

Chapter 13

Techniques for Counting Carbon-14 and Phosphorus-32 Labelled Samples of Polluted Natural Waters

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INTRODUCTION

There is considerable interest at present in aquatic biological systems, especially in the field of pollution where organic wastes, inorganic nutrients and algal productivity of natural waters are receiving particular attention. Problems with these are likely to throw an increasing strain on natural resources in the future as industrialization and urbanization continue to increase. Two key elements essential to all biological systems are carbon and phosphorus. In aquatic systems available carbon occurs as carbon dioxide, bicarbonate and carbonate, and in diverse organic compounds which may be present from polluting discharges, or from the breakdown of biological material. Phosphorus is intimately involved in all biological energy transformations, and in aquatic systems, where it is usually present in very low concentrations, it can be a limiting factor in biological production by photosynthetic or heterotrophic growth. The ability to measure accurately carbon and phosphorus, and their rates of uptake by microbial populations is thus essential to an understanding of any aquatic biological system.

Classical methods of analysis have serious limitations in the study of most natural aquatic systems where important substances are often present at levels less than 1 or 2 mg/l of water. In many waters the accurate estimation of soluble orthophosphate is difficult and tedious. A similar situation occurs with the chemical assay of most organic compounds likely to be present, and the test for biochemical oxygen demand (BOD) is non-specific for biodegradable organic material and is unreliable at best, especially at low concentrations. Additionally, for an understanding of aquatic ecosystems it is necessary to know both the standing crop (biomass) of the organisms, and the metabolic activity or productivity of the system. Using classical methods it is difficult to assess accurately the biomass of either autotrophic systems (algae and rooted plants) or heterotrophic ones (such as bacterial growths and slimes). Primary production — the photosynthesis of cellular carbon from carbon dioxide together with the release of oxygen — is due primarily to algae in deep water masses. This can be measured by three principal methods:

1. from the direct increase in suspended solids (a method which is unreliable when the weight of algae can be only a few mg/l);
2. from the rate at which dissolved oxygen is produced (this method is insensitive at low

- numbers of algae and can be erroneous when the water is supersaturated with oxygen);
3. from the change in carbon dioxide concentration (this may be chemically calculated from pH changes, the accuracy depending on the buffering capacity of the water and the accuracy with which the dissociation constants of carbonic acid are known).

The use of radionuclides as tracers provides a powerful technique for the study of substances present in low concentrations, and both carbon-14 and phosphorus-32 have been widely used in biological studies, though the assay techniques have been subject to limitations. Substances have been assayed using either end-window Geiger-Müller counting of biological materials and evaporated solutions (a method subject to errors from self absorption, the geometry of the counter and dead time), or by liquid scintillation counting of non-aqueous solutions (which is usually limited to the use of materials miscible with organic solvents and is subject to loss of efficiency and reproducibility owing to chemical quenching). The present paper discusses techniques involving Cerenkov counting of aqueous phosphorus-32 and solubilization techniques which allow liquid scintillation counting of biological materials, in particular of phosphorus-32 in biological slime growths and carbon-14 in algae. The techniques discussed could be applied to studies involving various aquatic systems.

MATERIALS AND METHODS

Automatic liquid scintillation counter. For phosphorus-32 assay a Tracerlab Coru/matic II automatic twin channel counter was used at an EHT setting of 360 (equivalent to 1100 V). The channel settings are tabulated in Table 1.

Table 1. Channel settings for phosphorus-32 counting.

Channel	Coarse gain	Fine gain	Threshold	Window
Scintillation	16	1.5	025	Out
Cerenkov	64	1.0	025	1000

Manual liquid scintillation counter. For carbon-14 assay an EKCO M5402 liquid scintillation head with a single photomultiplier was coupled, via an interface circuit, to an EKCO N610 B scaler/timer with pulse height analyser. Two reproducible channels were selected by fixing the threshold, and then counting with and without a higher energy discriminator. As shown in Fig. 1, this enabled the whole pulse spectrum to be counted in integral channel C_I, and only the lower energy region in channel C_{II}. The criteria of Bush¹ were used to select the actual channel widths which are set out in Table 2. To obtain steady consistent backgrounds on the instrument 'smoothing and filtering' of the mains electricity supply was necessary.

