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A METHOD FOR THE DETERMINATION OF CHLOROPHYLL IN SEA-WATER

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SUMMARY

Sampling and analytical methods for the spectrophotometric determination of chlorophyll a in sea-water are given. A cheap and easily constructed water sampler is described. It consists of a perspex tube which can be closed at either end by rubber flaps which are joined internally by a rubber spring.

A small continuous centrifuge for the removal of suspended matter from sea-water is also described. This centrifuge operates with an r.c.f. of 5,000 and sea-water is passed through it at the rate of 4 l/hr. A test of the efficacy of the instrument using marine bacteria shows that this centrifuge will retain any organisms of an equivalent density above 0.5μ diameter.

90 per cent. acetone is the most suitable solvent for the extraction of chlorophyll a from plankton when extraction is carried out over a period of eighteen hours.

Some inadequacies of this and other methods of chlorophyll analysis are discussed.

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I. INTRODUCTION

The concentration of chlorophyll in sea-water has been used by several workers as an indication of the standing crop of plant material in the ocean. All marine plants, except the pigmented bacteria, possess chlorophyll *a* which is essential for photosynthesis in these organisms.

Over the last twenty years the original method developed by Harvey (1934) has been modified by other workers so that chlorophyll determinations might be made with increased accuracy and in absolute rather than arbitrary units.

The principal difficulties encountered in chlorophyll determinations are caused by the presence of other plant and animal pigments which are extracted from the plankton along with the chlorophyll, and the lability of chlorophyll itself. Chlorophyll is very readily broken down both in the plant as for example by enzymic decomposition by chlorophyllase, and after extraction by decomposition to phaeophytin should the pH fall below five.

The method described below was developed to assist in this Division's studies in marine productivity. An analytical method was required which would give an indication of the standing crop of plant material. In the waters studied, the concentration of chlorophyll *a* is often low (<0.02 mg/m^3) and it was desirable therefore to find a method which would be sensitive enough to enable the volume of the water sample to be kept within practicable limits.

II. METHODS

Sea-water samples are collected from the desired depth. They are returned to the laboratory where the particulate matter is removed by centrifugation. This particulate matter, which contains the phytoplankton of the original sea-water, is extracted with acetone, and the chlorophyll and other pigments are brought into solution. The concentration of chlorophyll in the acetone extract is then determined spectrophotometrically.

(a) Preparation of 90 per cent. Acetone

Technical grade acetone is stored in contact with powdered $MgCO_3$ until required. The acetone is then decanted off and dried by the addition of CaO. The absence of water is tested for by the addition of 10 ml of CS_2 to 2 ml of acetone. A clear solution should be produced. The acetone is then purified by distillation. For dilution,

100 ml of water are placed in a one litre volumetric flask and made up to the mark with acetone.

(b) Apparatus

(1) Water Sampler.- The water sampler consists of a piece of perspex tubing, 30 inches long and 3-3/4 inches in internal diameter, closed at either end by flaps which are joined internally by a rubber spring. The mechanism for clamping the sampler to a wire or rope and releasing the end flaps is mounted on the outside of the cylinder. It consists of a central rod connected to two trips which can be actuated by the usual type of Nansen bottle messenger. A second messenger may be hung from the lower trip to operate a second sampler. (Plate 1).

The sampler takes one gallon of water and this is in contact only with perspex and rubber. It is cheap, easy to construct and can be used to take water samples at any depth; it is attached either to a rope or to the wire used for hydrological sampling.

