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Calibration of activity measurements in primary productivity studies using Geiger-Müller and liquid scintillation counters

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Abstract

The calibration of primary productivity determination using the ^{14}C method of Steemann Nielsen (1952) has been studied in detail using both the geiger counter and the liquid scintillation counter.

It is shown that the traditional ^{14}C-$BaCO_3$ method of assaying the ampoule activity will lead to errenous result, both because of uncertainty in extrapolation and because the phythoplankton activity does not lie on the surface of the filter but penetrates into it to a considerable degree. The apparent extrapolated efficiency of the ^{14}C-$BaCO_3$ precipitates was found to be 17.1%, the efficiency of a thin layer of ^{14}C-acryl film 13.1% and the counting efficiency of the phytoplankton filters 9-12%, depending on the degree of penetration.

Finally the merits of the geiger counting of the filters and liquid scintillation counting for primary productivity determination is discussed.

1. Introduction

The measurement of the primary productivity using the ^{14}C method of Steemann Nielsen (1952) can lead to an underestimation for several reasons. Some of the interfering factors can be evaded by using the scintillation counter instead of a geiger or a proportional counter for assaying the ^{14}C radioactivity. The majority of workers in this field are therefore now using the liquid scintillation counter.

Better knowledge of the factors interfering with the assay of the radioactivity on the filters when the geiger counter is used is, however, of considerable interest for two reasons: 1) older primary productivity determinations can then be compared to more recent results obtained with a liquid scintillation counter with greater confidence, and 2) it will allow a more reliable choice between the geiger counter and the liquid scintillation counter.

In the ^{14}C-$BaCO_3$ method of calibration it has been assumed that the phytoplankton cells are lying intact on the surface of the filters and self-absorption in the sample will therefore be negligable (zero thickness assumption). The present work started with a study of this assumption and it was proved that it was far from being correct (Theodorsson 1974). The result of this work will be given below. In continuation of this the calibration of the radioactivity measurements was studied in detail, using both the geiger counter and liquid scintillation counter.

The results obtained allow a better comparison to be made between these two counting methods. The choice of detector in ^{14}C primary productivity measurements is therefore discussed at the end of the paper.

2. Methods

Primary productivity measurements have been made in a conventional way. 4 μCi of ^{14}C-$NaHCO_3$ is added to 70 ml of sea-water and the sample is then incubated for 4 hours. Afterwards the sample is filtered with a membrane filter (active diameter 20 mm) with a pore diameter of 0.22μ.

A flow-counter working in the geiger region has been used to measure the filters. It has a mylar window with a thicness of 1 mg/cm^2

and a window diameter of 25 mm.

The filters have further been measured with a liquid scintillation counter. The filters are solubilized in 2 ml of ethyleneglycol-monomethylether and 5 ml of Instagel fluor (Packard) is added.

The activity of the ampoules was measured by diluting one ampoule to 100 ml with a buffer solution and taking one ml of this solution with 1-4 ml of inactive buffer solution and then add 5 ml of Instagel to this. Adding 3 ml of the inactive buffer solution gave the most reproducible results and stable counting rate so this was used for all further measurements. The efficiency of individual samples was always measured with the channel ratio method and the counting efficiency was further measured for a number of samples with the internal standard method.

3. Penetration of phytoplankton activity into filters

In the ^{14}C-$BaCO_3$ method of calibrating the activity measurements it has always been assumed that the newly assimilated organic matter is lying intact in a thin layer on the surface of the filter and that the self-absorption is therefore negligable. The validity of this assumption was studied by counting the filters from both sides, and this will be referred to as the double side counting method.

The filters are first counted in the conventional way with the front side facing the window, then with the backside. The ratio of the second counting rate (B) to the first (F) is called the back to front ratio , or B:F ratio.

If the ^{14}C activity is all lying in a thin layer on the surface of the filter the back counting rate will always be the same fraction of the front counting rate, determined by the thicness of the filter which is assumed to be the same for all filters. The filters used in this study were selected within the range of 5.4 ± 0.2 mg/cm^2 and the B:F ratio will be 0.19 ± 0.01. If the activity has, however, penetrated into the filter to some degree the front counting rate will be decreased because of absorption in the surface layer of the filter and the back counting rate increased as compared to an active layer on the surface of the filter. The B:F ratio will therefore be increased.

