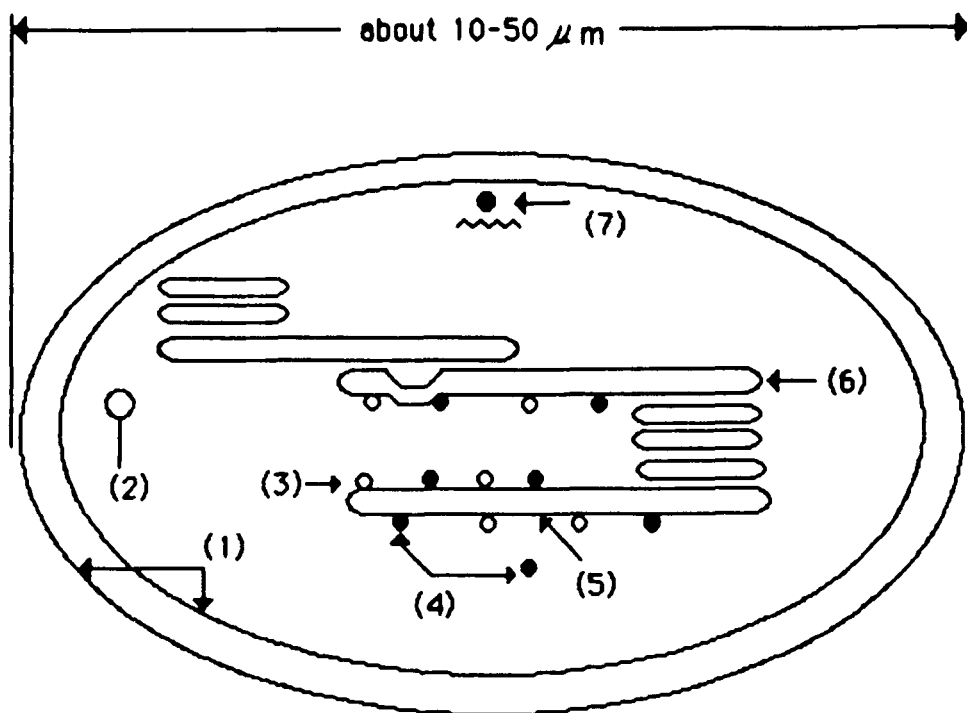


Fig. 4. A cartoon (not to scale) of a chloroplast, showing the lamellar thylakoids, the light-harvesting chlorophyll protein complexes (LH a/b) and various inclusions and membrane surface particles (Clayton, 1980, p. 116).

1. Two envelope membranes
2. Quinone globule
3. CF₁
4. RuDP carboxylase
5. LH a/b in unstacked regions
6. LH a/b in stacked regions
7. Ribosomes, DNA

chlorophyll molecules and other pigments, all bound to proteins. Duysens et al. (5) envisioned a special bacterial chlorophyll that traps energy from the antenna pigments to power a charge separation reaction. Kok (6) attributed an absorption peak in plants near 700 nm to a pigment designated chlorophyll a (*P*700) ("*P*" stands for "pigment" and the number refers to the wavelength of absorbed light expressed in nanometers).

Light Reactions

There are two distinguishable light reactions in plants. Emerson and coworkers (7,8) showed that at low-light intensities the rate of photosynthesis in combined far-red and shorter-wavelength light was greater than the sum of the rates driven by the beams separately. One reaction depends on a short wavelength form of chlorophyll a (Chl a), and the other requires pigments that include a long wavelength form of Chl a (9).

Hill and Bendall (10) proposed that the two light reactions operate in series and designated them photosystem I (PSI) and photosystem II (PSII). A schematic representation of the Hill-Bendall "Z-scheme" is shown in Fig. 5. Photosystem II is activated by light of wavelengths below 670 nm and is associated with noncyclic electron transport and oxygen evolution. Photosystem I, however, contain chlorophyll that absorbs light at a maximum of 700 nm and is associated with both cyclic and noncyclic electron transport and also with the reduction of NADP^+ . Cyclic photophosphorylation employs PSI, involves no net reduction of an electron acceptor or oxygen evolution, and produces ATP. These reactions require a series of electron and hydrogen carriers.

In the Z-scheme, PSI and PSII are equal. However, Melis and Brown (11) noted that the ratio of PSII to PSI varies considerably, depending on the organism and growth conditions. Anderson and Anderson (12) pointed out that the stacks of thylakoid membranes (grana) are rich in PSII, whereas the stroma (oppressed, double membrane) and margin regions of grana contain PSI. This raises questions concerning the mobility of large protein complexes within the membrane and long-distance electron transport between the two photosystems. It would seem that the original photosynthetic unit concept is too simple to accommodate all of the complex interactions between the two photosystems. Although phenomena are not fully understood, the Z-scheme has only a few serious challengers (13).

The early experiments of Ruben et al. (14) supported the argument that water was the source of O_2 in photosynthesis, but this has been challenged (15–17). Although there is general agreement that the ultimate source of O_2 is H_2O (18,19), Metzner et al. (20) and Stemler (17) believe that the immediate source of oxygen may be HCO_3^- or CO_2 . The role of CO_2 in water oxidation is presently unsettled, although it has been shown that CO_2 participates in electron transport between the two photosystems (21).

Energy trapped by the light-harvesting complexes is transferred to the specialized reaction center—chl a, P680, which becomes oxidized. The electron is then passed via a pheophytin molecule to a primary acceptor, referred to as "Q" because its identity is not fully defined. It may be a 550 nm-absorbing species of phenophytin (C550) or a 320 nm-absorbing species of plastoquinone-iron complex (X320). Chlorophyll at the reaction center is rereduced by a donor that is probably a quinone. This donor is itself rereduced by electrons derived from water. The splitting of two molecules of water to yield one O_2 releases four electrons in a four-step mechanism probably involving at least two manganese atoms. Joliot et al. (22) showed that when dark-adapted *Chlorella* cells are illuminated by a series of short flashes of light, the O_2 evolved/flash was not constant, but showed a periodicity of four (Fig. 6). Several hypotheses have been proposed that are consistent with Joliot's work (23). According to Kok's hypothesis, the reaction chain, which includes the O_2 evolving

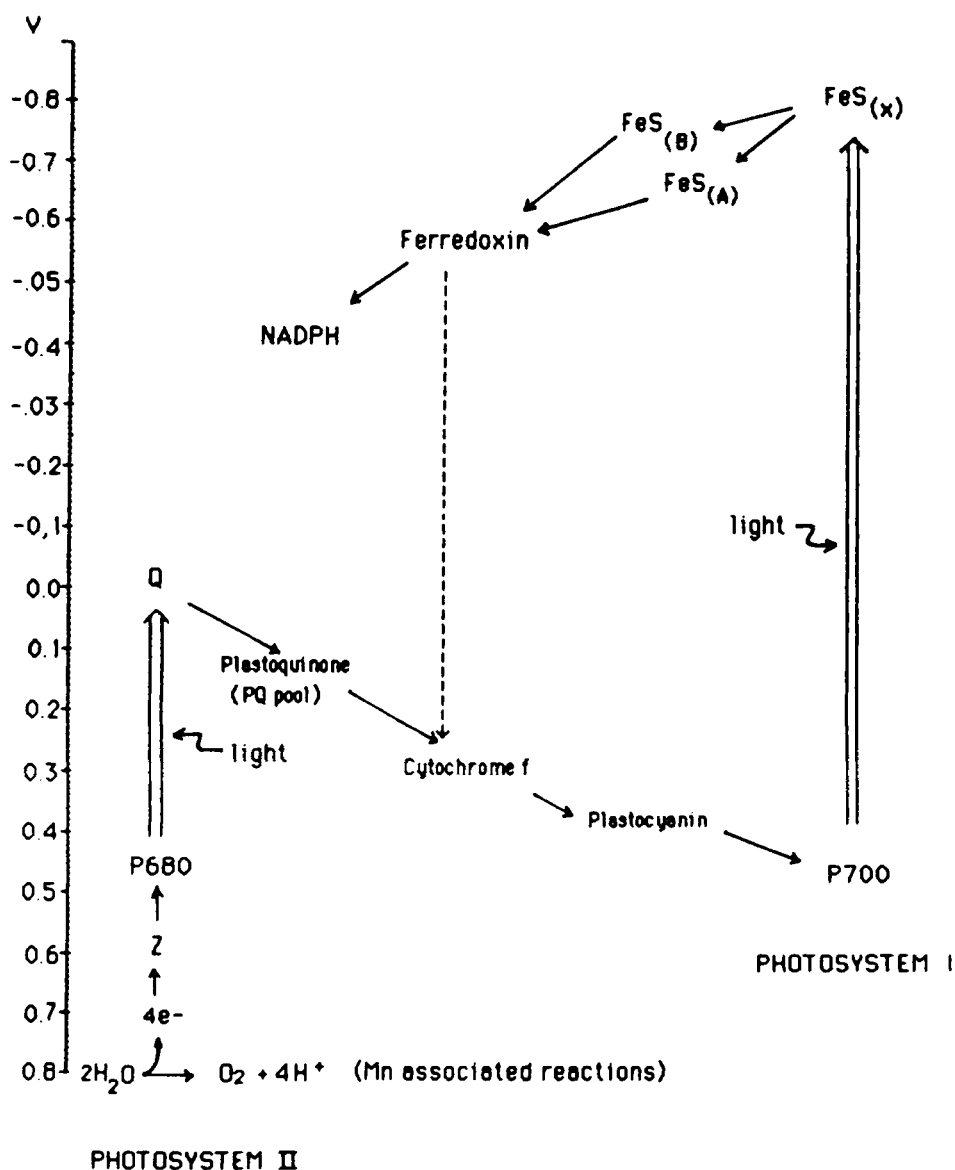
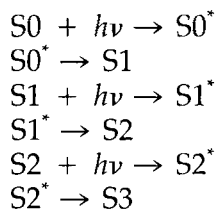


Fig. 5. Simplified Z-scheme of photosynthesis (see text for detailed discussions).

enzymes and PSII, cycles through five oxidation states and requires four successive quanta ($h\nu$):



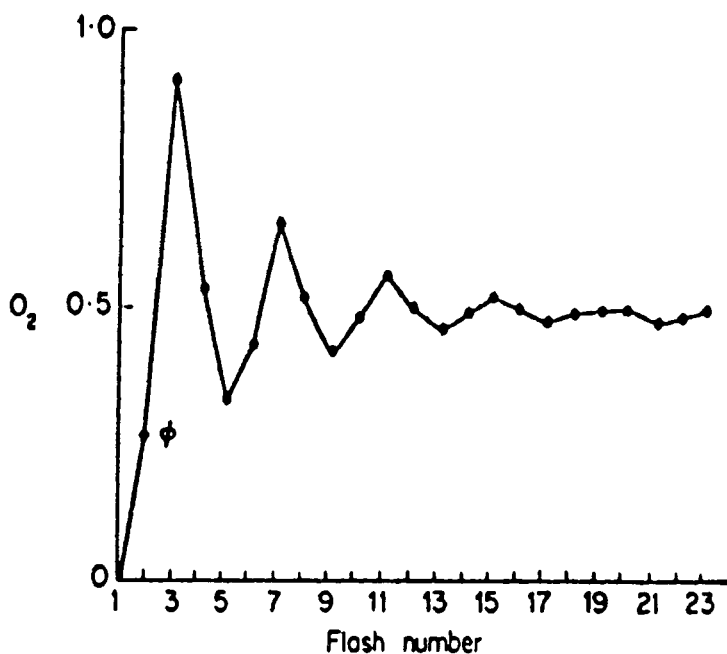
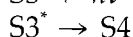
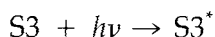


Fig. 6. Oxygen evolution by *Chlorella* cells excited by a series of short light flashes. Dark incubation for three minutes and dark interval between flashes was 300 milliseconds. (From Joliot et al., 1969).



