SIMULTANEOUS OBSERVATION OF OXYGEN AND CARBON DIOXIDE EXCHANGE DURING NON-STEADY STATE PHOTOSYNTHESIS

by

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In a previous article[1] we reported the observation of high rates of gas exchange in *Chlorella* following changes in light intensity. The results, obtained mainly by means of volumetric methods, pointed to the occurrence of large variations of $\gamma(CO_2/O_2)$ during such transitions.

In connection with current investigations on the time course of photosynthesis under anaerobic conditions[8], techniques were available for the simultaneous and independent measurement of oxygen and carbon dioxide exchange. It was therefore attractive to collect a few additional observations on the above subject.

TECHNIQUES

Oxygen determination. We used an adaptation of the method of Tödt (DAMASCHKE *et al.*[3]) for the polarographic oxygen measurement. Instead of the iron electrode, we used an external EMF source (1 volt) and a large mercury pool ($\pm$ 2 cm^2) as reference electrode. This was connected to the reaction vessel by means of a KCl-agar bridge of wide cross-section to minimize the electrical resistance in the circuit. The linearity of the instrument was checked by saturating the suspension liquid with various mixtures of N_2 and O_2. The polarograph proved rather inconstant at high oxygen pressures (*cf.* Fig. 2). Moreover, under these conditions, long illumination periods should be avoided, since the oxygen should not be allowed to appear as gas bubbles. Therefore, we made most measurements at concentrations of less than 5% of oxygen or after anaerobiosis.

Measurement of carbon dioxide. The algae were suspended in 0.05 *N* KCl solution. In some experiments, 0.001 *N* $NaHCO_3$ was added. Carbon dioxide exchange was observed by measuring pH-changes with the glass electrode, a method first introduced for the study of photosynthesis by BLINKS AND SKOW[4]. Polarographic current flowing through the algal suspension may interfere with the potentiometric pH-measurements, as it creates a potential gradient in the liquid. This difficulty could be avoided completely by placing the glass electrode and the calomel reference electrode at positions in the vessel which are equipotential with respect to the polarographic current. To this end, they were placed at right angles to the polarograph electrodes (Fig. 1).

Partial pressures of CO_2 in the suspension, ranging from 0.1% to 20% were used. Most measurements were made in the range of 0.1 to 2%, since the sensitivity increases with decreasing CO_2 pressure.

A microburette filled with a CO_2 solution of known concentration was connected to the vessel by means of a fine capillary. Before or after a period of photosynthesis, known amounts of carbon dioxide solution were added stepwise to the liquid, and the pH-changes were recorded (*cf.* Fig. 2). In this way, the pH-changes during the experiment could be related in a simple

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way to the volumes of CO_2 involved. This enabled us to avoid complications due to the incompletely known ionic composition of the liquid, such as were encountered by ROSENBERG[5]. Since the change in pH was small in most of our experiments, the response of pH was usually almost linearly related to the CO_2 concentration*.

Recording equipment. Changes in the polarographic current and the EMF of the glass electrode were recorded photographically, on a drum camera, with the apparatus described earlier[6]. A rotating switch first connected a galvanometer (response time 0.2 sec) to the polarograph circuit for 1.25 sec, then to the pH-electrometer, and was finally short-circuited to give a zero line. Each cycle, therefore, lasted 3.75 sec. The sensitivity of both polarographic and pH-measurements could be varied independently. In most experiments, a full scale deflection corresponded to about one pH-unit, and roughly to 5% O_2.

