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Determination of phytoplankton production by the radiocarbon method: a comparison between the acidification and bubbling method (ABM) and the filtration technique*

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Abstract. On the occasion of the workshop of the group on aquatic primary productivity (GAP) held in Konstanz (1982), ^{14}C-uptake rates were determined by two widely used, well-established procedures. In order to avoid any variation in the results caused by manipulation of the samples, subsamples for both determinations were withdrawn from the same bottles. The acid-bubbling method (ABM) yielded results which exceeded those of the filtration method by about 30%. Excretion of ^{14}C labelled dissolved organic matter was negligibly small and therefore cannot account for the observed differences. Based on available information also other possible explanations discussed can likely be dismissed. Hence additional effort is needed to identify and eliminate possible shortcomings in either method.

Introduction

Arthur and Rigler (1967) first showed that the specific activity of the labeled material retained on the filters (as cpm ml^{-1} filtered) may decrease with the amount of sample filtered. This was attributed to cell leakage with increasing filtering times and pressure. Algae release dissolved organic material which can be utilised by heterotrophic microorganisms. Therefore it is to be considered as primary production but is not measured by the filtration technique (Watt, 1966; Fogg, 1971; Berman, 1976; Mague *et al.*, 1980; Larsson and Hagström, 1982). Sharp (1977) attributed what previously had been termed 'extracellular release' to experimental artifacts such as cell leakage, organic contamination and release by cultural shock. Regardless of whether loss of ^{14}C labeled dissolved organic carbon (DOC) is due to artifacts or release by viable cells, primary production is underestimated if labeled DOC is not measured.

The acidification and bubbling technique (ABM) introduced by Schindler *et al.* (1972), offers several advantages over the conventional filtration technique. (i) Fragile organisms are not destroyed by filtration and photosynthates are not lost. (ii) It avoids contamination of the sample with dissolved (McMahon, 1973) or particulate inorganic ^{14}C which might occur at high pH values, when $CaCO_3$ precipitates. (iii) Activities of added inorganic ^{14}C and assimilated ^{14}C are measured with equal efficiencies and therefore no quench corrections are needed. Acidification has been shown not to interfere with ^{14}C counting efficiency. (iv) Because the liquid scintillation counting technique does not discriminate between

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homogeneously suspended ^{14}C labeled algal cells and dissolved ^{14}C, the phenomenon of self-absorption which might occur when algae are concentrated on filters (Pugh, 1973; Gargas, 1975) is negligible in the ABM (Gächter *et al.*, 1979).

The main shortcoming of the ABM as compared to the filtration technique is its lower sensitivity because only relatively small fractions of the entire sample (in our case 6%) are used for the determination. Therefore, relatively 'hot' labels are required which in turn may raise blanks. By careful handling moreover, several of the above mentioned shortcomings of the ^{14}C filtration technique can be avoided or at least minimised.

The objectives of our experiments were: (i) To compare the results of the ABM technique with those of the filtration technique routinely applied at the Limnological Institute at Konstanz. In order to avoid any variation in the results caused by the manipulation of the samples, subsamples for both determinations of the ^{14}C-uptake rate were withdrawn from the same bottles. (ii) To estimate the labeling rate of DOC. Our experiments were carried out in two systems that differed markedly with regard to total phytoplankton biomass and species composition (Lake Constance and experimental pond at the Limnological Institute, respectively). For details see Sakamoto *et al.*, 1984).

Methods

Two light bottles and one dark bottle of ~120 ml volume were used for the incubations. *In situ* exposure lasted for 4 h. The activity of the ^{14}C bicarbonate solution added to each bottle was 8 μCi. Dissolved inorganic carbon (DIC) was determined from alkalinity titrations (0.1 n HCl and methyl-orange-brome cresole-green indicator) and pH-measurements. The concentration of DIC was calculated by the following equation derived from Stumm and Morgan (1981):

$$\mathrm{DIC\,(mg\,m^{-3})} = \mathrm{Alk.(Meq\,l^{-1})}\,12{,}000\,\frac{1+\frac{10^{-\mathrm{pH}}}{K_1}\,1+\frac{K_2}{10^{-\mathrm{pH}}}}{1+\frac{2\,K_2}{10^{-\mathrm{pH}}}}$$

where $K_1\,(=10^{6.38})$ and $K_2\,(=10^{-10.38})$ are the dissociation constants of the carbonic acid at 20°C.