End-window counter. An EKCO N530F scaler/timer connected to a 2B2 thin end-window (window thickness 1.9 mg cm⁻²) Geiger-Müller tube.

Ultrasonic cleaning bath. This consisted of a KS 100 ultrasonic generator connected to a KS 101 cleaning bath, both items manufactured by Kerry Ultrasonics Ltd., Hitchin.

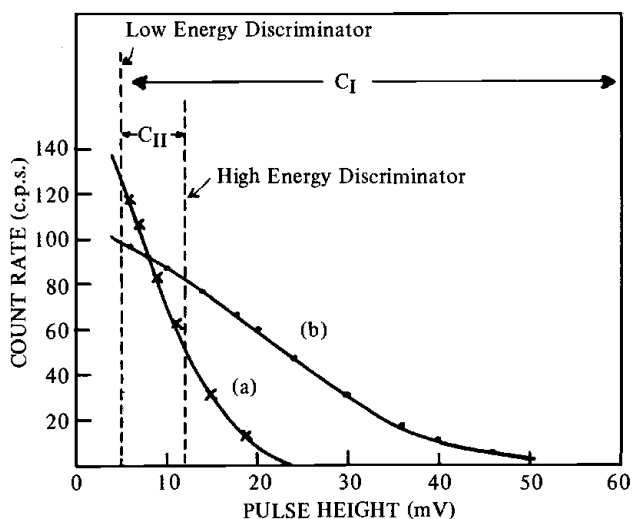


Fig. 1. Pulse spectra of (a) quenched and (b) unquenched carbon-14.

Table 2. Channel settings for counting carbon-14.

Channel	EHT	Amplifier gain	Threshold	Window
C _I	1255 V	1000	5 mV	Out
C _{II}	1255 V	1000	5 mV	7 mV

Reagents. (a) Scintillators:

- (i) KL 353*, a dioxan-based liquid scintillator miscible with up to 12% of water. To render this scintillator thixotropic the finely ground silica powder Cabosil* was used.
- (ii) Scintillation grade toluene* containing 15 g/l of Butyl-PBD†.

(b) Solubilizing agents:

- (i) Hyamine hydroxide*, 1 M in methanol.
- (ii) Soluene 100§.
- (iii) NE 520||.

Other chemicals used were of Analytical Reagent Grade.

Radiochemical materials. All were supplied by the Radiochemical Centre, Amersham, Buckinghamshire. Stock solutions were:

- (i) Phosphorus-32, supplied as high specific activity orthophosphate in dilute hydrochloric acid (1 mCi/ml).
- (ii) Carbon-14, supplied as Na₂ ¹⁴CO₃ having a specific activity of 20 mCi/mM and a concentration of 1 mCi/ml (cat. No. CFA 1).

* Koch-Light Laboratories, Colnbrook, Bucks., England.

† 2-(4-*t*-butylphenyl)-5-(4''-biphenyl)-1,3,4-oxadiazole, Ciba Chemicals, Duxford, Cambs., England.

§ Packard Instrument Co. Ltd., York House, Empire Way, Wembley, Middlesex, England.

|| Nuclear Enterprises Ltd., Edinburgh, Scotland.

Radiochemical standards were:

- (i) Phosphorus-32, supplied as a standardized solution of $\text{NaH}_2^{32}\text{PO}_4$ in water.
- (ii) Carbon-14, supplied as standardized n-hexadecane (carbon-14).

Dispensing techniques. Small quantities up to 1 ml were dispensed using Marburg micro-pipettes* with disposable tips. Quantities between 1 ml and 15 ml were dispensed with Aimer D-L Repeating Syringes†. The standard deviation on the reproducibility of these was within 1% of their calibrated volume.

Filtration equipment. Samples were vacuum filtered using Millipore § glass funnels and sinters. The glass funnels were coated with PTFE to minimize cross-contamination. Bacterial slimes, being mucilaginous, were filtered through Whatman GF/C discs; algae were filtered through 50 mm diameter 0.45 μm Millipore HA filters (cat. No. HAWP 04700).