The model is based on the following assumptions: (i) A manganese-enzyme center exists in any one of the five different oxidation S-states; (ii) oxygen evolution results with the absorption of a quantum at the S3 state; (iii) of the five states, the ground state, S0, and the first oxidized state, S1, are stable in the dark, whereas the higher states, S2, S3, and S4, are unstable and can deactivate in the dark (though the exact deactivation scheme is still a subject of debate); and (iv) the rate constant for oxygen production from flash experiments has been estimated to be approximately 800/s at 20°C corresponding to a half time of about 1.25 ms (24).

Extensive studies of water splitting have used algae and chloroplasts (25,26). Recently, several groups (27–29) have reported procedures yielding detergent-fractionated PSII preparations that contain an active water-splitting, manganese-containing enzyme. Lavorel and Seibert (30) and Seibert and Lavorel (31) reported patterns of oxygen evolution from O₂-evolving PSII preparations elicited by sequences of short flashes. Although their results are generally consistent with the S-state model, they observed some differences in the deactivation process, particularly the apparent stability of S2 and the dark deactivation pathway of S3.

Electron Transfer between PSII and PSI

Two photoreactions operate in series to raise electrons from the high-potential donor, water, to the low-potential acceptor NADP. The transfer is mediated by flavoproteins, iron-sulfur compounds, cytochromes, and cofactors. The electron pathway is characterized in general terms, but some details are still obscure. Table 1 summarizes properties of some of the electron carriers.

Exciting the photochemically active complex leads to the oxidation of a chlorophyll molecule in a reaction center. The transfer of an electron to the primary acceptor across the thylakoid membrane charges it electrically. The primary acceptors, Q, a "bound" plastoquinone, and FeS_x, that may be a "bound" iron-sulfur protein, are tightly coupled to their respective chlorophylls. Extremely rapid and essentially irreversible transfer of electrons competes with the rapid wasteful processes that deactivate singlet excited chlorophylls.

Both photosystems are rich in plastoquinones (PQ) that interconnect electron-transport chains and improve the reliability of the system under conditions in which some of the photochemical reaction centers may be damaged. Any reaction center I can be furnished via PQ with electrons from a nearby center II. Plastoquinones also transfer hydrogen ions across the membrane to contribute to the generation of the electrochemical potential. The oxidation of plastohydroquinone (PQH₂) by plastocyanin (PC) is the rate-limiting step of the whole chain (32) and controls the electron-transfer rate by the electrochemical gradient and the ATP/ADP ratio.

Plastocyanin, ferredoxin (Fd), and the flavoprotein ferredoxin-NADP-oxidoreductase (FNR) are essential because electron transfer is in-

TABLE 1
Characteristics of Electron Carriers

Component	Midpoint potential, E_m (V)	Absorption maxima, nm	Reference
P680		680	110
Pheophytin	-0.6	685	111
Q, C550, X320	-0.2	330, 550	112
	0.025-0.05		113
PQ pool	0.01-0.06		114
	0.12		115
		265, 295	116
Cyt f	0.33-0.39		117-119
PC	0.37-0.39		120, 121
P700	0.4-0.5	700	122, 123
FeS _x , P430	< -0.52	400-500	124
Fd	-0.41	430	125, 126
NADP	-0.32(est.)		

interrupted if they are extracted or blocked. Probably they function to enhance the velocity of electron transport.

The reducing power of photosynthesis can be used by the cell in several ways, and there are reactions that dissipate this energy. Although NADP is an efficient electron acceptor, electron drainage to O_2 (the Mehler reaction) persists at a low rate. Nitrogen fixation and hydrogen production can be powered by photosynthesis in some organisms.

The electron-transport chain is illustrated in Fig. 7 (33,34). The time constants for the reactions represent the reaction rates under nonlimiting conditions. Energy and electron transfer steps are very rapid and should not limit the rate of photosynthesis.

Photophosphorylation

Phosphorylation forms ATP from ADP. Light-driven phosphorylation was first demonstrated in the photosynthetic systems of certain bacteria by Frenkel (35). A conceptually simple explanation of the coupling of electron transport to phosphorylation and the suggestion that electron transfer results in the unidirectional translocation of protons across a membrane led to a Nobel prize (36). The resulting proton-gradients provide energy for the phosphorylation of ADP. Chloroplasts can exhibit light-induced pH changes that drive the synthesis of ATP. Chloroplasts in medium buffered at pH 4.0 had roughly the same pH at the inside of the thylakoids, but rapid transfer to a medium buffered at pH 8.5 established a pH gradient so that added ADP and phosphate synthesized ATP in the dark (37).

The Chemiosmotic Account

A mechanism proposed by Mitchell (38) has gained considerable experimental support, particularly in the laboratory of Witt (39,40). In the scheme shown in Fig. 8, it is assumed that both photoacts move an electron from the inside to the outside of the thylakoid membrane. The concerted action of many units can then establish a significant membrane po-

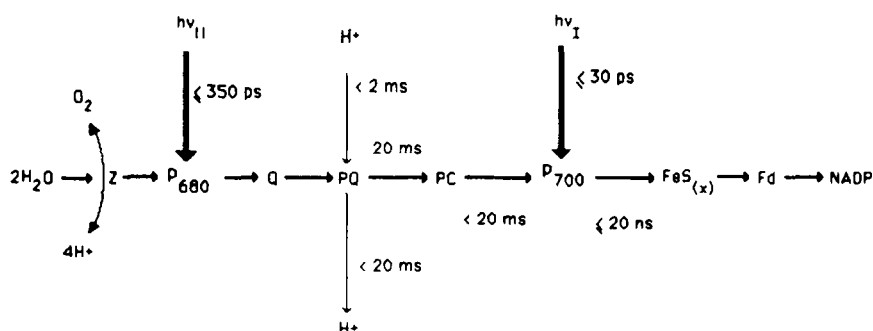


Fig. 7. Estimates of the characteristic time constants of the energy transfer into the reaction centers and for the electron transport.

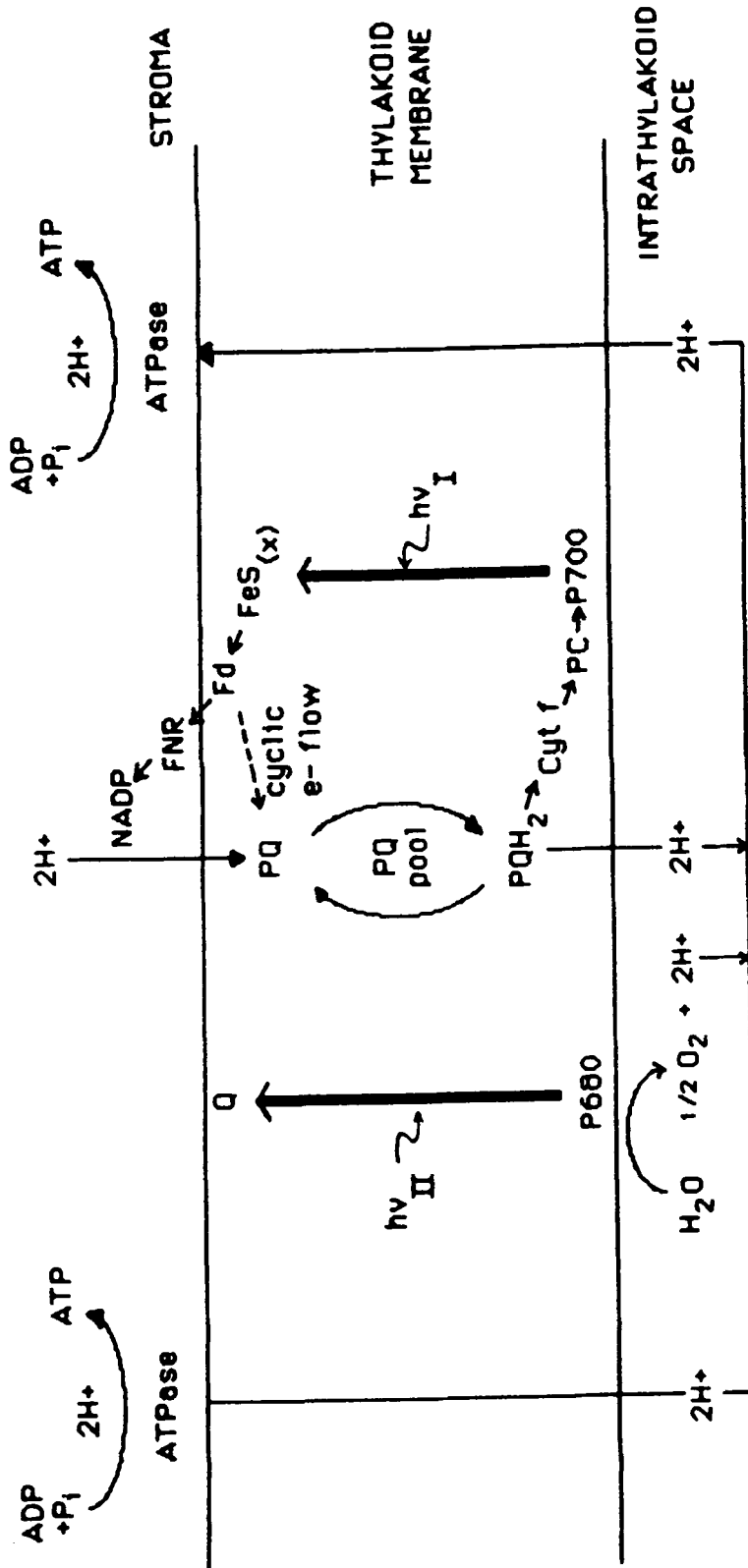
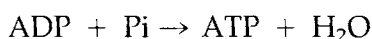


