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An evaluation of liquid scintillation counting techniques for use in aquatic primary production studies[1]

ABSTRACT

Methods evaluated for the liquid scintillation counting of samples from aquatic primary production studies include the counting of intact filters and cells in a toluene fluor, solubilization and counting of membrane filters and cells in various reagents, and counting of a suspension of cells mixed with the fluor. A 1:1 Triton X-100:toluene fluor was very effective for the suspension of cells and gave high counting efficiencies. Up to 5 ml of sample could be mixed with the fluor, but this was considered inadequate for most primary production studies.

The filter standardization method for the counting of intact filters was investigated further and found to be applicable over a wide range of conditions but only accurate when the weight of algae on the filters was small (< 1 mg).

Direct solubilization of the filters and cells in a naphthalene-dioxane or 2-methoxyethanol-toluene fluor were the simplest, most accurate, and economical methods for primary production studies; the latter dissolved both wet and dry cellulose nitrate membrane filters and gave excellent replicate counting.

The use of ^{14}C-tracer techniques in aquatic primary production studies has increased as a result of the need for more accurate measurements of the rate of production, both in situ and in the laboratory. The radiocarbon taken up by phytoplankton was originally measured (e.g. Steemann Nielsen 1952) with Geiger-Müller counters, which generally had low counting efficiencies; often the results were difficult to standardize. When attempts were made to increase the counting efficiences of the system, as by using windowless counters and very thin samples, the counting efficiency was higher than predicted by the normal zero extrapolation procedure (Jitts and Scott 1961), and this standardization method was thrown into disrepute (Goldman 1968). Wood (1970, 1971) reviewed previous problems with the Geiger-Müller counting system and concluded that the zero extrapolation procedure is applicable so long as the counting efficiency is kept below 25%, thus excluding the very weak β-activity (0–30 keV) from detection. Wood (1971) also pointed out other factors which contribute errors of some magnitude. For these reasons, together with the compulsory low counting efficiency in comparison with liquid scintillation techniques, the latter methods have been investigated in the hope that comparisons between the results of different workers may be made easier and more reliable.

In liquid scintillation counting for primary production studies, Schindler (1966) adapted a naphthalene-dioxane fluor to solubilize membrane-filtered algae, and Wolfe and Schelske (1967) counted the intact filters and cells in a toluene fluor. Lind and Campbell (1969) proposed some advances to this latter method but, as I

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pointed out earlier (Pugh 1970), neither took fully into account the effects of self-absorption of the cells on the filters. Bransome and Grower (1970) and Furlong (1970) also demonstrated a decrease in the counting efficiency of ^{14}C-labeled molecules small enough to penetrate the matrix of a solid support, so that the external standard channels ratio method could not be used for quench correction. Other methods for the solubilization of membrane filters and algae in the fluor solution have been proposed: Wallen and Geen (1968) used a 2-methoxyethanol-toluene fluor and Parsons and Anderson (1970) one of 2-ethoxyethanol-toluene.

It is my purpose here to review some of these methods for the liquid scintillation counting of ^{14}C primary production samples, primarily from the point of view of their accuracy, but also for their applicability to field studies, e.g. simplicity and avoidance of possible ^{14}C losses during storage, etc. Results would be most accurate if the samples could be wet combusted and the resultant ^{14}C-CO_2 absorbed and counted using liquid scintillation (*see* Smith 1969; Watson and Williams 1970). However, these methods are not applicable to field studies.

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METHODS

Counts were made with a Philips scintillation analyzer, except in experiments involving Triton X-100 when counts were recorded by a Packard Tri-Carb model 3314 counter as the samples could be kept at –5°C. The counts were recorded in two channels set for the optimal counting of homogeneous toluene solutions; one counted only weak β-activity while the other was set to record all the activity within the ^{14}C β-spectrum. The ratio of the counts in the first channel to those in the second was calculated automatically by the analyzer, and this value is called the *channels ratio.* Whenever possible the counts were made in triplicate and the average was taken.