The B:F ratio of about 150 filters has been measured. About half of these filters have been taken from a stock of filters that have been used to measure the primary productivity in the sea around Iceland, the rest of the filters comes from a study of various factors that affect the activity measurement in the ^{14}C method. None of the filters show a B:F value of 0.19 as one would expect if the zero thickness assumption is correct, but they lie in the range of 0.24 to 0.64 (Fig. 1). This proves that the phytoplankton activity penetrates to a considerable degree into the filters.

In order to study the penetration by an independent method the filters were also measured in the liquid scintillation counter after having been solubilized in 2 ml of ethyleneglycol-monomethylether. The counting rate will here be proportional to the activity of the filter, independent of the penetration.

The counting efficiency of individual filters in the liquid scintillation counter is found by the channel ratio method and it lies in the range 88-92%. The efficiency of the geiger counter with the front side of the filter facing the window can now be calculated. If the activity of the plankton is lying on the surface of the filter the geiger counting efficiency will be the same for all the filters. If the activity has, however, penetrated into the filter the geiger counting efficiency will be decreased, and the more the deeper the penetration is.

The geiger counting efficiency of individual filters varies considerably and lies in the range of about 9-12%. This confirms that the activity has penetrated into the filter. The geiger counting efficiency of all filters has been plotted against the B:F ratio in Fig. 1. As is to be expected there is a close correlation between the two values, the efficiency decreasing with increasing B:F value (increasing penetration).

4. The ^{14}C-$BaCO_3$ calibration

The uncertainty of this method lies both in the difficulty in precipitating very thin samples of the $BaCO_3$ and in extra-

polating to zero thickness as this must be done empirically. Mathematical models that have been used in the extrapolation have in reality little to offer over straight visual extrapolation.

A procedure recommended by The International Agency for ^{14}C Determination has been followed in making the precipitated samples. Until 1972 the calibration was made by precipitating samples in the range of 1-5 mg/cm^2 and plotting the logarithm of the counting rate against the thickness of the samples (in mg/cm^2) and then draw a straight line visually through the points. A typical self-absorption curve is shown in Fig. 2. It was estimated that the extrapolated value was reproduceable to about 5% if the precipitation was repeted in exactly the same manner. The extrapolated counting efficiency calculated from the result will be discussed in section 7.

Two years ago the calibration was studied in some more detail. ^{14}C-$BaCO_3$ samples of varying thickness were made with great care. As earlier it was found impossible to get reproducible results for thin samples (> 2mg/cm^2). The absorption curve fitted rather well a similar curve taken at The International Agency for ^{14}C determination in may 1964. It was therefore decided to fit the Copenhagen curve to our data and then apply the same correction as Steemann Nielsen (1964) had found necessary in his biological method of calibration.

Having fixed the absorption curve a number of ampoules were measured by precipitating a number of samples with a thicness of 6.0 mg/cm^2 and 0.46 devided to this value in order to calculate the extrapolated counting rate. Finally this value was reduced by 31% before the primary productivity was calculated. This extrapolated value was reproducible to about 1.5%.

Although the extrapolated counting rate is reproducible to this accuracy and the relative activity of the various ampoules can be compared with this precicion the true thin layer counting efficiency may deviate 15-25% from this value because of the uncertainty in the extrapolation.

5. Thin layer efficiency

The thin layer efficiency was measured by a method deviced by Jitts and Scott (1961). ^{14}C-acryl is dissolved in chloroform and a very thin film is made by letting a drop of this solution spread

on the surface of water. The film is then lifted out of the water with a membrane filter which is afterwards dried and trimmed.

The filter is then measured under the same counting conditions with the geiger counter as well as the liquid scintillation counter. 12 such filters were measured and the result of the measurements is shown in Table 1 and the efficiency is shown versus the B:F value on Fig. 1. This point is in very good agreement with the measuring points of the plankton filters.

6. Assay of ampoule activity

The activity of the ampoules was assayed by counting a known fraction of an ampoule in a buffer solution and with Instagel fluor as described in section 2. The six batches that had been measured with the ^{14}C-$BaCO_3$ method in 1972 were assayed with this technique as well as two older batches and one recent.

The aquaeous buffer solution with the ^{14}C forms a gel solution with the Instagel and the ^{14}C-$NaHCO_3$ is in the water phase but the ^{14}C-toluene used for internal standardization in the Instagel phase. The counting efficiency of the ^{14}C should be the same for both phases but in order to check this the ^{14}C was assayed in a different way, where the conting solution is in one phase, in order to confirm the result. The ^{14}C-CO_2 from 0.5 ml of the 100 ml ampoule solution was driven off in a closed vessel (counting vial) by adding 0.2 ml of 0.1 N H_2SO_4 and the ^{14}C-CO_2 absorbed in 0.5 ml of Hyamine. The Hyamine was then counted with 5 ml of Instagel and the counting efficiency found with an internal standard. The result of the assay of the ampoule activity of both methods is shown in Table 2.