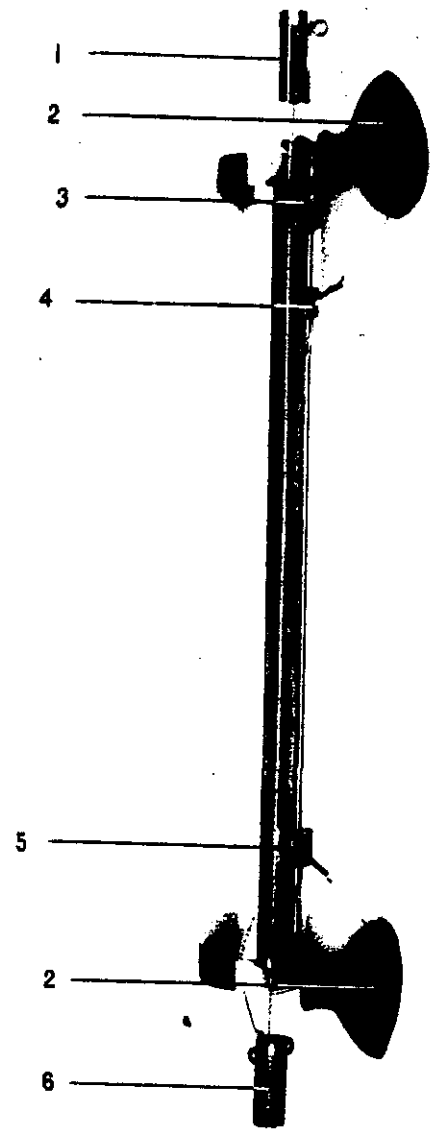
(2) Continuous Centrifuge.- Because of the low concentration of marine organisms which contain chlorophyll it is necessary to take large samples of sea-water and to separate the organisms from these samples. This is conveniently done by the use of a continuous centrifuge.

The continuous centrifuges used in these studies were made by adapting portable electric tool-post grinders. Two high-speed precision races were fitted to the centre spindle and housing. In the earlier model a water jacket was fitted so that cooling water could be circulated. In the later model, which is illustrated (Plate 2), it has been found more convenient to dispense with this and substitute a system of continuous drip feed lubrication.

The top of the spindle has been threaded to take the centrifuge cup and around this was secured a plastic shield with inlet and outflow openings. The centrifuge cup was turned from solid stainless steel. The internal measurements of the cup are.- height 1.389 in., diameter at top 1.527 in., diameter at base 1.822 in.

The water sample is run through the centrifuge at a rate of 4 l/hr. The relative centrifugal force (r.c.f.) of the instrument at 14,000 r.p.m. calculated on the bottom diameter is 5,000.

The efficacy of the centrifuge was demonstrated in the



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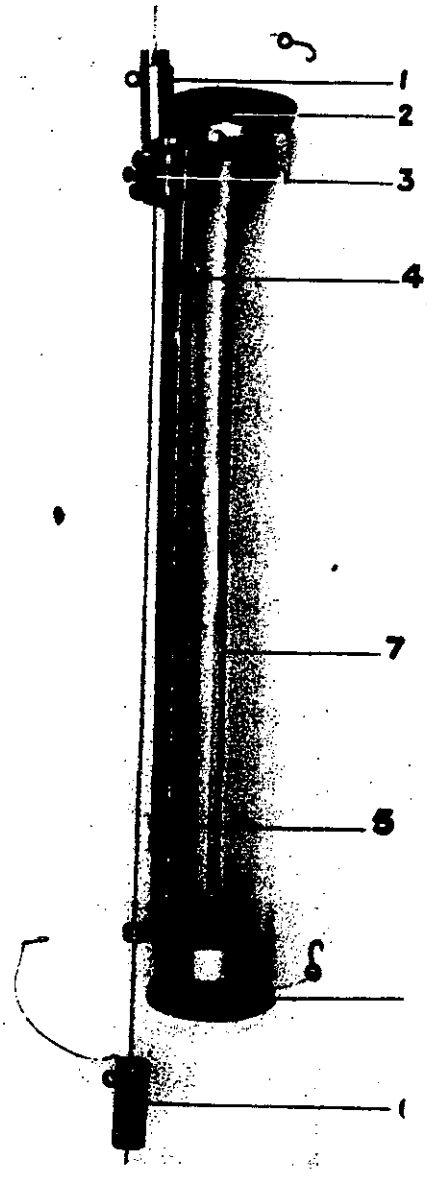