Table 1. The characteristics of the quench curves for the various methods of liquid scintillation counting. SD—the standard deviation of the counting efficiency; R*—the coefficient of multiple correlation;* n*—the number of observations*

Method		SD ±%	R	n	Fig. 1	
1(i)	GF/C	4.051	0.956	30	1A	a
	MF125	2.102	0.986	30		b
	Combined	3.986	0.957	60		c
1(ii)	GF/C	0.822	0.976	38	1B	a
	MF125	1.346	0.909	30		b
	Combined	1.382	0.942	68		c
1(iii)	MF	2.608	0.990	60	1C	a
2(iv)	Wet	0.288	0.9994	30	1D	a
	Dry	1.144	0.994	29		b
	Combined	1.097	0.994	59		
2(v)	1 + 2	2.216	0.865	90	1E	a
	3	4.176	0.869	50		b
3	--	0.933	0.991	84	1F	
1(i)	Pugh 1970	4.877	0.970	220	1C	b

The analyzer was also equipped with program boards set to "normalize" the counts for each sample and thus calculate its counting efficiency, i.e.

$$\text{Counting efficiency} = \frac{\text{cpm of sample}}{\text{dpm of standard}},$$

the dpm of the standard having previously been calculated using other internal standardization techniques. The samples were always chosen to give a wide range of counting efficiencies and channels ratios so that a quench curve of one against the other could be calculated. The best fit for quench curves resulting from ^{14}C-liquid scintillation counting is binomial, and an Elliot 903 computer was programmed to calculate the constants of this curve, together with the standard deviation (SD) of the counting efficiency, and the coefficient of multiple correlation (R). The actual formulae of the quench curves will not be given; they are characteristic of the experimentation and the analyzing machinery and are not otherwise applicable. However, the SD, R, and n, the number of results used in the calculation of the curve, are given in Table 1.

The methods investigated follow. Certain minor alterations, described below,

were made to the basic procedure of Pugh (1970).

1. The counting of samples on intact filter papers

The materials tested (weight range 0–20 mg) were cultures of the marine diatom *Phaeodactylum tricornutum*, the marine yeast *Metschnikovia zobelli*, and samples of phytoplankton and suspended matter from the North Sea (kindly supplied by Dr. P. C. Head).

The samples were filtered onto Whatman GF/C and Sartorius "Membran-filter" MF125 filter papers, dried labeled, and counted in 10 ml of a 0.5% Butyl PBD in AR toluene fluor. If excessive color quenching developed from the leaching of pigments during preparation of the vials, the samples were bleached for 24 hr under strong illumination and recounted.

2. Solubilization experiments

The membrane filters were solubilized in various reagents and subsequently counted. The reagents tested were (i) Soluene-100 (2 ml); (ii) Hyamine 10-X hydroxide (2 ml); (iii) NCS (1 ml); (iv) 1:2 v/v 2-methoxyethanol[2]: 0.5% Butyl PBD in toluene fluor; (v) naphthalene-dioxane fluor (100 g of naphthalene dissolved in 1 liter of 0.5% Butyl PBD in 1,4-dioxane). The solubilized samples from i, ii, and iii were diluted to 10 ml with a 0.5% solution of Butyl PBD in either AR toluene (dry filters) or 1:3 ethanol:toluene (wet filters), and then counted.

While method 1 gave excellent results for lower weight ranges of material, variations in results with heavier weights necessitated better ways of measuring the activity within the cells. The rapidity and degree of solubilization with different reagents and the accuracy of the counts obtained were therefore investigated.

All experiments were with *P. tricornutum*. Cells were filtered onto MF125 membrane filters, labeled, and placed in scintillation vials. The solubilization reagent was added immediately, or after the cells had been dried for 24 hr; a further 24 hr was allowed for solution and simultaneous bleaching of the samples. In certain experiments cells were labeled by preliminary incubation with ^{14}C-CO_3.