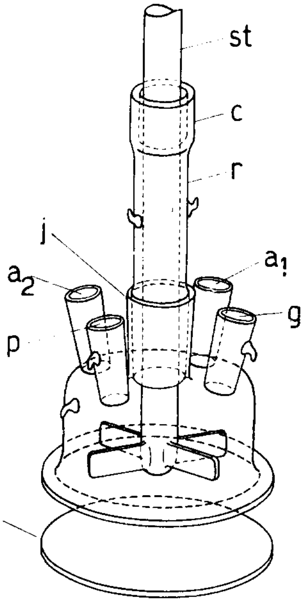

Fig. 1. Reaction vessel. *St.*: stirrer fitting into a cylindrical ground-glass bearing *r* (Jenaer "Rührwelle"). Cup *c* contains water acting as lubricant and seal. The stirrer bearing was connected to the vessel with a ground glass joint *j*. a_1: Agar bridge to polarographic reference electrode. *g*: Glass electrode, *p*: platinum microelectrode for polarography. The joint a_2 takes a cone with four capillary tubes, two of which are connected to microburettes, the third serves as an agar bridge for the glass electrode circuit, and the remaining serves as an overflow. The vessel has a flat-ground flange at the bottom, against which a circular glass plate *b* can be clamped by means of brass rings and rubber washers.

RESULTS

The behaviour of both O_2 and CO_2 exchange during transitions proved quite variable and seems to depend upon factors such as the previous history of the algal material used.

We have not attempted to make a detailed study of such conditions, and shall restrict ourselves to giving a few examples of the phenomena that can be observed. Since most of our measurements were made at low concentrations of both carbon dioxide and oxygen, the present results cannot be compared directly with those reported in the previous article[1].

Observations at high oxygen pressures

The first illumination in Fig. 2, performed at 20% O_2, followed a dark period of about half an hour. The response of both the O_2 and CO_2 exchanges showed a slight induction followed by a temporarily increased rate. Upon darkening and during the following cycles, oxygen responds immediately. On the other hand, the response of carbon dioxide upon darkening and illumination is more or less delayed as usual. In many cases the overshoot in the dark is followed by an increased rate of CO_2-production. In the experiment of Fig. 2 this high rate lasted from the 20th to the 75th second approximately. Throughout the exposures, γ appears to vary considerably. During the second dark period of experiment of Fig. 2, it oscillated between 0 and —5.3. This variation of γ is sometimes even more pronounced.

The experiment of Fig. 3 was performed at an oxygen concentration of about 5%, and low CO_2 ($\sim$2%) pressure. During a series of intermittent exposures, the rate

* It has not yet been demonstrated that the pH-changes are due to CO_2 exchange only. Especially under anaerobic conditions, there is a possibility that acids other than carbonic, contribute. We realise that this point requires further study.

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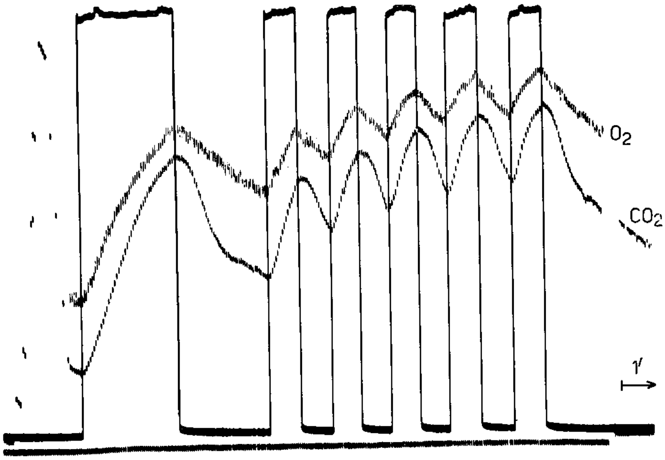

Fig. 2. Simultaneous recordings of polarograph and potentiometer. To the left: calibration points for CO_2 concentration. Solid black trace indicates light intensity (bottom: dark, top: light). A separate galvanometer actuated by a photronic cell yielded this trace.