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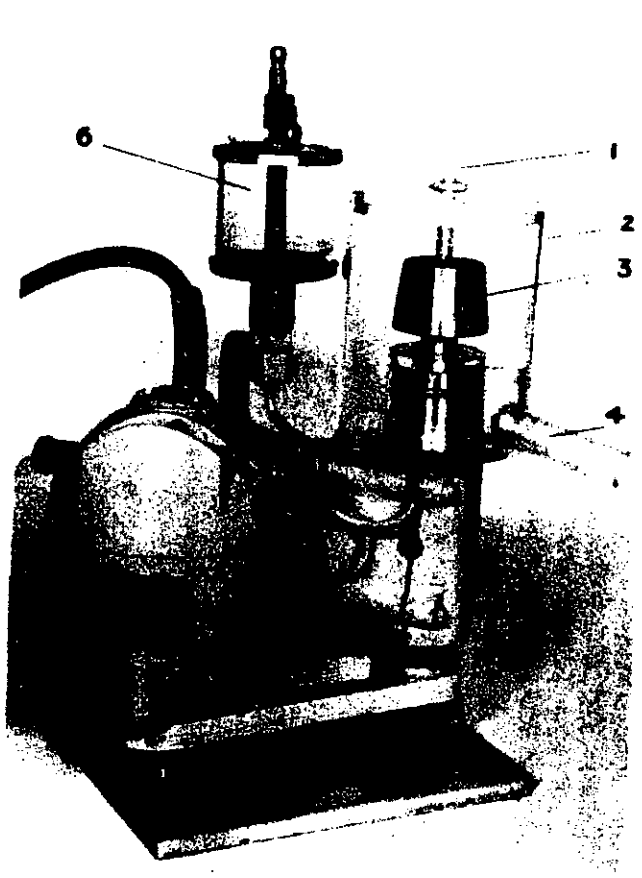
Plate 1: Plastic Water Sampler

1. Messenger
2. Rubber end flaps
3. Clamp for attaching sampler to wire
4. Upper trip
5. Lower trip
6. Second messenger
7. Rubber spring