Aquasol, a xylene-based fluor which will dissolve membrane filters slowly (Schindler and Holmgren 1971), was not tested.

3. Suspension of samples in a Triton X-100 fluor

Five milliliters of the labeled algal suspension in a 0.5% solution of Butyl PBD were emulsified with 10 ml of the 1:1 v/v Triton X-100:toluene fluor and counted in the Packard Tri-Carb instrument. It is important to use the right proportions of sample and fluor. This combination, when counted at –5°C, formed a stable monophasic gel which gave excellent replication and counting efficiencies in the region of 50–60%. The sample, however, tended to become bi- or triphasic when counted at the relatively higher temperature of the Philips machine.

4. Comparison of methods 1, 2 iv, and 2 v

The most useful methods in terms of simplicity, accuracy of counting, and economics were counting of the intact filter paper (method 1), and solubilization in either 2-methoxyethanol-toluene (method 2 iv) or in naphthalene-dioxane (method 2 v). To compare the relative accuracies of these methods, 3 ml, and multiples of this volume up to 21 ml, of a labeled culture of *P. tricornutum* (ca. 3×10^6 cells ml^{-1}) were dispensed from a Jencons "Zippette" into the filtration apparatus and the cells filtered off and washed with "cold" medium; 30 replicates of each volume were filtered and 10 dried overnight and counted intact (method 1); the other 20 were placed directly into either of the solubilizing reagents. All the vials were counted after bleaching for 24 hr, and the counts were converted to dpm with the relevant quench curve equations from the earlier experiments so that results of all methods could be directly compared.

[2] Also available as ethylene glycol, monomethyl ether, methyl cellulose, and methyl oxitol.

