
ALGAL PHOTOSYNTHESIS AS MEASURED BY ABSORPTION OF RADIOACTIVE CARBON FROM WATER

GEORGE P. TRYFIATES*

Department of Biology, Bowling Green State University, Bowling Green, Ohio

INTRODUCTION

Photosynthesis is perhaps the most fundamental process in nature. The green plant, using the energy of sunlight, is able to combine the carbon dioxide of the air with water from the soil and from them produce carbohydrates. The energy needed for the activities of most living matter comes directly or indirectly from these carbohydrates.

While in the past photosynthesis has been studied only sporadically, a new era of investigative work began with the availability of radioactive carbon. Marine physiologists and limnologists, especially, became greatly interested in oceanic and lake productivity.

This investigation is concerned with algal photosynthesis in Urschel's Quarry. The Steemann-Neilsen C¹⁴ method (1957) was employed for measuring the absorption of radioactive carbon by the algae, with a modification which allowed for comparison between the C¹⁴ concentration and yield and the duration of exposure and yield. The C¹⁴ method is very useful when applied to oceanic and lake waters because it is one thousand times more sensitive than other methods. Although a

*Present address: *Agricultural Biochemistry Dept., Rutgers, The State University, New Brunswick N.J.*

great deal of study has been conducted on oceanic productivity, this method has been little used in fresh waters. Therefore, more testing of this method in fresh water, as in this study, is needed.

I wish to acknowledge the assistance of Dr. Verduin, who served as adviser and suggested the problem. The Department of Biology, Bowling Green State University, furnished the necessary facilities for this study. The C^{14} was supplied gratis by Dr. Mikihiko Oguri, of the University of Hawaii. This work was supported in part by an Atomic Energy Commission grant: AT(11-1)-536.

METHODS

Between July, 1958 and January, 1959 water samples from Urschel's Quarry were collected and inoculated with radioactive carbon. They were subsequently suspended under water, on the same site from which they were collected, for a certain period of time. These samples were then analyzed in the laboratory and tested for radioactivity on an end-window Geiger-Müller counter. From these data average photosynthetic values were computed.

In order to measure algal photosynthesis, a modification of the Steemann-Nielsen C^{14} method was employed. This provided for comparison between the C^{14} concentration and the observed yield.

A set of three 35 ml dropper bottles was used, one of which was covered with masking tape and used as a control. Into each bottle were placed 25 ml of water from Urschel's Quarry. One ml containing $0.5 \mu\text{c}$ of radioactive carbon was then added to each bottle. The three bottles were tied with plastic tubing at 0.1 and at 0.6 meter depths as follows: the control (dark) bottle and one clear bottle were both suspended at 0.6 m depths while the other clear bottle was suspended at 0.1 m. The experiments were begun at 1100 o'clock and terminated at 1400 each day. Each sample was filtered on a Millipore filter and immediately washed with 25 ml of distilled water containing one drop of IKI (acetic) killing agent. The filters were allowed to dry at room temperature, and then were taken to an end-window Geiger-Müller counter for counting. The Geiger-Müller counter was manufactured by Nuclear of Chicago Incorporated and had an efficiency of 0.00415 for C^{14} .

In September, 1958, this method was modified and three sets of three 35 ml dropper bottles were used. While the basic procedure remained unaltered, both the duration of exposure and the concentration of C^{14} were changed.

First, the three sets were submerged under water at 0800 o'clock and one set was brought to the laboratory at 1100, at 1400, and at 1700, respectively.

Second, the concentration of radioactive carbon in each set was varied while the duration of exposure was kept constant. The three sets were suspended from 1100 to 1400. The first set contained $0.25 \mu\text{c}$, the second set, $0.5 \mu\text{c}$, and the third set, $2.0 \mu\text{c}$ of radioactive carbon. These two modifications were employed alternately each week.

Radioactive carbon, in the form of $\text{Na}_2\text{C}^{14}\text{O}_3$ solution in ampoules, was furnished by Maxwell S. Doty's research laboratory of the University of Hawaii. Each ampoule contained five μc of C^{14} . The stock solution of radioactive carbon was prepared as follows: tap water and a 35 ml dropper bottle were placed in a 250 ml beaker and boiled for 20 min. This was done in order to sterilize the bottle and kill the microorganisms present in the tap water. Nine ml of boiled water were added to the sterilized bottle. An ampoule of C^{14} ($5\mu\text{c}$) was then placed in the bottle and broken immediately. The bottle was stoppered and shaken vigorously. This represented the stock solution of radioactive carbon.

