APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1976, p. 75-79
Vol. 32, No. 1

Printed in U.S.A.

# Constant Release of Photosynthate from Marine Phytoplankton

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Received for publication 2 February 1976

The release rate of dissolved organic carbon (DOC) by unialgal cultures and natural phytoplankton assemblages was constant over a wide range of dissolved inorganic carbon concentrations. DOC release was not proportional to the particulate organic carbon production rate. We postulate that intracellular DOC, fated for release, exists either as a separate pool from that leading to particulate organic carbon production or that there is active metabolic control on one portion of a common pool.

In recent years investigators have demonstrated that marine algae in culture and from natural waters release some of the carbon fixed during photosynthesis into the medium (1, 3, 6, 8, 11). This release varies from less than 1% to greater than 40% of the total fixed carbon. As reviewed by Hellebust (8), a variety of compounds from vitamins to simple sugars have been reported to be released; both physical and biological conditions, including light spectra and intensity, dissolved inorganic carbon (DIC) and $O_2$ concentrations, physiological state of the cells, temperature, and salinity, affect the rate of dissolved organic carbon (DOC) release. Marine ecologists have been concerned with measuring the relative amounts of DOC released by phytoplankton, expressed as the percentage of the total fixed carbon released. Generally, release has been assumed to take place by passive diffusion through the cell plasmalemma, although evidence for active excretion has been reported for some algae under specific conditions (8). It has been shown that a variety of "stress" conditions such as high light intensity (7) or low DIC concentrations (12) affect the percentage of fixed carbon released by algal cells. In this paper we show that the rate of DOC release from both plankton samples and algal cultures is constant over a wide range of DIC concentrations and occurs independently of particulate organic carbon (POC) production.

## MATERIALS AND METHODS

Water samples, collected from the Port Hacking estuary on two different dates (experiment 1, 27 May 1975; experiment 2, 3 June 1975) were filtered through nylon mesh (124 $\mu$m), and the plankton was concentrated by continuous centrifugation (4). Microscopic examination revealed the concentrate to be composed mainly of diatoms and dinoflagellates. Flasks containing 125 ml of membrane-filtered seawater were inoculated with sufficient plankton concentrate to yield a cell density equal to that of the original seawater sample. The flasks were incubated for 20 min at ambient seawater temperature (18°C) in an Aquatherm water bath shaker (model G86), shaken at 180 rpm, and illuminated by a bank of five 40-W fluorescent lamps (daylight), which provided the samples with an average irradiation of 180 $\mu$ Einsteins/m$^2$ per s. The DIC concentration in each flask (measured as equivalents of $CO_2$ [10]) was adjusted by mixing varying proportions of membrane-filtered seawater and membrane-filtered seawater of low DIC concentration. Seawater of low DIC content was prepared by acidifying seawater with 1.0 N HCl (5 ml/liter) and sparging vigorously with a $CO_2$-free synthetic air mixture for 1 h. The seawater was then returned to pH 8.2 by addition of 1.0 M morpholinopropane sulfonate buffer, pH 8.3 (2.6 ml/liter), followed by 1.0 N NaOH. The same quantity of $^{14}CO_2$ was added to each flask ($2.44 \times 10^7$ dpm in experiment 1 and $2.62 \times 10^7$ dpm in experiment 2), and the time of tracer addition was recorded. After incubation with tracer for approximately 90 min, the samples were filtered onto membrane filters (0.45-$\mu$m pore size) under vacuum (<127 mm Hg). The POC samples residing on the filters were dried overnight in vacuo over KOH, placed in counting vials containing 15 ml of Permablend scintillation fluid (Packard Instruments Co.), and counted to 20,000 counts in a liquid scintillation spectrometer (Packard Instruments Co., model 3390). Filtrates, containing the DOC, were acidified with 1 ml of 37% (wt/vol) $H_3PO_4$ and sparged vigorously for 20 min with $CO_2$. A 10-ml portion of each filtrate was mixed with 10 ml of Instagel (Packard Instruments Co.) and counted to 5,000 counts.

Net radioactivities in the samples were obtained by subtracting the radioactivities of the control flasks in which the cells had been killed by adding 18% (wt/vol) formaldehyde solution (1 ml) prior to the addition of tracer. To normalize the samples for differences in incubation periods, the net radioactivities were expressed as disintegrations per minute

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incorporated per 1,000 s of incubation. The disintegrations per minute of the DOC and POC samples were obtained from quench correction curves by using the method of automatic external standardization. The quantities of DOC and POC (nanomoles of C per 1,000 s) were calculated from the samples' disintegrations per minute and the specific activities of the DIC in the sample flasks. Where samples were taken in triplicate, the range of observed values is shown by a pair of horizontal bars.

