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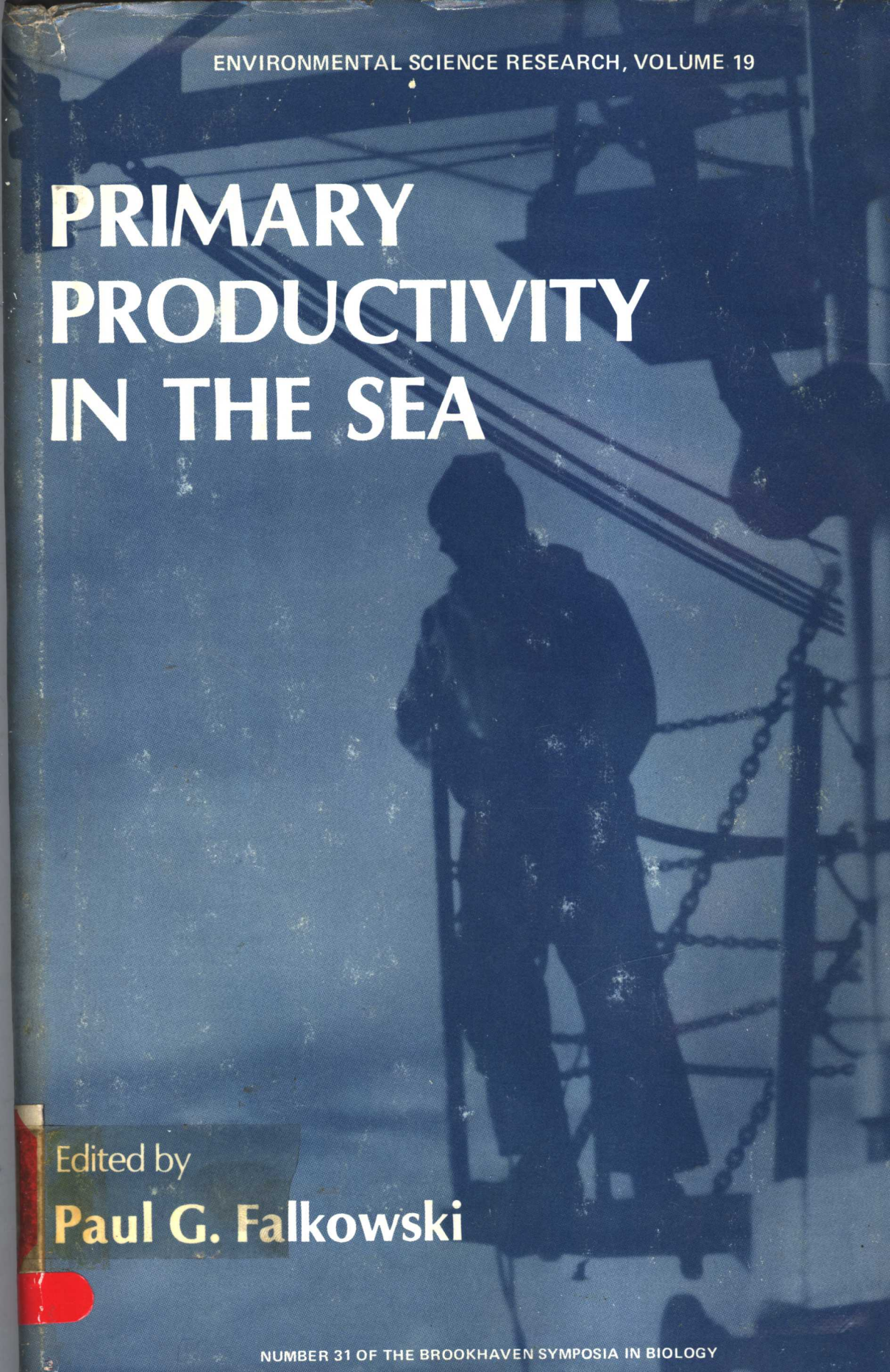
PRIMARY PRODUCTIVITY IN THE SEA