Carbon-14 method for measuring primary production. The carbon-14 method for measuring primary production, developed by Steemann Nielsen,² involves adding a known quantity of $\text{NaH}^{14}\text{CO}_3$ to a water sample, followed by incubation and subsequent removal of the algae by membrane filtration for carbon-14 assay. Total carbon assimilation is calculated from the concentrations of carbon-14 fixed, carbon-14 added, and all forms of natural inorganic carbon dioxide present (measured by methods outlined by Golterman³). A small correction is necessary as carbon-14 is fixed at a slightly slower rate than natural carbon.^{2,4} In the method used at the Water Pollution Research Laboratory a 1.5 ml aliquot containing between 2 and 6 μCi of sterile $\text{NaH}^{14}\text{CO}_3$ is added to the sample (160 ml in a round-bottom glass-stoppered bottle) using a repeating syringe. A replicate aliquot of the $\text{NaH}^{14}\text{CO}_3$ is diluted to 250 ml with 0.01 M Na_2CO_3 solution, and 0.5 ml aliquots are removed for calibration using the methods described later.

ASSAY OF LIQUID SAMPLES

Liquid samples were assayed using liquid scintillation techniques for carbon-14 and Cerenkov counting for phosphorus-32.

Cerenkov counting of phosphorus-32 in aqueous solution

Cerenkov radiation is produced as a bluish light when β -particles of energy greater than about 300 keV travel through a medium such as water. Cerenkov radiation forms only a small proportion (less than 1%) of the total energy losses and thus its use as an assay technique is limited to those radionuclides which are high energy β -emitters (phosphorus-32 has E_{max} of 1.71 MeV) and has only been possible since the development of automatic liquid scintillation counting equipment capable of detecting very low intensity light pulses.^{5,6} As no scintillator is required and chemical (but not colour) quenching is absent, sample preparation is extremely simple and the technique is ideal for the assay of phosphorus-32 in aqueous solution.

Samples were assayed in 25 ml opaque polythene vials, which were found to yield a higher counting efficiency than low background glass vials (see Fig. 2), presumably owing to diffusion of the directional Cerenkov emissions. Efficiency of counting was very dependent on volume for both types of vials (Fig. 2), and in any series of samples it was therefore necessary to standardize the sample volume carefully. In most cases a total sample

* Eppendorff Co., imported by Anderman & Co., Tooley Street, London S.E.1, England.

† Aimer Products Ltd., 56-58 Rochester Place, Camden Road, London N.W.1, England.

§ Millipore (U.K.) Ltd., Heron House, 109 Wembley Hill Road, Wembley, Middlesex, England.

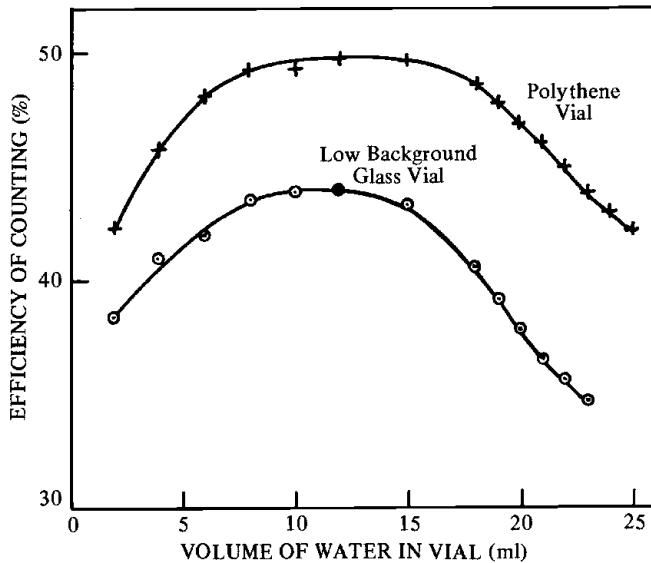


Fig. 2. Effect of sample volume on Cerenkov counting efficiency.

volume of 22 ml was used.

Counting efficiency for each series of samples was standardized by removing 2 ml aliquots for counting in a scintillator giving a known high and reproducible counting efficiency. For this purpose, KL 353 containing 6.3% ethyl alcohol, which raised the water capacity of the scintillator from 12% to over 20%, was used. Over the range 0 to 20% water the counting efficiency of this scintillator for phosphorus-32 was $98 \pm 0.5\%$ relative to RCC standard phosphorus-32 solution. Efficiency of Cerenkov counting 22 ml replicate samples was found to be $44.2 \pm 0.2\%$ by this means. Although this is much lower than the efficiency of liquid scintillation counting, the minimum detectable levels of activity are much less because the Cerenkov background activity is lower and the volume of the aqueous sample which can be counted is much larger. The comparison of performance is summarized in Table 3.