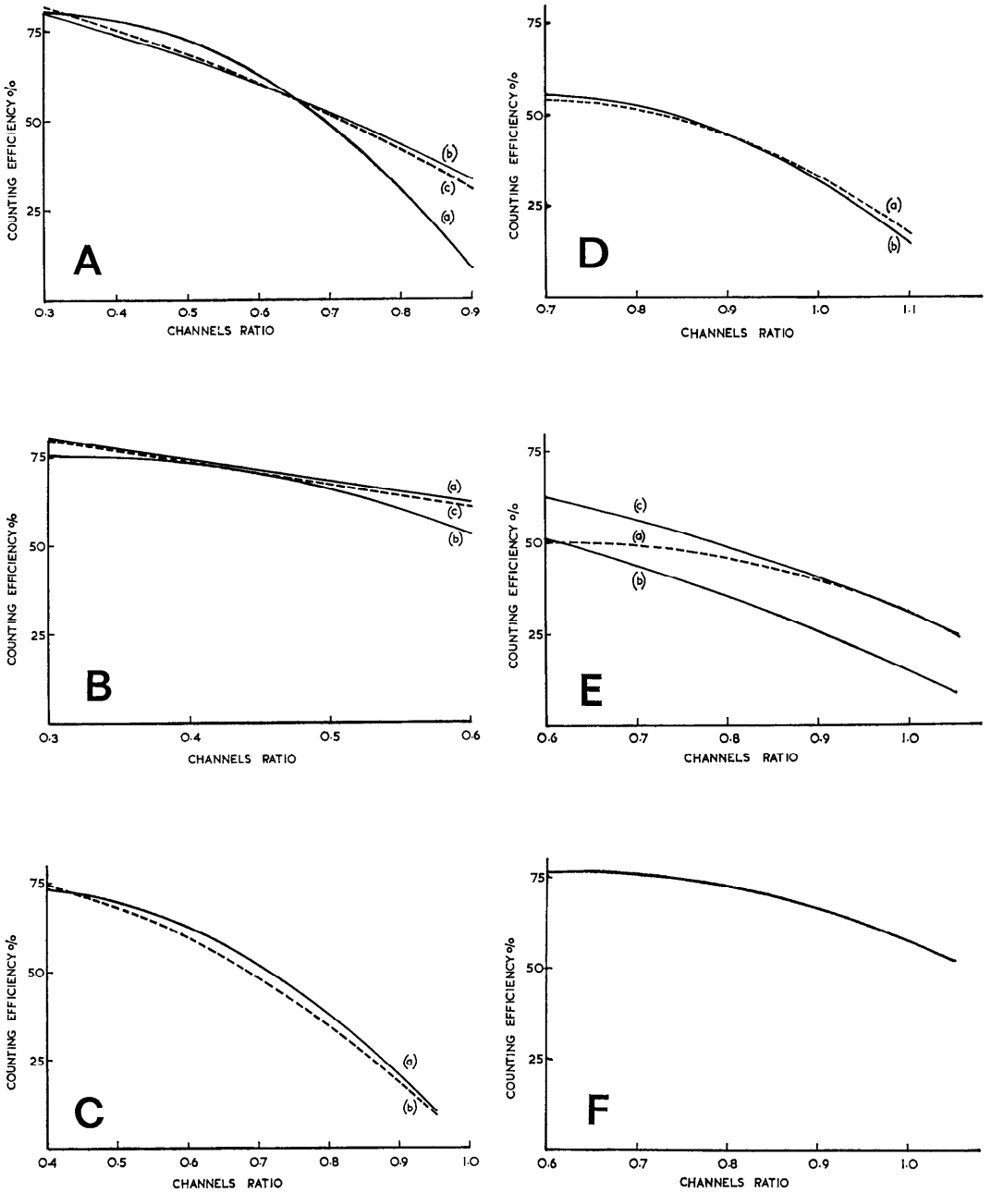

Fig. 1. Quench curves derived from the results for the liquid scintillation counting. A. Cells of *Phaeodactylum tricornutum* on intact (a) GF/C filters, (b) MF125 filters, and (c) the combined results of (a) and (b). B. Cells of *Metschnikovia zobelli* on intact (a) GF/C filters, (b) MF125 filters, and (c) the combined results for (a) and (b). C. Material from the North Sea on intact (a) membrane filters, and (b) the standard curve for cells of *P. tricornutum* (Pugh 1970). D. Cells of *P. tricornutum* solubilized in a 2-methoxyethanol-toluene fluor when (a) wet and (b) dry. E. Cells of *P. tricornutum* solubilized in a naphthalene-dioxane fluor: (a) experiments 1 and 2 combined, (b) experiment 3, and (c) internal standardization of experiment 3 with ^{14}C-toluene. F. Cells of *P. tricornutum* suspended in a Triton X-100-toluene fluor.

RESULTS AND DISCUSSION

1. The counting of samples on intact filter papers

The quench curves for the GF/C and MF125 filtered samples of cells of *P. tricornutum* and *M. zobelli*, and for the membrane-filtered North Sea samples are shown in Figs. 1A–C. The calculated SD, *R*, and *n* values for the curves are given in Table 1.

The quench curve for the combined results of the *P. tricornutum* samples (Fig. 1A, curve c) and for those published earlier (Fig. 1C, curve b) compare favorably when the channels ratio is not in excess of 0.7. At higher ratios there is an increasing divergence between the calculated efficiencies, reaching a level of 12.5% at a channels ratio of 0.9. Earlier, I had also shown a wide scatter in this region (Pugh 1970) but more data allowed a better curve to be fitted. The more highly quenched samples apparently cannot be accurately counted by this method. However, for most purposes the channels ratio can be kept below 0.7 with adequate pigment bleaching and accurate counts can be obtained.

The results for the yeast cells (Fig. 1B) were only spread over a small range of channels ratios as it was difficult to filter larger volumes. Within the range of the results, agreement between the two curves is excellent (maximum of 3% divergence). The curve obtained by the combination of the results is virtually identical with the GF/C curve, and fits closely with the standard *P. tricornutum* curve (Fig. 1C, curve b).

