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CHANGES IN PHOTOSYNTHETIC ACTIVITY DURING ALGAL GROWTH AND MULTIPLICATION

by

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INTRODUCTION

In a previous article (1) we described a continuous culture device, about 800 ml in volume, in which the optical density of the culture during growth was automatically kept constant by adequate dilution with fresh nutrient solution. Alternate light and dark periods gave one complete life cycle per day for *Scenedesmus* spec. at a temperature of 30 °C and a light intensity of about 17000 ergs.cm^{-2}.sec^{-1}.

During the light period of 14 hours, the volume of the single cell increased and the cell number per unit of volume suspension decreased, whereas in the subsequent dark period of 10 hours the mature cells divided into a number of daughter cells, so that the cell number increased again to the original value.

The present paper presents data describing the relationships between the photosynthetic activity during cell development and cell characteristics for the same strains of *Scenedesmus* as used in the above experiments.

EXPERIMENTAL METHODS

At different time intervals, during the light and dark periods a 20 ml sample was withdrawn from the continuous culture for the measurement of the maximum rate of photosynthesis and the examination of the cellular characteristics of the suspension.

For measurements of the saturating photosynthetic rate an aliquot of 1.5 ml of the sample was transferred to the reaction vessel of a small differential volumeter, as described by KOK (2). The population density of the suspension in the culture device was adjusted at 2.5 to 3.0 µl cells per ml suspension, which algal suspension could be used directly in the reaction vessel.

As a light source a 500 W incandescent projection bulb (PHILIPS Type 375 E) was used. The light was focussed upon the reaction vessel with the aid of a condensor (illuminated area 3 cm^2). The vessel was mounted in a thermostat bath of 30 °C. The light intensity used was 1.4×10^5 ergs.cm^{-2}.sec^{-1} of visible radiation.

Determinations of dry weight were obtained by centrifuging known aliquots of algal suspension in tared tubes of 15 ml in volume; the sediment was dried at 60 °C under vacuum.

Chlorophyll determinations were made by extraction with 5 per cent potassium hydroxide in methanol, as described earlier (3).

Measurements of packed wet cell volume per unit volume of suspension were made with calibrated TROMMSDORFF tubes; the results are furtheron mentioned as "TROMMSDORFF value". Division of the TROMMSDORFF value by cell number gives the volume of the single cell, mentioned as "calculated cell volume".

During growth and multiplication, measurements of the volume of the single cell have also been made by microscopic observation. Cell numbers were estimated by hemocytometer counts.

The light saturated photosynthetic activities expressed in µl O_2 per hour produced at different stages of development of the algae have been calculated in terms of unit dry weight, unit volume of packed wet cells (TROMMSDORFF) and unit cell number.

RESULTS

In fig. 1, the changes in photosynthetic activity are illustrated as dependent upon cell growth, ripening and multiplication, related to dry weight and