7. Comparison of ampoule activity assayed with geiger counter and liquid scintillation counter

Nine different batches from The International Agency for ^{14}C determination were measured in 5 ml buffer solution and 5 ml Instagel. Six of these were also measured by the ^{14}C-$BaCO_3$ method. From these results one can compare these two methods. The ratio of the two counting rates has therefor been calculated (Table 3). The inner

consistency of these two sets of measurements can be seen from the constancy of the ratio where the standard deviation from the mean of the six measurements is only 1.7%.

The apparent counting efficiency corresponding to the extrapolated counting rate of the ^{14}C-$BaCO_3$ precipitated samples measured with the geiger counter can now be calculated and the result is 17.1%. It should be remembered that the extrapolated counting rate was reduced by 31% before it was used to calculate the primary productivity, and this brings the counting efficiency down to 11,8 %, a value that will give correct value for the primary productivity for filters with shallow penetration of the activity.

These values are plotted on Fig. 1 at a B:F value of 0.193, corresponding to a thin layer on the filter used.

These results show clearly that neither the original $BaCO_3$ method nor the thin film method will lead to correct primary productivity figures. If the geiger counter is used for assaying the radioactivity of the filters the metod should be calibrated with a liquid scintillation counter by measuring the efficiency of the plankton filters and the disintegration rate of the ampoule activity as described here.

8. Comparison of the liquid scintillation counter and geiger counter for primary productivity determinations

Before discussing the relative merits of the two methods used here for determining the radioactivity a short review will be given of the sources of error that are maintained to interfere with the mesurement of radioactivity of the phytoplankton cells. These are the following:

1. Soluble organic compounds that are excreted from the cells during illumination as a part of their normal metabolism and will pass through the filter.

2. The first 5-10 ml filtered will according to Arthur and Rigler (1967) give higher counting rate per ml.

3. Loss of ^{14}C activity during drying of the filter (Wallen and Geen 1968, Nakanishi and Ward 1971).

4. Retention by filters of inorganic ^{14}C carbonate (McMahon 1973).

5. Penetration of algal activity into the filters.

6. Uncertainty in ^{14}C-$BaCO_3$ calibration.

The influence of these interfering factors on geiger and liquid scintillation assay of the radioactivity will now be discussed in the same order.

1. Neither method will detect this part of the activity. This is really not an error in the method, rather a different property is being measured.

2. According to Arthur and Rigler (1967) the filter will retain more activity per ml from the first 10-20 ml of sample being filtered. This phenomen has not yet been studied in detail. Personally I am of the opinion that the zero volume extrapolated value of Arthur and Rigler does not lead to a correct value for the primary productivity. This conclusion is based on comparative measurements made with the acidification and bubbling method of Schindler et al. (1972). In any case this interfering factor will have the same effect on both methods of counting the filters.

3. This loss has not either been studied yet in detail. Work at my laboratory shows that for our sea water samples show only an insignificant (about 2%) difference between wet and dry filters assayed with the liquid scintillation counter is found (Theodoorsson and Bjarnason 1974). According to Steemann Nielsen (1974) this loss can be eliminated by rapid drying of the filters. This is therefore probably not a serious factor for the geiger method.

4. This phenomen has not been studied in any detail and my experience does not indicate that this is normally of any importance. This source of error would affect both counting methods in the same way.

5. The penetration of the organic matter into the filters will only affect the geiger counting rate where a deviation of about 15% to either side of the correct primary productivity value can be expected. This error can, however, for large part be corrected for by counting the filters from both sides.

6. It is clear from the results that have been presented in this paper that the ^{14}C-$BaCO_3$ method of calibration should not be used. If a geiger counter is used for primary productivity determination it should be calibrated by counting in a liquid scintillation counter a number of normal phytoplankton filters that have already been measured with a geiger counter, preferably from both sides. The ampoule activity should then be assayed as has been described in this paper or by a similar method. The calibration of the geiger method and liquid scintillation method is therefore quite similar.

If the supplier of the ampouls gives the disintegration rate of the ampoules the efficiency of the geiger counter needed only be measured once. A ^{14}C-acryl standard would then be used to correct for minor variations in the efficiency of the geiger counter. The efficiency of the liquid scintillation counter must be monitored in a similar way.