Edited by
Paul G. Falkowski

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PRIMARY PRODUCTIVITY IN THE SEA

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PREFACE

Primary productivity in the sea accounts for ~30% of the total global annual production. Holistic understanding of the factors determining marine productivity requires detailed knowledge of algal physiology and of hydrodynamics. Traditionally studies of aquatic primary productivity have been conducted by workers in two major schools: experimental laboratory biology, and empirical field ecology. Here an attempt was made to bring together people from both schools to share information and concepts; each author was charged with reviewing his field of expertise. The scope of the Symposium is broad, which we feel is its strength.

We gratefully acknowledge financial support from the Department of Energy, the United States Environmental Protection Agency, the National Oceanic and Atmospheric Administration, including the NMFS Northeast Fisheries Center and the MESA New York Bight Project. Thanks are due to Mrs. Margaret Dienes, without whose editorial skills this volume could not have been produced, and to Mrs. Helen Kondratuk as Symposium Coordinator. Finally, we wish to record our indebtedness to Dr. Alexander Hollaender for his tireless efforts and valuable advice in supporting all aspects of this Symposium.

Symposium Committee

Paul Falkowski, Chairman
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ON THE ALGAE: THOUGHTS ABOUT PHYSIOLOGY AND MEASUREMENTS OF EFFICIENCY

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Though I am honored to be a keynote speaker for this symposium, I am going to duck the responsibility of providing a review. For any thorough review of algal physiology I would not have the temerity and you would not have the patience. Thirty years ago I attempted such a review (1) because one was then needed to define a discipline emerging at the interfaces between microbiology, phycology, and plant physiology. What I can say here most usefully will be directed toward two diverse targets. First, I shall consider the consequences or corollaries of the fact that the algae (at least the microalgae) are microbes. Secondly, I shall consider the ultimate limit of productivity, the maximum efficiency of photosynthetic cell synthesis.

THE ALGAE AS MICROBES

Within the discipline of microbiology the algae have been treated as poor relations of the other microbes. Recent recognition that the blue-green algae qualify as cyanobacteria seems to have made them more fashionable subjects for microbiologists. I find that my botanical colleagues chauvanistically view the development with alarm. I want no part of this territorial dispute. All that needs to be said is that converting the blue-greens into bacteria does not relieve them of their responsibilities for behaving like algae.

Actually, the eucaryotic algae have a long history of being treated as microbes. In 1890 the legendary microbiologist, Beijerinck, published a paper on his culture experiments (2). In a color plate he displayed some of his microscopic observations and

culture experiments. He found that Scenedesmus acutus liquefied gelatin. He observed that an alga, identified as Chlorosphaera limicola, isolated from a Hydra, accumulated starch grains when grown in the dark on sucrose. And the color plate showed that, though grown in the dark, the cells were green.

Now it is not true that I was once a colleague of Beijerinck. But it is true that his casual observation provided my own entree to the algae. In the literature of plant physiology, dedicated to higher plants, it was anomalous that algae should make chlorophyll in the dark. An obvious question was whether such chlorophyll was photosynthetically competent. The question required metabolic-type experiments and for them I turned to the work of Otto Warburg. In 1919 Warburg had introduced to the study of photosynthesis a new method of measuring gas exchange and a new experimental organism, Chlorella (3). It was reputed that his Chlorella strain had been isolated from Berlin tap water.

When it came to questions about metabolic or nutritional physiology, the biochemist Warburg provided a much better model for experimentation than did the current phycologists and microbiologists. Microbiologists did not then understand the light and CO_2 problems. Aerobic bacteria live very well at the O_2 level provided by diffusion through cotton plugs from 21% O_2 in air. For analagous autotrophic growth of an alga the problem of getting CO_2 from air is 600 times more difficult. The problem is that it takes about 1 cc of pure CO_2 to provide the carbon needed to produce 1 mg dry weight of an alga.