Fig. 8. Simplified scheme for photosynthetic electron flow coupled to ATP formation.

tential ($\Delta\psi$). This electrical field presumably causes absorption changes (electrochromic shifts) of some of the pigments in the membrane (41). The most pronounced of these changes, a red shift from 480 to 515 nm, originally observed by Duysens (42), is probably mediated by a carotenoid. Subsequently, the reduction of the PQ pool, which is mobile, uses protons from the outer phase, whereas its oxidation, as well as O_2 evolution, releases protons and, thus, the gradient functions in the reaction (43,44). Together, the proton gradient and the electrical potential form the proton motive force (PMF):

$$\text{PMF} = (\Delta\psi) - 2.303(RT/F)\Delta\text{pH}$$

where $2.303 RT/F = 59 \text{ mV}$ at 298 K, R is the gas constant, T is the absolute temperature, F is the Faraday constant, and the sign is correct for the internal pH minus the external pH. The pH difference across the photosynthetic membranes in chloroplasts in the light is approximately three. In most cases, the H^+ is largely balanced by an influx of Cl^- ions and an efflux of magnesium ions. Subsequently, proton flow through specific sites in the membrane causes the coupling enzyme or ATPase (45) to generate ATP. The synthesis of ATP is a condensation or dehydration reaction:



The mechanism of this energy translation remains unclear.

According to Izawa and Hind (46), noncyclic electron transport results in a $H^+/e^- = 1$. However, values of 1–2 H^+/e^- have been reported (47). Mitchell (48) proposed that a protonmotive quinone cycle (Q cycle) allows two protons to be transported for each electron passing through PQ, and thus, $H^+/e^- = 2$. These results and some others are collected in Table 2. The consensus seems to be that there is 1.0 proton for each electron passing from PSII to PSI, though there are enough reports of higher values to confuse the issue. Still another major disagreement comes in calculating the $\text{ATP}/2e^-$ ratio or H^+/ATP ratio. Values of 2, 3, and 4 for the H^+/ATP ratio have been reported, even from the same laboratory (Table 3). Some of these discrepancies can be attributed to nonuniform experimental conditions, but the possibility of variable ratios (depending on the environment) should not be ignored. The "correct" H^+/ATP value is also important in discussions of the range of the redox potential in the electron-transport chain that would be required in order to synthesize ATP.

Although there are many arguments supporting the concept that the proton gradient is essential for phosphorylation, several observations form the bases for other explanations (49,50). *In vitro*, NH_4Cl can abolish proton uptake and still allow ATP formation (45). Some chloroplast preparations that are rich in PSI can show high rates of cyclic phosphorylation, but no appreciable proton translocation (51). Such cyclic paths may involve the various cytochromes that have not been assigned clear-cut roles in the electron-transport system.

TABLE 2
Reported H^+/e^- Ratio for
Noncyclic Photosystem

System	H^+/e^- Ratio	Reference ^a
PSI + PSII	1	46
	1	127
	1	128
	1.7	129
	1	123, 130–132
	1	47 ^b
	1	133 ^b
	1	134, 135
	2	136
	2	137
	2	138
	2	139, 47 ^c
	2	140
	2	141, 142
	2	133 ^c
PSII	1	127
	0.5	129
	1	130
PSI	1	127
	1	143

^aSee references for experimental conditions.

^bHigh-light intensity.

^cLong-light intensity.

TABLE 3
Reported H^+/ATP Ratios
for Photosynthetic
Phosphorylation

H^+/ATP ratio	Reference ^a
2	144
2	137
2.4	145
3	146
2	138
3	147
4	148
3	149
2	129
4	150
3–4	151
1.7–1.8	152
2.4	153

^aSee references for experimental conditions.

The chemiosmotic theory is well-accepted, and other hypotheses for the coupling of ATP synthesis to electron transport are mainly of historical interest. In the chemical coupling hypothesis (52), electron transport generates a reactive chemical intermediate that has sufficient energy for ATP synthesis. The coupling intermediate is a molecule or complex that drives this and other energy-linked processes, such as proton pumping. In the conformational-coupling hypothesis (53,54), transport of electrons elicits a conformational change in certain macromolecules that shifts hydrolysis/dehydration reactions to favor ATP synthesis.

Energy Conservation Sites

Many early measurements of phosphorylation stoichiometry yielded values of 1 ATP/ $2e^-$ transferred to NADP. More recent reports indicate ratios of 1.0 (55), 1.3 (56), or 2.0 ATP/ $2e^-$ (57). Moreover, when NADP is replaced by electron acceptors (benzoquinone, oxidized *p*-phenylenediamine) that are presumably reduced directly by PSII, this ratio is decreased by one-half (58). Such data could be explained by two sites of proton uptake between O_2 evolution and NADP reduction, leading to ATP formation. One site is within the evolution process and the other is within the electron-transport chain between the photosystems. Carbon dioxide fixation requires 5–6 ATP for each carbon fixed, i.e., an ATP/ $2e^-$ ratio of 3. Thus, noncyclic electron-transport seems unable to meet the overall ATP demand. This apparent ATP deficit might be overcome with phosphorylations via some other energy conservation sites. The electron-transport chain between the two photosystems may conserve energy by pumping protons into the thylakoids, thereby forming a proton gradient. Oxygen evolution processes inside the membrane also accumulate protons to help form the gradient. Cyclic electron-transport between Fd and P700 could have two sites for energy conservation. Finally, a reaction path associated with a light-enhanced uptake of oxygen observed in whole cells may also be a site for energy conservation.

Carbon Fixation

A major development in carbon fixation was the discovery by Kortschak et al. (59) that sugarcane fixed carbon initially into a 4C rather than a 3C acid. This was followed by a delineation of the detailed pathways in many plants, now known as C_4 plants, by Hatch and Slack (60). Algae and cyanobacteria follow the C_3 pathway. This review will focus on C_3 pathways because of the microbial orientation of the authors. Figure 9 shows the pathway of carbon in C_3 plants. It is important to note the autocatalytic characteristic of the cycle. The regenerative formation of ribulose diphosphate (RuDP) leads to the autocatalytic buildup of pathway intermediates. This cyclic process makes the pathway self-sufficient in that it can produce and increase its own substrate.

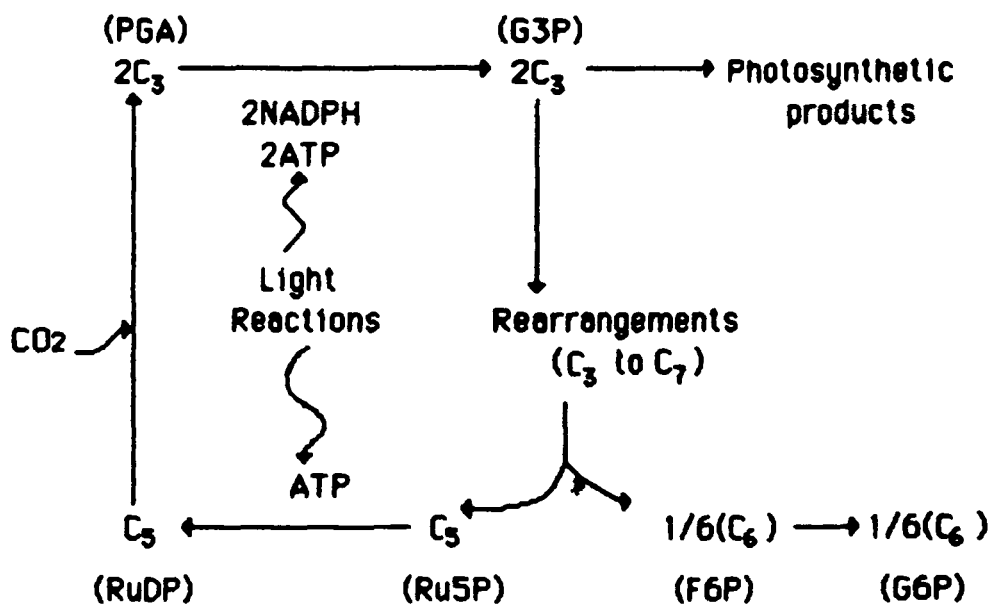


Fig. 9. A skeletal outline of the reductive pentose or Calvin cycle.

PGA: 3-phosphoglyceric acid;

G3P: glyceraldehyde-3-phosphate;

F6P: fructose-6-phosphate;

G6P: glucose-6-phosphate;

Ru5P: Ribulose-5-phosphate;

RuDP: Ribulose-1,5-diphosphate;

Details of the rearrangements involving 3- to 7-carbon compounds can be found in Bassham and Calvin, 1957.

Futhermore, this leads to the phenomenon of induction—activation of the enzymes that are essential for the activity of the cycle. These enzymes include fructose diphosphatase, sedoheptulose diphosphatase, and phosphoribulokinase. The physiological function of light-dependent reductive activation of enzymes is still a matter of debate (61–64).

Photorespiration

Kok observed that the net oxygen exchange of an illuminated algal suspension is not a linear function of light intensity (65). This non-linearity, now known as the "Kok effect," has been demonstrated in a number of unicellular organisms, including *Chlorella fusua*, *Anacystis nidulans* (a cyanobacterium) (66), and *Anabena variabilis* (67). Kok suggested that the effect resulted from a light-induced depression of respiration. Hoch et al. (67) employed isotopes to discriminate between production and consumption of oxygen in algal suspensions. Dark respiration was inhibited by light, but at higher intensities, oxygen uptake was dependent on light intensity. The term "photorespiration" describes

oxygen uptake resulting from reactions of the molecules formed by photosynthesis. The well-known "Warburg effect" of oxygen inhibition of photosynthesis is discussed by Gibbs (68).