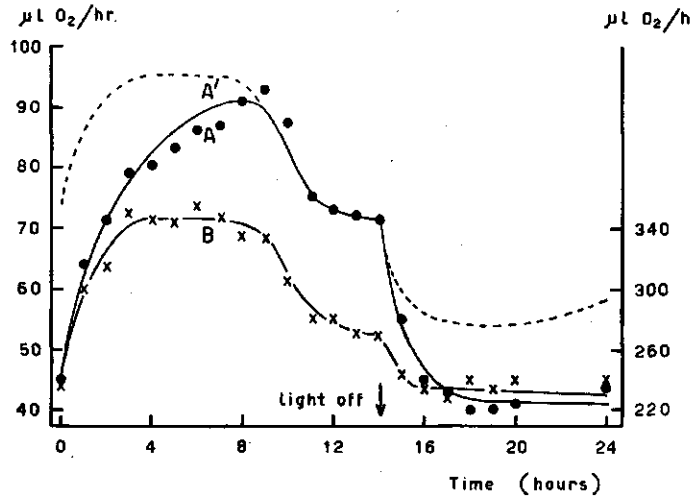

FIG. 1. Time course of changes in photosynthetic activity during cell development, measured at light saturation (1.4×10^5 ergs.cm^{-2}.sec^{-1}) and 30 °C.
Abscissa: Time in hours; 0–14 hours: continuous culture at a light intensity of $1.7 \times \times 10^4$ ergs.cm^{-2}.sec^{-1}; 14–24 hours: continuous culture darkened.
Ordinate to the left: Rate of oxygen evolution per µl of wet packed cells per hour (curves A and A').
Ordinate to the right: Rate of oxygen evolution per mg dry weight of cells per hour (curve B).

TROMMSDORFF value. Curve A (closed circles), shows the relationship between photosynthetic activity expressed in µl O_2 evolved per µl TROMMSDORFF, against the time of development. At the start of the illumination an increase in the rate

is observed, reaching a peak after 6 to 7 hours, which is about twice the rate found at the end of the preceding dark period of 10 hours. Then, the rate gradually decreases and becomes about constant at the end of the light period at a value, about 50 to 60 per cent higher than the value at the start. After illumination is stopped, a fast decrease in the photosynthetic activity is observed until a constant level is reached after 3 to 4 hours darkness.

Curve B (crosses), illustrates the relation of the photosynthetic activity evolved per mg dry weight versus time of cell growth and multiplication, and shows the same general picture. The maximum level, however, reaches only half the peak of curve A. This points to a discrepancy between dry weight and TROMMSDORFF value.

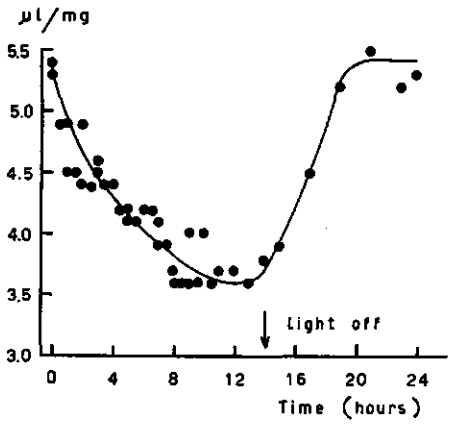

FIG. 2. Time course of changes in the volume of packed wet cells per mg dry weight. Light and dark periods of the continuous culture as in fig. 1.

The correlation between the changes in photosynthetic rates based on TROMMSDORFF values and the growth stages can only be determined accurately if the sedimentation of the algae is the same in their successive stages of development. Fig. 2 shows, however, that small cells, after a dark period, have a

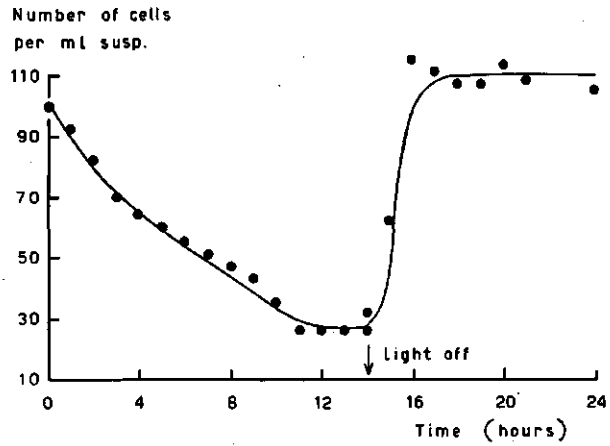

FIG. 3. Time course of cell counts per ml algal suspension. Light and dark periods of the continuous culture as in fig. 1.

considerably (about 50 %) higher TROMMSDORFF value per mg dry weight, than the large cells at the end of the light period. This means that the TROMMSDORFF value itself has been influenced by the size of the cells. Consequently, the peak of curve A has been influenced, either by the change in sedimentation properties of the algae, or by differences in water content of the different types of cells. A correlation between the photosynthetic activity per unit TROMMSDORFF and cell development, therefore, may be inadequate, at least in algal cultures with synchronized multiplication.