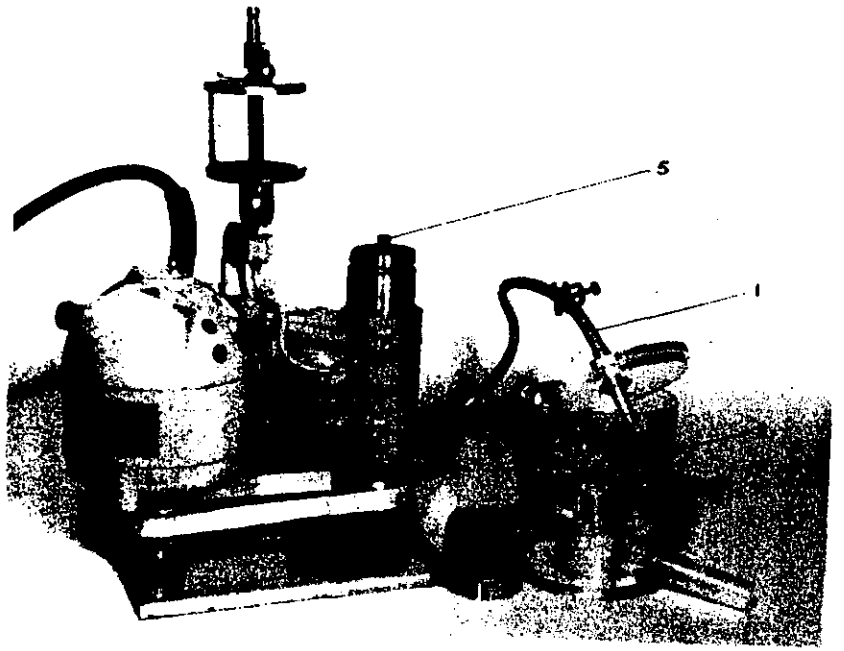


Plate 2: Continuous Centrifuge

1. Sample inlet
2. Plastic shield
3. Centrifuge cup
4. Overflow outlet
5. Spindle
6. Oil reservoir

following manner. A one litre mixed culture of marine bacteria, 0.5 - 1.0μ in diameter, growing in an enriched artificial sea-water medium, was run through the centrifuge under the conditions stated above. The concentration of the culture was 10^8 organisms per ml. Culture of the centrifugate on the same medium failed to give any growth of the test organisms. It is concluded that the centrifuge will retain any organisms of an equivalent density above 0.5μ diameter.

(c) Sampling and Analytical Procedures

One or two gallon water samples are brought to the surface in the water sampler. The water is poured into one gallon plastic bottles which are stored in the dark until they are returned to the laboratory.

Usually the centrifugation of the water samples is completed within thirty six hours of their collection.

In the laboratory the particulate matter is removed by the continuous centrifuge as described above. The compacted sediment and the last few ml of sample remaining in the bowl are transferred to a 25 ml graduated, round bottomed centrifuge tube. The bowl is then washed successively with small amounts of supernatant. The suspension is centrifuged and the clear supernatant is decanted off and discarded; the tubes are inverted and allowed to drain for fifteen minutes. Fifteen ml of 90 per cent. acetone are then added, the tubes are corked and stored in the dark without any subsequent shaking. The extraction is allowed to proceed for 12 - 18 hours. After centrifugation, the total volume of pigment solution is read off the graduated tube and sufficient of the supernatant extract is pipetted off and transferred to a 40 mm cuvette. The optical density at 665 mμ is then determined using a Unicam SP500 spectrophotometer. The maximum slit width used is 0.06 mm corresponding to a bandwidth of 2.7 mμ.

(d) The Effect of Acetone Concentration on Efficiency of Pigment Extraction

The following experiment was carried out to determine the concentration of acetone to be used in pigment extraction.

Several 5 ml aliquots of a mixed culture of microscopic marine algae were taken. The algae were centrifuged out and the suspending fluid decanted. The algae were extracted for eighteen hours with acetone-water solutions of varying compositions. At the end of this time the optical densities

of the extracts were measured at 665 mμ. The results which are represented graphically in Figure 1 show that 90 per cent. acetone gives maximum extraction of chlorophyll over a period of eighteen hours.

(e) Beer's Law

Following the work of Richards and Thompson (1952), it was confirmed that in the range of optical density 0.0-0.8, the readings were proportional to chlorophyll concentration. The pigment extracts were diluted, if necessary, to bring them within this range, thus permitting the application of Beer's Law.

(f) Calculation of Results

In accordance with the laws of Beer and Lambert the following relationship is used

$$D = k\ c\ d$$

where D is the observed optical density,

k is the specific extinction coefficient of chlorophyll a in 90% acetone measured at 665 mμ,

c is the concentration of chlorophyll a in the acetone solution used in the spectrophotometer (g/l),

d is the length of absorbing path of the sample, (in this case 4 cm).

Richards and Thompson (1952) give the value of k as 66.7

$$\therefore\ D = 66.7 \times 4 \times c$$

Let x = concentration of chlorophyll a in the sea-water sample (mg/m^3)

Vs = volume of water sample (ml)

Va = volume of acetone extract (ml)