During photorespiration, oxygen is taken up and carbon dioxide is formed. Photorespiration increases with increasing light intensity, temperature, and oxygen concentration and with decreasing carbon dioxide availability. Consumption of oxygen and release of carbon dioxide during photorespiration are generally underestimated because the opposite reactions of photosynthesis masks their true magnitudes.

The main functions of photorespiration appear to be protection of the photosynthetic apparatus and regulation of growth by utilizing excess energy, since photorespiration dissipates newly formed ATP, NADPH, or reduced Fd. Photorespiration is probably a protective mechanism against light and oxygen toxicity. In the atmosphere, photosynthesis is severely limited by low carbon dioxide (0.033%) and high oxygen (21%), so photorespiration may help to maintain a delicate balance in photosynthetic organisms. Photorespiration can be viewed as glycolate biosynthesis and metabolism. Oxygen inhibits CO_2 fixation because O_2 itself is a substrate for the first enzyme of the Calvin cycle, ribulose diphosphate carboxylase, that actually fixes the CO_2 . Oxygen and carbon dioxide compete for the same sites on the enzymes. Ribulose diphosphate can thus be oxygenated in an essentially irreversible reaction to form 3-phosphoglycerate and 2-phosphoglycolate (69,70). During photosynthesis, algae produce uniformly labeled glycolate and glycine in substantial amounts that are increased by high oxygen concentrations and lowered levels of carbon dioxide or low pH (71). A schematic diagram of the mechanism of glycolate metabolism and possible reaction sites of oxygen is shown in Fig. 10. This reaction is largely negated in C_4 plants in which the carboxylase is compartmentalized in a region of high CO_2 concentration in the bundle sheath cells.

Recent evidence suggests that photorespiration may provide a protective mechanism that recycles CO_2 in conditions in which the concentration of CO_2 is low relative to O_2 . The NADP pool may be largely converted into its reduced form. Oxidized NADP would therefore not be as readily available to accept reducing equivalents from PSI; as a result, over-reduction of the electron-transport system would occur. This situation would favor the production of free radical derivatives and other active oxygen species harmful to the photosynthetic apparatus. Photorespiration may protect by maintaining a high concentration of CO_2 in the cellular environment (12). Figure 11 illustrates the key relationship of the photosynthetic carbon reduction and the photorespiratory carbon oxidation pathways. Since CO_2 and O_2 compete for the same active site on the enzyme, the rates of the two reactions are functions of the relative concentrations of the two gases. The actual rates depend on many factors, but under normal conditions the carboxylase activity could be ex-

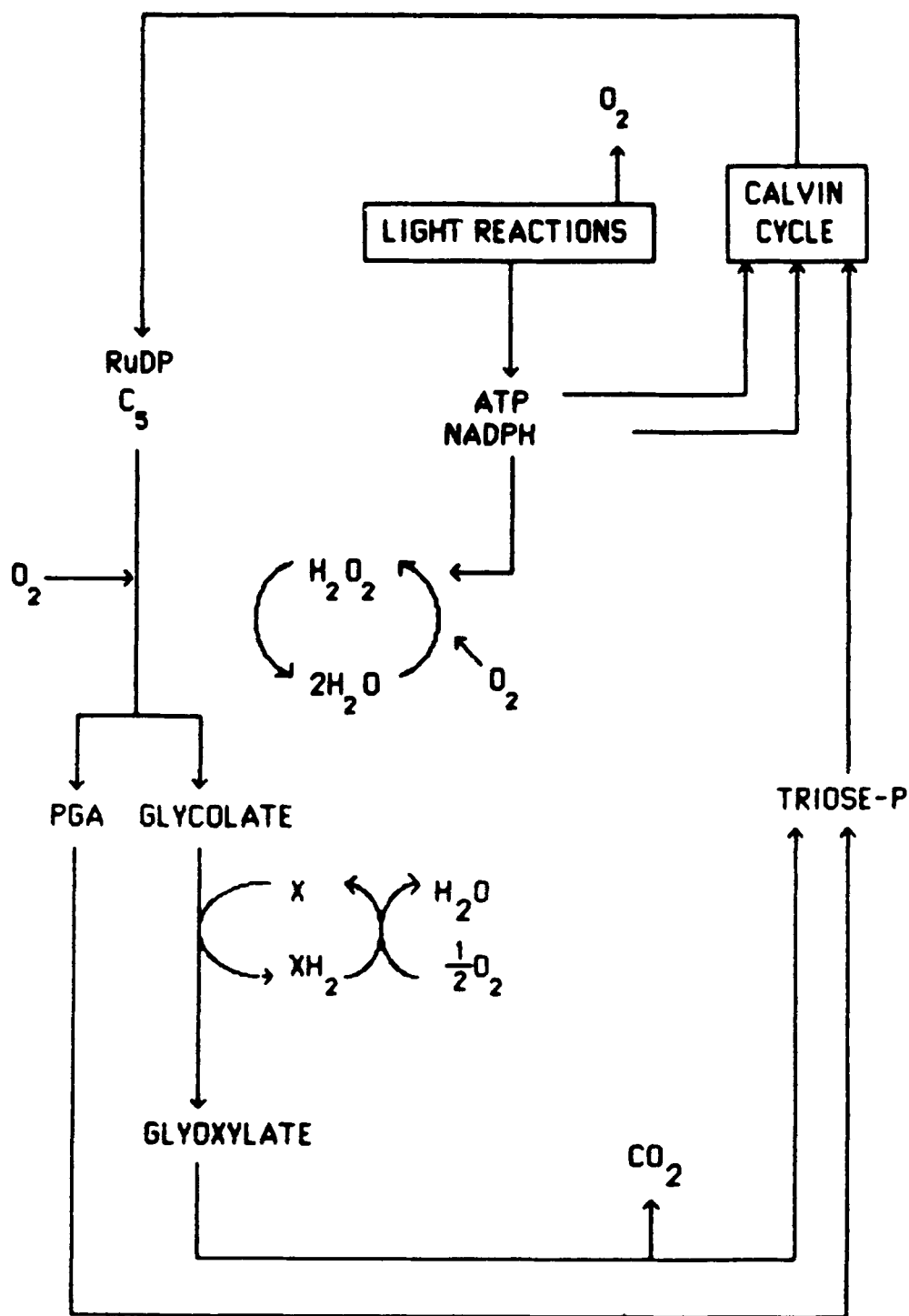


Fig. 10. Glycolate metabolism and the possible reaction sites of oxygen (Adapted from Harris, 1977).

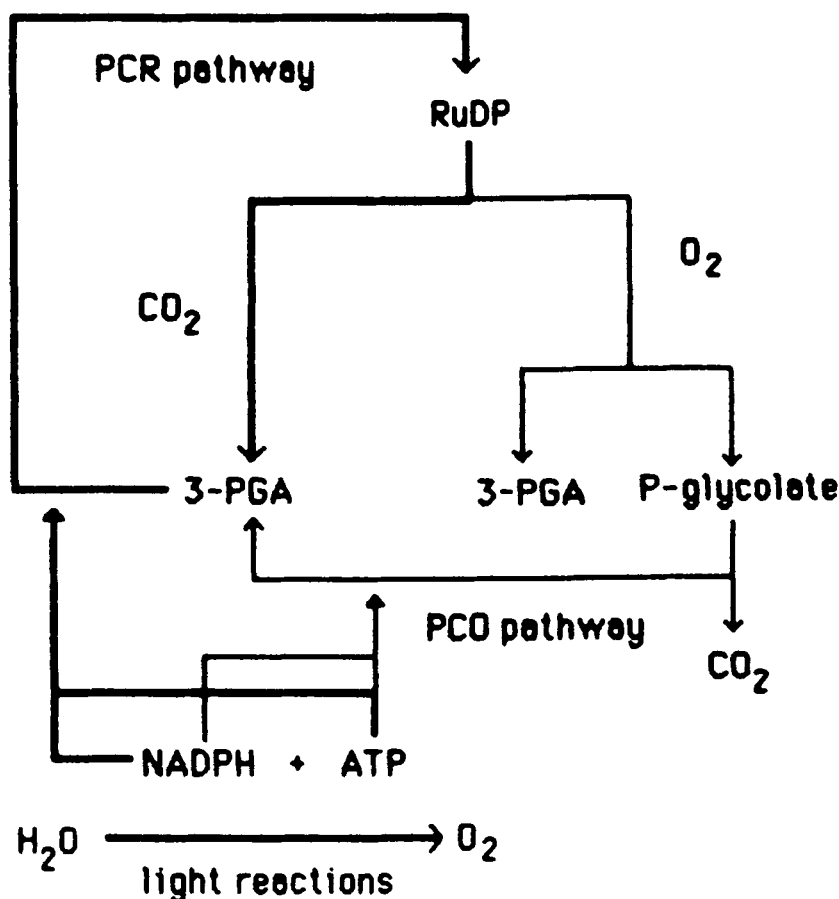


Fig. 11. Schematic diagram of the two competing pathways for Ribulose Diphosphate (RuDP)

PCR: photosynthetic carbon reduction

PCO: photosynthetic carbon oxidation

3-PGA: 3-phosphoglycerate

P-glycolate: 2-phosphoglycolate

pected to be from three to five times greater than the oxygenase activity (64,73).

Photorespiration consists of oxygen uptake during photosynthesis. Mehler (74) observed that isolated illuminated thylakoids slowly reduced O₂ in the absence of added electron acceptors. This reaction, in which oxygen is both evolved and taken up by the action of the electron-transport chain, is called the Mehler reaction or pseudocyclic electron flow (Fig. 12). Oxygen is reduced by the electron-transport chain during CO₂ fixation in plants and algae. Even though the reduction of NADP is highly efficient, electron drainage to O₂ continues at a low, but constant, rate. Oxygen is not an efficient electron acceptor, but could be necessary to divert electrons to O₂ at times when NADP levels are low. Light-

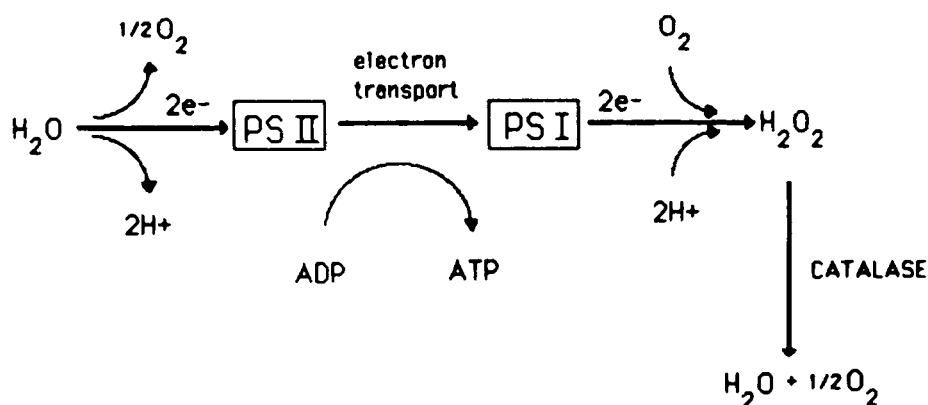


Fig. 12. A schematic diagram of hydrogen peroxide formation by a Mehler type reaction.

scattering measurements with intact leaves have shown cyclic electron flow only after electron flow to O_2 has been largely saturated. At low-light intensities, CO_2 reduction is slow, and electron transport to O_2 could be sufficient to supply the additional ATP needed for CO_2 reduction. When NADP is largely reduced and electron flow to O_2 is near saturation, electrons are diverted into the cyclic pathway (75). Even during cyclic photophosphorylation, electron drainage to O_2 may serve to prevent overreduction of the electron carriers. Thus, oxygen reduction prevents overreduction of the electron transport system while remaining a coupled process leading to ATP synthesis (76).

