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Chapter A4

METHODS FOR COLLECTION AND ANALYSIS OF AQUATIC BIOLOGICAL AND MICROBIOLOGICAL SAMPLES

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Book 5

LABORATORY ANALYSIS

PRIMARY PRODUCTIVITY (PRODUCTION RATE)

Introduction

Bodies of water differ greatly in their populations of plants and animals, and these differences may be used in the interpretation of water quality. Biological differences may be expressed qualitatively and quantitatively. For many purposes, however, the factor of greatest interest is the rate at which new organic matter is formed and accumulated in the system being studied. Organic matter can be produced by photosynthesis and chemosynthesis. In most environments, chemosynthesis is not an important component of primary productivity. Through photosynthesis, organic compounds are synthesized from water (H_2O) and carbon dioxide (CO_2) using energy absorbed from sunlight by chlorophyll. Light energy is used to convert carbon dioxide to reduced carbon compounds. This process can be summarized by

$$6\ CO_2 + 6\ H_2O + \text{light} \rightarrow C_6H_{12}O_6 + 6\ O_2.$$

This implies that primary productivity could be determined by measuring any of the following parameters: (1) Uptake of carbon dioxide, (2) production of oxygen (O_2), or (3) increases in pH. In addition, changes in biomass or nutrient concentrations per unit time also can be a measure of primary productivity.

The underlying assumptions in the following methods are that the change in oxygen and dissolved carbon concentrations is a result of photosynthesis and respiration. As described in the preceding paragraph, photosynthesis involves uptake of carbon dioxide and production of oxygen. Respiration is the reverse of this process.

Two general approaches are described for the estimation of primary productivity. In the first, the organisms are isolated in suitable containers, and the production and respiration rates are estimated from changes in the dissolved-oxygen concentration or from changes in carbon dioxide concentration as measured by uptake of radioactive carbon [carbon 14 (^{14}C)]. If the rate of primary production is sufficient for accurate measurements to be made within 24 hours, the oxygen method is preferred. Vollenweider (1974) indicates that the oxygen method is impractical when there is less than a 7-mg (O_2/m^3)/h photosynthetic rate for a 3-hour exposure. Alternatively, if the chlorophyll concentration is less than 1 mg/m^2, the oxygen method should not be used. Therefore, the ^{14}C method, which is of greater sensitivity, is preferred for use in oligotrophic (low-productivity) water. In the second approach, production and respiration rates for nonisolated natural communities are estimated from changes in the dissolved-oxygen concentration of the open water.

The metabolism of aquatic plants and animals may result in changes in the concentrations of dissolved substances in the environment. The diel (24-hour) rise and fall of dissolved oxygen or carbon dioxide has been used to determine the productivity of biological communities in streams (Odum, 1956, 1957; Hoskin, 1959; Edwards and Owens, 1962; Gunnerson and Bailey, 1963; Edwards, 1965; O'Connell and Thomas, 1965; Wright and Mills, 1967; Hornberger and Kelly, 1972, 1974) and in standing water (Talling, 1957; Odum and Hoskin, 1958; Park and others, 1958; Odum, 1959; Verduin, 1960; Odum and Wilson, 1962; Lyford and Phinney, 1968; Welch, 1968; Eley, 1970; Cory, 1974; Hornberger and Kelly, 1974). The following methods use oxygen changes because of the ease with which they can be determined, but the principles are applicable as well to changes in total carbon dioxide (Vollenweider, 1974; Hall and Moll, 1975).

In the first approach, diel changes in the in-situ concentration of dissolved oxygen caused mainly by photosynthesis and respiration are used to estimate the primary productivity of the entire aquatic plant community. The advantages of this method are: (1) Unnatural effects of enclosures are eliminated, (2) phytoplankton and attached plants are included, and (3) observations can be of long duration or can be adapted for continuous monitoring. The disadvantages of the method are: (1) Limited sensitivity; (2) the unknown effects of transient conditions between sampling intervals; (3) the exchange of oxygen between the air and the water requiring calculation or measurement; and (4) in the graphical analysis, the necessity of assuming that the respiration rate is the same during the night as during the day. In standing water, unmeasured horizontal exchange (advection) may cause errors.

Changes in the dissolved-oxygen concentration in a reach of stream or in a standing body of water are results of photosynthesis, respiration, diffusion, and inflowing surface and ground water. If how these factors affect the oxygen concentration in the study area is known, a dissolved-oxygen curve can be drawn, and the primary productivity can be determined. The equation for the oxygen curve (Odum, 1956; Owens, 1965) is

$$Q = P - R + D + A, \qquad (1)$$

where

Q = rate of change (gain or loss) of dissolved oxygen per unit area;

P = rate of gross primary production per unit area;
R = rate of oxygen use (respiration) per unit area;
D = rate of oxygen uptake or loss by diffusion per unit area, depending on whether the water is undersaturated or oversaturated with oxygen when compared to the air; and
A = rate of supply of oxygen from drainage accrual.

If possible, select an area for study in which accrual has a negligible effect on the dissolved-oxygen concentration when compared with the other components.

The rate per unit area of the diffusion of oxygen into or out of the water, D, is the product of the gas-transfer coefficient, K, and the percentage-saturation deficit of oxygen between the water and air, S, or

$$D = K\frac{S}{100}, \qquad (2)$$

where D and K are in grams per square meter per hour. If equations 1 and 2 are divided by the depth, z, in meters, then the terms are expressed as volume, or grams per cubic meter per hour. Conventionally, capital letters are used for quantities defined on an areal basis and lowercase letters are used for quantities defined volumetrically (Odum, 1956). Thus, k is the gas-transfer coefficient, in grams per cubic meter per hour.

Various equations for obtaining K and D, as well as example values, are described in Odum (1956), Odum and Hoskin (1958), Churchill and others (1962), Odum and Wilson (1962), and Owens and others (1964). Procedures for measuring and predicting the reaeration coefficient of open-channel flows are evaluated by Bennett and Rathbun (1972).

In the methods described in this section, the diffusion rate either is obtained directly by the plastic-dome technique (Copeland and Duffer, 1964) or is calculated from measurements of hydraulic (mean flow) parameters (Churchill and others, 1962). The determination of K and D during the study period by one of these methods is preferable, but if that is not possible, a value for K may be estimated from the following data (Odum and Hoskin, 1958, p. 20):

Water type	Gas-transfer coefficient, K (grams per square meter per hour at 0 percent saturation)
1 Quiet water less than 0.5 meter deep or shallowly stratified	0 1-1
2. Bay and lakes that have gentle circulation and small waves	1-3
3. Rivers, streams, and open tidal water that have strong circulation and large waves	≥3

The presence of sewage and surfactants in the water tends to decrease the K value when compared with the pure-water K value; whereas, winds tend to increase the K value when compared with the quiescent-air K value (Bennett and Rathbun, 1972, p. 56-58).

A possible source of error when estimating gross primary productivity from changes in dissolved-oxygen concentration is the loss of oxygen to the atmosphere in the form of bubbles. Losses of 1 to 6.5 percent of the total oxygen production have been reported (Odum, 1957; Edwards and Owens, 1962). Although the rate of gas loss may be slow for many environments, estimates can be made of the quantity of oxygen produced during photosynthesis that is lost in this way (Owens, 1965).

The procedures for graphical analysis of the diel oxygen curve are described for streams (single-station and upstream-downstream methods) and for stratified water.

Collection

For oxygen light- and dark-bottle and ^{14}C methods, determine the depth of the euphotic zone (the region that receives 1 percent or more of the surface light) using an irradiance meter or submarine photometer. Quantum radiometers also are used for measurement of photosynthetically active radiation (Fee, 1976). If no other method is available, an estimate of the bottom limit of the euphotic zone is obtained by multiplying the Secchi disk depth by 2 (Dillon and Rigler, 1974; Vollenweider, 1974). Select sampling depths equivalent to 100-, 50-, 25-, 10-, 3-, and 1-percent light-penetration depths using the following equation:

$$\text{Depth at } (x)\text{-percent light} = \frac{ln(100/x)}{K},$$

where, for example, depth at 25-percent light = $ln(100/25)/K$; and K = extinction coefficient (Vollenweider, 1974) and is determined by

$$K = \frac{ln(Is/Iz)}{z},$$

where
Is = irradiance at the surface;
Iz = irradiance at depth, z; and
z = photometer depth.

In-situ incubations for oxygen and ^{14}C should be no longer than 4 hours, and the incubation period should be at midday (1000–1400 hours). For further details, refer to Schindler and Holmgren (1971) or Hall and Moll (1975).

If a 4-hour incubation is too short to measure oxygen changes, then ^{14}C should be used. In studies where more than one site must be sampled in 1 day, an on-board incubation technique can be used for the ^{14}C method (Fee, 1973a and b, 1976). A similar technique for multistation investigations of primary productivity using the oxygen light- and dark-bottle method is described by Megard (1972).

Collect a water sample, using an opaque, nonmetallic sampler, from each preselected depth. The sample volume should be sufficient to rinse and fill three incubation [biochemical oxygen demand (BOD)] bottles and a sample bottle for determination of alkalinity. After collection, all

samples should be kept in the dark at sample water temperature during the following procedures to avoid light injury to the organisms. Samples preferably should be collected in early morning. This procedure allows for measurements of light penetration and water sampling during daylight and for an incubation period from 1000 to 1400 hours (Schindler and Holmgren, 1971).

Oxygen light- and dark-bottle method for phytoplankton

Transfer the water sample collected from each depth to an 8-L polyethylene bottle, and let it stand for 15 to 30 minutes (but not more than 1 or 2 hours) at a temperature slightly higher than the in-situ water temperature. Shake the bottles occasionally to eliminate oxygen supersaturation. Supersaturation is most likely to occur in extremely productive water or in samples that have warmed several degrees.