Log-phase cultures of *Amphidinium carterae*, *Chlorella vulgaris*, and *Phaeodactylum tricornutum* (obtained from the culture collection of the Marine Biochemistry Unit, Division of Fisheries and Oceanography, CSIRO, Sydney, N. S. W., Australia) were grown in medium F (10); 1-ml portions of the cultures were suspended in seawater of varying DIC concentrations at cell densities of $0.81 \times 10^4$, $2.6 \times 10^4$, and $6.1 \times 10^4$ cells/ml, respectively. Incubation temperature was 20°C. The experimental design was identical to that used for the plankton samples, except the initial centrifugation step was omitted.

## RESULTS AND DISCUSSION

As part of our study of secondary production in plankton communities, we set out to measure the assimilation and dissimilation of DOC by planktonic heterotrophs. For this work we wanted to use radioactive DOC produced by natural phytoplankton assemblages. To attain high radioactivity in the DOC, we concentrated plankton from several liters of water and resuspended them in water in which the DIC concentration had been greatly reduced. In this way we could increase the specific activity of the $^{14}C$-labeled DIC and thus proportionally increase the radioactivity of the DOC released. As it appeared likely that the very low DIC concentrations would limit the photosynthetic rate, initial experiments were conducted in which the concentrated plankton cells were resuspended in seawater of varying DIC concentrations. In this way we could determine the conditions for the maximum yield of radioactive DOC.

The results of two experiments using phytoplankton samples (Fig. 1) show the rate of production of DOC and POC plotted versus both the relative specific activity (the specific activity of seawater, containing 90 mg of DIC per liter, being equal to 1.0) and the total DIC in the medium. In each case the rate of DOC release remained constant over the entire range of DIC concentrations; that is, a doubling of specific activity of the DIC yielded a doubling in radioactivity of the DOC. In contrast, the rate of POC production decreased as the DIC concentration was lowered. Both the dark and killed cell controls, which were included for each DIC concentration, contained negligible radioactivity compared to flasks incubated in the light; thus, photosynthetic fixation was responsible for the observed radioactivities of DOC and POC.

The constant rate of DOC production and simultaneous decline of POC production rate with increasing DIC specific activity furnish evidence that the DOC radioactivities did not arise from cell lysis during filtration. If the DOC radioactivity arose from cell fragmentation, then an increase in DOC radioactivity would have had to originate from an increase in POC radioactivity; our results show that this is not the case.

To determine whether the results we obtained were a consequence of using a mixed plankton population or could be obtained by using algal cultures, we examined three unicellular marine algal species. Results (Fig. 2) agree with the data obtained from the experiments using plankton samples. *A. carterae* (Hulbert), a dinoflagellate, *C. vulgaris* (Beijerinck), a green alga, and *P. tricornutum* (Bohlin), a pennate diatom, each showed a constant rate of DOC release over all DIC concentrations and a decrease in the rate of POC production at lower DIC concentrations. Whereas in all cases the DOC release rate was constant, there was a large variation in the absolute amount of DOC released. *A. carterae* (Fig. 2) released an order of magnitude more DOC than either of the other cultures or the plankton samples, indicating that the constant release of DOC is not solely associated with species that excrete only minute quantities.

It has been suggested (13) that reports of constant DOC production could arise from an experimental artifact, the employment of $^{14}C$-labeled DIC contaminated with $^{14}C$-labeled organic compounds. The criticism is not relevant to our results for the following reasons.

First, the labeled DIC used in our experiments was obtained by trapping the $^{14}CO_2$ liberated from acidified solutions of $Na_2{}^{14}CO_3$.

Second, the radioactivities observed in the dark control flasks and in flasks containing formalin-killed cells were only a few counts per minute above background, three orders of magnitude less than the greatest radioactivities observed for light-incubated flasks.

Third, the $^{14}C$-labeled DIC could be quantitatively removed from the acidified filtrate by sparging with carrier $CO_2$.

Finally, although the same quantity of tracer was added to each flask, the radioactivity observed in the DOC was directly proportional to the DIC specific activity. If the results we obtained were due to the presence of $^{14}C$-labeled