The early phycologists were preoccupied with the problems of just getting algae into culture. After that their inclinations led them to concerns for details of life history, taxonomy, and ecology. They tended to develop media designed to mimic natural habitats (4). This was fine for some purposes but a complete misconception for others. The trouble was that for questions about physiology we usually needed cell concentrations much higher than those of natural waters.

Otto Warburg cut through all these problems. He used a Knop's solution of remarkably high salt concentrations, still used sometimes today in the fine tradition of ancestor worship which governs algal media. He provided aeration with 4% CO_2 in air. He replaced the classical "light of a north window" with artificial light. And from among the algae he chose the weed, Chlorella.

And so it came to pass that algae like Chlorella and Scenedesmus were adopted by biochemists and plant physiologists as standard organisms for study of photosynthesis. The consequences of microbial character got lost. What I take to be the essential feature of a microbe is this: an organism small in size with a

minimum of skeletal crud, rich in protein as cellular machinery, and with potential for an intrinsically high metabolic rate.

The microbial world has its own distinctive features which go beyond the fact that its members are small in stature. For the animal world it is fashionable nowadays to talk about "Foraging and Reproductive Strategies." I think we could sound equally erudite by discussing such strategies for any particular alga. What would we say in general terms about the strategies of microbes? I think their game plans must be to survive by frugal use of resources when times are bad (nutrients are scarce) and to be ready to accelerate their metabolism and make new cells rapidly when times are good. A special case arises when only one nutrient is limiting. What then? I suppose it would be sensible to go on synthesizing all possible cell constituents, diluting out those that require the limiting nutrient. Then, when that nutrient is again available, the limiting parts of cell machinery can be rapidly synthesized. There are real-life illustrations of this kind of behavior. And in the particular case of algae, for which light is the energy nutrient, the challenges of good times and bad times alternate in a daily cycle.

I shall cite some support for my thesis that the algae should be viewed as microbes. There is an interesting anomaly simply in the fact that the alga Chlorella was selected for study of photosynthesis. I like anomalies. So for this one I offer a favorite scenario as follows.

Photosynthesis has long been regarded as a synthesis of carbohydrate. The early evidence was mainly that a CO_2/O_2 exchange ratio close to 1.0 was obtained from long-time measurements on higher plants. Actually, no other value could have been obtained and it had little relevance to any biochemical details of photosynthesis per se. A higher plant is mostly cellulose (skeletal crud). Its CO_2/O_2 exchange ratio necessarily reflects its predominantly carbohydrate metabolism. If algae rather than higher plants had been used in exactly analagous experiments, we would have decided that photosynthesis must be mostly a synthesis of protein. Hence the anomaly: for study of photosynthesis, a process firmly believed to be a synthesis of carbohydrate, Otto Warburg selected a predominantly protein synthesizing microbe, the alga Chlorella.

Actually, Chlorella turned out to be an excellent experimental organism, mainly because (like other algae) it could be managed to perform under different metabolic conditions. Warburg trained his cells by a period of starvation or very low light, after which they did have a predominantly carbohydrate synthesis. Another condition of high carbohydrate synthesis occurs when algae are transferred from low to high light. The

suddenly increased rate of photosynthesis leads to an overflow metabolism into storage carbohydrate. Overflow metabolism is a feature found widely among the microbes and especially evident in the molds where it results in excretion of various organic acids. In most algae excretion is minimal and overflow product results in accumulation of storage material.

An extreme case of metabolic variability is that demonstrated by Spoehr and Milner (5). Under nitrogen starvation Chlorella showed a remarkable accumulation of lipid. In the context of the physiology of higher plants this was so remarkable that a patent on the process was applied for -- and, in fact, awarded. Evidently the patent office considered the finding something new to the art as judged by the literature of plant physiology. However, as judged by the art of microbiology, the finding was just another illustration of an old principle -- in fact so old as to be traceable back to Beijerinck.