For each depth sampled, fill four light and two dark BOD bottles by letting the well-mixed sample flow gently through a rubber tube inserted into the bottom of the bottle. Allow the water to overflow for about three bottle volumes and slowly withdraw the filling tube while the water still is flowing into the bottle. Immediately stopper the bottle, taking care to avoid entrapment of bubbles. All bottles from each depth must have the same initial dissolved-oxygen concentration. This requirement can be met during filling by adding successive increments of sample to each of the bottles in rotation until all are filled and flushed about three times. Place all bottles in a dark storage box until used.

The sequence of the following two steps may be altered as required. The determination of the initial dissolved-oxygen concentration should be started as soon as incubation begins.

Immediately add the reagents for the azide modification of the Winkler method to two light BOD bottles from each depth. These samples, designated IB, are used for determination of the initial dissolved-oxygen concentration. Titration may be delayed several hours, if necessary, if the samples are kept cool and dark.

Secure the stoppers in the BOD bottles that are to be incubated. The method of securing may be part of the suspension system, or stainless-steel or aluminum wire may be wound around the neck of the bottle and looped over the stopper. Do not use copper wire. Cover the stopper and neck of the dark bottles with several layers of aluminum foil. Attach pairs of light and dark bottles to a bottle holder attached to a wire cable (fig. 59). Lower the holders to the depth corresponding to the original sample depth. The wire cable can be attached to a surface float or suspended from a supporting arm attached to a pier or similar structure. Care must be taken not to shade the bottles with opaque floats or nearby structures. Begin the incubation, and prepare any remaining IB samples for dissolved-oxygen determination. At the end of the incubation period, raise the bottles and place them in a darkened box.

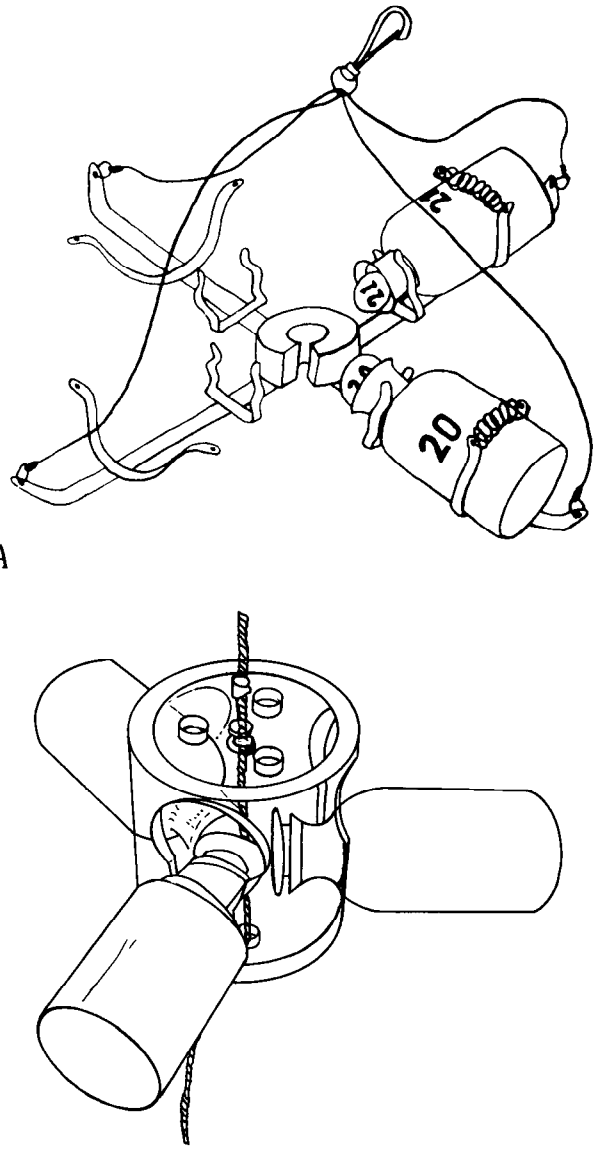

Figure 59.—Devices for holding light and dark bottles in a horizontal position: (*A*) Metal suspension frame (modified from Saunders and others, 1962); (*B*) polyethylene-bottle holder. (Sketch based on photograph courtesy of Schindler and Holmgren, 1971.)

Carbon-14 method for phytoplankton

Transfer the contents of ^{14}C bicarbonate stock ampoules to a 50-mL Erlenmeyer dispensing flask (see e in Analytical Problems in the "Supplemental Information" section for alternative method). Remove an ampoule of radioactive solution from storage. Carefully snap the ampoule neck. Using a clean, dry pipet, or syringe, that has a 7.5- or 10-cm needle, transfer the ^{14}C bicarbonate to the dispensing flask. The volume of ^{14}C bicarbonate in the dispensing flask should be sufficient to inoculate all BOD bottles and three inoculant standards. Swirl the contents to provide a homogeneous bicarbonate solution. Shake the sample thoroughly. Rinse each BOD bottle using a small volume of sample water.

Shake the sample thoroughly again. Fill one dark and two light BOD bottles with water from the sample depth. Also collect a sample for alkalinity determination from each depth. Place the light and dark BOD bottles in a plastic tray to confine possible spills and to minimize the potential for radioactive contamination of the working area. Alkalinity bottles that contain sample water should be capped and stored until analyzed in the laboratory. Alkalinity determinations for the available carbon-12 (^{12}C) value used in primary productivity calculations are limited. Stainton (1973) describes the use of IR or gas-chromatographic techniques, especially for water that has small carbonate concentrations.

Inoculate each BOD bottle using ^{14}C bicarbonate solution. The radioactivity of the sample after incubation is dependent on standing stock of the phytoplankton, growth rate, length of incubation, and volume of sample counted. Initially, the radioactivity of the sample should be increased by adding about 3 μCi ^{14}C bicarbonate per 100 mL of sample. With experience, one can decrease the strength of the inoculant so the resultant radioactivity is sufficiently high, but the natural alkalinity of the sample has not been altered unnecessarily.

Using a 1-mL precision volumetric pipet, dispense a 1-mL aliquot of ^{14}C bicarbonate inoculant into each light and dark BOD bottle. The tip of the pipet should be inserted well into the bottle. As the inoculant is added, the pipet tip is withdrawn from the bottle. Following inoculation, cap and shake each bottle well. Place the bottles in a darkened box until incubation begins. Cover the cap and neck of each dark bottle with black electrical tape.

The concentration of ^{14}C bicarbonate inoculant must be checked by preparing standards onsite. Using the precision volumetric pipet, dispense a 1-mL aliquot of ^{14}C bicarbonate inoculant into a clean volumetric flask, and dilute to 100 mL using distilled water. Transfer 0.1 mL of the diluted ^{14}C bicarbonate inoculant into each of three vials. Add 1 mL of liquid scintillation-grade phenethylamine to each vial of ^{14}C bicarbonate standard. Cap, shake well, and let stand for 5 minutes. To each vial of standard, add 10 mL Aquasol^{R} scintillation cocktail.

When all BOD bottles are ready for incubation, place one dark and two light bottles from each sampling depth into a bottle holder attached to a wire cable (fig. 59). Lower the holder to a depth corresponding to the original sample depth. The wire cable can be attached to a surface float or suspended from a supporting arm attached to a pier or similar structure. Care must be taken not to shade the bottles with opaque floats or nearby structures. At the end of the incubation period, raise the bottles and place them in a darkened box.

Oxygen light- and dark-enclosure method for periphyton

Samples for periphyton primary-productivity determinations may be obtained either from natural or from artificial substrates. The best results will be from direct in-situ measurements of undisturbed periphyton.

Periphyton measurement sites should be selected on the basis of study objectives. If successive measurements are needed to determine primary-productivity changes with time for a selected reach of stream, each measurement must represent the same habitat. Similarly, if measurements are needed to compare periphyton among different reaches or different streams, the measurements must represent comparable habitats. Factors, such as water depth, current speed, degree of sedimentation or erosion, and exposure to sunlight, must be similar if meaningful comparisons are to be made. The same attention to habitat applies to lake environments for which depth, sediment type, and presence of macrophyte beds are significant factors in site selection. The proximity of each measurement site to outfalls, marinas, bridges, or other effects of man must be considered.

Measurements of primary productivity of stream periphyton in static cultures may provide useful comparative values but undoubtedly are too small in absolute terms because of suppression of photosynthesis in the absence of current (Wetzel, 1964; Bombowna, 1972; Rodgers and others, 1978). To correct for the lack of current, methods have been developed for measuring primary productivity in plastic chambers in which water is circulated using a pump (McIntire and others, 1964; Thomas and O'Connell, 1966; Hansmann and others, 1971; Bombowna, 1972; Pfeifer and McDiffett, 1975; Rodgers and others, 1978; Gregory, 1980).

Circulating chambers are not available commercially; as a result, designs have varied. Three recent designs are shown in Gregory (1980) and Rodgers and others (1978), based on McIntire and others (1964). Some chambers have been miniaturized and use battery-operated pumps. The small size is convenient particularly in remote areas, but it has the disadvantage of collecting small samples; and the small pool volume may result in rapid oxygen supersaturation and nutrient depletion in water in the chamber. Large chambers that have large pool size are much more effective. The chambers made of Plexiglas are expensive to build and bulky to move. Because the most reliable pumps require line voltage, a generator usually is required. Because the chambers are submerged for temperature control, care is required when handling them because of the electrical hazard. Despite the many problems, the chamber (flowing enclosure) is a reliable method for obtaining estimates of primary productivity of periphyton.