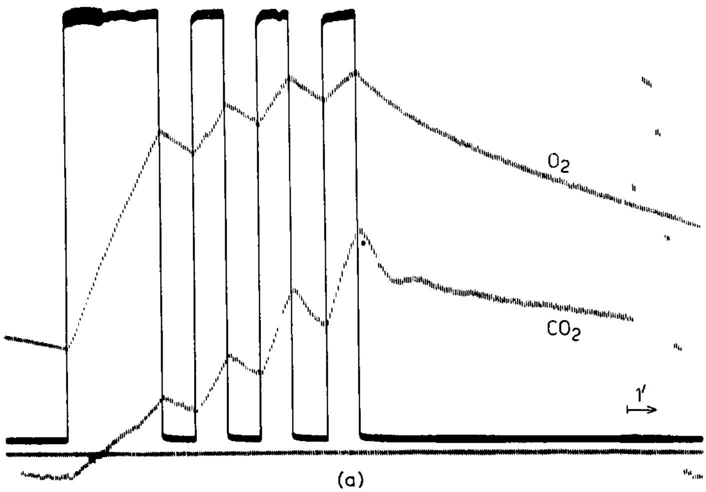

Fig. 3a.

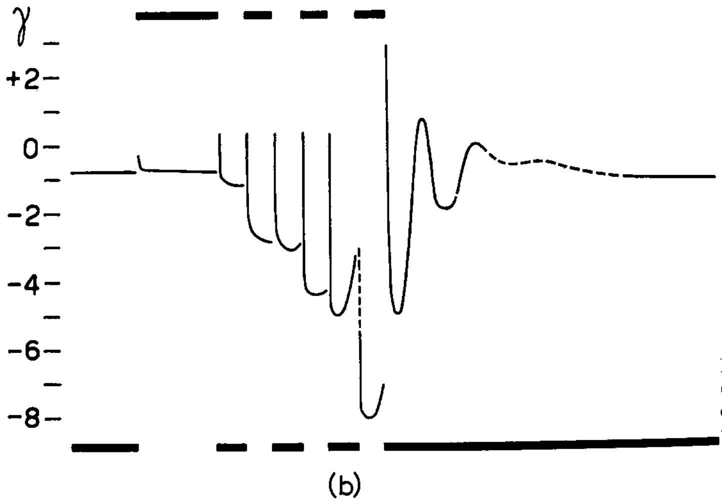

Fig. 3. Time course of (a) oxygen and carbon dioxide, (b) photosynthetic quotient γ, calculated from (a).

of oxygen exchange, in both the light and the dark periods, decreases. But the opposite effect is observed for the CO_2 exchange. Therefore the γ values show increasing deviations from unity (down to —8, *cf.* Fig. 3b). The delayed CO_2 response moreover is responsible for a momentary change of γ to positive values immediately after each change in the light intensity, regardless of in which direction.

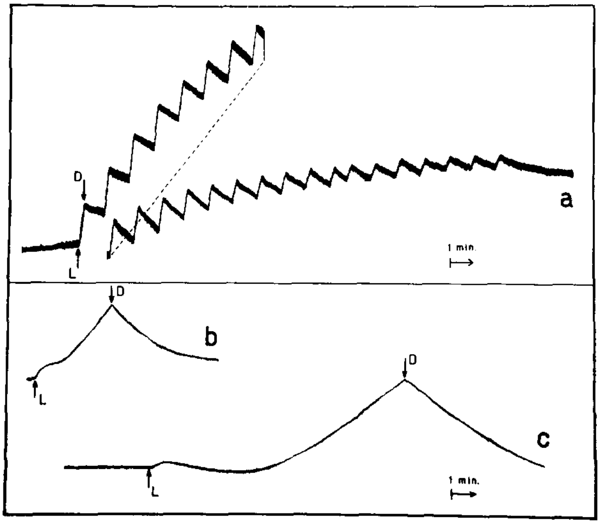

Fig. 4. Volumeter recordings. Carbonate buffer, anaerobic conditions. (a) Intermittent exposures of 50 sec each during about 40 minutes. (b) and (c) Initial outbursts of gas (probably mixtures of oxygen and hydrogen).

Fig. 4a gives a similar experiment made with the recording volumeter[7] with cells suspended in bicarbonate buffer under initially anaerobic conditions, leading to positive gas production by fermentation in the dark (hydrogen[8]). In a series of 25 cycles of 50 sec light and 50 sec dark each, the rate of pressure change in both light and darkness continually decreases.