What I have said somewhat redundantly can be summarized more succinctly. The essential principle is that the algae are microbes, powered by a photosynthetic metabolism. As microbes they are mostly cell machinery, which goes about its business in synthesis of new cell machinery. In that endeavor they are highly adaptive. Response to an environmental factor depends upon past history for that factor. This has been documented by many illustrations for temperature, for nitrogen, for light, and now more recently for CO_2 (6). There is no fixed or static machinery. This is the particular challenge of algal physiology.

EFFICIENCY OF ALGAL CELL SYNTHESIS

The limiting or maximum efficiency of algal cell synthesis is not a conceptually exciting problem. The difficulty is that the answer is just a number. And everything hinges on the validity of that number, i.e., on the nitty-gritty experimental details of the way it is measured. However, the maximum efficiency is important as an ideal or limiting value on which real-life light conversion efficiency, and hence productivity, depends. Further, it just turns out that I have some pertinent measurements which have not been properly published.

Kok's Measurements. What I regard as the best published data on efficiency are those of Kok (7), subsequently extended by Oorschot (8). Kok had had long experience with the problem of measuring the quantum yield of photosynthesis. For that problem conditions are arranged to obtain a synthesis of carbohydrate, the cheapest possible product. Further, there is a complicating and not entirely resolved question of just how to handle a respiration correction. Any study of photosynthesis per se seeks to isolate

it as a partial process separated out from all other metabolic processes. Kok recognized that in utilization of light for growth the efficiency in synthesis of complete algal cells is a different question. For efficiency of total cell synthesis the cost of respiration losses is included, the product is somewhat more reduced than carbohydrate, and the product includes a lot of protein and other biochemically expensive components.

Kok used the methodology of manometry, but with oversized Warburg vessels containing 100 cc of a culture of Chlorella vulgaris and presenting 64 cm^2 of surface to light from a sodium lamp. Experiments typically lasted 4 days with a 6X to 10X increase in dry weight. During most of that time the vessels were aerated with 3% CO_2 in air. At intervals light absorptances were measured in an Ulbricht sphere and vessels then were closed for short periods of manometric measurement of rate of O_2 exchange. The periodic measurements allowed integration with time for the total experiment. At termination the cells were subjected to analysis for C, H, N, and ash. On one representative sample bomb calorimetry gave a heat of combustion (ΔH) of 5.77 kcal/g for total cells containing 6.2% ash. From calculation of the O_2 required for combustion Kok deduced an O_2 equivalent of 112 kcal/mol which he used on all other samples in evaluating ΔH from cell analysis.

Kok wrote synoptic equations (cf. below) for cell synthesis from CO_2, H_2O, and NO_3^- and found that his measured O_2 evolution was in good agreement with that predicted from cell synthesis. (No estimate or allowance was made for synthesis of organic material not recovered as cells). He estimated efficiency as the calculated ΔH of harvested cells divided by calories of 589-nm light absorbed. His 31 determinations, plotted as a bar graph for efficiencies in 3% increments, cover a range of values from 6 to 24%. However, he included experiments purposely designed to test effects of varied conditions (e.g., N deficiency). His own judgement seems to be a value of 20%.

Oorschot (8) extended Kok's measurements, mostly with concern for effects of higher intensities and other varied conditions. He did inquire about effects of nitrogen source, NH_4^+ or NO_3^-, but did not really document his conclusion of a 30% higher efficiency with NH_4^+.

Our Experimental Design. I now consider some of our data on efficiency of cell production of Chlorella pyrenoidosa (10). Our experimental design was in some respects better, in others poorer, than that of Kok. We used a steady-state culture (turbidostat mode) containing 110 cc of cell suspension about 3 cm thick and held in a water bath at 25°C (Fig. 1). Constant volume of suspension (A) was maintained by overflow and accumulation of effluent (E) in an aerated reservoir. At daily intervals effluent was withdrawn to a reference mark, its volume determined, and its cells centrifuged, washed, dried by lyophilization, weighed, and saved for analysis.