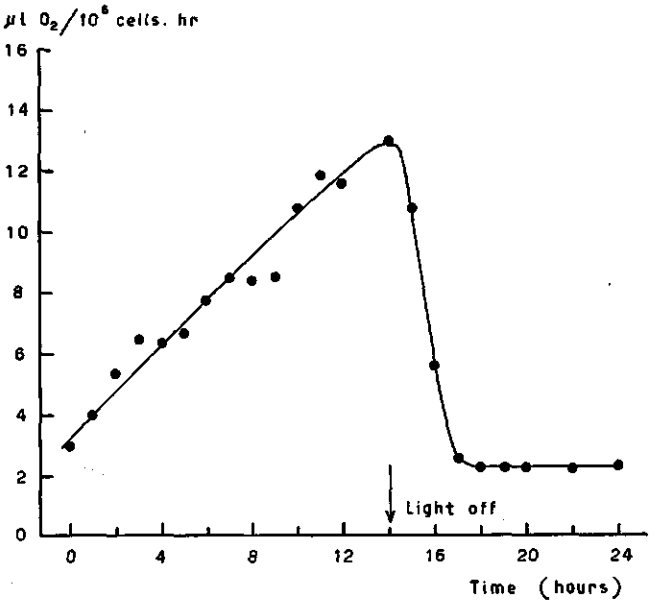

FIG. 4. Time course of photosynthetic activity, at light saturation (1.4×10^5 ergs.cm^{-2}.sec^{-1}) and 30 °C, during cell development.
Abscissa: Legend as in fig. 1.
Ordinate: Rate of oxygen evolution expressed in µl O_2 per hour and 10^6 cells.

We, therefore, recalculated curve A by taking the TROMMSDORFF value per unit of dry weight of large cells as a reference value. These recalculated photosynthetic rates, correlated to cell development are shown in curve A' (broken line, fig. 1). This recalculation influenced the starting rates to a larger (increase of about 50 %), and the maximum rates to a lower extent. The recalculated curve shows that the increase in photosynthetic activity as a consequence of cell development is about one half of curve A, which renders curve A similar to curve B, showing the same extended flat maximum.

Darkening of the culture is accompanied by a fast decrease in photosynthetic activity (fig. 1), and a strong increase in TROMMSDORFF value per unit dry weight and in cell number (*cf.* figs 2 and 3). It looks as if decrease in photosynthesis and cell multiplication are correlated, and that the photosynthetic activity is restored again in the light period.

The photosynthetic activities expressed in μl O_2 evolved per 10^6 cells per hour versus time of cell growth and multiplication are shown in fig. 4. The general picture is that at the start of an illumination the photosynthetic activity increases linearly with time reaching about 4 times the initial rate after 14 hours of light. In the following dark period the photosynthetic activity decreases again to the original value. After 2 to 3 hours darkness this value is already attained, remaining constant until illumination starts again.

The curve of fig. 4 reflects the increase in photosynthetically active cell components in 10^6 cells versus time of cell development.

It is of interest to compare this curve with the curves represented in figure 5 in which cell volume (a, b) and cell dry weight (c) are plotted versus time. Both these curves show a linear increase with time of cell volume and dry weight; besides this, after 8 to 10 hours of illumination the steepness of the curves increases sharply.

From this comparison it follows that the sharply increased rate in growth of cell weight and volume is not accompanied by a proportional increase in photosynthesis. This means that after 8 to 10 hours of illumination the rate of formation of photosynthetically inactive cell components increases, whereas the rate of formation of photosynthetically active material remains equal (*cf.* fig. 4), also during prolonged illumination. This leads to the curve of fig. 6 (closed circles) which shows the photosynthetic activity in relation to the volume of the single cell. The curve illustrates an initial, linear relationship between photosynthetic activity and cell volume in the small cells, followed by a gradual relative decrease in the photosynthetic activity.