In experiments involving the assay of phosphorus-32 in natural waters by Cerenkov counting, various factors have to be considered, the most important of which are:

1. adsorption of phosphorus-32 onto vial walls;⁷
2. precipitation of $\text{Ca}_3(^{32}\text{PO}_4)_2$ from solution in hard natural waters;
3. microbial activity. (After sampling, phosphorus-32 can be rapidly removed from solution by micro-organisms in non-sterile samples; there may be uptake by organisms already present, or growth of micro-organisms on vial walls and in solution).

To control these factors, a stopping solution was added to the vials prior to sample collection; a solution containing EDTA to chelate Ca^{2+} ions, KH_2PO_4 to dilute $^{32}\text{PO}_4^{3-}$ and thus prevent its adsorption onto surfaces, and formalin to prevent uptake by micro-organisms was used for this. In most experiments sample volumes of 21 ml were added to

Table 3. Performance of counter for aqueous samples.

	Maximum sample volume (ml)	Counting efficiency (%)	Efficiency \times volume	Background (c.p.s.)	Minimum detectable activity (nCi/l) ^a
Liquid Scintillation	4	98	3.92	1.3	0.70
Cerenkov	20	44.2	8.80	0.25	0.12

^a 20 min counting period.

Table 4. Effectiveness of stopping solutions in Cerenkov counting of phosphorus-32.

Period from start	5 h	1 day	2 days	5 days
% activity retained in vial after washing	0.03 \pm 0.03	0.07 \pm 0.03	0.15 \pm 0.02	0.25 \pm 0.04
Activity in control vials (0 h = 100 \pm 2.6)	—	—	101 \pm 2.4	99 \pm 1.4

1 ml of stopping solution. Concentrations in the final stopped solution were: EDTA . 2Na, 2.74 g/l (to allow for a Ca²⁺ content of 300 mg/l in the sample); KH₂PO₄, 12.1 mg/l; and formalin, 1%. To determine the effectiveness of this solution, a series of vials was prepared containing these concentrations with a phosphorus-32 sample in local (Stevenage) hard water. At intervals of 5 h and 1, 2 and 5 days replicate samples were decanted and the vials were washed twice with tap water, filled with tap water, and recounted. Control samples without washing were counted at each interval. Results are shown in Table 4.

Carbon-14 assay by liquid scintillation

The method involves assay of 0.5 ml of a known dilution of the aqueous Na₂¹⁴CO₃ inoculum by homogeneous liquid scintillation counting using either the internal 'channels ratio' method^{1,8} or an internal standard to calibrate the efficiency of counting.

A channels ratio calibration was prepared by quenching samples containing known quantities of carbon-14 (as n-hexadecane) with increasing quantities of either acetone, water, or methyl acetate; Fig. 1 shows normal and quenched pulse spectra of carbon-14 in KL 353 and Fig. 3 shows the efficiency of counting in each of the channels C_I and C_{II} as a function of the ratio of the count rate in C_{II} to that in C_I. The curves were independent of the quenching or diluting agents, in agreement with the findings of Baillie⁸ and Bush.¹ Efficiency in C_I is a linear function of channels ratios over a wide range and is represented by the equation:

$$\text{Efficiency} = 103.5 - 85.0 \times \text{channels ratio}$$

This equation together with background values and counting times is programmed into a desk calculator (IME Digidorder) to facilitate data processing.

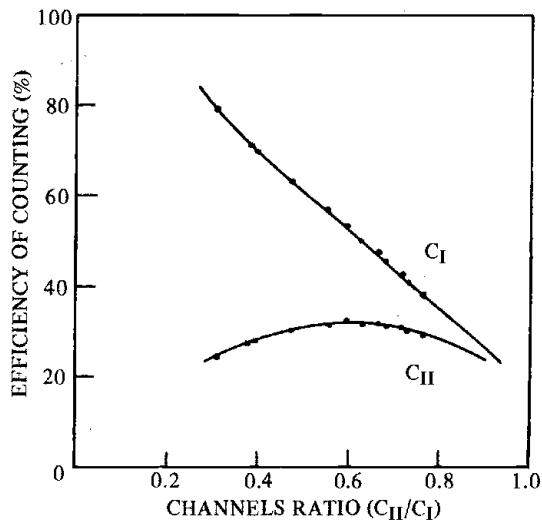


Fig. 3. Typical calibration of efficiency of counting against ratio of count rates in overlapping channels.