Natural substrates

Rocks or other substrate material of suitable size may be placed into circulating chambers, or the chambers may be constructed to enclose an undisturbed area of periphyton-covered substrate. If the periphyton is moved from its original depth, keep the samples in subdued light to avoid light injury.

Using a nonmetallic water-sampling bottle, collect a water sample from the same depth from which the periphyton was collected. The volume should be sufficient to rinse and fill all the circulating chambers and to determine the initial dissolved-oxygen concentration. For light-bottle and dark-

bottle studies, samples preferably should be collected in the morning. This procedure allows for a 4-hour incubation period (Schindler and others, 1973).

Filter the required volume of water, and allow the filtrate to stand at a temperature slightly higher than the in-situ water temperature for 15 to 30 minutes. Shake the flask occasionally to eliminate oxygen supersaturation.

Enclose a known area of substrate containing living periphyton in a light and a dark circulating chamber containing a known volume of freshly filtered water. Fill the chambers and at least one BOD bottle so the chambers and the bottle(s) all have identical dissolved-oxygen concentrations. This requirement can be met during filling by adding successive increments of sample to each container in rotation until all are filled and flushed about three times. Keep all containers in the dark until used. Prevent entrapment of bubbles.

Place circulating chambers at the original depth from which the periphyton was collected, and incubate the samples for about 4 hours. In extremely productive water, where oxygen supersaturation is likely, an incubation period of 1 to 3 hours during midday may be sufficient.

Prepare the BOD bottle sample(s) for determination of the initial dissolved-oxygen concentration by using the methods of Skougstad and others (1979) or the American Public Health Association and others (1985). Titration may be delayed for several hours, if necessary, if the samples are kept cool and in the dark.

Diel oxygen-curve method for estimating primary productivity

The sample-collection method for estimating stream primary productivity will be determined by the type of environment being studied. In general, the objective is to determine the concentration of dissolved oxygen that is representative of the study area for each sampling interval. In well-mixed water, one or two determinations for each sampling period may be representative of the entire water mass. Even in well-mixed streams, the investigator must watch for spatial changes in dissolved-oxygen concentration. A consistent increase in dissolved oxygen toward the banks, when compared to the center of several rivers, was reported by Churchill and others (1962), and the effects of incompletely mixed tributary inflows can persist far downstream. Macrophytes frequently are distributed unevenly, which results in nonuniformity of water chemistry.

Sampling procedures are described for two types of stream conditions and for three methods of determining the diffusion rate, D. If the incoming water has metabolic characteristics similar to the outflowing water, follow the procedure for the single-station analysis. If the metabolic characteristics of the inflowing water are unknown or are not similar to the outflowing water, follow the procedure for the two-station analysis. Additional discussions of these methods are reported in Vollenweider (1974, p. 110-126) and Hall and Moll (1975).

Single-station analysis

Select a representative reach of stream in which surface- and ground-water accrual are negligible and in which similar conditions exist upstream. In such a stream, a second station would have a diel oxygen curve identical with that of the first station (Odum, 1956). Determine the cross-sectional mean velocity and the mean depth of flow to obtain stream discharge (Buchanan and Somers, 1969). Sufficient measurements must be made to determine the mean stream discharge for the 24-hour observation period.

Determine the dissolved-oxygen concentration, in milligrams per liter, and the temperature of the streamflow continuously, or at 1-, 2-, or 3-hour intervals for at least 24 hours. Make measurements at or near sunrise and sunset. Determine the barometric pressure.

If the Winkler method is used for dissolved-oxygen determination, collect duplicate or triplicate samples at each sampling time, and average the results from replicate samples. Collect the samples using a threefold-displacement sampler or using a water-sampling bottle to protect the water from contact with the air. If a water-sampling bottle is used, fill one or more BOD bottles by letting the sample flow gently through a rubber tube inserted into the bottom of the BOD bottle. Allow the water to overflow for about three bottle volumes, and slowly withdraw the filling tube while the water is still flowing into the bottle. Immediately stopper the BOD bottles, taking care not to entrap bubbles. Add the reagents for the azide modification of the Winkler method. Titration may be delayed several hours, if necessary, if the samples are kept cool and in the dark. Measure water temperature to ± 0.5 °C at each sample time and location.

For small streams, a single sample at the centroid of flow may be adequate. For large streams, samples may be required from several verticals at centroids of equal flow (Guy and Norman, 1970; Goerlitz and Brown, 1972).

If an oxygen meter is used, determine the dissolved-oxygen concentration at the sampling times and locations described in the preceding paragraphs. When using a portable recording system, place the temperature sensor and electrode at the centroid of flow, and ensure that sufficient water current is maintained past the membrane of the oxygen electrode. For stream velocities less than 0.6 m/s at the electrode, increase flow to the membrane surface using a submersible stirrer. Many oxygen electrodes are photosensitive, and the membrane-covered surface needs to be protected from bright light during calibration and use. Determine the diffusion rate, D, by one of the methods described in the "Diffusion Rate" section.

Two-station analysis

Select an upstream and a downstream station on a representative reach of stream in which surface- and ground-water accrual are negligible. Determine the cross-sectional mean velocity and the mean depth of flow to obtain stream discharge (Buchanan and Somers, 1969). Sufficient measurements must be made to determine the mean stream discharge

for the 24-hour observation period. Measure the surface area, in square meters, and the mean depth, in meters, for the reach between the stations, and determine the average time required for water to travel between the stations. If the flow rate of the stream cannot be determined directly, it can be estimated from the time required for a spot of dye to pass from the upstream station to the downstream station and from the mean cross-sectional area of the reach.

Determine the dissolved-oxygen concentration, in milligrams per liter, and the water temperature at each station as described in the "Single-Station Analysis" section. Determine the diffusion rate, D, by one of the methods described in the following section.

Diffusion rate

Determination of the rate at which oxygen enters or leaves the water when the concentration is not in equilibrium with the air is a critical step in the use of the oxygen-curve method for water. The rate at which oxygen diffuses in or out of the water increases as the degree of undersaturation or oversaturation increases. Moreover, in controlled streams that have open water or variable discharge, different gas-transfer coefficients, K, may need to be used at different times of day to explain changes in flow or in wind speed and direction (Odum and Wilson, 1962). The correction for wind does not need to be used for relatively protected areas.

Any of the following methods can be used for determining D. For the two-station analysis, D should be representative of the reach between the stations.

Hydraulic-parameter method

A detailed study of reaeration of rivers downstream from Tennessee Valley Authority reservoirs indicated that water depth and velocity were the most important factors affecting K (Churchill and others, 1962). To calculate K and D, values are required for the cross-sectional mean velocity, the mean depth of flow, the water temperature, and the dissolved-oxygen concentration and percentage saturation continuously, or at 1-, 2-, or 3-hour intervals for at least 24 hours. The measurements for these determinations are described in the "Single-Station Analysis" section.

Floating-diffusion-dome method

D is determined directly by measuring changes in the concentration of oxygen in a plastic dome filled with air and floating on the water surface (Copeland and Duffer, 1964) (fig. 60). The changes in oxygen inside the dome with time are attributed to diffusion. Measurements of oxygen inside the dome are made at night to avoid errors resulting from greenhouse effects and to eliminate photosynthetic oxygen production.

Fill the dome with fresh air and float it on the water surface. Record the volume of air in the dome, the area of the dome in contact with the water, and the time of the initial measurements. At intervals of 2 to 5 hours during the night, measure the temperature and the fraction (percentage) of oxygen inside the dome using an oxygen meter capable of measuring gaseous oxygen. Record as in table 14. Simultaneously measure the dissolved-oxygen concentration and

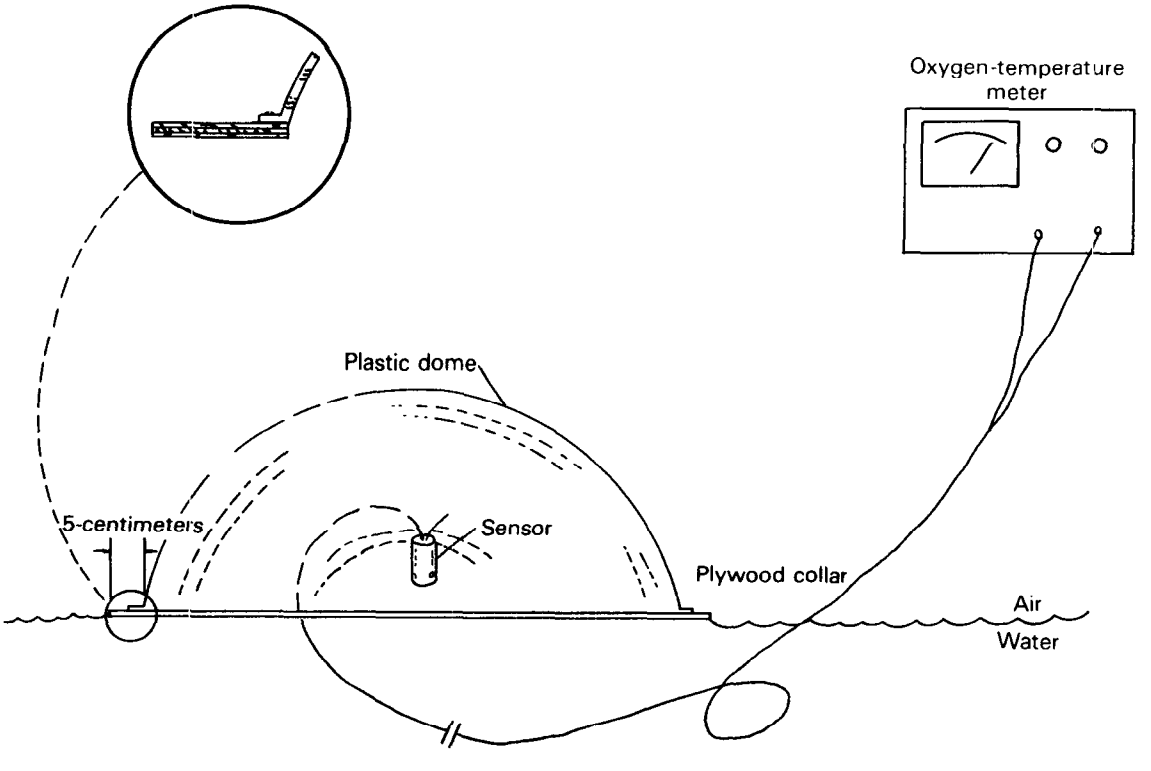

Figure 60.—Floating-diffusion-dome apparatus (modified from Hall, 1971).