Direct photoreduction of O_2 by the electron-transport chain is inevitable in all organisms carrying out oxygenic photosynthesis. Since water is the donor for electron transport, the thylakoid membranes exist in an environment rich in oxygen. This oxygen competes with NADP for reducing equivalents (Fig. 7), so that fairly complete reduction of NADP stimulates electron transport to O_2 . Electron flow to O_2 is generally very low and at most can only account for 16% of the net steady-state rate of O_2 evolution during CO_2 reduction by intact chloroplasts at high light intensities (64). However, significant rates of O_2 reduction can be observed, particularly when CO_2 fixation is limited, as in the initial lag phase of photosynthesis (77,78). From the onset of illumination, O_2 evolution is maximal, but is totally or partially offset by the rate of oxygen uptake so that no net O_2 is produced. After this induction phase, the rate of O_2 uptake diminishes to a low level and the rate of O_2 evolution increases. Observed rates of O_2 reduction during steady-state photosynthesis in algae appear to be somewhat greater than those observed in higher-plant chloroplasts. In intact cells of the cyanobacterium *Anacystis nidulans* the onset of illumination results in a transient burst of hydrogen peroxide production (79). Hydrogen peroxide is sometimes excreted from isolated intact chloroplasts during photosynthesis (80).

There is some uncertainty about the importance of photorespiration in the overall carbon and oxygen balance in algae. However, Tolbert (81) has pointed out that much of the earlier literature that disregards photorespiration was based on experiments carried out at low light intensities and high carbon dioxide concentration, conditions that do not favor photorespiration.

Dark Respiration

Aerobic respiration oxidizes carbohydrates to generate energy for the cell. The overall equation is:



For some time there has been a controversy about whether dark respiration continues in the light. Much confusion exists because of the difficulty of distinguishing between the respiratory and photosynthetic pools of oxygen and carbon dioxide and because of the problems of refixation of respired carbon dioxide and photosynthetically produced oxygen (82).

The metabolic balance photosynthesis and respiration has been explored by several investigators in a number of ways. Hoch et al. (67) observed the effect of photophosphorylation on cellular ATP levels and on rates of oxidative phosphorylation. They proposed that cyclic photophosphorylation mediated by PSI caused the ATP:ADP ratio in the cytoplasm of eukaryotic algae to rise either from direct movement of ATP or the involvement of a shuttle system that eventually depressed respiration in the mitochondria. This theory is supported by the apparent inhibition of glycolysis (83,84) and the failure of recently fixed ^{14}C to enter the tricarboxylic acid cycle in the light (85). However, in an illuminated algal cell, the tricarboxylic acid cycle (TCA) appears to be operational and functioning as a carbon skeleton pathway (82,86). Ried and Setlk (86) speculated that photophosphorylation controls the flux through the glycolytic pathway by allosteric inhibition of phosphofructokinase by ATP. This proposal relates the oscillations in oxygen transients to glycolytic oscillations in yeast, observed by Chance et al. (87). The energy demands of algae in the dark must be met by glycolysis. In the light it would seem reasonable that photosynthetic ATP production suppresses respiration, and in darkness this control is lifted (67,88).

Analyzing respiration in the light is further confused by evidence for two more light effects that may be independent of photosynthesis (89). Blue light exerts two pronounced effects on dark respiration. At low intensities, with a wavelength dependence corresponding to the absorption spectrum of flavins or carotenoids (460 nm), blue light can stimulate respiration. At high intensities corresponding to the absorption spectrum of heme proteins (370 nm), blue light can cause inhibition. The consequences of either phenomenon are uncertain, and a connection be-

tween enhanced dark respiration and the formation of photosynthetic apparatus has been proposed by Kowallik (89).

ENGINEERING RELEVANCE

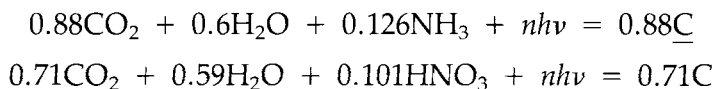
At this point, focus will shift to aspects of photosynthesis with more direct implications to engineering. Energetics and simple mass balance consideration define what is possible and supply a framework for the economics. Identifying the bottleneck steps would lead to an attack on these specific limitations. For example, a rate impairment can be circumvented by suppressing an alternate pathway, stimulating production of a needed enzyme, damaging a control that slows synthesis of a key intermediate, or other biological manipulations. In some cases, the answer can be strictly engineering, as with better aeration, maintaining a high concentration of carbon dioxide, or better control of pH, redox potential, temperature, or the like.

Solar Energy Bioconversion Efficiency

Photosynthetic efficiency is the ratio of the free energy of the biomass produced to the amount of light energy absorbed or the number of photons ($h\nu$) required/mol of oxygen released or of carbon dioxide assimilated. Photosynthetic efficiency sets standards for commercialization. The values reported in the literature for the minimum quantum demand have ranged from 4–13 $h\nu/O_2$ (90–92), and no definitive value has emerged. Knowing whether the minimum quantum demand is less or greater than 8 $h\nu/O_2$ is important because the widely accepted Z-scheme has a minimum quantum demand not less than 8 $h\nu/O_2$. The problem is optimizing the maximum growth yield from the available light energy. In a recent review, Pirt (93) highlighted some fundamental causes of the disagreement between values for the photosynthetic efficiency and deduced values to be preferred. He attributed discrepancies to different methods of culturing and suggested that maintenance should be part of the calculation of efficiency.

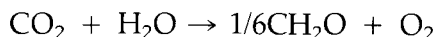
Stoichiometry

The reactions for photosynthetic production of biomass (94) are:



where \underline{C} , a mol of biomass, contains 1 mol carbon, and the empirical formula of the ash-free biomass is taken to be $CH_{1.8}N_{0.143}O_{0.423}$ (94). The number of quanta needed for the reaction is n . The heat of combustion of the biomass represents the chemical energy stored in the process. Conversion of the heats of combustion to Einsteins (at 700 nm, 1 Einstein =

171 kJ) indicates a maximum demand of $3 h\nu/O_2$ released. The simplified representation of photosynthesis as:



does not significantly alter the overall energy balance; however, it obscures the fact that during biomass growth the quantum requirement for CO_2 assimilation exceeds that for O_2 release.

Energetics

According to the Z-scheme, photosystems PSI and PSII must act in series, and each utilizes one photon for transferring an electron from water to NADP via Fd. This means that there should be at least 8 photons/molecule of O_2 . However, there are discrepancies that should not be ignored (95). Arnon et al. (13) proposed that Fd can be reduced by PSII without the intervention of PSI, with other photoacts required to provide enough NADPH and ATP, resulting in a total quantum demand of $12 h\nu/O_2$.

For each mol of CO_2 fixed, 2 mol of NADPH and 3 mol of ATP are required. Again there is controversy about the energetics of ATP generation (96). Mitchell's chemiosmotic theory (37) seems to account well for photophosphorylation. Although the concept is widely accepted, there are many arguments over the $H^+/2e^-$ ratio (Table 2). If the $H^+/2e^-$ ratio is 2, and the resulting $ATP/2e^-$ ratio is 1, there is an ATP deficit because the ratios for $NADPH/2e^-$ and $ATP/NADPH$ are 1.0 and 1.5, respectively, for CO_2 fixation. The extra ATP may come from cyclic photophosphorylation or oxidative pathways. However, if the $H^+/2e^-$ ratio is 4 and the resulting $ATP/2e^-$ ratio is 2, then there is an excess of ATP. This may explain the phenomenon of photoinhibition of respiration (67,83).

Measurement of Photosynthetic Efficiency

The total O_2 production rate of an algal cell suspension will be designated as P_t . The photosynthetic efficiency is given by P_t/Q_L where

$$P_t = P_n + R$$

$$Q_t = Q_1 + Q_2 + Q_L$$

where P_n is the net oxygen production rate, R is the respiration rate, Q_1 is the rate of light utilization by PSI, Q_2 is the rate of light utilization by PSII, and Q_L is the rate of light absorption by nonphotosynthetic systems in the cell. The problem here is the measurement of the true photosynthetic O_2 production rate. Account must be taken of light-induced O_2 uptake and photosynthetic inhibition of respiration when estimating photosynthetic efficiency. Figure 13 is a provisional scheme for reactions in the total O_2 exchange of a photosynthetically active cell.