SAMPLE COMPUTATION

Some of the data recorded during August, 1958 are shown in table 1. In order that the rate of carbon uptake be computed correctly, the following conditions should be present. First, $C^{14}\text{O}_2$ should be incorporated in organic compounds

only through photosynthesis. Second, the rate of assimilation of C¹⁴O₂ should be the same as that of C¹²O₂. Third, C¹⁴O₂ should not be lost by respiration taking place simultaneously with photosynthesis. Fourth, no organic matter should be lost by excretion (Steemann-Nielsen, 1957). Although none of these conditions could be absolutely assured, the difference between the control bottle and the clear bottle was assumed to represent photosynthesis. Steemann-Nielsen postulated that correction factors ranging from 5 to 155 percent are applicable, depending on the ratio of photosynthesis to respiration. Because the ratio of photosynthesis to respiration is not known, and the influence of bacterial synthesis also is not known, the computations in the present study did not include such correction factors.

TABLE 1
Sample data recorded during August, 1958

Date	Depth (m)	Time in	Time out	Count/min	Methyl Orange Alk.
8/8/58	0.1	1100	1400	92	4.225 (mEq of base)
	0.6			87	or 4225 (μmole of CO ₂)
8/12/58	Control	1100	1400	56	3.844 (mEq of base)
	0.1			106	3844 (μmole of CO ₂)
8/20/58	Control	1100	1400	58	3.970 (mEq of base)
	0.1			95	3970 (μmole of CO ₂)
	0.6			93	or 3970 (μmole of CO ₂)
	Control			25	CO ₂

The photosynthetic rates were computed as follows: from table 1, no. 1, the count of the control bottle was subtracted from the count of the clear bottle. The difference represented the C¹⁴ absorbed due to photosynthesis.

$$(1) \quad \frac{92 \text{ count/min} - 56 \text{ count/min}}{36 \text{ count/min}}$$

This value was divided by the product of (0.00415 x 1.1 x 10⁶), where 0.00415 was the efficiency of the Geiger-Müller counter, and 1.1 x 10⁶ was the disintegrations per min contained in 0.5 μc of C¹⁴. This yields the fraction of the C¹⁴ absorbed by the phytoplankton.

$$(2) \quad \frac{36}{0.00415 \times 1.1 \times 10^6} = 79 \times 10^{-4}$$

Formula two was multiplied by the value of total CO₂ in the water as indicated by the titratable base (Methyl Orange Alkalinity). This yields micromoles of CO₂ per liter of water. Methyl orange alkalinity tests were run each day as described by Welch (1952). For example: if the titratable base is 4.225 mEq per l and the pH is about 8.2, then bicarbonate predominates and each calcium ion will be associated with 2HCO₃⁻ ions; hence, total CO₂ is 4225 μmole per l.

$$(3) \quad 79 \times 10^{-4} \times 4225 = 33.4 \text{ } \mu\text{mole CO}_2/\text{l.}$$

Formula three in turn was divided by the number of hours of exposure to give micromoles of CO₂ per liter of water per hour of time.

$$(4) \quad \frac{33.4}{3} = 11.1 \text{ } \mu\text{mole CO}_2/\text{l/hr.}$$

RESULTS AND DISCUSSION

Computations were made for each experiment performed. Average values of micromoles of CO_2 absorbed per liter of water per hour were found for 0.1 and 0.6 m. An overall average value of micromoles of CO_2 absorbed per liter of water per hour was also computed. The same procedure was used for 0.25 μc , 0.5 μc , 2.0 μc and also for three-hr, six-hr, and nine-hr exposures. The results found during the summer of 1958 revealed an average hourly yield of 13 $\mu\text{mole/l}$ at 0.1 m and 11 $\mu\text{mole/l}$ at 0.6 m. Data for the period between September, 1958 and January, 1959 are tabulated in tables 2 and 3.