Table 14.—*Hypothetical data for determining the diffusion rate, D, in a stream by the floating-diffusion-dome method*

[The dome has a volume of 2.5 liters and an area of 0.038 square meter in contact with the water; ---, not applicable]

Time interval (hour)	Dome			Water			Gas-transfer coefficient, K (grams per square meter per hour at 0-percent saturation)
	Percent oxygen[1]	Temperature (degrees Celsius)	Volume oxygen (milliliters)	Temperature (degrees Celsius)	Average saturation deficit[2]	Oxygen diffusion rate, D (grams per square meter per hour)	
Beginning (0000)----	99.0	29.5	519.8	29.5	-26.6	0.82	3.1
End (0500)----	74.8	25.0	392.7	25.0			
Beginning (2000)----	99.4	30.0	521.8	30.0	-19.4	.64	3.3
End (2400)--	84.8	29.0	445.2	29.0			
Average K for study period----	---	---	---	---	---	---	3.2

[1]Fresh air = 100 percent.
[2]From table 15.

water temperature as described in the "Single-Station Analysis" section.

For lakes, the objectives of sampling are to determine the diel changes in the average concentration and percentage saturation of dissolved oxygen in the euphotic zone and the oxygen demand in the benthic zone. Total community metabolism of the water body then may be estimated on an areal basis.

Sampling stations should be located in areas representative of the water body if values are to be averaged to yield metabolism of the entire water body. Local hours of sunrise and sunset, as well as average barometric pressure during the study, are required; and phytoplankton standing crop and chlorophyll *a* are useful supportive data.

Determine the depth of the euphotic zone using a submersible photometer. If no other method is available, an estimate of the bottom limit of the euphotic zone is obtained by multiplying the Secchi disk depth by 2 (Dillon and Rigler, 1974; Vollenweider, 1974). Select sampling intervals equal to one-tenth of the depth of the euphotic zone. Respiration in the deepest part of the lake (hypolimnion) can be estimated by including one or more sampling depths between the euphotic zone and the bottom of the lake. A computer-analysis method requires that depth intervals be constant.

At 1-, 2-, or 3-hour intervals for each increment of depth, determine water temperature, dissolved-oxygen concentration, and if appropriate, salinity or conductivity. Determine *D* as described in the preceding paragraphs, or by the following method.

Nighttime rate-of-change method

Odum (1956) and Odum and Hoskin (1958) developed this method to estimate reaeration gains or losses during darkness in the absence of photosynthesis. It assumes that there is no photosynthetic production of oxygen and that respiration is constant during the nighttime measurement interval.

Individual values for *K* corresponding to a nighttime measurement interval may be used to correct the surface-water layer value for nighttime diffusion. An arithmetic average of the nighttime values can be used to provide the daytime diffusion correction.

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Oxygen light- and dark-bottle method for phytoplankton

(B-8001-85)

Parameters and Codes:
Productivity, primary, gross [mg(O_2/m^3)/d]: 70959
Productivity, primary, gross [mg(O_2/m^2)/d]: 70960
Productivity, primary, net [mg(O_2/m^3)/d]: 70963
Productivity, primary, net [mg(O_2/m^2)/d]: 70964
Respiration [mg(O_2/m^3)/d]: 70967
Respiration [mg(O_2/m^2)/d]: 70968

1. Applications

The method is applicable to standing or slowly moving water. Best results are obtained in eutrophic water in which the production rate is about 3 to 200 mg(C/m^3)/h during the photoperiod (Strickland and Parsons, 1968, p. 263). The smaller limit for measurable oxygen production occurs when there is less than a 7-mg(O_2/m^3)/h photosynthetic rate for a 3-hour exposure (Vollenweider, 1974, p. 93).

2. Summary of method

Light (clear) and dark (blackened) bottles filled with water samples are suspended at several depths in the euphotic zone for a known period of time. The concentration of dissolved oxygen is measured at the beginning and at the end of the incubation period. Changes in the dissolved-oxygen concentrations of the enclosed samples are interpreted in terms of photosynthesis and respiration. Productivity is calculated on the basis of one carbon atom assimilated for each oxygen molecule released.

3. Interferences

3.1 The method uses isolated phytoplankton samples to indicate the response of the natural system. Care must be used when collecting the sample, handling the sample, and exposing the sample to light to prevent interference with the life requirements of the organisms. Water-sampling bottles or devices should be made of plastic or glass, and the essential metal parts should be made of stainless steel. Copper, brass, and bronze fittings on water-sampling bottles or on suspension equipment should not be used. The water-sampling bottles should be opaque to decrease the risk of light injury, and biochemical oxygen demand (BOD) bottle filling should be done in the shade or in an enclosure to avoid exposure of unadapted algae to full sunlight. Light leaks into the dark bottles must be prevented. The formation of bubbles in the BOD bottles results in errors during the determination of dissolved-oxygen changes; microbial activity and chemical oxygen demand cause losses of oxygen when incubation times exceed a few hours (Vollenweider, 1974; Hall and Moll, 1975).

3.2 Interferences with the chemical determination of dissolved oxygen were described by Skougstad and others (1979) and American Public Health Association and others (1985).

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies. All materials must be free of agents that inhibit photosynthesis and respiration.

4.1 *BOD bottles*, numbered, 300 mL, Pyrex or borosilicon glass, that have flared necks and pointed ground-glass stoppers. A supply of light and dark bottles is required. The dark bottles may be prepared by painting the bottles black and covering the paint with overlapping strips of black plastic tape. The exposed parts of the stoppers should be similarly blackened, and a hood of several layers of aluminum foil should cover the stopper and neck of the bottle during use (Note 1).

Note 1: To prepare the BOD bottles, fill with the acid cleaning solution and let stand for several hours. Rinse thoroughly using distilled water. Traces of iodine from the Winkler analysis should be removed by rinsing the bottles and stoppers using 0.01*N* sodium thiosulfate solution followed by thorough rinsing using distilled water. Do not use phosphorous-based detergents.

4.2 *Dark box*, preferably insulated, for storing filled BOD bottles until ready for incubation.

4.3 *Equipment for determination of dissolved oxygen*, by the azide modification of the Winkler method (Skougstad and others, 1979; Golterman, 1982; American Public Health Association and others, 1985).

4.4 *Polyethylene bottles*, 8-L capacity, that have cap and bottom tubulation.

4.5 *Suspension system*, for holding light and dark bottles in a horizontal position at various depths (fig. 59).

4.6 *Underwater light-measurement equipment.* A quantum/radiometer/photometer measures photosynthetically active radiation (400–700 nm). If a submersible photometer is not available, a Secchi disk may be used.

4.7 *Water-sampling bottle*, Van-Dorn type or equivalent.

If a clear acrylic bottle is used, care should be taken to avoid light shock to dark-adapted organisms. Depth-integrating samplers are described in Guy and Norman (1970).

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Acid cleaning solution*, 20 percent. Mix 20 mL concentrated hydrochloric acid (HCl) (specific gravity 1.19) with distilled water and dilute to 100 mL.

CAUTION.—Use rubber gloves, safety goggles, and protective clothing when handling concentrated HCl.

5.2 *Distilled or deionized water.*

5.3 *Reagents for the azide modification of the Winkler method*, for dissolved oxygen (Skougstad and others, 1979; American Public Health Association and others, 1985).

5.4 *Sodium thiosulfate solution*, 0.01*N*. Dissolve 2.5 g sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) in distilled water and dilute to 1 L.

6. Analysis

6.1 After suitable incubation, remove the BOD bottles from the suspension system; and, as quickly as possible, add the first two Winkler reagents to each bottle to arrest biological activity and to fix the dissolved oxygen. Complete the Winkler determination of dissolved oxygen for all samples; average the results from duplicate samples.

7. Calculations

Primary productivity is expressed as the quantity of oxygen released, or of carbon assimilated, per unit time. Adjust the following calculated values for the appropriate incubation period. Gross or net primary productivity is calculated on the assumption that one atom of carbon is assimilated for each molecule (two atoms) of oxygen released.

7.1 Gross primary productivity [$mg(O_2/m^3)/t$]

$$= \frac{LB - DB}{t} \times 1{,}000,$$

where

LB = dissolved-oxygen concentration, in milligrams per liter, in the light bottle after incubation;
DB = dissolved-oxygen concentration, in milligrams per liter, in the dark bottle after incubation; and
t = incubation period, in hours or days, and 1,000 converts liters to cubic meters.

7.2 Gross primary productivity [$mg(C/m^3)/t$]

$$= \frac{LB - DB}{t} \times \frac{12}{32} \times 1{,}000,$$

where

LB, DB, t, and 1,000 = as in 7.1;
12 = atomic weight of carbon; and
32 = molecular weight of oxygen.