After incubation two 7 ml samples were withdrawn from each flask for the ABM technique and 50 ml for the filtration technique. The samples were processed as follows:

Acidification and bubbling method (ABM)

Determination of ^{14}C available and ^{14}C assimilated. Two subsamples were pipetted into glass scintillation vials. In order to determine the activity of the added ^{14}C (^{14}C available) one subsample was mixed with 10 ml of Luma-Gel® , a ready for use scintillator. At this water to fluor ratio a stable monophasic gel is produced, keeping ^{14}C labeled particles in suspension. The other subsample was acidified with 0.1 ml of 0.1 N HCl and bubbled with air for 1 h as shown in Figure 1 prior to addition of Luma-Gel® . This procedure removes inorganic ^{14}C

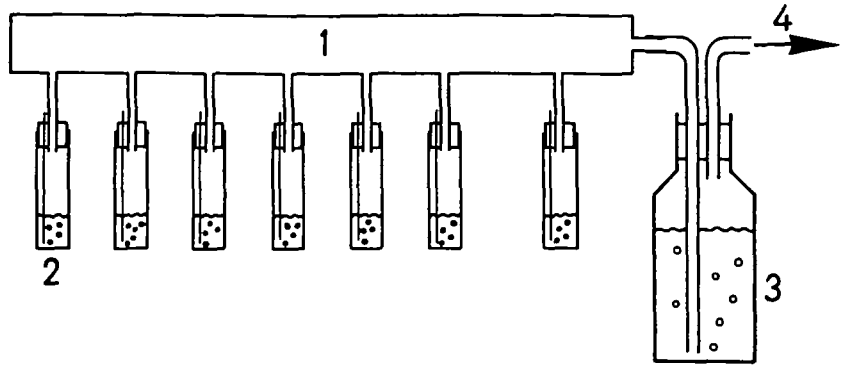

Fig. 1. Apparatus for bubbling acidified samples. (1) Plexiglas tubing with attached silicone rubber stoppers, each of which is bored through with a hypodermic needle; (2) liquid scintillation counting vials; (3) CO_2 absorber; and (4) line to vacuum pump.

quantitatively and the remaining activity (^{14}C assimilated) is due to labeled organic ^{14}C in either particulate or dissolved form.

Determination of ^{14}C activity in the filtrate (^{14}C filtrate). To estimate the production of ^{14}C labeled dissolved organic matter, particles were removed by low vacuum filtration (Sartorius membrane filters, pore size 0.2 μm). Then 7 ml aliquots were acidified and treated as described above.

Determination of background activity (^{14}C background). To consider possible trace impurities of organic ^{14}C in the ^{14}C stock solution, lake water was passed through a Sartorius membrane filter (pore size 0.2 μm) to remove algae and bacteria. Then 8 μC ^{14}C/100 ml were added. Ten 7 ml aliquots were acidified immediately after labeling, bubbled for 1 h and mixed with 10 ml Luma-Gel® as described above. These samples yielded an activity of 40.1 ± 3.0 cpm.

Calculation of results. Total primary production (PP) and the production of ^{14}C labeled DOM (^{14}C-DOM) were calculated as follows:

$$PP = \frac{^{14}C \text{ assimilated} - \text{background}}{^{14}C \text{ available} - \text{background}} \text{ DIC}$$

$$^{14}C\text{-DOM} = \frac{^{14}C \text{ filtrate} - \text{background}}{^{14}C \text{ available} - \text{background}} \text{ DIC}$$

where DIC is the concentration of dissolved inorganic carbon. No correction for isotope discrimination in carbon uptake rates was considered. ^{14}C-DOM may underestimate the true rates of ^{12}C-DOC excretion because during the 4 h exposure period the specific activity of excreted DOC is not constant and not equal to that of the available DIC, but increases from zero at the beginning to an unknown value at the end of the experiment. However, observations of Mague *et al.* (1980) indicate, that ^{14}C labeling of the pool supplying ^{14}C-DOM may occur very quickly after incubation with $H^{14}CO_3^-$. Excretion of DOC may further be underestimated due to uptake and partial mineralisation of labeled exudates by heterotrophic organisms.

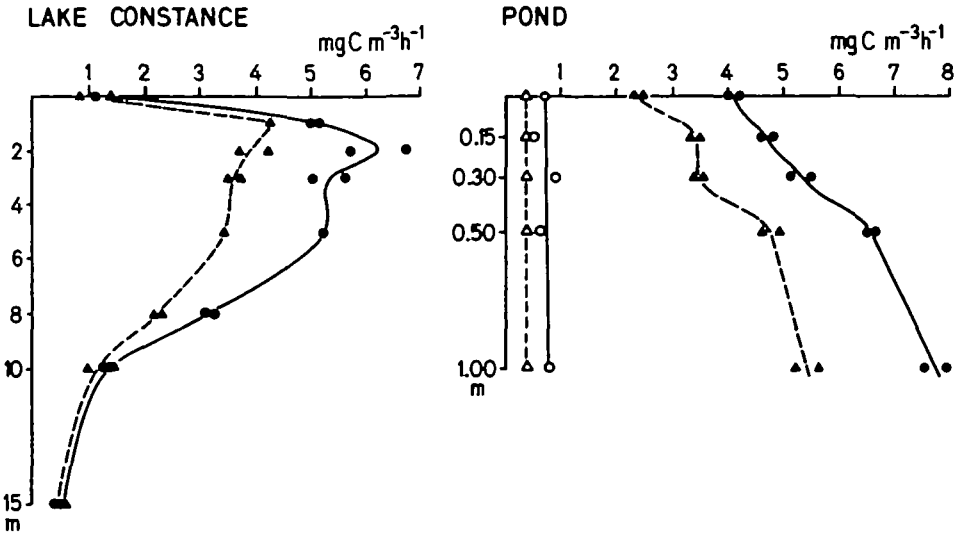

Fig. 2. Comparison of primary production measurements in (a) Lake Constance and (b) experimental pond. (●) light and (○) dark bottles ABM. (▲) and (△) dark bottles filtration technique.