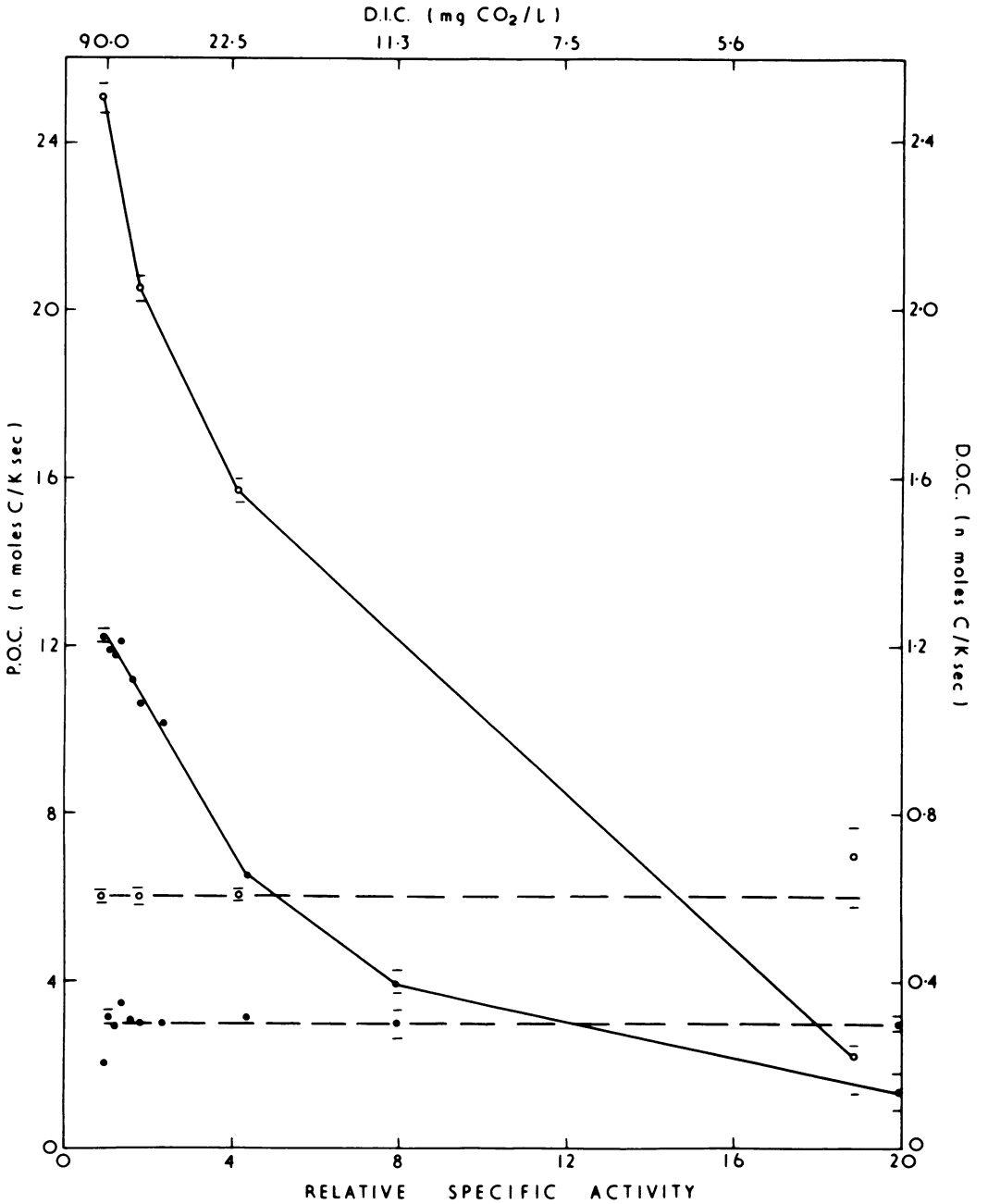


FIG. 1. *Effect of DIC concentration on DOC and POC production by plankton. Symbols: Experiment 1 (○), 27 May 1975; experiment 2 (●), 3 June 1975; DO (- - -); POC (——).*