In the experiment of Fig. 3, a remarkable effect can be noticed during the final dark period, in which the CO_2 concentration shows oscillations. Such oscillations are observed quite generally (*cf.* Fig. 2, 5). As a consequence, γ also oscillates during the first few minutes of darkness.

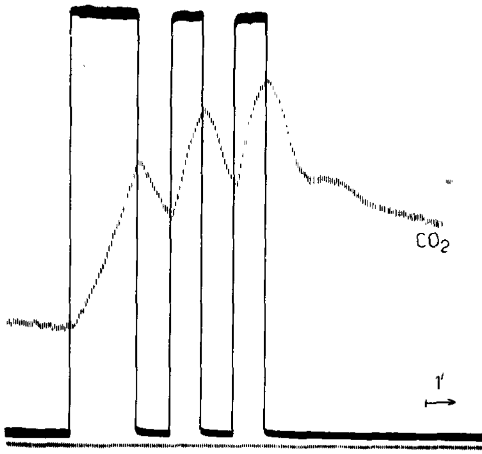

Fig. 5. Recording of pH during intermittent illumination. For further details about this and the subsequent figures see text.

Observations at low oxygen pressures

As soon as the oxygen tension in the medium becomes low, *e.g.* below 1%, still other phenomena become more pronounced. In the experiment of Fig. 6, the cells were kept in darkness overnight, so that they had been deprived of O_2 for a long period. During the first illumination, after a short induction, the rate of oxygen evolution is initially increased. During the subsequent dark period, oxygen uptake is first retarded after which the oxygen, produced in the preceding light period, is rapidly consumed completely. In the first illumination period, carbon dioxide uptake shows an even more pronounced induction than did oxygen evolution. The most remarkable phenomenon is the failure of carbon dioxide to reappear during the removal of oxygen by respiration in the following dark period ($\gamma = 0$) except for a slight initial production. Another example of this behaviour is shown in Fig. 7. A similar phenomenon was also reported by GAFFRON[9].

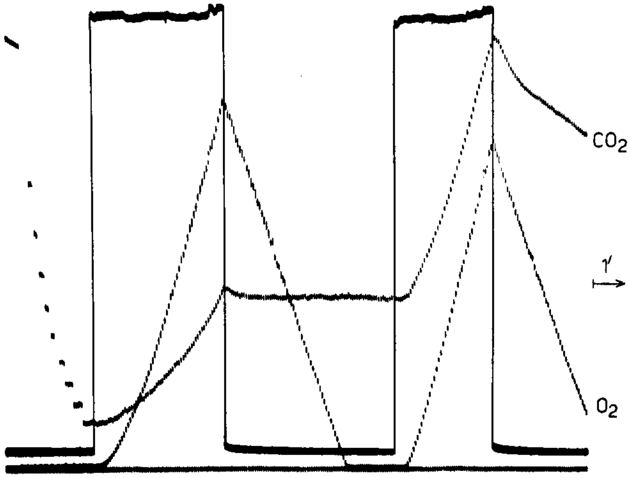

Fig. 6. Simultaneous recordings of pH and polarographic current.

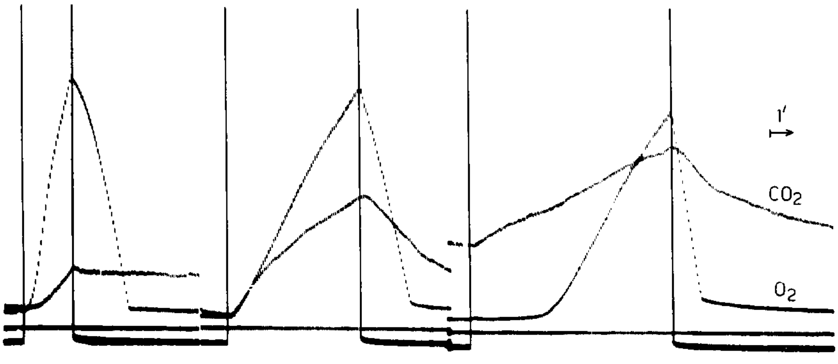

Fig. 7. Simultaneous recordings of pH and polarographic current.