7.3 Net primary productivity [$mg(O_2/m^3)/t$]

$$= \frac{LB - IB}{t} \times 1{,}000,$$

where

LB = dissolved-oxygen concentration, in milligrams per liter, in the light bottle after incubation;
IB = initial dissolved-oxygen concentration, in milligrams per liter, in the light bottle before incubation; and
t = incubation period, in hours or days, and 1,000 converts liters to cubic meters.

7.4 Net primary productivity [$mg(C/m^3)/t$]

$$= \frac{LB - IB}{t} \times \frac{12}{32} \times 1{,}000,$$

where

LB, IB, t, and 1,000 = as in 7.3;
12 = atomic weight of carbon; and
32 = molecular weight of oxygen.

7.5 Respiration [$mg(O_2/m^3)/t$]

$$= \frac{IB - DB}{t} \times 1{,}000,$$

where

IB = initial dissolved-oxygen concentration, in milligrams per liter, in the light bottle before incubation;
DB = dissolved-oxygen concentration, in milligrams per liter, in the dark bottle after incubation; and
t = incubation period, in hours or days, and 1,000 converts liters to cubic meters.

7.6 The gross or net primary productivity of a vertical column of water, 1 m^2 in cross section (milligrams oxygen per square meter per time or milligrams carbon per square meter per time), is determined by a summation of the productivities in successive cubic meter volumes, from top to bottom, in the euphotic zone at each study site. However, the maximum value in the euphotic zone for primary productivity, expressed on a cubic meter basis (pmax), has much more meaning for data interpretation than does an integrated square meter value (Megard, 1972). Therefore, the maximum cubic meter value should be reported in addition to the square meter integral value for primary productivity. On a graph of depth versus productivity (fig. 61), plot the experimentally determined productivity value for each incubation depth, and draw a line of best fit through the points. Integrate the area under the productivity-depth curve to obtain a total productivity value for the euphotic zone. An example of the vertical distribution of daily primary productivity in a lake is shown in figure 61.

8. Reporting of results

Report primary productivity as follows: less than 10 mg, one decimal; 10 mg and greater, two significant figures.

9. Precision

The following precision estimates were reported by Strickland and Parsons (1968, p. 263) for aliquots from a single, large sample and do not include variabilities from sampling. For precision at the 100-mg(C/m^3)/h level, the correct value lies in the range: Mean of n determinations $\pm 15/n^{1/2}$ mg(C/m^3)/h (6-hour incubation). For precision at the 10-mg(C/m^3)/h level, the correct value is in the range: Mean of n determinations $\pm 1.5/n^{1/2}$ mg(C/m^3)/h (6-hour incubation).

10. Sources of information

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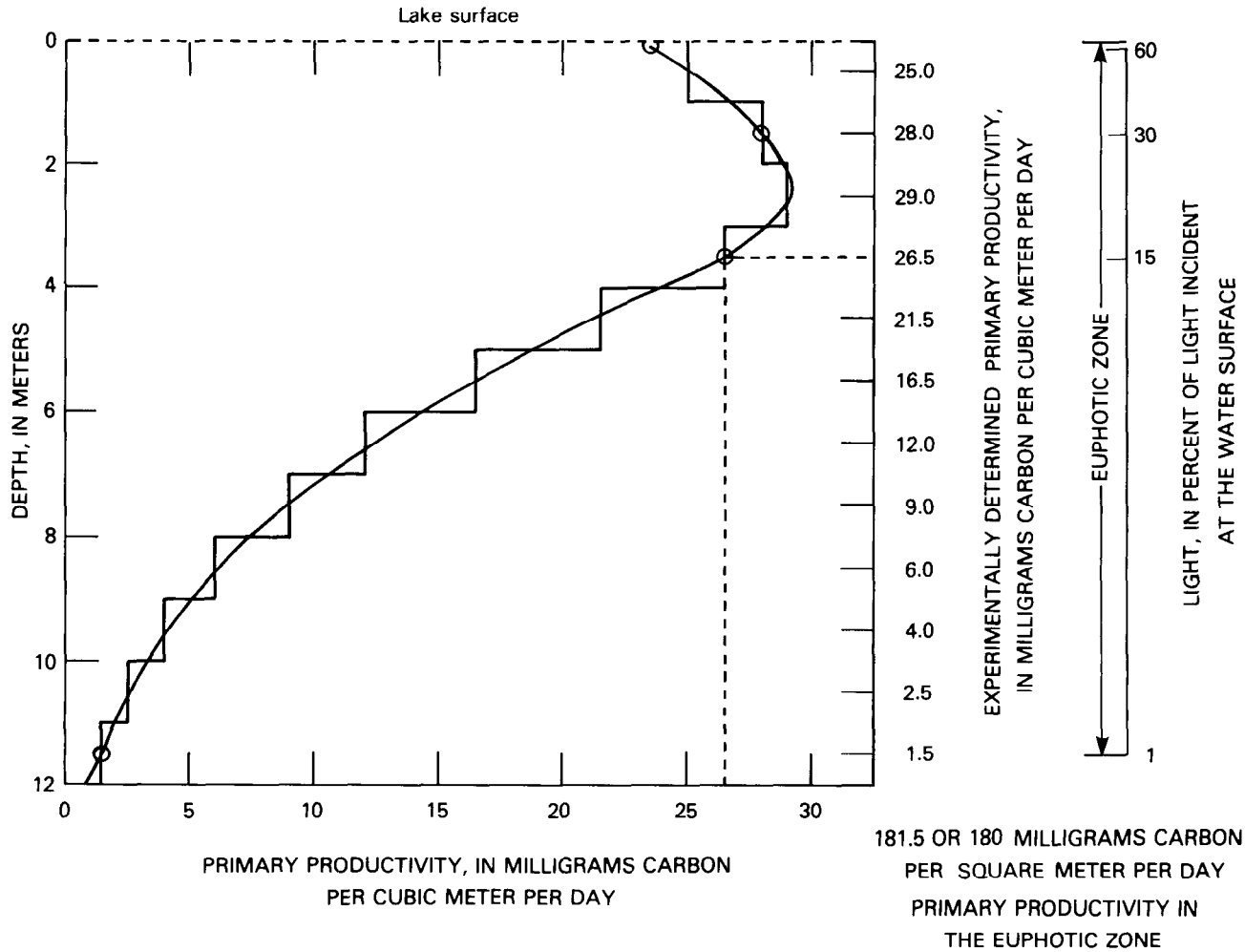

Figure 61.—Example of the vertical distribution of daily primary productivity in Koocanusa Reservoir, Mont. The circled points are values of primary productivity (milligrams carbon per cubic meter per day) calculated from contents of light and dark bottles suspended at those depths. The smooth curve was fitted by eye, and the area under the primary productivity-depth curve (milligrams carbon per square meter per day) was estimated by summing the values at 1-meter intervals through the euphotic zone (modified from Janzer and others, 1973).

Carbon-14 light- and dark-bottle method for phytoplankton

(B-8020-85)

Parameters and Codes:

Productivity, primary, gross [mg(C/m^3)/d]: 70961
Productivity, primary, gross [mg(C/m^2)/d]: 70962
Productivity, primary, net [mg(C/m^3)/d]: 70965
Productivity, primary, net [mg(C/m^2)/d]: 70966

Phytoplankton primary productivity as determined by the ^{14}C light- and dark-bottle method measures the rate of assimilation of carbon dioxide (CO_2) into particulate organic material by contained algal populations. The ^{14}C method measures productivity by determining the rate of incorporation of a radioisotope tracer, $^{14}CO_2$, into organic material.

The ^{14}C method was used first by Steemann-Nielsen (1952). Originally, radioactivity of incorporated ^{14}C was measured using Geiger-Müller (GM) counters, but this measurement technique is rarely used because GM counters are susceptible to considerable back scatter and self-absorption and can have inaccurate counting efficiencies. Comparisons of the merits of GM measurements and liquid-scintillation measurements (Schindler, 1966; Wolfe and Schleske, 1967; Wallen and Geen, 1968) indicated that liquid-scintillation measurements do not have many of the drawbacks inherent with the use of GM counters. Pugh (1970, 1973) reported that counting efficiency as calculated by internal or external standardization can result in serious errors if applied to a heterogeneous sample, for example, a filter that has attached phytoplankton. High levels of self-absorption caused by dense layering of particulate material on filters can be corrected accurately only by using a filter standardization technique (Pugh, 1973). Many investigators proposed the use of solubilizers, emulsifiers, and bleaching to provide a homogeneous sample that has accurate counting efficiency. Schindler and others (1972) proposed acidification and bubbling of the sample to eliminate errors and uncertainties associated with filtration techniques (Arthur and Rigler, 1967). Further modifications of the acid bubbling method (Smith, 1975; Theodorsson and Bjarnason, 1975; Mague and others, 1980) have resulted in a technique that eliminates many problems inherent in ^{14}C-filtration methods (Goolsby, 1976; Gachter and Mares, 1979), particularly problems caused by filtration artifacts, accurate determination of counting efficiency, and excretion of dissolved organic material.

1. Applications

1.1 The ^{14}C method is applicable to standing or slowly moving eutrophic and oligotrophic water in freshwater or saline environments. In very eutrophic water, the rate of photosynthesis may be so rapid that adjustments in experimental procedure may be necessary (see "Supplemental Information" section). Lean and Burnison (1979) warn of possible insensitivity of acidification and bubbling techniques in water that has greater than 1,500 to 3,000 μm dissolved inorganic carbon.

1.2 Although radioisotope techniques seem to be straightforward, exactly what is being measured by ^{14}C techniques has never been determined precisely. Measures of gross or net productivity typically are of interest. But, because the technique cannot directly measure respiration, photorespiration, or the rate of ^{14}C movement through the cellular carbon pool, accurate determinations of whether gross or net productivity is being measured cannot be made. Studies by Hobson and others (1976) and Gieskes and others (1979) indicate that incubations of 2 to 4 hours are needed to measure gross carbon uptake; whereas, incubations of 24 hours are required to measure net productivity.