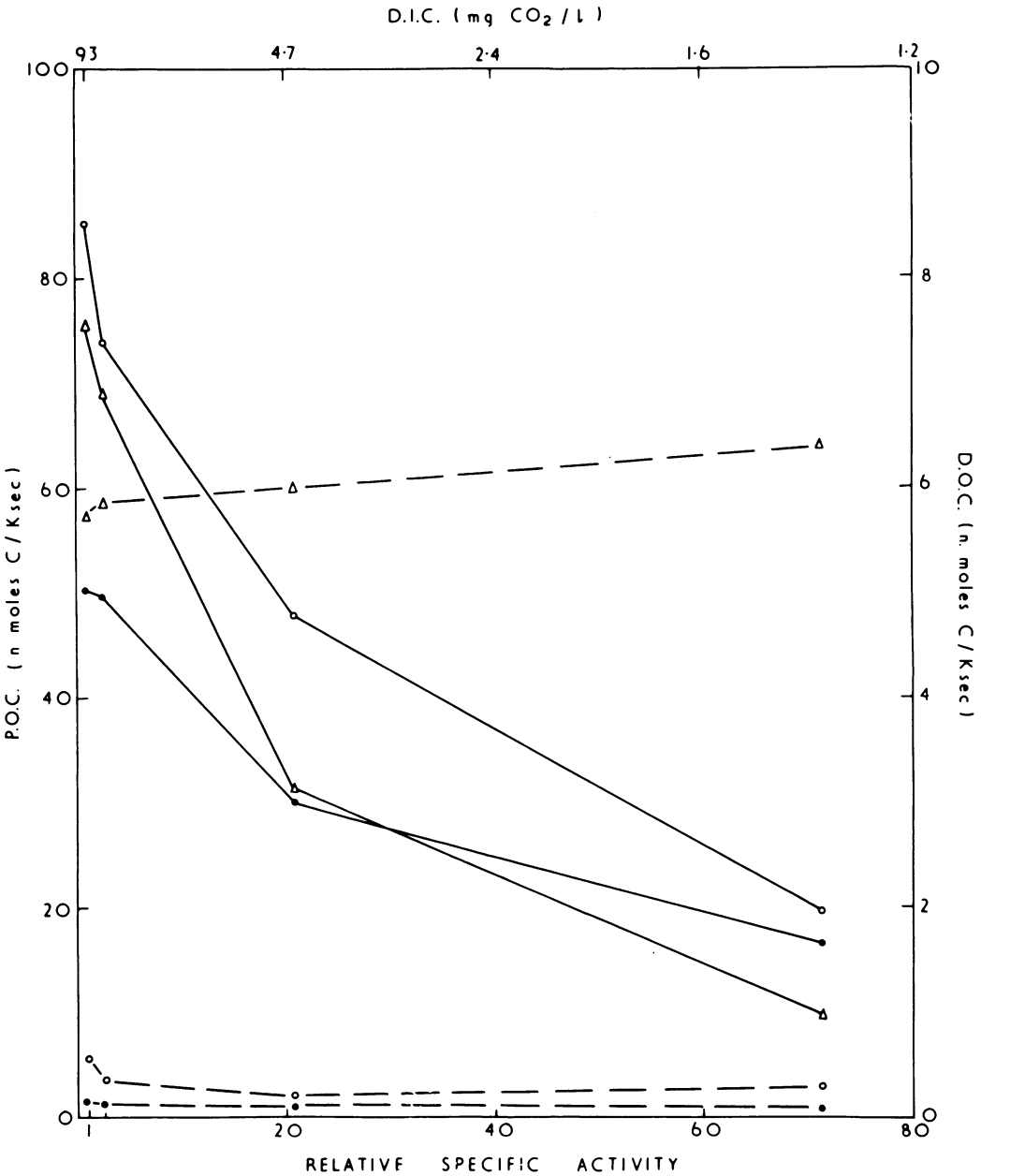


FIG. 2. *Production of DOC and POC by unialgal cultures at varying inorganic carbon concentrations. Symbols: A. carterae (△); C. vulgaris (○); P. tricornutum (●); DOC (- - -); POC (——).*

organic compounds in the tracer bolus, then the observed radioactivities of the DOC would have remained constant.

The hypothesis that DOC release occurs chiefly by diffusion through the cell plasmalemma cannot account for our results. If DOC originated from the passive leak of intracellular POC precursors, then the DOC could not be released at a constant rate while POC production decreased. There are two possible alternative explanations. First, two or more discrete internal photosynthate pools could exist, one destined for DOC release and one for POC production. Secondly, there could be a single photosynthate pool, in which release is controlled to assure a constant release rate.

The constancy of the DOC rate of release (nanomoles per 1,000 s), observed throughout the range of DIC concentrations used in this study, suggests that there is a constant chemical composition of the DOC. For if this is not the case, i.e., if the chemical composition changes while the total carbon release is constant, one must postulate an intracellular control that can monitor the rate of release of carbon atoms irrespective of the molecular species in which they reside.

Our observation that DOC is released at a constant rate is in agreement with the reports of other workers who have noted variation in DOC release, expressed as the percentage of the total $^{14}C$-labeled DIC fixed during primary productivity measurements (2, 7, 9, 11).

If DOC is released at a constant rate, the percentage of carbon fixed as DOC will vary if only the amount of carbon fixed as POC is altered.

The variation in the percentage of $^{14}C$ released as DOC, observed by Berman (2) and by Nalewajko and Schindler (9), is an inevitable consequence of the changes in POC production that they observed. In light of our findings, we would urge the abandonment of reporting DOC production in terms of percentage of the total carbon fixed in favor of expressing DOC production directly as grams of carbon fixed per unit of time.

In summary, we have shown that DOC is released from three marine algal species and phytoplankton at a constant rate over a wide range of DIC concentrations and that release is not directly related to the rate of POC production. We postulate that intracellular DOC fated for release exists either as a separate pool from that leading to POC production, or that there is active metabolic control on one portion of a common pool.

ACKNOWLEDGMENTS

We wish to express our gratitude to G. F. Humphrey, H. Jitts, S. W. Jeffrey, and R. R. Parker for discussions and review of this manuscript, and to thank H. Higgins for technical assistance.

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