A metal bellows pump recirculated gas between the algal chamber and a 9-liter ballast bottle via metal and glass tubing and a few short butyl rubber connections. The CO_2 and O_2 volumes (STP) were obtained from Haldane absorption analysis (20 cc samples), temperatures, and pressures. For 7 samplings taken during an 11-day dry run, starting with 6% CO_2 and 10% O_2, and correcting for sampling volumes, the contained gas volumes were 577 $\pm$ 2 (SD) for CO_2 and 914 $\pm$ 4 for O_2.

The optical arrangement (Fig. 3) delivered a reasonably collimated beam from a mercury lamp (L) with filtering by 15 cm of water (W) and a Corning cut-on filter (F) and attenuation by copper screens (S). A fraction of the beam, diverted by a glass plate onto selenium cell P_2, provided comparison with transmitted light seen by photocell P_1. Photocell balance was set to control dilution so as to maintain desired constant density of cell suspension. The front face of the algal chamber was protected by a diaphragm slightly smaller than the cross section of algal suspension (Fig. 2). The back and sides of the chamber were covered by a stainless steel cover (reflectance about 0.6) with a 3.2-cm hole for P_2.

Incident irradiance on the suspension was measured at the beginning and end of each experiment by a Moll large surface thermopile. Readings with and without a Schott RG8 filter cutting on at 700 nm allowed subtraction of a small infrared component. Because the thermopile opening of 5.3 cm^2 was smaller than the diaphragm area, the entire diaphragm area was scanned with a photocell behind a 5 x 5-mm aperture. The scan allowed correction for small non-uniformity of the beam and estimate of total energy through the diaphragm.

Choice of Irradiance. Before beginning efficiency measurements we sought to ask about desirable choice of irradiance and specific growth rate. For this purpose we used dilute cell suspensions controlled at constant density (absorptance < 25%) and measured rate of dilution compared to 110-cc constant chamber volume. Resulting specific growth rates vs irradiance are shown

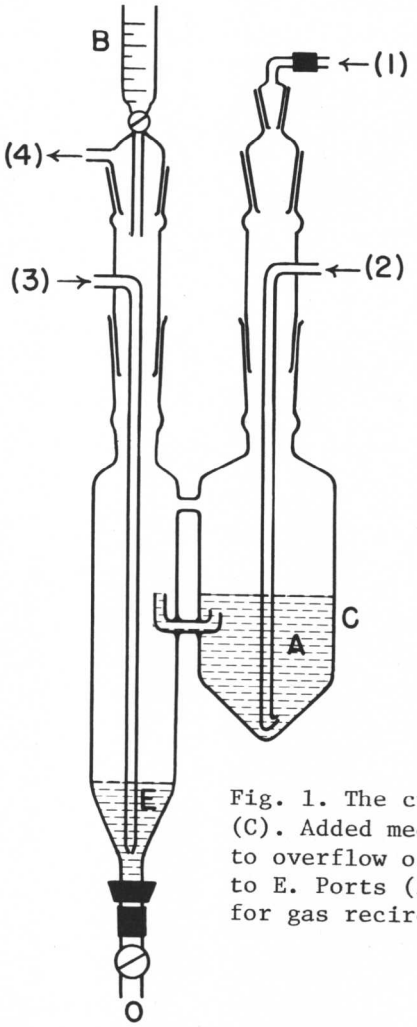

Fig. 1. The culture chamber (C). Added medium via (1) led to overflow of suspension A to E. Ports (2), (3), (4) were for gas recirculation.

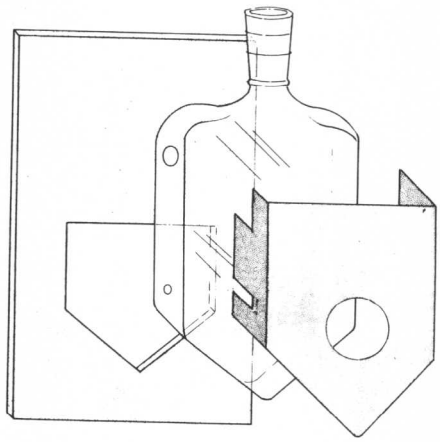

Fig. 2. The algal chamber shown with diaphragm and reflecting shield.