2. Summary of method

Measurements of primary productivity of organic matter using the ^{14}C method (Steemann-Nielsen, 1952) require adding radioactive bicarbonate, $NaH^{14}CO_3$, to an enclosed water sample. After incubation (either in situ or in an incubator), photosynthesis is stopped by chemical means before further processing. An aliquot of the fixed sample then is acidified and bubbled (Schindler and others, 1972) to separate the inorganic $^{14}CO_3^{-2}$ from the organic fraction. Following acidification and bubbling, an unfiltered subsample and a filtrate subsample are used for subsequent scintillation counting. After a volumetric subsample of the filtrate is acidified and bubbled, a known quantity is put into a scintillation vial and a light-sensitive scintillation fluor is added to the vial. As the ^{14}C atom decays, an energized β particle is emitted, which causes the scintillation solution to fluoresce pulses of light. Very sensitive photomultiplier tubes in a scintillation spectrometer record the light pulses. The ^{14}C activity in the sample is proportional to the frequency of light pulses. The uptake and reduction of CO_2 to organic matter is assumed to be proportional to the uptake of ^{14}C bicarbonate. Primary productivity, as the quantity of carbon fixed per unit time, is calculated from the proportion of ^{14}C fixed to ^{14}C available and total CO_2 in the sample.

3. Interferences

Some interferences are inherent in the ^{14}C method and cannot be avoided. The "Supplemental Information" section

at the end of the description of this method indicates commonly occurring problems and the procedures that minimize their effects.

4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies. All materials used must be free of agents that inhibit photosynthesis and respiration.

4.1 *Bags*, polyethylene, about 30×60 cm, for solid radioactive wastes.

4.2 *Black tape*, to cover cap and neck of dark bottles after inoculating using ^{14}C bicarbonate.

4.3 *BOD bottles*, numbered, 300 mL, Pyrex or borosilicon glass, that have flared necks and pointed ground-glass stoppers. A supply of light and dark bottles is required. The dark bottles may be prepared by painting the bottles black and covering the paint with overlapping strips of black plastic tape. The exposed parts of the stoppers should be similarly blackened, and a hood of several layers of aluminum foil should cover the stopper and neck of the bottle during use (Note 1).

Note 1: To prepare the BOD bottles, fill with the acid cleaning solution and let stand for several hours. Rinse thoroughly using distilled water. Traces of iodine from the Winkler analysis should be removed by rinsing the bottles and stoppers using 0.01*N* sodium thiosulfate solution followed by thorough rinsing using distilled water. Do not use phosphorous-based detergents.

4.4 *Carboy*, waste, 20 L, polyethylene.

4.5 *Dark box*, preferably insulated, for storing filled BOD bottles until ready for incubation.

4.6 *Filtration assembly*, 20-mL syringe that has the plunger removed, attached to a 25-mm filter unit. The sample is filtered through a 25-mm filter, and the filtrate is collected in a temporary holding vial.

4.7 *Glass-fiber filters*, 47-mm diameter disks, or membrane filters, white, plain, 0.45-μm mean pore size, 47-mm diameter.

4.8 *Micropipet*, automatic, precision volumetric, 1 mL.

4.9 *Needles*, hypodermic, 7.5 or 10 cm, Luer taper.

4.10 *Pipet*, automatic, adjustable, volumetric, 1 to 5 mL.

4.11 *Pipet tips*, disposable, 1-mL capacity.

4.12 *Pipet tips*, disposable, 5-mL capacity.

4.13 *Repipettor.*

4.14 *Sample bubbler*, for agitating the sample while stripping $^{14}CO_3^{-2}$ from the solution. A number of designs have been employed (Theodorsson and Bjarnason, 1975; Gachter and Mares, 1979). A system proven to be effective is shown in figure 62. After acid is added to the sample vial and the stopper is in place, air, which agitates the solution and mixes the sample and acid, is drawn through the inlet tube. The $^{14}CO_2$ is drawn away by vacuum and vented outside the laboratory.

4.15 *Spectrometer* (spectrophotometer; fig. 57), that has a band width of 2 nm or less so absorbance can be read to ±0.001 units. Use cells that have a light path of 1 cm.

4.16 *Suspension system*, for holding light and dark bottles in a horizontal position at various depths (fig. 59).

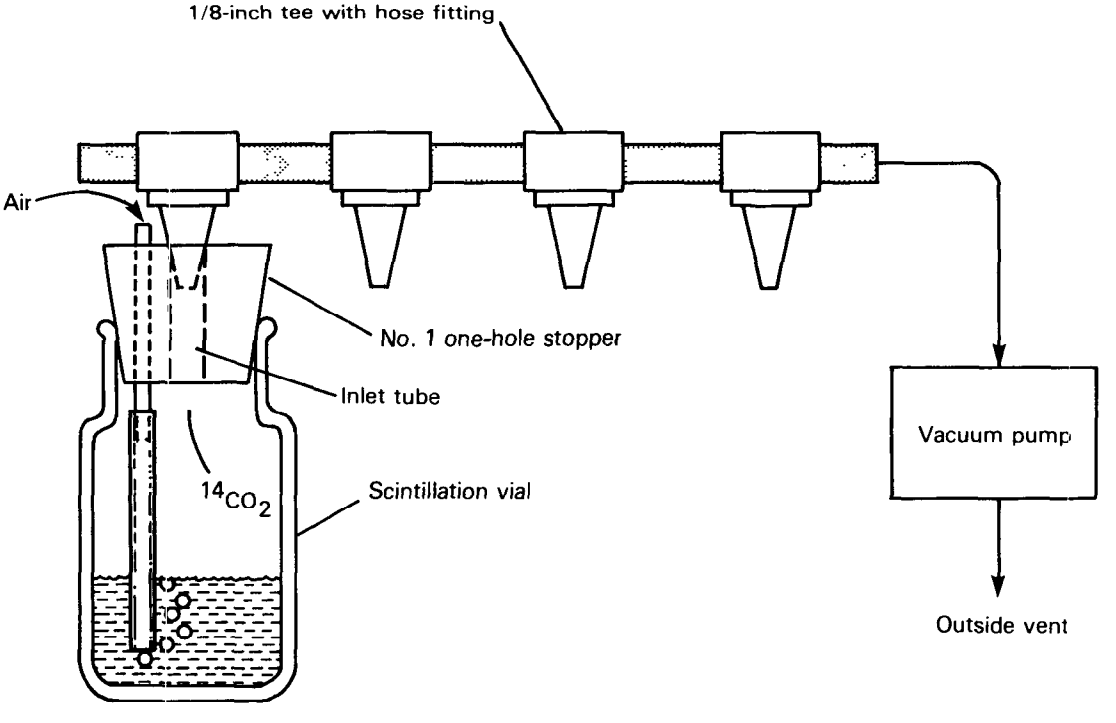

Figure 62.—Sample bubbler that has sample vial attached. The stopper is a No. 1 (one-hole stopper). An air vent is made from a 3-centimeter section of a No. 20 hypodermic needle to which is attached a short length of tygon tubing.

4.17 *Syringe*, 10-mL Luer taper.

4.18 *Underwater light-measurement equipment*. A quantum/radiometer/photometer measures photosynthetically active radiation (400–700 nm). If a submersible photometer is not available, a Secchi disk may be used.

4.19 *Vacuum pump*.

4.20 *Vials*, liquid scintillation, 20-mL capacity, that have plastic-lined screwcaps (Note 2).

Note 2: Place identifying marks on the caps and not on the sides of the vials.

4.21 *Water-sampling bottle*, Van-Dorn type or equivalent. If a clear acrylic bottle is used, care should be taken to avoid light shock to dark-adapted organisms. Depth-integrating samplers are described in Guy and Norman (1970).

5. Reagents

Most of the reagents listed in this section are available from chemical supply companies.

5.1 *Acid cleaning solution*, 1*N*. Mix 82.6 mL concentrated HCl (specific gravity 1.19) per liter of distilled water.

CAUTION.—Use rubber gloves, safety goggles, and protective clothing when handling concentrated HCl.

5.2 *Ammoniacal barium chloride solution*. Dissolve 50 g $BaCl_2 \cdot 2H_2O$ in approximately 1 L lakewater or tapwater, add 75 to 100 mL concentrated NH_4OH (specific gravity 0.90), and place in the 20-L polyethylene waste carboy.

5.3 *^{14}C bicarbonate solution*, $NaH^{14}CO_3$ or equivalent. Specific activity of 0.1 μCi/μg. Standard solutions of 1, 5, 10, or 20 μCi/mL are available. The activity necessary for a particular environment should be established by the researcher.

5.4 *^{14}C labeled toluene standard*, certified calibration standard of toluene (^{14}C) that has a specific activity of 4×10^5 DPM/mL.

5.5 *Distilled or deionized water*.

5.6 *Hydrochloric acid*, 0.1*N*. Mix 8.3 mL concentrated hydrochloric acid (HCl) (specific gravity 1.19) with distilled water and dilute to 1 L in a repipettor that has 0.1-mL graduations.

5.7 *Liquid-scintillation solution*. Aquasol^R scintillation cocktail has been a satisfactory fluor. PCS Solubilizer premixed liquid-scintillation cocktail also has been satisfactory (Janzer and others, 1973).

5.8 *Reagents for determining total alkalinity* (CO_2, HCO_3^{-1}, and CO_3^{-2}) (Skougstad and others, 1979; American Public Health Association and others, 1985).