In the second illumination period of Fig. 7, there is only a short induction ($\pm$ 10 sec). Thereafter, the initial rate of both oxygen and carbon dioxide exchange is high. First the rate of CO_2 uptake declines and after about one minute a similar decrease of the rate of oxygen evolution occurs.

During the following dark period, oxygen consumption is initially decreased, whereas carbon dioxide evolution, after a short "overshoot", is temporarily enhanced, while this time there is no "plateau" as in the preceding dark period. After an additional period of one hour, a third illumination was given. There is little overshoot (*ca.* 10 sec) in the response of CO_2. The oxygen, however, shows a very long induction period of 3 minutes after which the rate is high initially and declines later on. Atten-

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tion is directed to a small transient oxygen outburst, occurring about 10 sec after the start of the illumination. In all probability this is the same phenomenon as was observed already by FRANCK *et al.*[10]. It occurs often more markedly and can also be found volumetrically, *cf.* Fig. 4b and c. The latter figures represent measurements made with the same sample of algae as used for experiment Fig. 4a. The curves are very similar to the one in Fig. 7, but it is likely that the initial gas outbursts observed in the volumeter represent mixtures of oxygen and hydrogen (*cf.*[8]).

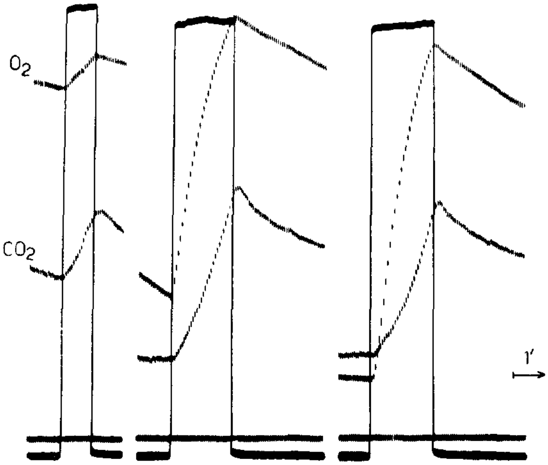

Fig. 8a. Simultaneous recordings of pH and polarographic current.

In the experiment of Fig. 8a, cells were kept in darkness long enough to consume the greater part of the available oxygen. The first exposure was made when the oxygen concentration was still about 5%. As was generally found at higher O_2 pressures, oxygen exchange was linear with time. The following two exposures were made after the oxygen concentration had decreased to *ca.* 0.3% and 0.1% respectively. The sensitivity of the oxygen measurement was readjusted accordingly. Under these conditions, the initial rates of oxygen production were found to be increased. The experiment of Fig. 8b, made under similar conditions (sensitivity of the polarograph the same in the three exposures), shows that at the lower oxygen pressures the rate of oxygen consumption in the dark may also be increased initially. In other cases, both in air and at low O_2 pressures, the rate of oxygen consumption in the dark was perfectly constant until the oxygen was completely exhausted (*cf.* Fig. 6).

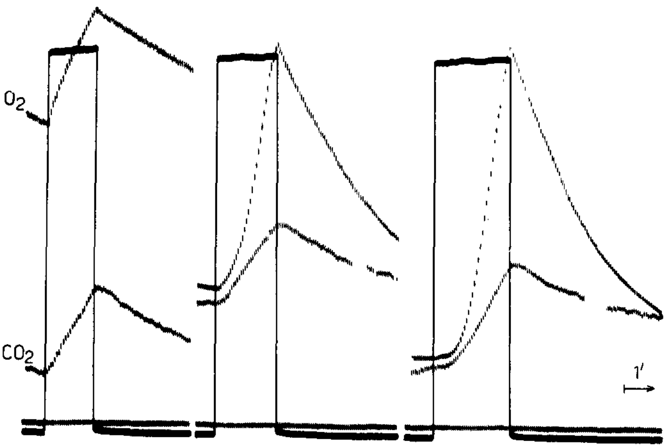

Fig. 8b. Simultaneous recordings of pH and polarographic current.