Filtration technique

Determination of ^{14}C added and ^{14}C assimilated. To determine the activity of the added ^{14}C solution, after incubation, 1 ml was withdrawn from each flask with a precision pipette, and injected into a scintillation vial that contained one drop of 2 N NaOH to prevent escaping of $^{14}CO_2$. Thereafter 2.5 ml of unisolve gel (Zinsser, Frankfurt/M.) were added. Filtration onto 0.8 μm cellulose acetate membranes (Sartorius, Göttingen) was performed at dim light. The vacuum was -35 mm Hg. By using two 6-place manifolds the filtration time was minimised. The filters were rinsed with distilled water three times and thereafter dissolved in 3.0 ml of dioxane cocktail (Riedel de Haen No. 24224). No fuming with HCl as proposed by Berman (1973) was applied because of consistently low blanks. Counting was performed in a Beckman LS 7500 liquid scintillation counter. The counting efficiencies determined by using toluene standards were 80% in the 1-ml water samples in unisolve gel and 90% for the dissolved filters in Dioxane cocktail.

Calculation of results. Results were calculated by using the following equation:

$$C_{ass} = DIC\,(mg\,C\,m^{-3}) \cdot \frac{cpm_F\ f_1^{-1}\ 1.06}{cpm_W\ f_2^{-1}\ 50}$$

Where cpm_F are the counts of the filters, cpm_W counts of the water samples and $f_1 = 0.90$ and $f_2 = 0.80$ are the counting efficiencies of the dissolved filters in dioxane and of 1 ml of ^{14}C solution in Unisolve cocktail, respectively, 1.06 is the isotope discrimination factor and 50 is a factor to relate the activity of the added ^{14}C to the volume that has been filtered. Calculations are routinely performed on a HP 9825 computer by using a program developed by Dr. H. Kausch, Hamburg.

Photosynthetic rates are given as hourly incubation averages by subtracting the dark value at each depth from the values obtained in the light bottles.

Results and Discussion

Both in the lake and in the pond experiment the acid bubbling method resulted in consistently higher photosynthetic rates than the filtration technique (Figure 2). The results obtained by the filtration technique in the lake were 70 ± 13% and in the pond 74 ± 10% of the results of the acid bubbling method (mean values and s.d. of the measurements at different depths of the profile). In the lake the relative discrepancy was highest at near-optimum light-levels. In the pond no consistent vertical trend was apparent.

This is in sharp contrast with results obtained by Theodorsson (1975) who found that results obtained by the two methods deviated <5%. Gieskes and Kraay (1980) found excellent correspondence between the two methods on one occasion. On two other occasions however, they observed in near surface layers, in agreement with our findings, deviations of up to 40%.

Since in our case no additional experiments were performed we can only speculate about the reasons of this discrepancy. Possible explanations include the following: (i) Incomplete removal of inorganic ^{14}C-label from the sample by the acid bubbling procedure which could lead to an overestimation of photosynthesis by this method. (ii) Extracellular release and/or leakage of labeled organic material during filtration by cell rupture or osmotic-shock. (The activity in the filtrate of the Lake Constance samples was 40.3 ± 7 cpm and thus was identical to the background. This indicates that stripping of $^{14}CO_2$ after acidification was complete and that release of ^{14}C-DOM was undetectably small. In the pond, extracellular release and/or lysis of DOM was only 1 – 2% of total primary production. Therefore excretion cannot account for the observed differences.) (iii) Filtration errors caused by passing of small algal cells through the filters. (Filtration errors are not likely since the smallest diameter of phytoplankton species in Lake Constance exceeded 1 μm.) (iv) Incorrect quenching corrections in one or both scintillation cocktails used in the filtration technique. (Quench correction factors f_1 and f_2 have frequently been estimated to be in the order of 0.9 and 0.8, respectively. Thus it is very unlikely that inaccurate quench correction could account for the observed discrepancy.)

Filtration differed in the two methods in so far as in the ABM pore size of filters was 0.2 μm and in the filtration technique it was 0.8 μm. In addition filters were rinsed three times with distilled water in the filtration technique.

Experiments performed on other occasions using either lake water or distilled water for rinsing yielded equal results so that ^{14}C losses due to osmotic shock are highly unlikely. However, we cannot exclude the possibility that cell membranes became partly destroyed when sucking the filters three times to dryness.

Peterson (1978) emphasised that organic carbon budgets for phytoplankton will be subject to large uncertainties as long as we do not better understand what ^{14}C uptake measures. This experiment has shown that production of particulate carbon determined from identical samples by two widely used methods can differ far beyond the standard error within either method. This demonstrates that dif-

ficulties in the interpretation of ^{14}C measurements already begin at a purely technical level. The sobering fact that the reasons responsible for the observed discrepancy could not be clearly determined demonstrates that additional experimental effort is needed to identify and eliminate possible short-comings in either method. Only if all basic methodological problems are solved can we address the question which processes are actually measured by the ^{14}C method.

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