The curve for the North Sea samples (Fig. 1C, curve a) is here directly compared with this standard diatom curve. Weight of material used was between 0.1 and 6.2 mg and most of the results fell into the 0.8–0.9 channels ratio range. The curve within this range tends to be about 1–3% higher than the diatom curve, but is within the limits of experimental error.

From these three sets of results it is concluded that the method for counting intact filters and cells can be applied to several different systems and, provided that the effects of self absorption are kept to a minimum, produce accurate and reproducible results. Although the quench curves for the three systems studied here are similar, the system should always be calibrated for each individual case, using the filter standardization method, preferably with labeled cells (*see* section 4), and not by using internal standard ^{14}C-toluene or the like.

2. Solubilization experiments

(i) Soluene-100; (ii) Hyamine 10-X; (iii) NCS: All these reagents were moderately good solubilizers for both wet and dry filter papers, although there was always some insoluble residue—probably the diatom's silica frustules which are resistant to solution but will have little effect on the results. However, after the pigments were bleached under strong illumination, the residual color and chemical quenching of the fluor solutions, even with bleaching agents such as benzoyl peroxide (Hansen and Bush 1967), were so great that accurate counts could not be made. This quenching probably is due to the solution of the ca. 100 mg of membrane filter, for when the cells were centrifuged down and solubilized, the resulting solutions could be counted directly.

These digestants thus had several drawbacks: the necessity for preliminary dilution before accurate counting, and the apparent loss of counts from labeled cells dissolved in NCS when increasing volumes of cells were filtered. Similar losses of counts with these reagents have also been reported to me by others. These chemicals are, incidentally, expensive and would make analysis of a large number of samples very costly (*see* table 3: Stevens et al. 1970). These methods were thus abandoned in favor of more accurate and more economical ones.

(iv) 2-methoxyethanol-toluene fluor: Quench curves are shown in Fig. 1D, and the SD, *R*, and *n* values are given in Table 1. These two curves are similar and the

counting errors involved are very small. The high channels ratios are basically due to the chemical quenching effect of the 2-methoxyethanol. The liquid scintillation counter was set for the optimal counting of ^{14}C material dissolved in a toluene fluor and possibly could be adjusted to give better counting efficiencies for these other fluor solutions, but conditions were kept standard so that direct comparisons could be made.

Solution in this fluor was always excellent, even with the higher weight ranges, and color quenching, with adequate bleaching, was usually no problem. Wallen and Geen (1968) do not appear to have achieved quite such complete solution, as they recommended the use of Cab-O-Sil to suspend any undissolved material; also their results with dried filters were erratic. My results for dried filters are not as good as those for wet ones, but they are by no means erratic. The degree of dissolution of the membrane filters does, in fact, depend on what sort of filter is used. Cellulose acetate filters (e.g. "Oxoid" membrane filters) do not dissolve in this fluor, either wet or dry. Cellulose ester filters (e.g. the main range of Millipore filters) may not dissolve completely (Wallen and Geen 1968 used Millipore HA filters). Cellulose nitrate filters (Sartorius "Membran-filter"), used in all the above experiments, dissolve readily and are thus to be preferred for this sort of work. PVC filters also readily dissolve in this solvent and could be used as an alternative to cellulose nitrate ones.

This method of counting radioactive cells was the most accurate of all those tested. Parsons and Anderson (1970) used 2-ethoxyethanol-toluene fluors in their production studies, but the 1:1 v/v ratio of the two solvents must have resulted in very high chemical quenching of the solution.

Brief experiments with 2-ethoxyethanol gave results similar to those for 2-methoxyethanol. Both solvents have a maximum water content of about 5%, depending on their ratio with toluene, which is lower than for other fluors (e.g. dioxane) but quite sufficient for most needs.