A dynamic equilibrium exists between photosynthetic O_2 evolution and three antagonistic O_2 consumptive processes related in different

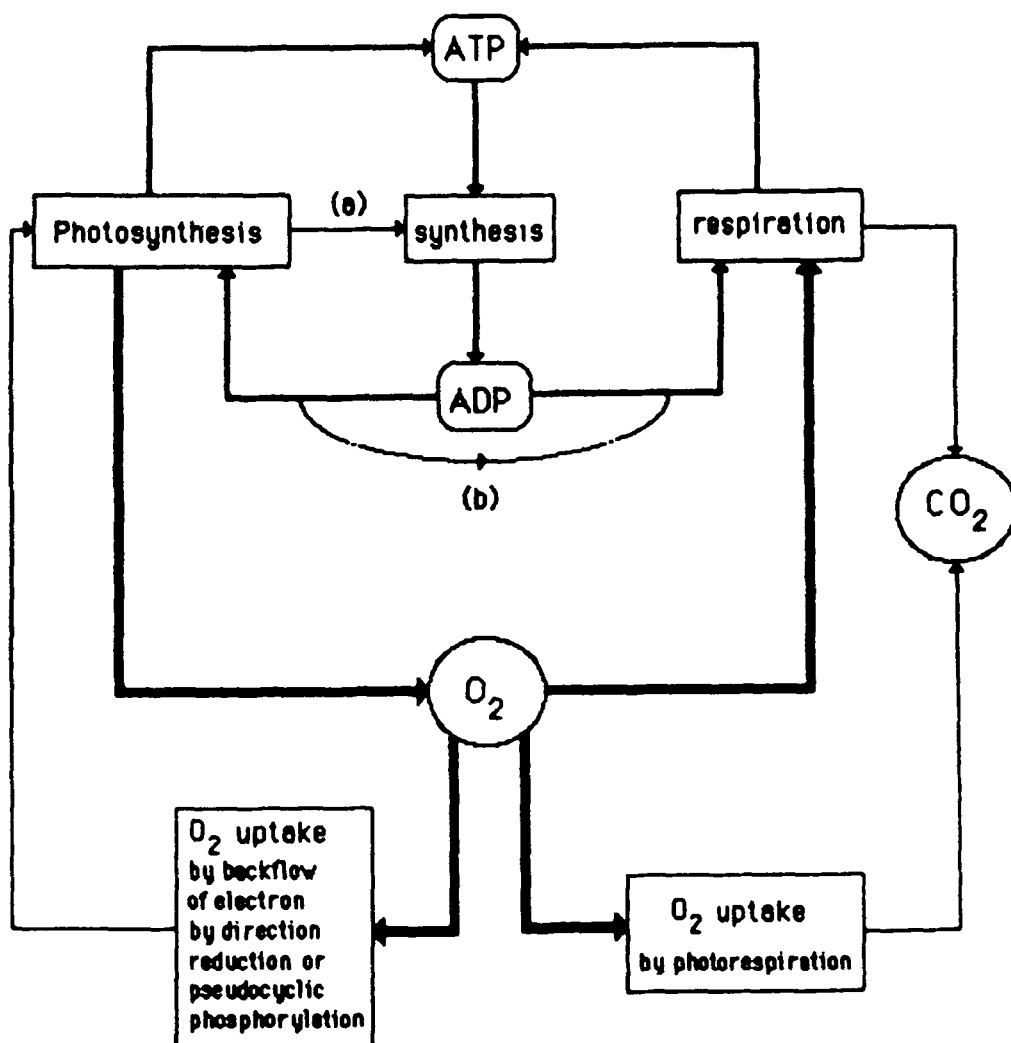


Fig. 13. Main reactions involved in the total exchange of oxygen in a photosynthetically active cell. (a) acceleration; (b) inhibition.

ways to the photosynthetic apparatus: respiratory O₂ uptake; O₂ consumption by photorespiration; and consumption by back reactions within the electron-transport chain. To evaluate the maximum photosynthetic efficiency, the dynamics of these processes should be fully understood.

The rate of photosynthesis is controlled by a large number of external and internal factors, such as oxygen toxicity and concentrations of ions. Returning to whether the minimum quantum demand is less than or exceeds the value of $8 h\nu/\text{O}_2$, excluding values less than eight is highly arbitrary. A value less than eight could mean that PSI and PSII work in parallel. The two photosystems may act in series under some conditions in which maximum photosynthetic efficiency is not required, as when some factor other than light limits photosynthesis. The observation by

Brackett and coworkers (90) that the culture conditions can cause the quantum demand of algae to vary from 6 to 13 $h\nu/O_2$ has great potential significance, but appears to have attracted little attention. Unfortunately, the essential conditions for achieving the maximum photosynthetic efficiency were not identified.

Responses of Photosynthetic Systems to Fluctuating Light

Light and dark zones in a photobiological reactor, with varying illumination to cells as they circulate, may be unavoidable. Understanding the effects of intermittent illumination is therefore crucial to the design and control of photobiological reactors. It is also important in estimating oxygen concentrations and primary productivities in natural ecosystems.

Time Scales

The relationships between photosynthetic responses and light are influenced by the time scale of change in the light field. For studying the initial steps in photosynthetic oxidation–reduction reactions, resolution on a milli- to picosecond time scale is important. Although such studies provide a mechanistic basis for understanding individual steps, they shed little light on the overall performance. Figure 14 shows the approximate time scale for various reactions comprising photosynthesis.

The shortest naturally occurring time scale of change in light fields is probably on the order to 0.1–1 s; this is the time scale of the so-called “flicker” effect (97,98), which consists of variations in light intensity resulting from the alternate focusing and defocusing of light by waves at the air–water interface. Similar effects occur in photobiological reactors because of turbulence (99). Variations in light of periods on the order of 1–100 s are created primarily by the passing of clouds across the sun.

Physiological Responses

It has long been known that the efficiency of light utilization can be markedly increased by exposing the cells to alternating periods of light and dark (3,4,100,101). A number of investigators have explored this phenomenon both experimentally and theoretically (99,102–106). Although early work indicated that flash periods as short as 10 μ s were needed to take maximum advantage of the flashing light effect, it is now clear that flash periods as long as 70 ms may still result in significant enhancements of photosynthetic efficiencies (104). Marra (105), reported increases of up to 87% in photosynthesis by simply modulating the light intensity of algal cells on a time scale ranging from minutes to hours. From the work of Seibert and Lavorel (107), increases for periods in the approximate range of 0.1–10 s can be inferred for PSII membranes. Walsh and Legendre (108) reported that fluctuating light could result in lower, equal, or higher photosynthetic capacity or efficiency than for a stable light regime, depending on the frequency of the fluctuation. It should be noted that they did not obtain their data under steady state oscillations.

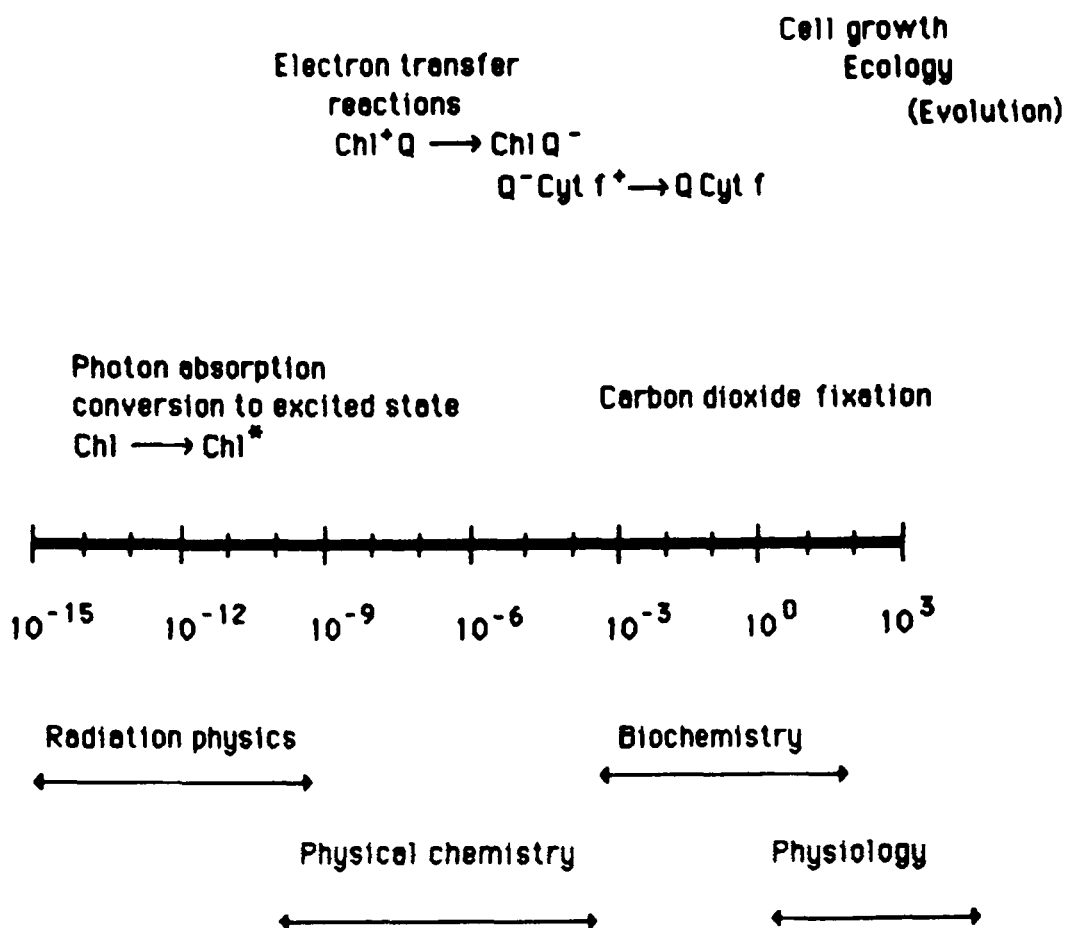


Fig. 14. Time scale for biological activity [Adapted from Kamen (154)].

Engineers commonly consider oscillating systems as reaching steady state when the signal repeats exactly. Frechette and Legendre noted an adaptation to fluctuating bright-light regimes that resulted in a daily trend of increased efficiency. It seems reasonable that the physiological mechanisms responsible for these observed photosynthetic enhancements differ substantially over the wide range of time scales involved. Regardless of the physiological mechanisms, it is clear that cell mass/unit of light energy may be enhanced by a factor of two or more by modulating or flashing the incident light on an appropriate time scale.

ENGINEERING RESEARCH

Research results will be published separately (Lam et al., in press), but some Bode diagrams and their interpretations are germane. Briefly, samples of green algae and of cyanobacteria were excited with light of

selected wavelengths. One polarizer was fixed and another rotated, so that the intensity of illumination was sinusoidal. Measurements were made of cell fluorescence, dissolved oxygen, and pH. Capillary micro-electrodes that respond in less than 0.1 s were employed because the response times of conventional electrodes are too slow.