Table 5. Quenching of four similar samples of 10 ml KL 353 containing 0.5 ml water with standard n-hexadecane (carbon-14).

Sample	Efficiency of counting carbon-14 in aqueous solution (%)	Decrease in counting efficiency on adding 15 mg n-hexadecane (%)
1	67.7	4.2
2	72.0	2.5
3	69.5	1.3
4	68.5	2.2

It was found, using the channels ratio method, that small volumes of water and standard n-hexadecane, which cause little quenching in the dioxan scintillator when used separately, cause increased quenching when mixed. Table 5 shows that this change in overall efficiency is not easily quantified and can be greater than 4% (a relative change of 6%).

Thus, under these conditions n-hexadecane is not recommended as an internal standard for aqueous carbon-14 solutions, and the determination of counting efficiency by channels ratio gives more reproducible results. Table 6 shows the results of six determinations of a $\text{NaH}^{14}\text{CO}_3$ stock solution by this method.

LIQUID SCINTILLATION COUNTING OF BIOLOGICAL SAMPLES

Biological tissues have presented serious problems with respect to assay of radioactivity. The simplest method is to count the dried material using an end-window Geiger-Müller counter, but this suffers from inherent disadvantages such as self-absorption and geometry. Assay by liquid scintillation is a much preferred method and can be attempted

Table 6. Replicate determinations of the activity of a standard $\text{NaH}^{14}\text{CO}_3$ solution.

Sample	Weight of aliquot (mg)	Count rate (c.p.s.)	Counting efficiency by channels ratio (%)	Absolute activity of inoculum (disintegrations/s)
1A	597	319.5	63.0	2.12×10^5
1B	592	325.4	62.5	2.20×10^5
2A	594	315.6	62.7	2.12×10^5
2B	607	314.2	61.5	2.10×10^5
3A	595	317.9	62.5	2.14×10^5
3B	586	314.3	62.3	2.15×10^5

Mean activity of inoculum $(2.14 \pm 0.01) \times 10^5$ disintegrations/s.

by two general techniques:

1. solubilization of tissues, and
2. the production of suspensions (especially with unicellular algae etc.).

Tissue solubilization

Various solubilizing agents have been used in attempts to prepare toluene solutions of tissues, proteins etc. Hyamine hydroxide, a lipophilic quaternary nitrogen base, was the first such agent to be used.⁹ It could dissolve various biological materials, even those with a high protein content, but was ineffective for dissolution of bacterial cell walls.¹⁰ Bacterial slimes are frequently encountered in studies of river pollution and a method for their solubilization into scintillator solutions would have wide application. Screening tests were therefore carried out on a number of possible solubilizing agents including a recently marketed product 'Soluene 100', using a range of aquatic plant materials and bacterial slimes.

Materials were added in the proportion of 1 ml solubilizing agent to 100 mg fresh weight of tissue (washed, blotted, and air dried) or the corresponding dry weight (10 to 25 mg depending on the ratio of dry to fresh weight of the particular tissue). Formamide was included in the screening test in the proportion of 10 ml/100 mg fresh weight or corresponding dry weight because it has been found to solubilize certain bacterial cells.¹¹ The results are shown in Table 7.

Soluene 100 is obviously the best general solubilizing agent for the range of tissues used, and for the bacterial slime it was the only one to be at all effective.

A mixture of Soluene-dissolved tissues with scintillator resulted in a yellow colouration and a very high count rate in the sample regardless of the presence of any radioactive isotope. This count rate decreased on removal from light, but approached a constant value much higher than could be attributed to radioactive emission (see Fig. 4). This phenomenon of chemiluminescence* has been observed with hyamine solutions of biological tissues.¹² Part of the effect may be due to the presence of biological tissue (Debye and Edwards¹³ found that proteins in alkaline solution exhibited chemiluminescence which they attributed to tyrosine and tryptophan), but a mixture of solubilizer and scintillator alone also results in some chemiluminescence. Dunn¹⁴ noted the interaction of Butyl-

* Probably a combination of chemiluminescence and phosphorescence.