(v) Naphthalene-dioxane fluor: Since Schindler (1966) noted that filters only dissolved completely if they were wet, solubilization in these experiments was only attempted when the filters were wet or had been rewetted after drying the ^{14}C-sucrose standard onto them. Three replicate experiments were carried out to standardize this method. The quench curves resulting from the first two were similar and the results have been combined (Fig. 1E, curve a). Some of the data were widely scattered, which resulted in a regression coefficient lower than that of the previous results, and so the third calibration was made. This quench curve is shown in Fig. E (curve b); SD, R, and n values for the curves are given in Table 1.

This third curve varied from between 5 and 15% below the quench curve for the first two experiments, within the range of the results, and the several possible reasons for this difference were investigated. Standardization of the ^{14}C-sucrose was checked and verified, confirming that there was no error due to normalization of the results. As dioxane, due to its chemical instability, can give variable results from batch to batch, all the samples were internally standardized using ^{14}C-toluene. The resultant curve for the samples from the third calibration experiment is shown in Fig. 1E (curve c). This curve closely resembles the standard curve for this fluor obtained by the usual method of chemical quenching and so it was concluded that any variation in the fluor from batch to batch would only result in a predictable chemical quenching.

Theoretically dioxane is an excellent solvent for membrane filters, but complete solution of the samples used in these experiments was unfortunately never obtained. Nicoll and Ewer (1972) have pointed out that dioxane-based fluors have a very low tolerance to salt solutions, which precipitate on addition to the scintillator causing some quenching. This also seems to affect the solubilizing property of the

fluor, resulting in incomplete dissolution of the filter and cells. Schindler always obtains excellent solution of his samples (personal communication), but these are derived from freshwater.

3. Suspension in Triton X-100-toluene fluor

Triton X-100 is a nonionic surface-active reagent which is an excellent emulsifier for scintillation counting purposes (Patterson and Greene 1965; Turner 1968; Fox 1968).

The quench curve for the combined results for the standardization of the method using an unlabeled cell suspension or medium and adding either ^{14}C-sucrose or ^{14}C-toluene is shown in Fig. 1F; SD, R, and n values are given in Table 1. The curve is similar to that for the counting of homogeneous toluene in the Packard machine and thus indicates that there was little effect of the excess volume of the sample. This method is thus an excellent one for counting filtrates especially (as in studies of extracellular products) and represents a considerable advance over the Geiger-Müller methods where only very small amounts could be dried onto planchettes and counted, possibly with considerable error. In my experiments 5 ml of medium could be counted, but, for instance, Samuel et al. (1971) were only able to count 0.4 ml of filtrates. 1,4-dioxane could also be used in freshwater studies as it can dissolve as much water as the Triton mixtures as well as solubilizing the filters; with marine studies precipitation of salts may affect the results too much.

4. Comparison of methods 1, 2 iv, and 2 v

The three more efficient methods are compared in Fig. 2. The average dpm from the 10 replicate counts for each volume filtered and for each method was plotted against the milliliters of culture filtered. The quench curve calculated for the first two sets of results for the naphthalene-dioxane fluor was used here to correct the counts for samples counted by that method.