A periodic response of dissolved oxygen is not a perfect sine wave (Fig. 15), but gain and phase shift can be estimated with reasonable accuracy. Bode diagrams for these data have smooth curves resembling those for two first-order systems in series. Typical Bode diagrams are shown in Figs. 16–18 for a green alga, a cyanobacterium, and a natural photosyn-

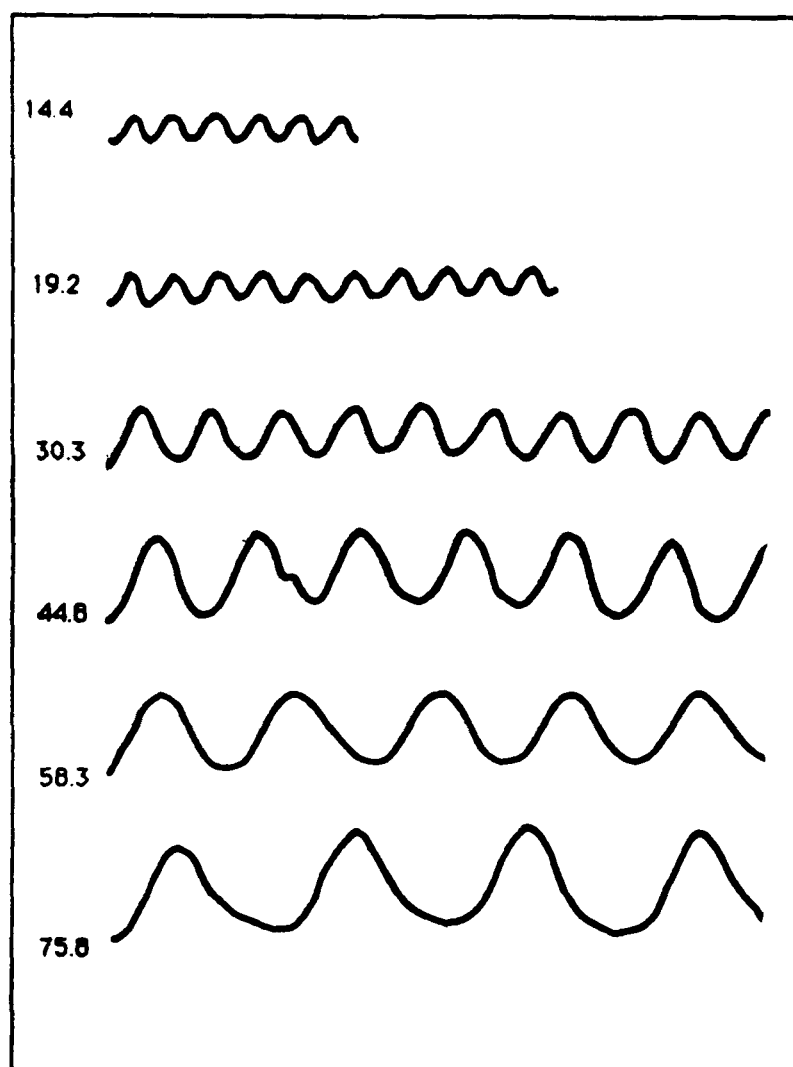


Fig. 15. Sample dissolved oxygen responses of sinusoidal forcing experiments. Number shown are periods of oscillation in seconds.

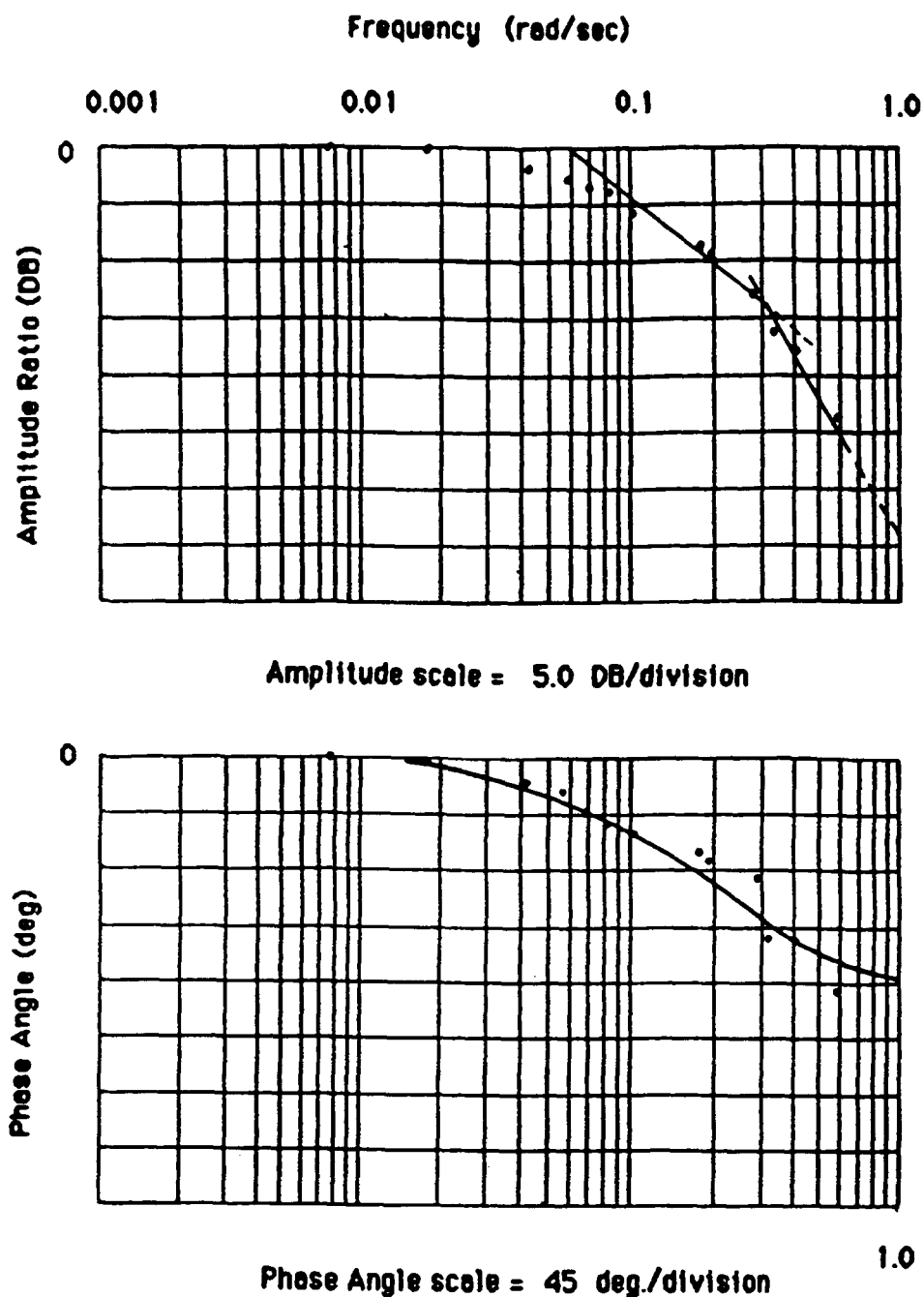


Fig. 16. Bode diagram of oxygen response for *Chlorella* film.

thetic slime taken from a shallow, unpolluted stream. These diagrams all show a phase shift approaching -180° at high frequencies, and asymptotes to the gains curves are roughly superimposable. Time constants derived from the inflections in these Bode diagrams should be no worse than $\pm 20\%$ and this is adequate for drawing some conclusions about the block diagram. Time constants are summarized in Table 4.

TABLE 4
Time Constants of Dissolved Oxygen Response to Variation of Light Intensity

Photosynthetic system	Time constant,	
	T_1	T_2
Chlorella biofilm ¹	3.3	17
Chlorella suspension ²	4.3	58
Scenedesmus biofilm ¹	3.3	15
Scenedesmus biofilm ¹	3.8	18
Anacystis suspension ²	4.5	58

¹Open system.

²Partially closed system.

Cell fluorescence depends on the ratio of NADP:NADPH and on light-trapping pigments. The block diagram for photosynthesis is redrawn in Fig. 19 to show where the signals relating to the Bode diagrams fit into the control scheme. Participation of NADP species is denoted by N, dissolved oxygen signals by D, and proton transfer steps by H. When a species is involved at more than one location in the block diagram, one reaction may dominate, or there may be severe interference, such that the time constant is compounded.

We think that the long time constant probably relates to the mass transfer of oxygen. The shorter time constant is probably fundamental to the photosynthetic system because it does not change significantly with the strains that were tested or with the experimental conditions. Further experiments are needed for more positive identification. From the order of magnitude of this time constant, the very fast reactions of photochemistry can be dismissed as being responsible. A reasonable cause of this time constant is the biochemical reactions in the cycle for fixation of carbon dioxide. Further support and discussion can be found with the details of our research.

CONCLUSION

Engineers with their emphasis on performance have a mind-set quite different from that of the usual bioscientist. Applying conventional systems analysis to photosynthesis could have made some contributions earlier, but rapidly responding microelectrodes for pH and dissolved oxygen are rather recent developments. The frequency response analysis shown here is but a first attempt to unravel some unanswered questions. However, the power of this approach is evident, and there are many obvious extensions to measuring other signals and to modifying the systems to alter the time constants.

Our provisional and oversimplified block diagram should be a guide to further experimentation. It is particularly important to determine