5.9 *2-phenethylamine, scintillation grade*. Phenethylamine is used to form carbonates, which are stable in Aquasol, to eliminate loss of radiocarbon from the acidic fluor.

6. Analysis

6.1 After incubation is completed, process the samples in a work area that has subdued lighting. After shaking the sample well, dispense a 3-mL aliquot of sample into a scintillation vial using a precision volumetric pipet. Add 0.2 mL of 0.1*N* HCl to decrease the pH to 2.5 to 3. Immediately insert a stopper (fig. 62) and attach the vial to the sample bubbler. Repeat in triplicate for each light and dark bottle.

6.2 Gravity filter 5 to 10 mL of each sample through a 0.45-μm glass-fiber filter. Pour the sample water into a 20-mL plastic syringe filtration unit. The filtrate is collected in a temporary holding vial from which a 3-mL subsample is dispensed into a scintillation vial. Add 0.2 mL of 0.1*N* HCl and bubble.

6.3 After aerating each sample for 10 to 15 minutes, remove the vial from the sample bubbler and replace the stopper with a scintillation vial cap. When convenient, add to each vial 10 mL liquid-scintillation solution, using a volume sufficient to produce a stable emulsion suitable for holding particulates dispersed throughout the medium.

6.4 Filter the remaining contents of all BOD bottles through a 0.45-μm glass-fiber filter. Dispose of the glass-fiber filters in the solid-waste disposal bag. Pour the collected filtrate into the 20-L polyethylene waste carboy to react with the ammoniacal barium chloride solution; ^{14}C bicarbonate in solution will be precipitated as barium carbonate, which is allowed to settle (see "Supplemental Information" subsection following references at the end of this section).

6.5 Temporary holding vials are reused after being washed, soaked in 1*N* HCl, rinsed, and dried.

6.6 When the vials are returned to the laboratory, wipe the outside of each vial using an acetone dampened tissue to remove dust and finger marks.

6.7 Dark adapt all vials until their activity drops to a consistent level. The time required for dark adaptation will vary but can be determined by counting a representative sample until little variation between successive counts is observed. Typically, a few hours is sufficient for dark adaptation.

6.8 Using a liquid-scintillation spectrometer, count each vial in series for 20 minutes. Repeat the counting procedure three times.

6.9 Determine the counting efficiency for each sample by internal standardization. After counting, add 100 μL of ^{14}C labeled toluene standard to two samples from each sampling depth. Repeat counting as described in 6.8.

6.10 Determine the counting efficiency for these spiked samples using the equation

$$E = \frac{(\bar{R}_{s'} - \bar{R}_s)}{S} \times 100,$$

where

E = the counting efficiency, in percent (Note 3);

$\bar{R}_{s'}$ = the average counting rate of the sample, in counts per minute after the addition of the ^{14}C labeled toluene standard;

$\bar{R}_s$ = the average counting rate of the sample, in counts per minute; and

S = the total activity of the ^{14}C labeled toluene standard added, in disintegrations per minute.

Note 3: Experience indicates that a variation of 2 percent in the counting efficiency is acceptable. If the variation is greater than 2 percent, the counting efficiency for all samples

in light and dark bottles from the location(s) in question should be checked and count-rate corrections made, if necessary.

6.11 Activity of ^{14}C bicarbonate standards are determined in a similar manner. Because the activity of standard samples is intense, counting time should be decreased to 1 minute to prevent overloading the scintillation spectrometer's counting mechanism. After counting each standard three times, add 1 mL of ^{14}C labeled toluene standard to two samples. Repeat the counting procedure for the spiked samples. Counting efficiency for spiked standards is calculated as outlined in 6.10.

7. Calculations

7.1 Primary productivity is expressed as the quantity of carbon assimilated per unit time. Gross photosynthesis, based on incubations of 2 to 4 hours, should be reported as productivity per hour (milligrams carbon per cubic meter per hour). Net photosynthesis, based on 24-hour incubations, should be reported in milligrams carbon per cubic meter per day.

Net primary productivity = total $carbon_{fixed}$ − excreted $carbon_{fixed}$.

Gross primary productivity = total $carbon_{fixed}$.

$$Carbon_{fixed} = \frac{(\bar{B}_l - \bar{B}_d) \times W \times (V_i/V_a) \times 1.064}{\bar{S} \times D},$$

where

Total $carbon_{fixed}$ = unfiltered sample fixation rate;

Excreted $carbon_{fixed}$ = 0.45-μm filtrate sample fixation rate;

$\bar{B}_l$ (DPM) = average light-bottle counting rate ($\bar{R}_x$) divided by sample counting efficiency (E) (see C in analytical problems in "Supplemental Information" section);

$\bar{B}_d$ (DPM) = average dark-bottle counting rate ($\bar{R}_s$) divided by sample counting efficiency (E);

W (mg/L) = alkalinity (actually ^{12}C-total inorganic carbon). Conversion of alkalinity data to inorganic carbon values is discussed in Vollenweider (1974);

V_i (mL) = volume incubated;

V_a (mL) = volume of aliquot acidified and bubbled;

1.064 = isotopic preference factor (Steemann-Nielsen, 1952);

$\bar{S}$ (DPM) = average ^{14}C bicarbonate standard counting rate ($\bar{R}_s$) × counting efficiency (E); and

D = unit time.

7.2 The primary productivity of a vertical column of water, 1 m^2 in cross section (milligrams carbon per square meter per time), is determined by a graphical summation of the productivity in successive cubic meter volumes, from top to bottom, in the euphotic zone at each study site. On a graph of depth versus productivity (fig. 61), plot the experimentally determined productivity value for each incubation depth, and draw a line of best fit through the points. Integrate the area under the productivity-depth curve to obtain a total productivity value for the euphotic zone. In addition, report the maximum cubic meter value of primary productivity (pmax) measured in the euphotic zone. LaBaugh (1979) and Smith (1979) have reported the usefulness of pmax in the interpretation of water-quality data related to primary productivity measured by the ^{14}C method. Kerekes (1975) describes why square-meter primary-productivity data are less suitable for interpretive studies than cubic-meter primary-productivity data. An example of the vertical distribution of daily primary productivity in Koocanusa Reservoir is shown in figure 61.

8. Reporting of results

Report primary productivity as follows: two significant figures.

9. Precision

Estimates of precision of primary-productivity measurements based on replicate samples from in-situ incubations seldom are reported. Hager and others (1980) reported the precision of replicate ^{14}C samples to be 5 to 10 percent. Precision of the acid bubbling technique is reported by Gachter and Mares (1979) to range from 0.7 to 2.4 percent ($n = 10$).

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Supplemental information

Interferences and limitations

Toxins

Any substance on the collecting apparatus or BOD bottles that is foreign to the natural-water sample may have a deleterious effect on the productivity of the sample. All equipment and glassware must be cleaned between sampling. All traces of HCl cleaning solution must be rinsed from the BOD bottles to eliminate loss of the inoculant. Liquid-scintillation vials and preservatives, such as Lugol's and formalin, are very toxic. Such chemicals should be restricted from the sample preparation area.

Contamination of samples by bare metal may have detrimental (Doty and Oguri, 1959) and stimulatory (Goldman, 1963) effects on the sample. To decrease either effect, plastic, stainless-steel, or plastic-coated metal parts should be used when possible.

Analytical problems

Since Steemann-Nielsen's (1952) description of the method, techniques for more accurate measurement of β-particle activity have led to many refinements in methods.

a. Counting methods. Originally, Geiger-Müller (GM) counters were used for measuring the frequency of β emissions. Although the equipment is less expensive than liquid-scintillation counters, the efficiency of GM counters is minimal (less than 20 percent), and there are serious errors that may be due to self-absorption and backscatter. GM counters require that the material be dried, a process that can result in a 30 to 50 percent loss in carbon (Wallen and Geen, 1968; Ward and Nakanishi, 1971). Liquid-scintillation counters have come into common use because of their more accurate counting efficiencies and ability to count wet filters and aqueous samples when a suitable fluor is used.

b. Quench. A decrease in the efficiency of a scintillation counter's detection of β emissions is caused by quenching of the sample. Of the three types of quench in liquid-scintillation samples—chemical, color, and physical—the last is the most difficult to correct when using phytoplankton samples. Large quantities of solid phytoplankton and filter material physically block the emission of light from the sample fluor.

c. Counting efficiency. Essential to an accurate estimation of the total activity of a sample is knowledge of the efficiency with which the scintillation spectrometer detects β emissions. Three common techniques for measuring counting efficiency are internal standardization, external standardization, and channels ratio. Specific techniques for implementing each of these methods are outlined in manuals supplied by manufacturers of scintillation spectrometers. These techniques for determining counting efficiency are limited in accuracy because they are suited ideally only for a homogeneous solution, one without particulate matter. This is especially true for the external-standardization and channels-ratio techniques, which are based on efficiency curves of standard solutions that may not accurately represent the factors causing quench in a heterogeneous sample. Pugh (1970) has reported serious errors in measuring efficiencies using these techniques when attempts are made to count filters heavily laden with particulate material. Pugh (1970,

1973) developed a filter standardization technique for ^{14}C-sucrose incorporation onto membrane filters, as long as the weight of sample algae on the filters was small (less than 1 mg). Solubilizers have been used to dissolve the filter and attached algae, which results in a homogeneous sample whose counting efficiency can be determined by one of the standard techniques. The digests of such samples may be very dark and require bleaching with either peroxide (Gargas, 1975) or intense ultraviolet light to decrease color quenching. The efficiency of dissolution varies with the fluor used. Undissolved particles still may cause self-absorption and may require the addition of an emulsifier (Schindler, 1966), such as NCS or Protosol, to prevent settling of particulates.