The first exposure in Fig. 9 shows a particularly clear example of an initially increased rate of oxygen production. This experiment was performed under conditions similar to those of the experiments of Fig. 8b. The cells were first allowed to consume the oxygen in the medium in the dark. This experiment is also noteworthy for the pronounced "outburst" and "intake" of carbon dioxide connected with the first illumination period. After the photosynthetic oxygen was consumed again for the

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greater part during a subsequent dark period, renewed illumination was again followed by outburst and intake phenomena, declining gradually during the following cycles of light and darkness.

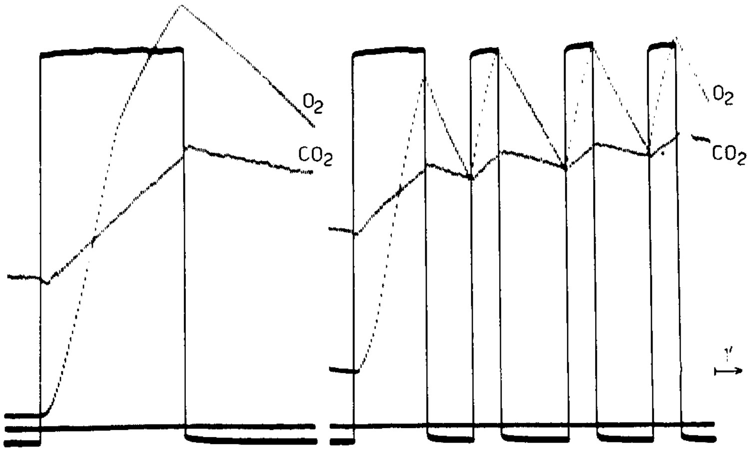

Fig. 9. Simultaneous recordings of pH and polarographic current.

DISCUSSION

The variety of effects that can be observed is such, that only a few positive conclusions can be drawn.

The occurrence of initially enhanced oxygen evolution appears to be related to conditions of low or very low oxygen pressure. The high initial rates of oxygen exchange found by DAMASCHKE *et al.*[3] may well have been caused by the use of similar conditions.

Intermittent illumination with periods in the order of one minute tends to influence the rates of carbon dioxide and oxygen exchange in the opposite way.

The main conclusion to be drawn from our experiments is, that under non-steady state conditions, the value of the photosynthetic quotient can vary greatly. The results demonstrate that under such conditions oxygen and carbon dioxide exchange are largely independent.

SUMMARY

A method is described for simultaneously recording carbon dioxide and oxygen concentrations in suspensions of algae. This method has been applied to the study of transitory rates of gas exchange in *Chlorella* suspensions during intermittent illumination.

A correlation was observed between high initial rates of oxygen evolution and partially anaerobic conditions.

The photosynthetic quotient was found to deviate considerably from —1 during non-steady state conditions.

RÉSUMÉ

Les auteurs décrivent une méthode d'enregistrement simultané des concentrations en gaz carbonique et en oxygène présents dans des suspensions d'algues. Cette méthode a été appliquée à l'étude des vitesses transitoires des échanges gazeux dans des suspensions de *Chlorella* soumises à un éclairage intermittent.

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Une corrélation entre des vitesses initiales élevées d'un dégagement d'oxygène et des conditions d'anaérobiose partielle a été observée.

Le quotient de photosynthèse s'écarte beaucoup de —1 au cours des périodes intermédiaires non-équilibrées.

ZUSAMMENFASSUNG

Es wird eine Methode zur gleichzeitigen Bestimmung der Kohlendioxyd- und Sauerstoffkonzentrationen in Algensuspensionen beschrieben. Diese Methode wurde auf die Untersuchung der vorübergehenden Gasaustauschgeschwindigkeiten während intermittierender Beleuchtung in *Chlorella*-Suspensionen angewandt.

Eine Korrelation zwischen hohen Anfangsgeschwindigkeiten der Sauerstoffentwicklung und teilweisen anaerobischen Bedingungen wurde beobachtet.

Bei Nicht-Gleichgewichtsbedingungen konnte eine bedeutende Abweichung des photosynthetischen Quotienten von —1 festgestellt werden.

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