An idealized line for the linear increases

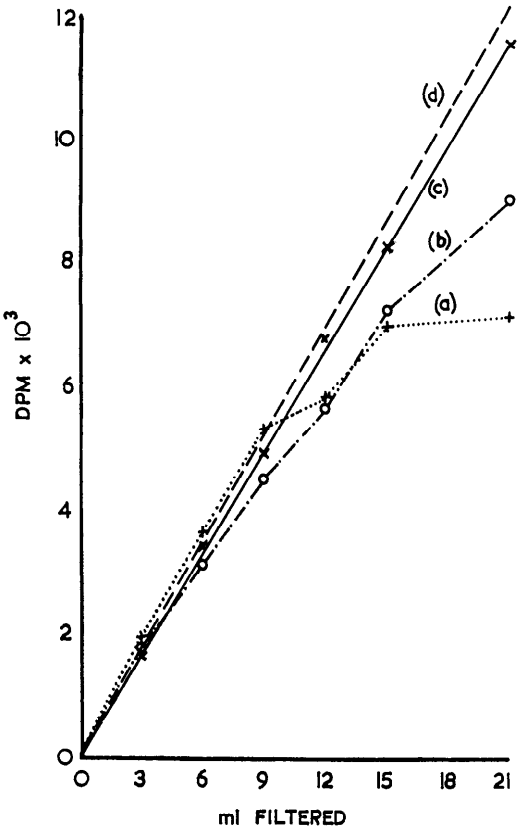

Fig. 2. A comparison between three liquid scintillation counting techniques. (a) Filter standardization technique, (b) naphthalene-dioxane fluor, (c) 2-methoxyethanol-toluene fluor, and (d) idealized result.

in dpm with volume filtered was calculated by taking the mean of the results from all three methods for 3 ml of culture filtered and extrapolating this, after suitable background correction, for all other volumes. This line (d) is also shown in Fig. 2. The results calculated for the 2-methoxyethanol-toluene fluor (line c) are excellent and indicate a linear increase in dpm with volume filtered. This line has a formula:

$$y\,(\text{dpm}) = 549.97x + 50.95;$$
$$R = 0.99969,\ n = 60,\ p \ll 0.001.$$

The intercept at 50.95 dpm approximates the background count of the machine. This regression line also only differs by 3% from the idealized line. These results thus indi-

cate that, in this case, there is no apparent loss of radioactive material from the filters as the volume filtered is increased (Arthur and Rigler 1967).

The naphthalene-dioxane fluor results (line b) diverge increasingly from the idealized result as the volume filtered increases, as also did the results for the filter standardization method (line a). It is concluded that these divergences are due to an inability of the standardization procedure to simulate fully the counting conditions. With the intact filters the addition of ^{14}C-sucrose to the surface of the filters is adequate in simulating the effects of self-absorption in the cells up to fairly dense precipitates (i.e. 9 ml filtered = ca. 3×10^7 cells or 1 mg of material) but not above this level. However, for most experiments in primary production less than 1 mg of material will be used and so the method is adequate, although the resulting counts can still be erratic, difficult to reproduce, and not as accurate as those derived from other methods.

There was no apparent loss of activity from the filters dried and stored overnight at 70°C. The results for the counting of these intact filters, up to 9 ml (30 determinations), closely resemble the results when the filters were immediately dissolved in the fluor solution. Once the samples had been prepared for counting, no significant losses of activity were ever noted, so that counts made immediately after the addition of the fluor, without any preliminary bleaching, and one made several weeks later gave similar results for the specific activity of ^{14}C within the cells. Some of the loss of activity from dried filters during 24-hr storage reported by Wallen and Geen (1968) can probably be accounted for by variations in their counting procedures. They apparently used 2-methoxyethanol-toluene fluor to determine the activity of the wet filters directly after filtration, but counted the dried stored filters in a toluene fluor, and internally standardized the samples using ^{14}C-toluene. This could lead to erroneous results, the degree of which would depend on the weight and type of material on the filters (Pugh 1970). However, as Wallen and Geen suggest, this situation could be resolved by placing some CO_2 absorbant, e.g. ethanolamine or even NCS, in a desiccator where the filters were being stored and seeing if there was any buildup of radioactivity within it.

In the case of the filters dissolved in the naphthalene-dioxane fluor, its appears that the increasing divergence from the idealized line is the result of an increased retention of salt water on the filter relative to the volume of culture filtered. The salts precipitate on contact with the fluor, causing increasing quenching and the incomplete dissolution of the filters, and resulting in the observed self-absorption. Any variation in the amount of salt water retained on a set of similar filters could thus cause their counting to be erratic. The deviation from the idealized result is noticeable even with the smaller volumes filtered and is quite marked (14%) with the 9-ml samples.