Then $x = c \frac{V_a}{V_s} \cdot 10^6$

$$\therefore\ x = \frac{D}{66.7 \times 4.0} \cdot \frac{V_a}{V_s} \cdot 10^6$$

$$\therefore\ x = D \cdot \frac{V_a}{V_s} \cdot 3750\ mg/m^3$$

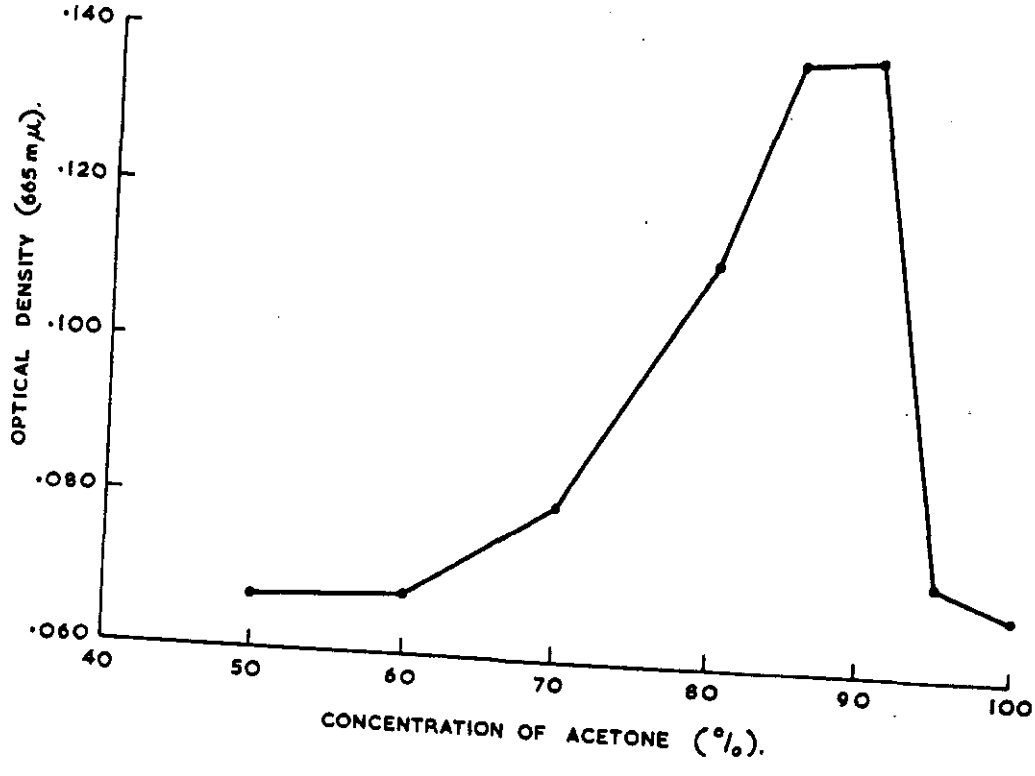


Fig. 1: Effect of acetone concentration on the efficiency of pigment extraction from a mixed culture of microscopic marine algae.

(g) Precision of Measurements

As is usual with photometric measurements at low absorbencies the instrumental error becomes appreciable. The readings at low absorbencies are reproducible within ± 0.002 units. To keep the instrumental errors within 5 per cent., the absorbency must be 0.04 or greater.

III. DISCUSSION

Following the practice of Atkins and Jenkins (1953), Richards and Thompson (1952), and Marshall (1956) it has been assumed that in plankton extracts only the chlorophylls contribute to light absorption at the wavelength at which measurements were carried out. The results are given in terms of chlorophyll a and assume further that at 665 mμ the contribution to the light absorption by the plankton extract, of chlorophylls b and c, is negligible. This assumption is not entirely true but results given thus can be compared with those of other workers and do not rely on an arbitrarily assigned absorption coefficient of chlorophyll c.

It is desirable to be able to report analytical results in absolute units. This has been done, using the figure of Richards (1952) for the specific absorption coefficient of chlorophyll a. However, his work is based in turn on the determinations made by Zscheile (1934). As Smith and Benitez (1954) point out "such discrepancies exist between the published values (of absorption data for chlorophylls) as to warrant their redetermination." Until the position has been clarified the specific absorption coefficient actually used should be stated to permit comparison of results between different workers and, if necessary, subsequent recalculations of results in the light of future more accurate data.

Considering the lability of chlorophyll and the difficulty of obtaining a pure preparation, it seems preferable to use a specific absorption coefficient rather than a chlorophyll standard of doubtful purity.

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