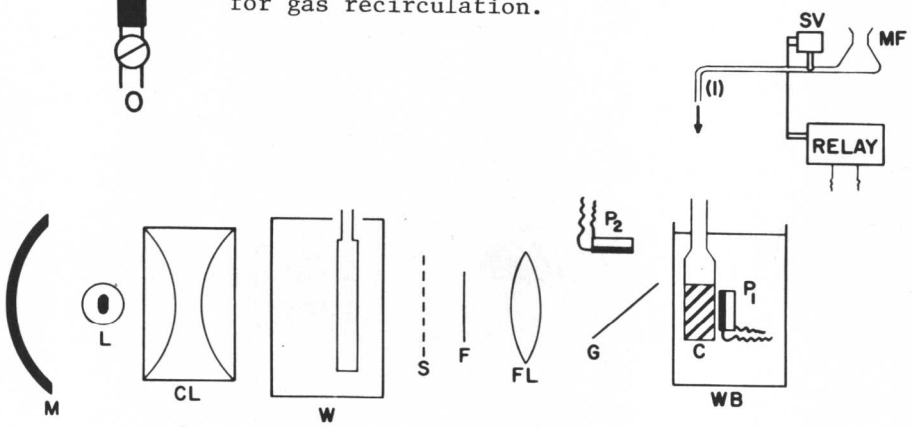

Fig. 3. The optical system with parts self-evident or described in text.

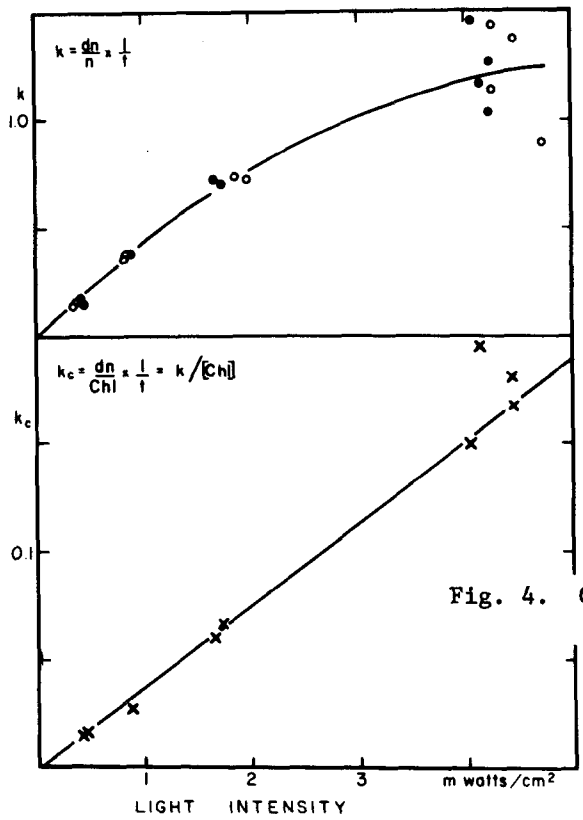

Fig. 4. Growth rate vs irradiance for a thin suspension.