Table 7. Effect of solubilizing agents.

Tissue material	Solvent	Solution of fresh material		Solution of dried material	
		(a)	(b)	(a)	(b)
Filamentous alga (<i>Cladophora</i>)	NE 520	None	None	None	None
	Hyamine	+	+	+	+
	Soluene	+	++	+	+
	Formamide	None		None	
Rooted plant (<i>Potamogeton</i>) leaves	NE 520	None	None	None	None
	Hyamine	+	+	None	None
	Soluene	+++	+++	None	None
	Formamide	None		None	
Rooted plant (<i>Potamogeton</i>) stems	NE 520	None	None	None	None
	Hyamine	+++	+++	+	+
	Soluene	+++	++++	+	+
	Formamide	None		None	
Bacterial slime (sewage fungus)	NE 520	None	None	None	None
	Hyamine	+	+++	+	+
	Soluene	+++	++++	++++	++++
	Formamide	None		None	

Degrees of solution: + (little);

++ (approximately 50%);

+++ (almost complete);

++++ (complete).

Treatment (a) involved heating at 60° for 12 h.

Treatment (b) involved treatment (a) followed by addition of 0.1 ml concentrated hydrochloric acid; 10 ml of scintillator was then added and the mixture immersed in the ultrasonic bath for 20 min.

Treatment (b) was not applied to formamide.

PBD scintillator and Soluene 100 and concluded that their use in combination was not possible. However, it was found in the present work that complete instantaneous quenching of this chemiluminescence could be achieved by the addition of 0.1 ml concentrated hydrochloric acid. This resulted in a slight phase separation but there appeared to be no coprecipitation of phosphorus-32 when this occurred. When phosphorus-32 was added to the Soluene-scintillator samples recovery of radioactivity was 99±1%, whether the phosphorus-32 was added before or after admixture of the acid with scintillator.

Counting of high activity (several hundred c.p.s.) phosphorus-32 slime samples by the end-window Geiger-Müller counter followed by Soluene liquid scintillation counting showed both techniques to be very reproducible; assay by Geiger counter was 23.1±2.4% of that achieved by liquid scintillation (98±0.5% efficiency). However, at lower levels there was less reproducibility, presumably owing to errors in the Geiger counting (self-absorption of phosphorus-32 and also adsorption of phosphorus-32 onto the glass fibre discs onto which the slime was filtered). At very high activities the dead time of the Geiger counter (300 μs) had to be taken into account.

Assay of algal suspensions

Recent workers have used liquid scintillation assay to determine carbon-14 in algae

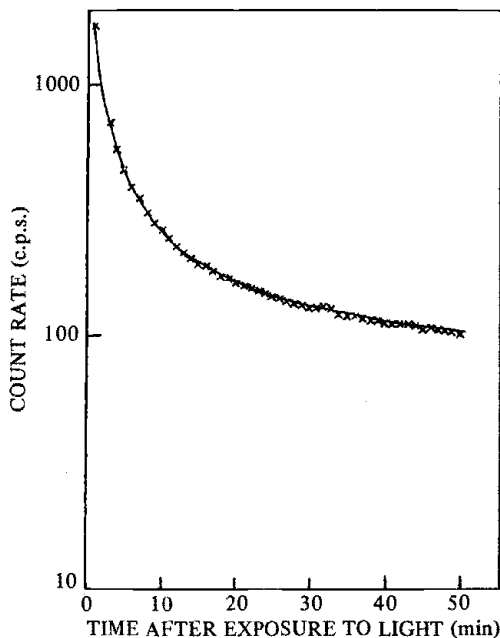


Fig. 4. Chemiluminescence and/or phosphorescence produced by Soluene, slime, and scintillator, and its partial decay on removal from light. True sample count rate after quenching with acid, 1.5 c.p.s.

on a membrane filter by two principal methods. In the first, the membrane is added to a counting vial containing scintillator (toluene based), which renders the membrane transparent. It has been claimed^{15,16} that the algae are counted on the membrane with the same efficiency as carbon-14 in the liquid phase, but Pugh¹⁷ showed that, in certain cases, this was not so, and that a correction for self-absorption, using the channels ratio, was required. In the second method, the algae are suspended directly in scintillator by dissolving the membrane. Krishnamoorthy and Vaswanathan¹⁸ used acetone to dissolve the membranes which were then mixed with scintillator. Schindler¹⁹ found that damp (but not dry) membrane filters were directly soluble in dioxan-based scintillators leaving the algae in suspension, but in his use of internal standardization with soluble standard toluene (carbon-14) it is not clear how the efficiency of counting of particulate carbon-14 is obtained.