TABLE 2
Average micromoles of CO_2 per liter of water per hour with respect to C^{14} concentration recorded from September, 1958 to January, 1959

μcuries per 25 ml	At 0.1 m	At 0.6 m	Overall average
0.25	16.2	13.2	14.7
0.5	13.5	11.7	12.6
2.0	13.9	11.8	12.8

TABLE 3
Average micromoles of CO_2 per liter of water per hour with respect to time of exposure recorded from September, 1958, to January, 1959

Duration From-To	At 0.1 m	At 0.6 m	Overall average
0800-1100	19.6	10.9	15.2
0800-1400	21.4	14.5	17.9
0800-1700	19.1	13.1	16.1

From these data certain definite conclusions could be derived. First, the summer data indicated that photosynthesis was more active on the average at the surface of Urschel's Quarry water than at the 0.6 m depth. Some variations, however, were encountered during the course of the experiments. It was noticed that during the brightest summer days photosynthesis was more active at the 0.6 m depth. Excess light appears to inhibit photosynthesis at the surface layer, while at 0.6 m, better photosynthetic conditions occur. Also, the control bottle occasionally gave high counts (see table 1). This was probably due to microbial growth in the stock bottle. A stock bottle was used for three consecutive days, and bacteria introduced on the first day might make considerable growth. Using a dark bottle provides a correction for such growth in the stock solution. The actual C^{14} as fixed in the stock solution was of the order of one percent of that present; hence, it represented a negligible error in the estimate of C^{14} concentration.

The data in table 3 indicate that the uptake of carbon dioxide was not influenced by the C^{14} concentration. In figure 1, the C^{14} concentration per bottle is plotted against the observed yield estimates at 0.1 m. The yield estimate is shown to be relatively independent of the C^{14} concentration. A similar graph for the 0.6 m data would appear essentially like that in figure 1, but the points would be near the value of 12 on the ordinate.

As indicated from the data of table 4, slightly higher yields were obtained during an exposure of six hr. This may be attributable to better light supplies during midday (Doty, 1957). Also, the yields obtained during a three-hr exposure and a nine-hr exposure are identical. It is believed that respiration occurring actively during the afternoon hours compensated for the higher yield obtained during the midday hours. In figure 2, the duration of exposure was plotted

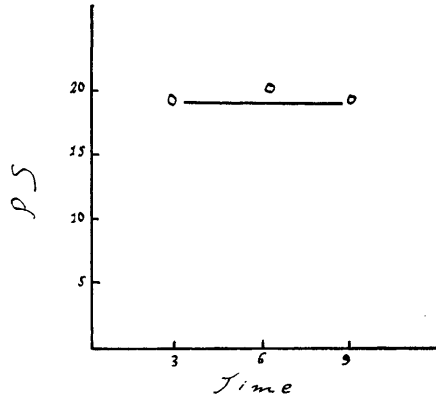
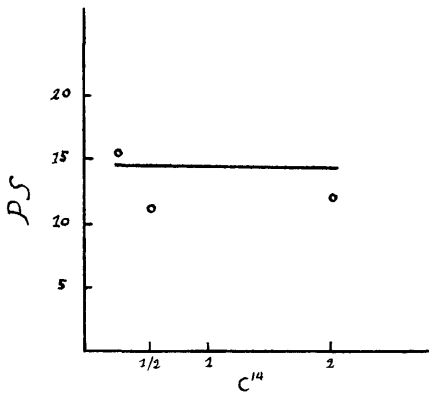


FIGURE 1. Relationship between C¹⁴ concentration ($\mu\text{c}/25\text{ ml}$) and photosynthesis (micromoles of CO₂ absorbed per liter of water per hour) at the 0.1 m depth.

FIGURE 2. Relationship between duration of exposure (in hours) and micromoles of CO₂ absorbed per liter of water per hour at the 0.1 m depth.

against the observed yields. Photosynthesis, represented by the straight line, is shown to be relatively independent of the duration of exposure within the range of time studied. A similar graph for the 0.6 m data would appear essentially like that in figure 2, but the points would be near the value of 12.5 on the ordinate. These data show that in situations where a nine-hr exposure is necessary to provide a significant count per minute, such exposures will yield results equally valid with those of shorter exposure.