From the above discussion it can be seen that there is little reason to prefere one method over the other as regards accuracy. Other factors are of more importance.

The liquid scintillation counter has the very important advantage that it is a powerful tool in modern biological research. The liquid scintillation counter can usually handle 200-400 samples automatically and it can in most cases also be used for much other work.

The geiger counter has much more limited use and in most cases it would not be used much for other work. It has, however, the advantage that the counting equipment is much simpler and less expensive. The inherent simplicity of the geiger counter is far from being fully exploited in modern equipment. The author is at present working on the development of a modified type of flow conter, a multicounter, that should be well suited for this type of work. Four counter elements will be built into a single plate of perspex and a single guard counter will eventually reduce the background by a factor of 5-10. Two multicounters can be used with the same guard counter. Modern integrated circuits can make such multicounters very attractive. The whole system can be made so compact that it could rather

easily be taken from the laboratory to a research vessel when immediate results of primary productivity determinations are desirable. A rather loose comparison will finally be made of such counting systems of multicounters with a conventional liquid scintillation counter

	Number of detectors	Background cpm	Counting efficiency	Price	Price per 1000 samples
LSC	1	18	90%	$10000	$100
Geiger	4-8	1-2	25%	$ 5000	Nil

This table could serve as a reminder that the geiger counter might still be suitable for some applications.

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TABLE I

Determination of thin layer counting efficiency of geiger counter with ^{14}C-acryl film

Number of samples	14
Geiger counting efficiency	0.132
Standard deviation from mean	0.001
B:F mean value measured	0.207
B:F value calculated from mean thickness of filters	0.197

TABLE II

Assey of ampoule activity of batch C-294 from The Internationa Agency for ^{14}C Determination

	Activity of ampoule 10^6 dis/min	Number of samples counted	Standard deviation
^{14}C-CO_2 absorbed in Hyamine	6.82	8	1.1%
Aquaeous buffer solution	6.81	14	1.1%

TABLE III

Assay of ampoule activity of batches from The International Agency for ^{14}C Determination and geiger counting efficiency of ^{14}C-$BaCO_3$ precipitaions (zero thickness extrapoæated value).

Batch	LSC 10^6 dis/min	Geiger 10^6 c/min	LSC/geiger
C-112	7.66		
C-133	8.06		
C-143	8.21		
C-294	6.82		
C-153	8.82	1.446	6.10
C-170	5.36	0.922	5.81
C-204	8.22	1.385	5.96
C-225	8.72	1.491	5.85
C-249	7.43	1.247	5.95
C-250	7.72	1.305	5.93
MEAN VALUE			5.93
Standard deviation from mean			1.6%
Geiger extrapolated ^{14}C-$BaCO_3$ conting eff.			17.1%

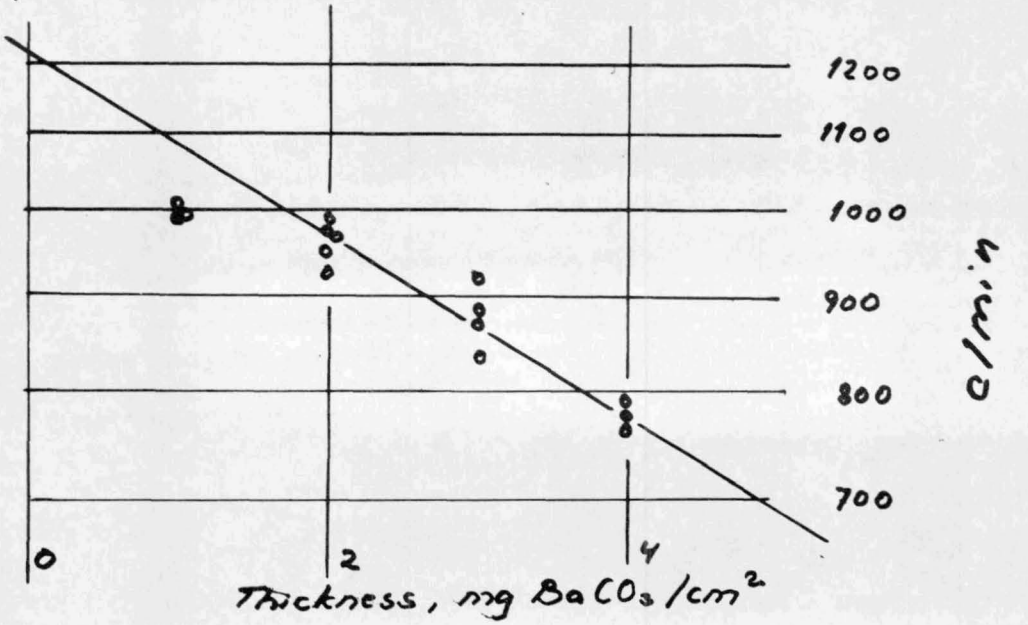

Fig.1 ^{14}C-$BaCO_3$ self absorption curve of batch 143 with straight line extrapolation