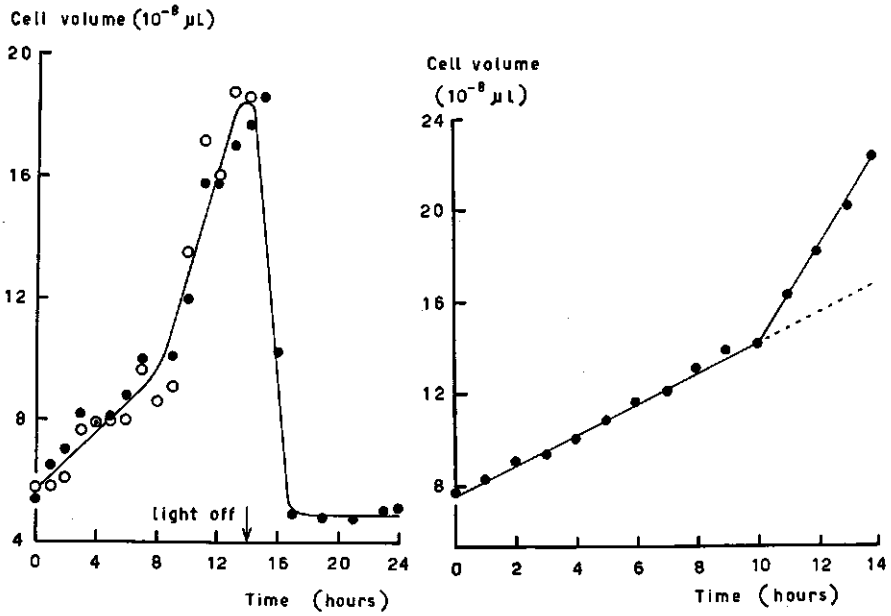

FIG. 5. Time course of the volume of the single cell and of the dry weight of the single cell.
a. (left) Cell volume calculated by dividing the volume of the packed wet cells per ml suspension by the cell counts per ml suspension.
b. (right) Cell volume determined by microscopic measurement of 100 cells.

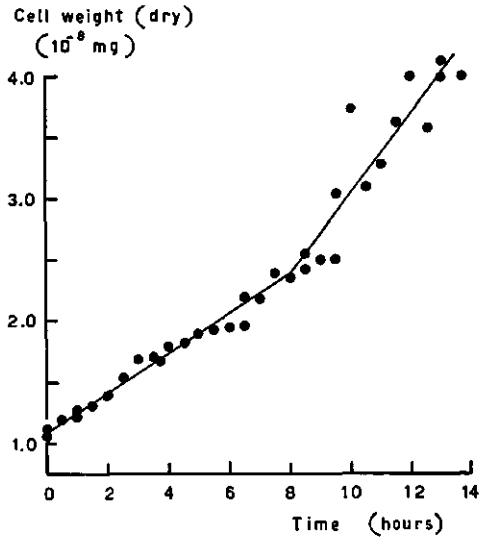

FIG. 5. *c*. Cell dry weight calculated by dividing the dry weight per ml suspension by the cell counts per ml suspension.

Suppose the increase in cell volume versus time was linear during the whole time of illumination – as the dashed line in fig. 5b shows – the relation between photosynthetic activity and cell volume would remain linear, also after 8 to 10 hours of illumination (fig. 6, open circles, obtained from the closed circle values assuming that the dashed line relation from fig. 5b holds).