Although it has been shown²⁰ that self-absorption can be neglected when assaying suspensions of algae, Hayes²¹ showed that when self-absorption did occur, the lower energy region of the pulse spectrum was degraded first (*cf.* quenching where the higher energy region is degraded preferentially). Therefore it is to be expected that the channels ratios, outlined earlier, will be increased by quenching and decreased by self-absorption.

For the assay of phosphorus-32 and other high energy β -emitters in algae, the simple suspension technique of Schindler¹⁸ can be recommended, using KL 353 rendered thixotropic with an equal volume of Cabosil. No self-absorption should occur and efficiencies of at least 95% should be obtained.

Table 8. Effects of shaking on the counting properties of algal suspensions.

Sample	Method of shaking	Change in count rate caused by shaking (%)
<i>Microcystis</i> suspensions	Mechanical	+3.3±0.5
<i>Microcystis</i> suspensions	Ultrasonic	+20.6±0.9
Soluble carbon-14 with non-radioactive <i>Microcystis</i> suspension	Ultrasonic	-1.9

To study the situation with carbon-14, a suspension of ^{14}C -labelled *Microcystis* (a blue-green alga forming microscopic gelatinous colonies) was formed in KL 353 as follows. The algae were filtered onto a membrane; this was then dissolved in 4 ml of non-quenching methyl acetate leaving a suspension of the colonies which was mixed with 10 ml of KL 353. To prevent settling (which is very marked with algae in liquid scintillators) the suspension was rendered thixotropic by the addition of 2 ml of 15% w/v Cabosil in KL 353. The suspension was counted in the two channels C_I and C_{II} before and after the addition of n-hexadecane internal standard. The channels ratio of the suspended carbon-14 at 0.67 was almost 0.10 higher than that of the soluble carbon-14, implying that quenching processes specific to suspended carbon-14 predominated over self-absorption.

Further samples of *Microcystis* colonies labelled with carbon-14 were suspended in scintillator and counted in the two channels before and after mechanical shaking, and after varying intervals of immersion in the ultrasonic cleaning bath. Table 8 shows that, in contrast to mechanical shaking, ultrasonic shaking had a marked effect on the count rate, raising this by about 20%.

The optimum time of shaking was about 25 min, after which there was no significant increase in count rate or efficiency. After this period it was found that the colonies had been broken up into individual cells of less than 5 μm diameter; these should have no self-absorption.²² It was then confirmed, by the addition of internal standards, that soluble and particulate carbon-14 fractions were being counted with the same efficiencies; from Fig. 3, the values of the channels ratios were used to calculate the absolute activity of the algae, and the efficiency of counting as a function of time of shaking was plotted (Fig. 5). The slight decrease in solution counting efficiency shown in Table 8 presumably results from the increased opacity of the fine suspension.

Using the calculated absolute activity of the algae, the efficiency of algal suspension counting as a function of channels ratios was then plotted (see Fig. 6). This shows the slope of the line to be almost twice that for chemical quenching, and similar to the colour quenching curve of Bush,¹ and to that for algae attached to membranes.¹⁷ It can be concluded that the loss in count rate and counting efficiency, and the anomalous channels ratios for these algal suspensions, are probably the result of very localized colour quenching, i.e. the very close proximity of a scintillation to a large 'black' particle of algae may result in absorption of up to half of the emitted light energy. This effect can be eliminated by ultrasonic shaking, and then the normal chemical quench correction curve can be used. Self-absorption from individual algal cells should be negligible; however, if its occurrence is suspected the effect can be decreased by discriminating against the lower pulses at the