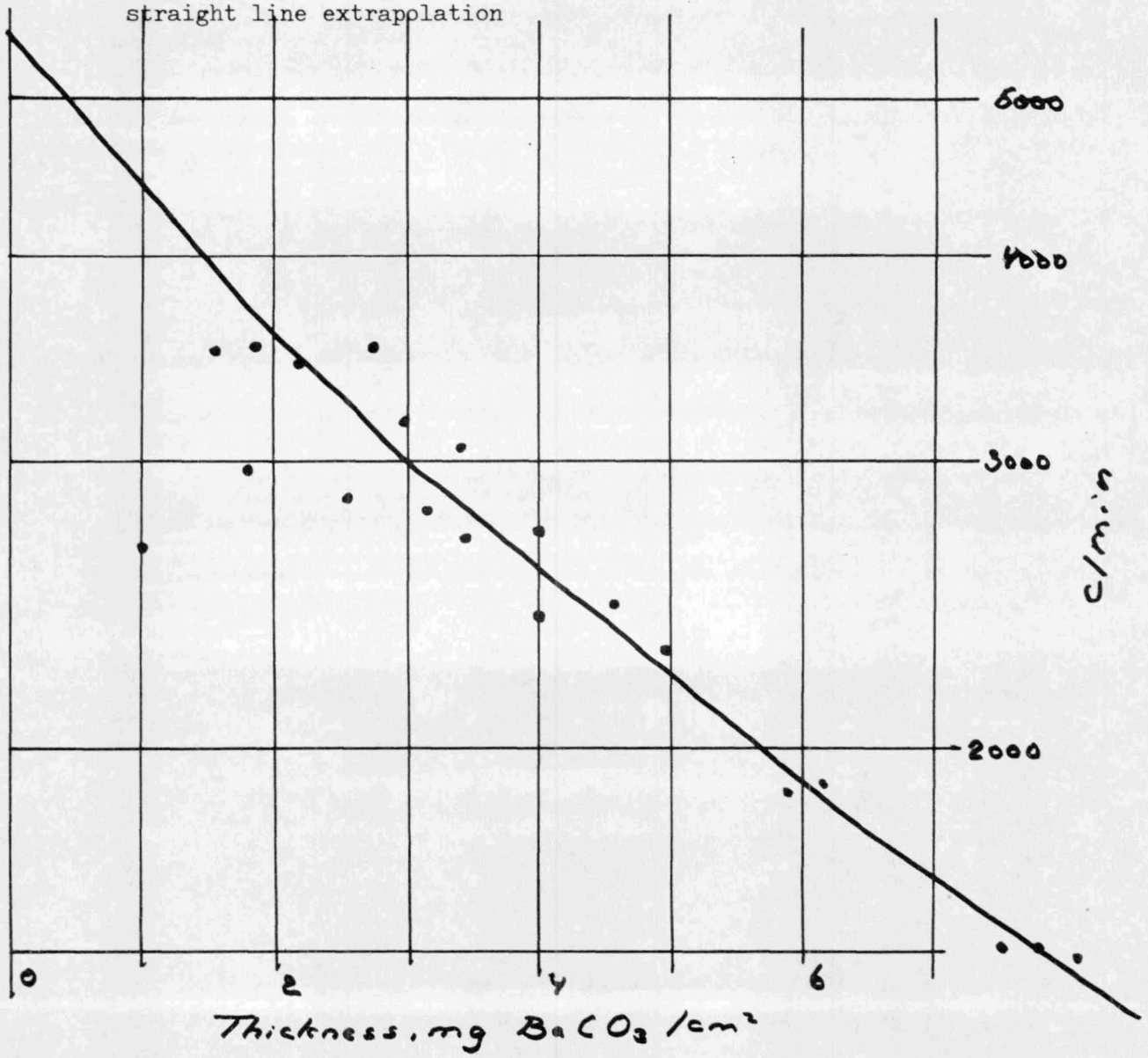

Fig. 2 ^{14}C-$BaCO_3$ self absorption curve of batch C204. Curved zero thickness extrapolation line.