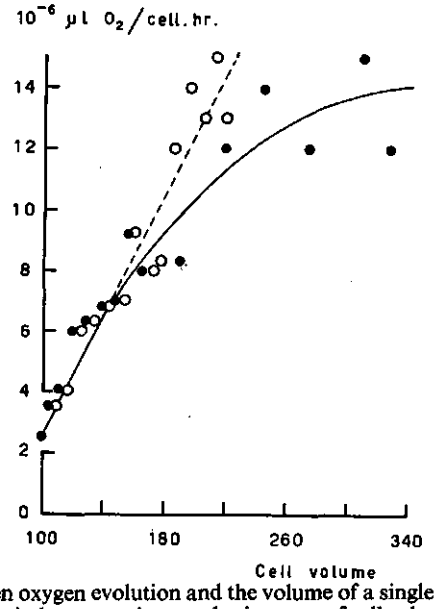

FIG. 6. Relation between oxygen evolution and the volume of a single algal cell (closed circles, *cf.* fig. 5a). Open circles: assuming regular increase of cell volume throughout the entire illumination period, (*cf.* fig. 5b, 0–10 hours: full drawn line, 10–14 hours: dotted line).
Ordinate: Oxygen evolution per hour, per cell.
Abscissa: The volume of a single cell in relative units.

DISCUSSION

Changes in photosynthetic activity during cell development were described by WINOKUR (4), TAMIYA *et al.* (5), NIHEI *et al.* (6),and SOROKIN (7). In WINOKUR'S experiments, maximum rates of photosynthesis were measured in aging cultures. The picture of his curves is an increase in photosynthetic activity on basis of dry weight and packed wet cells, during the first three days, followed by a gradual decrease to 20 or even 10 per cent of the starting value after 32 days of exposure to light. Also in his experiments, the TROMMSDORFF value per mg dry weight decreased from about 6.5 after the start of an experiment to 3.5 at the end of his experiments, resulting in different changes of photosynthetic activity on dry weight or cell volume basis. The conditions of these experiments, however, differ so much from ours that a comparison seems hardly feasible.

In the experiments of TAMIYA and NIHEI the light saturated rates of photosynthesis decline regularly during illumination to about 25 per cent of the value found at the beginning of the exposure to strong light, and increase again in the subsequent dark period. In their cultures the small cells were prepared at low light intensity and 25 °C. These cells were used in the synchronized growth experiments, and exposed to strong light at a lower temperature (15 °C). It might be assumed that under these conditions a normal growth and production of photosynthetically active cell components is counteracted by photooxidation phenomena, (see also KOK (10)). The restoration of the photosynthetic activity in the subsequent dark period emphasises such an assumption. In some preliminary experiments, we observed the same decrease in photosynthetic activity, by synchronizing algal cells in strong light after they had grown at low light intensity. At all events, these observations demonstrate that changes in photosynthetic activity during cell growth and multiplication depend strongly upon the conditions of pretreatment and illumination.

The results of SOROKIN (7), obtained under conditions of illumination and synchronization comparable to those applied in our continuous culture device, show changes of the same order of magnitude in photosynthetic activity per unit packed cells as we have presented in fig. 1 (curve A). SOROKIN, however, appears not to have taken into consideration the changes in packed cell density as a consequence of cell development. As was pointed out in the preceding section, the water content of the different types of cells changes (fig. 2), so that the unit volume of packed cells can hardly be used as a reliable index of the photosynthetically active material in algal cells of different age. The same holds for measurements of the rate of photosynthesis per unit dry weight. In this case changes in the ratio photosynthetically active over photosynthetically inactive cell components may suggest divergences in photosynthetic activity per unit of dry weight, though in fact the rate of photosynthesis may remain constant. A change in the proportion, *e.g.*, of cell wall over protoplasmic material may occur per excellence in cells, large in size, preparing the daughter cells. One might expect that in a culture, synchronized as pointed out, the formation of the cell walls of the daughter cells starts within the mother cell at the end of the light period. The ratio of the average surface of a sporulated mother cell to the average surface of 4 daughter cells is 1.3 to 1, whereas the ratio of the average volume of a sporulated mother cell to the average volume of 4 daughter cells is 0.75 to 1. The decrease of the ratio of the photosynthetically active over

the photosynthetically inactive cell material might be responsible for the decline in photosynthetic activity per unit dry weight at the end of the light period, as plotted in fig. 1, curve B.