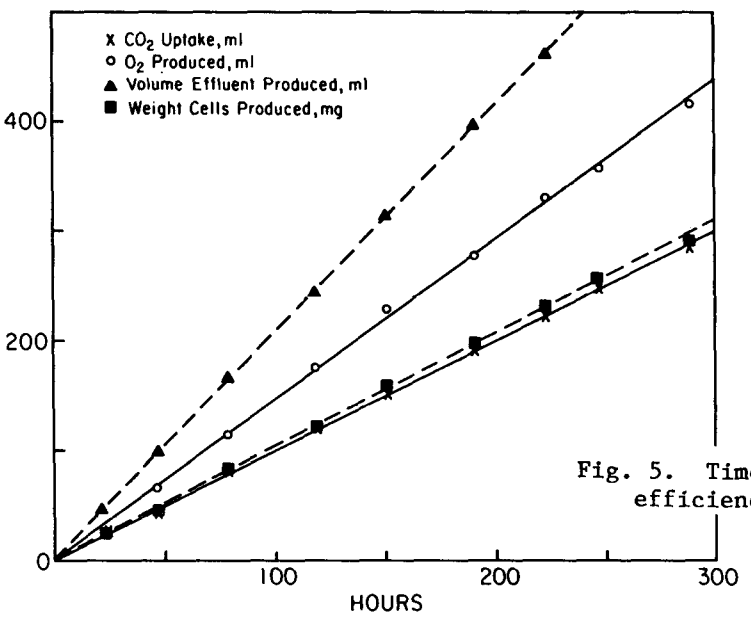

Fig. 5. Time course of an efficiency experiment.

in the upper part of Fig. 4. The data are not good enough to determine the light intensity intercept for zero growth (here drawn probably too close to zero irradiance). We wondered about the nonlinearity, which also has shown up in other such plots. In one series of experiments (solid points) we also measured cell chlorophyll concentrations. This allowed calculation of a rate of cell production per unit chlorophyll, which is plotted as the lower curve. I conclude that nonlinearity in the upper curve is caused by a cell chlorophyll content decreasing with increasing light intensity. And I take linearity of the lower curve to mean that specific growth rate is actually close to linear vs absorbed irradiance, at least up to about half of its maximum value. As a practical matter, incident irradiances in the experiments to follow were about 2 mW/cm^2 or less.

Experimental Protocol. For experiments designed to estimate efficiencies we used more dense cell suspensions (about 0.5 mg/cc and absorptance 93%). Each experiment was started and brought to steady state under several days flow-through of 5% CO_2 in air. Contained gas was then adjusted to about 5% CO_2 and 10% O_2 and the system was closed. Thereafter dry weight of cells produced and contained volumes of CO_2 and O_2 were monitored by daily sampling. The monotonous course of a typical experiment is shown in Fig. 5.

Estimates of actual algal gas exchange in cc STP required a number of small corrections for gas volumes: (a) dissolved in the algal suspension, (b) gained in influent medium, (c) lost in effluent suspension, and (d) lost in gas samples taken for analysis. Increasing pH in the algal suspension (6.4 to about 7.2, due to decreasing CO_2 concentration) required correction for changing amount of CO_2 bound as bicarbonate. We also estimated a correction for respiration of cells which accumulated linearly in the overflow before each daily sampling. At the end of each of several experiments we transferred samples directly from A to Warburg vessels and measured gas exchange for the following 24-hour period. Total O_2 uptakes were about 45 $\mu l/mg$-day at RQ = 1.0. The correction factors computed from these measurements were small, scattered around values of 1.028 for cells, 1.020 for CO_2 and O_2, and were applied uniformly in all experiments. (Note that these are not "respiration corrections" in the conventional sense. They merely correct for the experimental imperfection that non-working cell suspension of the overflow is held for some time within the system.)

We measured efficiencies in a total of 13 experiments using various combinations of Hg lines, the 578 Hg line plus 644 Cd line, and filtered tungsten light. There were no unexpected wavelength effects. I will report here the results of all five experiments with 578-nm light chosen because they (a) were reasonably representative, (b) allowed simplest calculation of

quantum yields, and (c) are at a wavelength close to the weighted average of quantum size for the usable portion of the sunlight spectrum. Further, at this wavelength we had three experiments with nitrate and two experiments with urea as a nitrogen source.