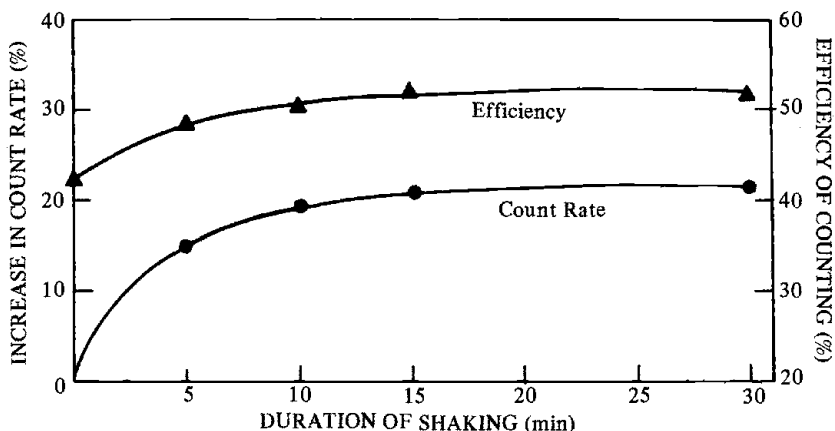


Fig. 5. Effect of ultrasonic shaking on the counting of carbon-14 in algal suspensions.

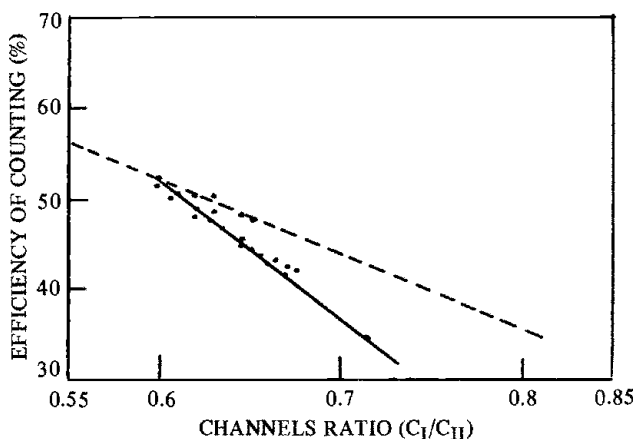


Fig. 6. Efficiency of counting of suspensions of *Microcystis* as a function of channels ratio. Solid line — particulate carbon-14 quench curve; broken line — chemical quench curve from Fig. 3.

expense of a slight decrease in counting efficiency.

For routine measurements, the dampened membrane is placed in a counting vial containing 10 ml of KL 353 rendered thixotropic with an equal volume of Cabosil. This is followed by immersion for 25 min in the ultrasonic bath. The vial is then assayed by counting the carbon-14 in the two channels.

APPLICATION AND CONCLUSIONS

Cerenkov counting of phosphorus-32 has been used to obtain quantitative estimates of biological slime growths. Initial uptake of phosphorus from water is primarily due to

the micro-organisms present. In an organically polluted reach of water the micro-organisms are almost entirely attached heterotrophs (i.e. slime organisms) and thus uptake of phosphorus (or phosphorus-32) gives a measure of slime biomass. This method was used to measure slime biomass in a polluted effluent channel. Liquid scintillation techniques have been used to assay carbon-14 in primary productivity studies on a multi-purpose water-supply reservoir subject to periodic blooms of algae.

The techniques described should prove suitable for other similar studies of production and biomass. Cerenkov counting especially, with the advantages of high sensitivity and little sample preparation, seems well suited to studies in aquatic systems. Because of the key role of phosphorus in biological systems, phosphorus-32 lends itself to the study of a variety of problems, but chlorine-36 and krypton-85 are other available isotopes which should prove amenable to Cerenkov counting.

ACKNOWLEDGEMENTS

The authors wish to thank Miss Angela Smith for sample preparation and processing of results. Crown copyright. Reproduced by permission of the Controller of H.M. Stationery Office.

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DISCUSSION

B. E. Gordon: How did you establish that the disappearance of $^{32}\text{PO}_4$ from the polluted channel was due only to slime uptake and not for example due to precipitation of salts?

E. J. C. Curtis: Samples of slimes, surface sediments and aquatic rooted plants were removed for assay from the whole length of the channel. From these data it was calculated that, of the total phosphorus-32 removed, less than 4% was present in rooted plants and sediments (which would include any precipitated salts).