The results for the three methods of liquid scintillation counting compared here indicate that in most cases where there is little self-absorption or quenching, i.e. for most natural phytoplankton levels, all three could be used with varying degrees of accuracy. However, results for the counting of filters either intact or solubilized in the naphthalene-dioxane fluor have high standard deviations (Table 1) especially in comparison with the third method. Also the divergence of the counts from the true for both these methods is a drawback which cannot be corrected for by the usual internal or external standardization procedures. If solution of the filters is complete, then either of these standardization procedures can be applied. If external standardization is to be used it should be noted that Takahashi and Blanchard (1970) found that with heavily quenched samples the quench curves for color and chemical quenching were different. It would be difficult in an ordinary sample to estimate the relative proportions of each.

Schindler and Holmgren (1971) have

discussed the relative advantages of 1,4-dioxane and toluene as the basic fluor. A direct comparison between the naphthalene-dioxane and 2-methoxyethanol-toluene fluors, however, shows that both are equally flammable, but the former has an extremely toxic vapor and is chemically unstable, sometimes forming explosive peroxides. The higher freezing point (ca. +4°C) of the naphthalene-dioxane fluor could preclude its use in some scintillation counters; the facts that, unlike the other fluor, it does not attack polythene vials and is miscible with up to 30% of water could be advantageous. Incidentally, the naphthalene-dioxane fluor is about four times the price of the 2-methoxyethanol-toluene one.

The conclusion thus reached here is that the 2-methoxyethanol-toluene fluor is to be recommended for use in marine primary production studies, provided that the samples are filtered onto cellulose nitrate filters or the like. This method was the most accurate and one of the simplest of those tested and presents no difficulties under field conditions.

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Membrane filter retention—a source of error in the ^{14}C method of measuring primary production

ABSTRACT

The relationship between volume of water filtered and apparent specific activity of phytoplankton in which the latter decreases (cpm ^{14}C ml^{-1}) with increasing volume filtered, previously attributed to cellular damage to phytoplankton during vacuum filtration, is now considered mainly due to retention of unfixed radiotracer on the membrane filter. The retention capacity for ^{14}C by the filter, expressed as radioactivity per milliliter filtered, is maximum for small sample volumes ($\leq$ 1 ml), decreasing to a constant value when sample volumes are larger than 100 ml. This is attributed partially to absorption in the filter and probable retention of ^{14}C bound to unknown substances in the water or on the filter which are eluted or exchanged by passage of volumes of water of 100 ml or greater. This "washing" effect permits almost all of the ^{14}C not fixed in organisms to pass through the filter.

A possible source of error in the ^{14}C method for measuring primary productivity is the rupturing of phytoplankton cells during separation from the water by vacuum filtration (Lasker and Holmes 1957; Guillard and Wangersky 1958; McAllister 1961; Kuenzler and Ketchum 1962). To compensate for ^{14}C losses associated with cellular damage Arthur and Rigler (1967) proposed use of a correction curve which gave an extrapolated estimate of plankton ^{14}C activity.

Nalewajko and Lean (1972) offered an alternative hypothesis for the Arthur-Rigler observations: the retention of dissolved substances (^{14}C-labeled organic and inorganic materials) by membrane filters. However, they failed to eliminate the error or to demonstrate that cellular damage during filtration was not responsible for at least some portion of the observed ^{14}C losses.

The variability in extrapolated correction factors between different lakes (Schindler and Holmgren 1971) also suggested retention by the filter of different amounts of ^{14}C-labeled materials, depending on their concentration in a lake, or, as suggested by Arthur and Rigler (1967), variability in rupturing of different species of algae during filtration.

These facts prompted me to reexamine the Arthur-Rigler phenomenon. A. E. Docherty provided indispensable help with production measurements, ^{14}C counting, and field assistance.

METHODS

Water samples were collected from 2 m below the surface of Maskinonge Lake, an oligotrophic shield lake on the Atomic Energy of Canada Limited property at Chalk River, and from 25 cm below the surface of the highly eutrophic Grenadier