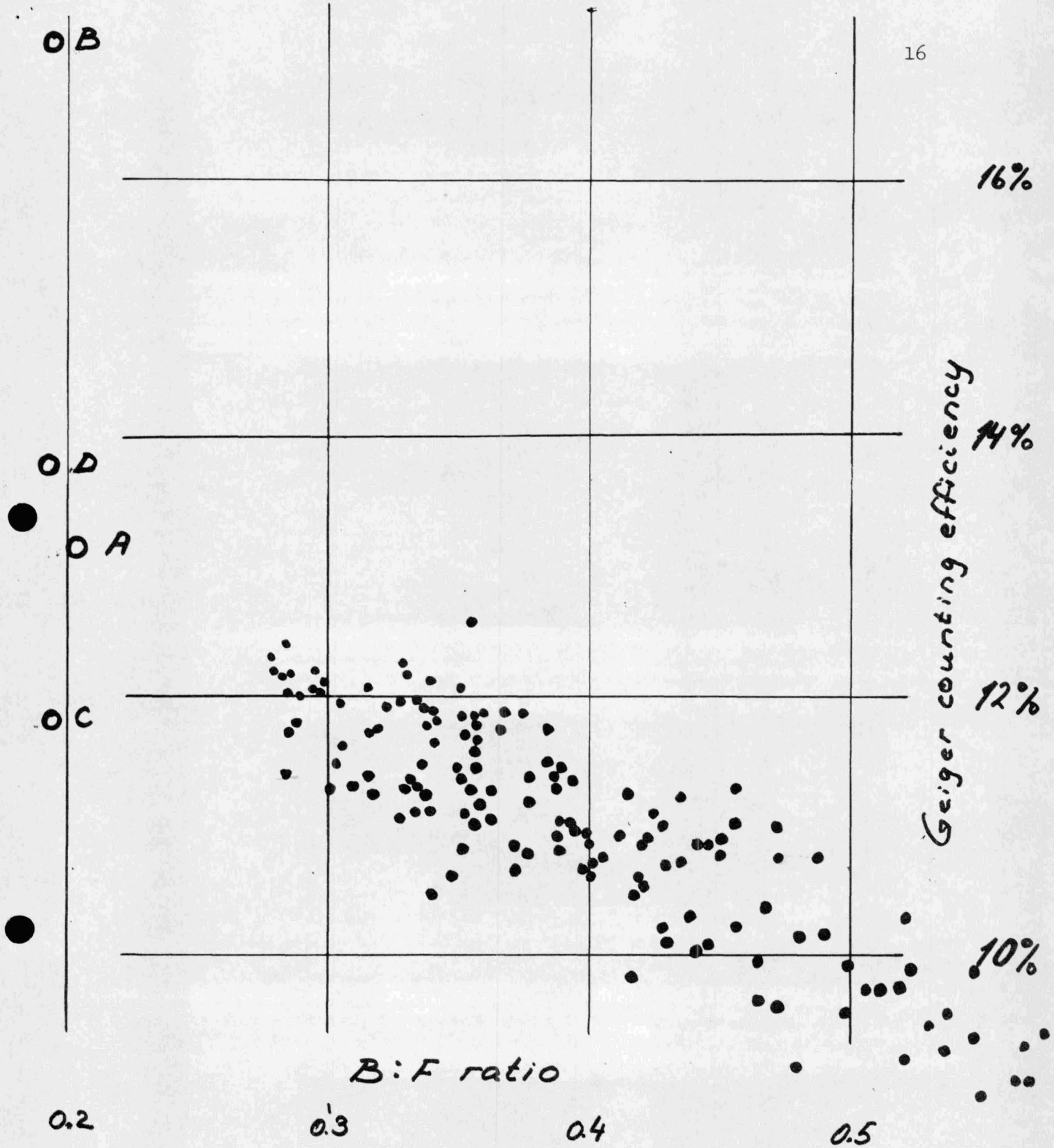

Fig. 3

Geiger counting efficiency versus B:F value. Filled circles: Algal filters. Open circles: A: thin layer ^{14}C-acryl film. B: ^{14}C-$BaCO_3$ extrapolated by curved line (Fig 2). C: B value reduced by 31%. D: ^{14}C-$BaCO_3$ extrapolated by straight line (Fig. 1).