Data on the nitrogen and chlorophyll content of small and mature cells show only small variation (*cf.* Table 1). In TAMIYA's experiments (5) the variations in N-content were a little higher, (8.0 to 5.5 % N), whereas in NIHEI's (6) and IWAMURA's (8) experiments no significant variation was observed. Decrease in N-content may decrease the photosynthetic rate per unit dry weight, as

TABLE 1. Changes in chlorophyll and nitrogen content, on dry weight basis, at the start (small cells, after 10 hours darkness), and at the end of the light period (mature cells, after 14 hours light).

Time of ill. (hours)	% N	% chlorophyll
0	8.7	3.8
14	7.8	3.1

described previously (3). In those experiments we observed a decrease in the photosynthetic rate of 20 per cent, while the N-content decreased from 8.8 to 6.1 per cent nitrogen. In the present experiments, the decrease in N-content is about one per cent; this may decrease the photosynthetic rate to less than 10 per cent. In fig. 1, curve B, at the end of the light period the photosynthetic rate is about 20 per cent lower than the maximum photosynthetic rate. This is about twofold the value to be expected from the decrease in N-content. At all events, the small decrease in N-content might account partly for the falling off of curve B, which, however, finds no full explanation in the data on nitrogen content, as yet present.

Presumably, measurements of the photosynthetic activity during algal devel-

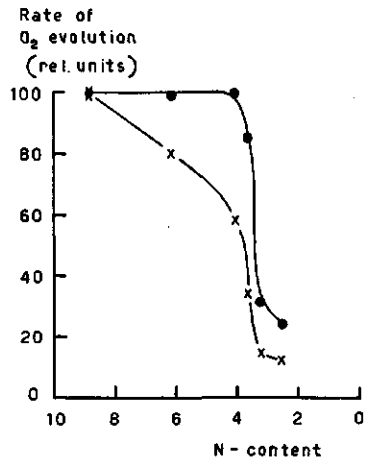

FIG. 7. Relation between oxygen evolution (relative units) and the percentage of cell nitrogen on dry weight basis.
Closed circles: Oxygen evolution per unit cell nitrogen.
Crosses: Oxygen evolution per unit dry weight.
Data from (3).

opment per unit cell protein provide more direct data to describe the observed phenomena (*cf.* 9). Measurements of the photosynthetic rate per unit cell nitrogen gave virtually constant values in nitrogen starved algae within the range of 9 to 4 percent N on dry weight, as shown in fig. 7 (replotted from (3)).

The sharp decrease in photosynthetic activity per unit packed cell volume (fig. 1, curve A) at the start of the dark period finds a partial explanation in the changes in water content of the daughter cells (*cf.* fig. 2); on the other hand also the recalculated curve A′ (broken line, fig. 1), shows a decrease. The decrease in photosynthetic rate as shown in fig. 1 after the end of the light period, might either be connected with the multiplication of the cells, or be induced by darkness. The last possibility can only be excluded if synchronization is brought about in continuous light. We have made some preliminary experiments related to this question. It was found that algal cells adapted to a rhythm of 14 hours light and 10 hours darkness, showed the same rhythm in multiplication in continuous light during several days. These experiments are being continued.

SUMMARY

Some problems in comparing photosynthetic activities of algal cells of different growth stages have been discussed.

In terms of unit volume of packed wet cells large variations in photosynthetic activity were observed. These changes are in part attributed to differences in the degree of hydratation of the algal cells in different stages of development cycles.

On dry weight basis, only small changes in photosynthetic activity were found, which are partly understandable by considering the changes in the ratio of photosynthetically active over photosynthetically inactive cell components in the algae in different stages of cell development.

Time course measurements of the rate of photosynthesis per number of cells showed an increase in photosynthetically active cell components which was linearly related to the time of the increase in cell volume. This suggests that the photosynthetic activity during cell development and multiplication remains constant, if referred to a reliable index of photosynthetically active cell components.

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