TABLE 4
Comparison of average μmoles of CO₂ per liter per hour under ice with summer data, 1958

	At 0.1 m	At 0.6 m	Overall average
Winter	14.3	8.3	11.2
Summer	13.0	11.1	12.0

TABLE 5
*Photosynthesis per square meter per day for various waters as compared with that of Urschel's Quarry**

Place	Photosynthesis M. Mole
Lake Erie	512†
Gaynor Lake	450
Walvis Bay (Ocean)	316‡
Boulder Lake	255
Sandusky Bay	200
Base Line Lake	170
Urschel's Quarry	168
Beasley Lake	162
Haydens Lake	152
Kansas Ditches	151
Allens Lake	145
Kossler Lake	122

*Verduin, 1956.

†Verduin, 1959.

‡Stemann-Nielsen, 1957.

TABLE 6
Average $\mu\text{moles of CO}_2/\mu\text{liter per hour}$ in summer and winter, 1958

	At 0.1 m	At 0.6 m	Overall average
Summer	1.32	1.13	1.22
Winter	0.78	0.45	0.61

Table 4 reveals that photosynthesis was as active during the winter months as it was during the summer months. This, then, provides evidence that aquatic plankton is actively photosynthesizing during the winter. Light penetration data obtained during the winter months showed that the amount of light at the 0.6 m depth is about 5 percent of the surface light. In spite of the dim light at 0.6 m, phytoplankton is shown to photosynthesize actively during the winter months at this depth.

In table 5, a comparison is presented between the photosynthetic rate per square meter in Urschel's Quarry and that of other waters as measured by different methods. It is evident that the productivity of Urschel's Quarry as determined by C^{14} methods is similar to the value reported for other aquatic environments.

Table 6 shows the hourly average micromoles of CO_2 photosynthesized per unit phytoplankton volume during the summer and winter, 1958. The plant volumes were determined by Cowell and Sechriest, who kindly provided the data (unpublished) for this computation.

Since photosynthesis per liter was almost identical (table 4) during the summer and winter of 1958, it took twice the volume of winter phytoplankton to photosynthesize as much CO_2 as the summer phytoplankton did. The value of 0.78 is close to the value of 1.2 determined by Verduin for Urschel's Quarry phytoplankton studied with the pH method of determining CO_2 change, during the winter of 1957-58 (unpublished data). Also, yields varying from 0.2 to 1.4, with an average yield of 0.5 at optimal light conditions, were reported by Verduin (1956) for Lake Erie. These values are closely similar to the value of 0.61 for Urschel's Quarry winter phytoplankton.

SUMMARY

The Steemann-Nielsen C^{14} method was employed to measure algal photosynthesis in Urschel's Quarry. It was adapted to permit comparison between the C^{14} concentration and yield, and the duration of exposure and yield. It was found that the C^{14} concentration did not influence the observed yield importantly. While slightly higher yields were obtained during a six hr exposure, the yields obtained during a three and a nine hr exposure were almost identical. It is postulated that the favorable light conditions during the midday hours influenced the photosynthetic rate during this time. Also, active respiration during the afternoon hours may account for the identical yields obtained with three and nine hr of exposure. In general, the yield obtained was independent of duration of exposure within the range of time studied.

Photosynthesis was found to occur actively during the winter months in the Urschel's Quarry phytoplankton.

Results of the present investigation are generally in agreement with the other work conducted on lake and oceanic productivity.

LITERATURE CITED

- Doty, M. S. 1957. Current Status of Carbon-Fourteen Method of Assaying Productivity of the Ocean. Univ. Hawaii. 100 pp.
- Steemann-Nielsen, E. and A. E. Jensen. 1957. Primary oceanic production. Galathea Rept. 1: 49-69.
- Verduin, J. 1956. Energy fixation and utilization by natural communities in western Lake Erie. Ecol. 37: 40-49.
- . 1956. Primary production in lakes. Limnol. and Oceanog. 1: 85-91.
- . 1959. Maximal photosynthetic rates in nature. Science 133: 268-69
- Welch, P. S. 1948. Limnological methods. McGraw-Hill Book Co., New York. 381 pp.