Cell Analyses. We estimated heat of combusion of the cells in the same fashion used by Kok: calculation of the oxygen required for combustion. Our cells were purposely lyophilized because of previous experience with variable ash contents of cells dried from a slurry at 105°C. We suspected that some salts leached out of these cells and were not uniformly recovered in the hard and glassy residue. Lyophilization apparently solved this problem but introduced another: the powdery product was hygroscopic. A first series of analyses was suspect because the analyst observed but failed to guard against the hygroscopic problem. In a second series of analyses (from a new analyst) samples from 5 experiments for cells grown on nitrate were, in %: 51.67 C, 7.28 H, 8.20 N, 5.75 ash. A sample from one of the experiments with urea gave a composition, in %: 51.89 C, 7.33 H, 9.07 N, 5.38 ash. I consider the difference in composition (higher % N, lower % O for cells grown on urea) as significant because it had been seen also in the first series of analyses. From the cell analyses (taking O by difference) there may be derived the cell formulas, the heats of combustion, the synoptic equations for cell syntheses, and the equivalencies shown in Table I.

Efficiencies. The essential data of 5 experiments are detailed in Table II. Efficiencies are calculated in terms of cells and in terms of O_2 produced. Efficiency for O_2 is calculated, e.g., in Experiment 71, as 56.9 cc/day x 4.37 cal/cc x 1/1319 cal input/day = 0.189. For cell production we generally observed recoveries (cell C/CO_2 C) somewhat less than expected and inferred some loss of cells in the lyophilization drying procedure. Hence I have corrected cell production to 100% recovery, e.g., in Experiment 71, as 36.8 mg/day x 1/0.923 x 5.82 cal/mg x 1/1319 cal input/day = 0.176. In terms of actually recoverable cells this is an overcorrection. Further, it really means efficiency of synthesis for cell C as measured by CO_2 uptake. However, it is chosen to best represent total productivity and it reasonably matches efficiency for O_2 production.

The comparison of efficiencies for cell production from urea and from nitrate at least qualitatively confirms expectations and the less well documented finding of Oorschot (8). Urea represents a practically useful way to provide ammonia without the difficulty of severe pH decrease accompanying ammonia uptake. It does include some danger of extraneous urea hydrolysis affecting CO_2; however, we saw no indication of this either in pH changes or in observed AQ.

Table I. Calculations from Cell Analyses

For nitrate-grown cells $C_{7.35}H_{11.63}O_{2.73}N$

Formula Weight: 161.1 ash free; 170.9 cells (incl. ash)

*Calculated ΔH: 994.6 kcal/mol cells; 5.82 kcal/g cells

Cell Production:

$$HNO_3 + 7.35\ CO_2 + 5.32\ H_2O \rightarrow C_{7.35}H_{11.63}O_{2.73}N + 10.15\ O_2$$

O_2 value = 994.6/10.15 = 98.00 kcal/mol O_2

= 4.37 cal/cc O_2

AQ = 7.35/10.15 = 0.724

Equivalence Cells/CO_2 = 170.9g/7.35 mols CO_2

= 1.038 g cells/cc CO_2

For urea-grown cells $C_{6.67}H_{11.23}O_{2.54}N$

Formula Weight: 146.1 ash free; 154.4 cells (incl. ash)

*Calculated ΔH: 919.5 kcal/mol; 5.95 kcal/g cells

Cell Production:

$$0.5\ N_2H_4CO + 6.17\ CO_2 + 4.62\ H_2O \rightarrow C_{6.67}H_{11.23}O_{2.54}N + 7.46\ O_2$$

O_2 value = 919.5/7.46 = 123.3 kcal/mol O_2

= 5.50 cal/cc O_2

#AQ = 6.17/7.46 = 0.827

#Equivalence cells/CO_2 = 154.4 g/ 6.17 mols CO_2

= 1.117 g cells/cc CO_2

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* Calculated for combustion to CO_2, H_2O and N_2 with oxygen equivalent of 112 kcal/mol.

For gas exchange only. Actual total CO_2 reduced includes 0.5 mol from urea.