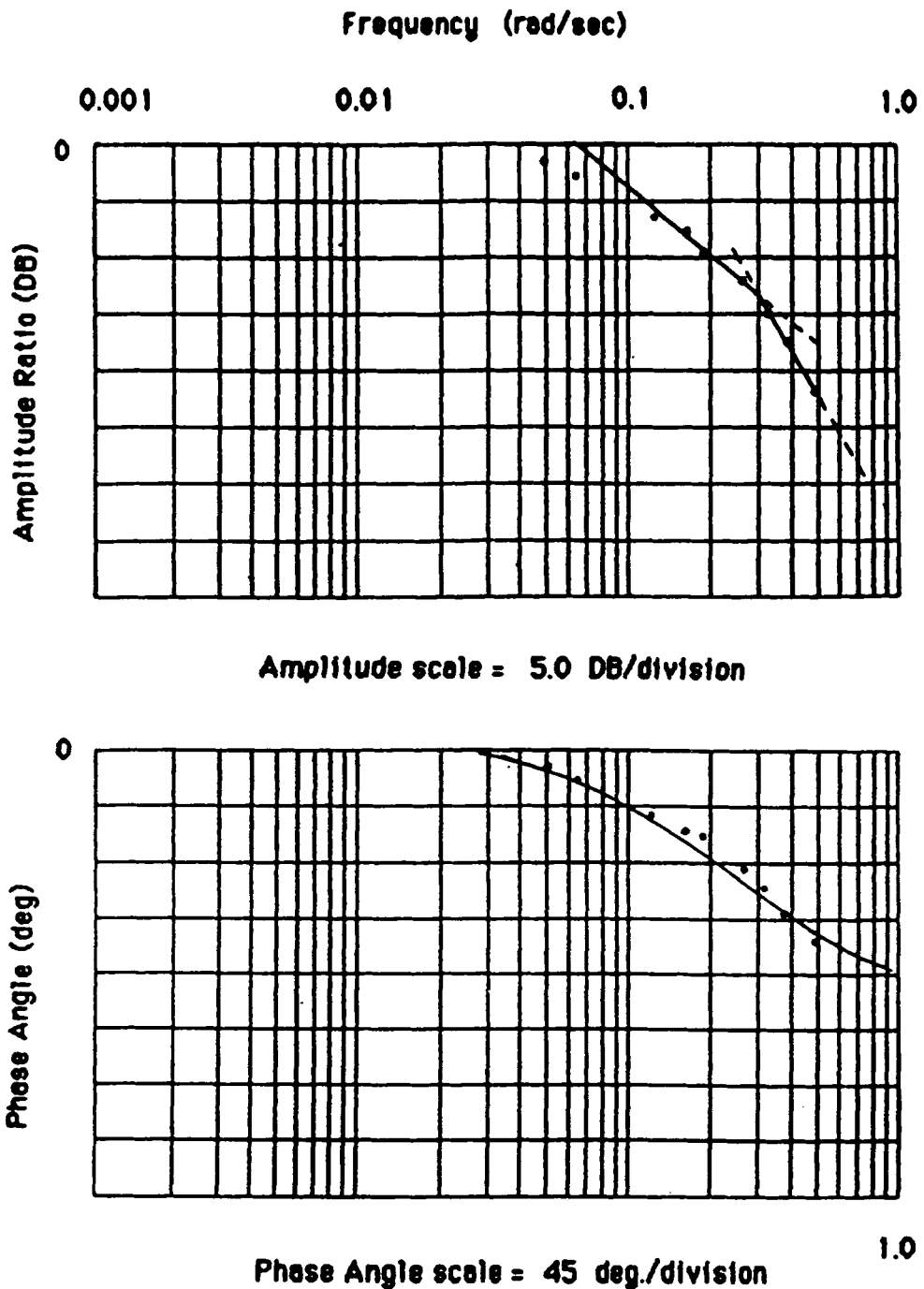


Fig. 17. Bode diagram of oxygen response for *Scenedesmus* film.

whether these time constants are roughly the same for all algae. If some organisms have different time constants, finding explanations should challenge the bioscientists. The ultimate goals of future research are a better understanding of photosynthesis, the factors that establish the

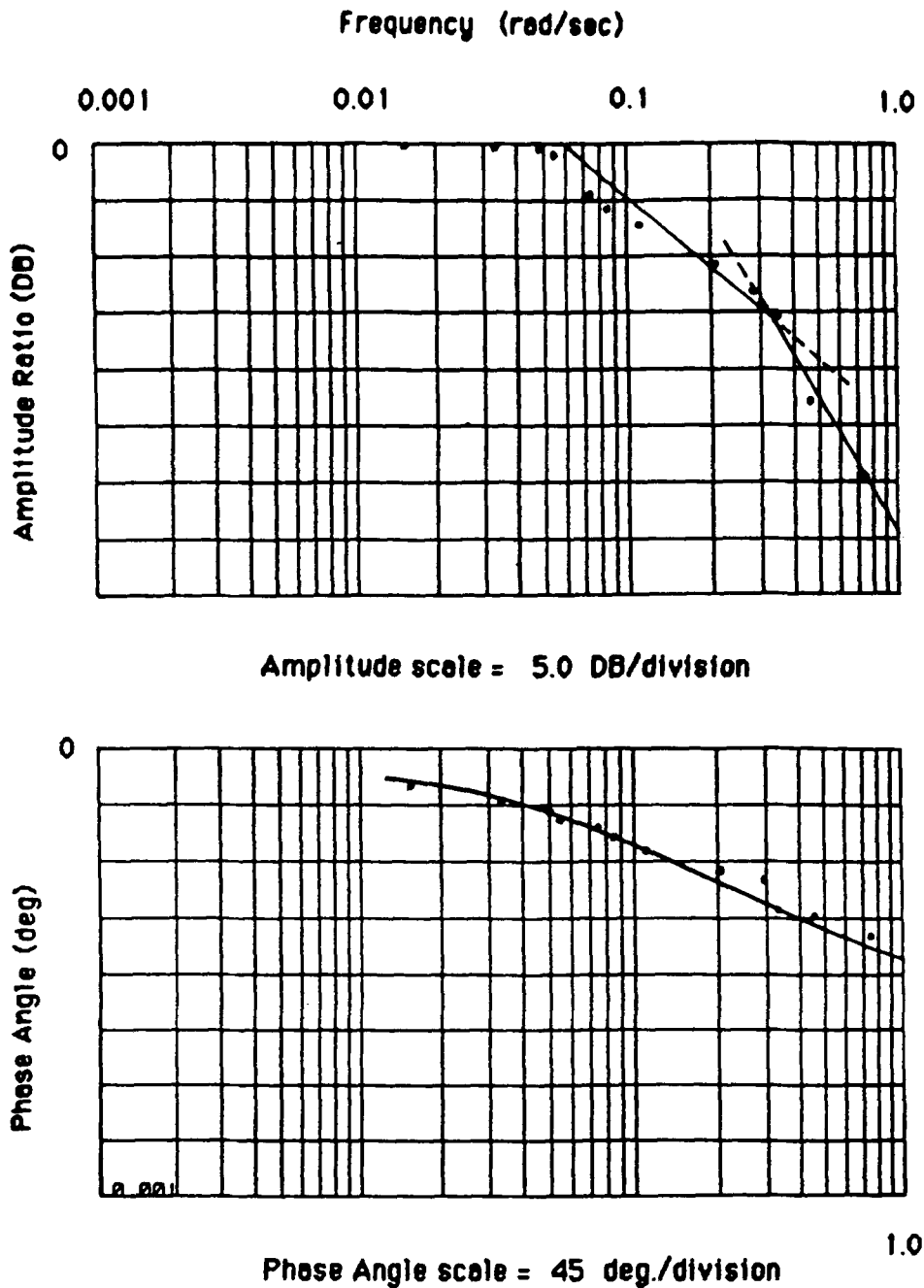


Fig. 18. Bode diagram of oxygen response for stream biofilm.

time constants, and ways to use the information for designing practical systems.

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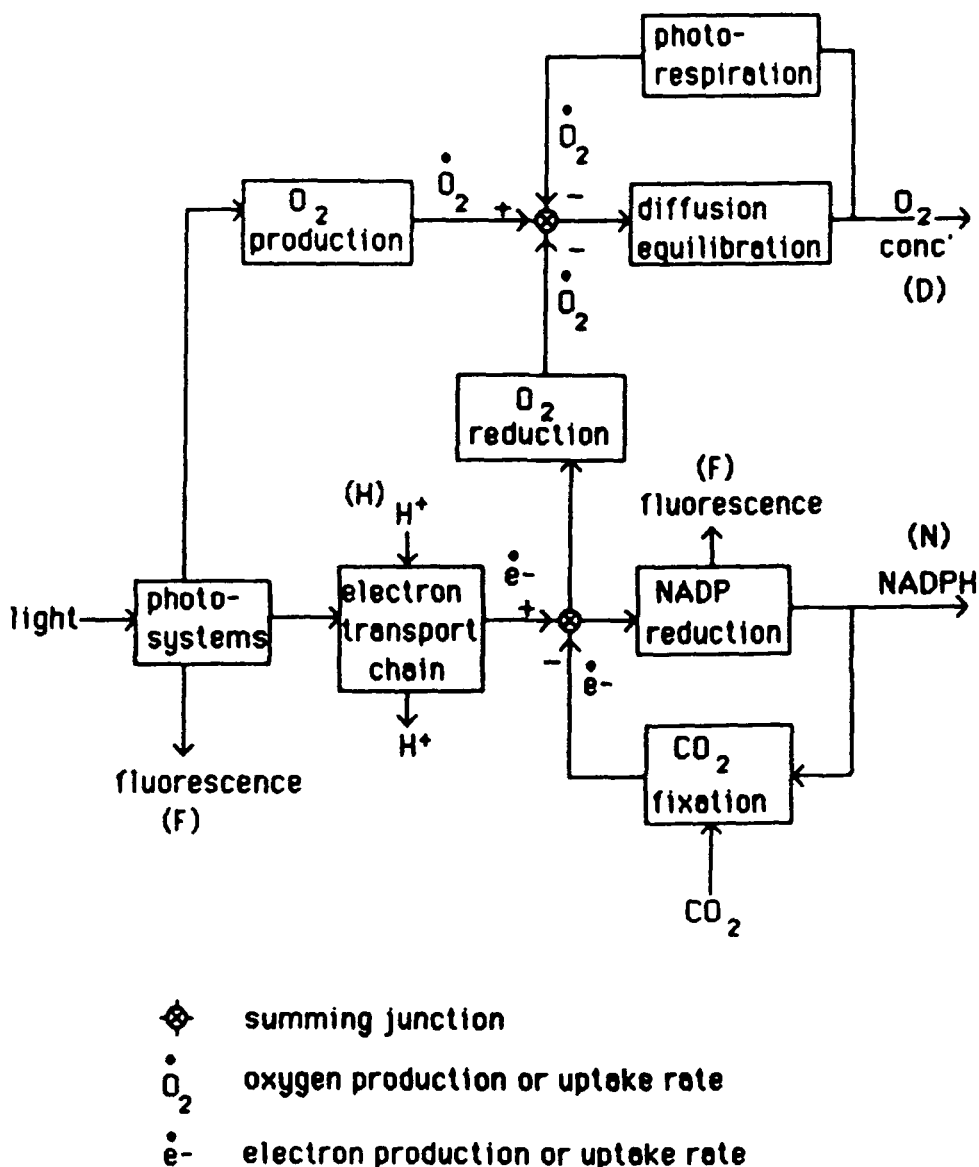


Fig. 19. Block diagram of photosynthesis.

synthesis. Dr. Lenore Clesceri helped in the culturing of algae. Dr. Michael Seibert of the Solar Energy Research Institute found errors in the original manuscript and graciously suggested improvements. However, errors that may persist are solely the responsibility of the authors. Our research was supported by the National Science Foundation through Grant No. CPE82-17676.

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