d. *Standardization of inoculant.* Measurement of the activity of the ^{14}C bicarbonate inoculant can be inaccurate if the liquid-scintillation vial used is acidic. Iverson and others (1976) reported the loss of ^{14}C activity when $NaH^{14}CO_3$ was added to Aquasol^R, a xylene-based fluor. They advised the addition of an organic base, such as phenethylamine, to stabilize the ^{14}C and to achieve complete retention of the radioisotope in the scintillation vial. Other compounds that have been found suitable in toluene-based fluors include Bio-Sol, PCS tissue solubilizer, and monethylamine. The efficiency of retention of inorganic ^{14}C in any scintillation vial should be evaluated prior to onsite studies.

e. Commerical ^{14}C bicarbonate solutions. The purity of commercially supplied $NaH^{14}CO_3$ has been questioned by a number of investigators (Gargas, 1975). Large concentrations of silica, which might be stimulatory to diatom growth, have been reported (Gieskes and Van Bennekom, 1973). Contamination by known organics also has been noted (Sharp, 1977). Use of these inoculants might result in anomalously large excretion rates resulting in small estimates of net productivity. These dangers can be minimized by preparing the ^{14}C bicarbonate solution in one's own laboratory by dilution of a commercial solution using large specific concentrations (1–5 mCi/0.5–2 mL) or from solid $Ba^{14}CO_3$ (Gargas, 1975). Irradiation of the ^{14}C bicarbonate solution using intense ultraviolet light has been used to oxidize all of the organic material to $^{14}CO_2$.

f. Filtration. An integral component of the ^{14}C method as used by early investigators was filtration to concentrate the particulates, enabling the GM counter, which has questionable counting efficiency, to measure the level of sample activity. The process of filtration can cause cell rupture and loss of intracellular carbon if the differential pressure is too great. Although Nalewajko and Lean (1972) and McMahon (1973) attribute the filtration artifact reported by Arthur and Rigler (1967) to filter retention of unfixed radiotracer, pressure differentials should be less than 100 mm of mercury to minimize cell breakage. The acid bubbling technique (Schindler and others, 1972) prevents the uncertainties due to possible absorption, cell rupture, and filtration corrections.

The presence of a filter in the scintillation vial adds to the difficulty of accurate determination of counting efficiency (Pugh, 1970, 1973). Solubilizers have been used to dissolve the filter. Unfortunately, the degree of dissolution attained depends on the filter and the fluor used (Schindler, 1966; Wallen and Geen, 1968; Pugh, 1973; Gargas, 1975). Solubilization of the filter can cause color quench that may be decreased by the addition of 1 to 2 drops of 30-percent hydrogen peroxide (Gargas, 1975) or by heating or suspending the samples in quartz tubes in strong ultraviolet light and adding peroxide (Schindler and others, 1974).

g. ^{14}C bicarbonate elimination. Decontamination of ^{14}C bicarbonate is necessary to remove residual inorganic ^{14}C from the sample. Steemann-Nielsen (1952) suggested exposing the filter to fumes of concentrated HCl. For greater speed, convenience, and safety, a few milliliters of dilute HCl were poured through the filter. The concentration of acid rinse ranged from 0.001*N* (Ryther and Vaccaro, 1954) to 1*N* (Smith and others, 1960). Which concentration is the most efficient is not clear. Williams and others (1972) and McMahon (1973) suggested simply washing the filter using nonradioactive, filtered sample water. Other investigators believed that the filters should not be washed with filtered sample water or dilute acids (McAllister, 1961; Gargas, 1975). Lean and Burnison (1979) suggested placing the filter in a scintillation vial, adding a few drops of 0.5*N* HCl, and fuming for 2 to 3 hours. Using acid bubbling techniques, ^{14}C bicarbonate is stripped from the aqueous sample after the addition of dilute acid. Efficiency of removal using acid bubbling is about 99.99 percent (Sharp, 1977; Mague and others, 1980) at pH 3.

Environmental variables

Accurate measures of primary productivity and an evaluation of their significance is dependent on an understanding of how environmental variables may affect the measured results.

a. Light. Light preconditioning, adaptation, and shock can have a dramatic effect on primary productivity. When using population sites where the light is dim, light shock must be minimized (Steemann-Nielsen and Hansen, 1959; Goldman and others, 1963). Short-term incubation productivity measurements particularly are susceptible to light shock. A satisfactory way to minimize light shock is to make dawn-sunset incubations. Cells preconditioned to dim light and then exposed to bright light have increased excretion rates when compared with those kept under dim light (Nalewajko, 1966; Watt

and Fogg, 1966; Ignatiades and Fogg, 1973). Hellebust (1965) suggests increased rate of excretion in bright light without dim-light preconditioning. Increases in excretion also are reported when samples are preconditioned to bright light and then are incubated in dim light.

An assumption made by many investigators is that for short incubation periods (for example, 2 hours) or long incubation periods (for example, 24 hours) the ^{14}C method measures the same type of productivity, gross or net. A second assumption is that for a specific incubation period, the method measures the same type of productivity, even when cells are exposed to varying irradiances (incubation depth). Neither assumption is correct. Hobson and others (1976) report that incubations for 24 hours are the minimum required for net productivity to be measured by ^{14}C techniques, and estimates of gross productivity can be calculated best after short exposure to ^{14}C. Their findings support those of McAllister and others (1961), Antia and others (1963), Bunt (1965), Ryther and Menzel (1965), and Paerl and Mackenzie (1977) that net productivity is measured in 24-hour experiments. Data from Hobson and others (1976) also indicate that the rate of passage of ^{14}C through the cellular carbon pool is dependent on irradiance. The incubation time required for measurement of net productivity is greater than 24 hours when samples are exposed to dim light. After 24 hours, productivity in the bright-light incubation bottle will more closely approximate net values while that in dim-light incubation bottles will approximate gross values. The integration of primary productivity when compared to depth, therefore, results in an overestimate of net production per unit area.

b. Temperature. Changes in temperature during sample handling or incubation can cause physiological stress on sensitive phytoplankton. All sample handling should be completed as quickly as possible after sample collection. Variation between the natural temperature of a sample and incubation temperature can seriously affect measured productivity. If it is necessary to incubate at a temperature different from the collection temperature, one can correct the data by application of Van't Hoff's law (Gargas, 1975)—an increase in temperature of 10 °C doubles the rate of an enzymatic process.

c. Nutrients. Nutrients may include carbon, trace minerals, chelators, and vitamins in addition to nitrogen, phosphorus, and silica. Primary productivity can be enhanced or inhibited depending on the concentrations of the nutrients involved. Samples from an oligotrophic system may be particularly sensitive to slight perturbations of the nutrient regime (Eppley and others, 1973). The concentration of a nutrient in a bottle may become limiting to photosynthesis during the course of incubation so the measured productivity does not represent accurately the natural system. Ambient nutrient concentrations may not be adequate evidence of the capacity of natural water to sustain intense productivity. Containment of a water sample for a prolonged period restricts interactions between the sample and the mixing and regeneration processes that normally replenish nutrients in the water. Although Eppley (1968) reported nutrient depletion in 36 samples contained for more than 24 hours, recent studies by Steemann-Nielsen (1978) and McCarthy and Goldman (1979) report that, even in oligotrophic systems, enough nutrients for rapid near-optimal growth are constantly available to phytoplankton by heterotrophic processes.

Nutrient contamination of sampling gear or incubation glassware can affect dramatically the results of an experiment. For example, Gieskes and Van Bennekom (1973) report dissolved silica in ^{14}C ampoules at concentrations of 800 to 1,000 μg-atoms/L caused by dissolution of silicate from the glassware wall during autoclaving. One could minimize this source of error by purchasing ^{14}C bicarbonate that has an intense specific activity (for example, 5 mCi/mL), and then diluting the ^{14}C bicarbonate to the desired activity (for example, 5 μCi/mL). Ultraviolet irradiation rather than autoclaving could be used to sterilize the solution.

Processes taking place in the sample bottle also may affect the speciation of a nutrient. In a very eutrophic system, photosynthesis by a contained population might enable the pH to increase to 9 to 10. As a result, NH_4^+ may be converted to the toxic form NH_3.

d. Zooplankton. At times, zooplankton can be so abundant that their grazing pressure might decrease the measured net primary productivity of a sample; therefore, productivity might be measured more accurately if the zooplankton are removed by filtering the sample through a screen. McCarthy and others (1974) reported that prescreening the sample to eliminate grazers had no effect on measured productivity, but production in 16 percent of the screened samples exceeded production in those not screened. They attribute the increased production in screened samples to decreased grazing pressure. Venrick and others (1977) also could not attribute any decline in productivity to prefiltration. However, the phytoplankton population must not be decreased simultaneously with the zooplankton population. If the sizes of the algae and grazing population overlap, the researcher will have to decide whether inclusion of zooplankton in the sample or the exclusion of a part of the phytoplankton community from the sample will bias the results. Simultaneous incubation of screened and unscreened samples may be required.

e. Dark-bottle fixation. The effects of heterotrophic carbon fixation on primary productivity measured by the ^{14}C method are difficult to assess. Although phytoplankton can assimilate CO_2 independent of light energy (Kreb's

Cycle), this is only 1 percent of the photosynthetic rate of CO_2 uptake. The incubation of a dark bottle is included in the ^{14}C method to correct for abiotic processes and heterotrophic uptake that will bias productivity calculations. Dark-bottle fixation, which is a biotic and an abiotic process (Petersen, 1978; Gieskes and others, 1979), is not related to light-bottle fixation, but to other factors and thus must be determined for each experiment. Although the processes involved in assimilation of CO_2 in the dark are not well understood, they account for 10 to 100 percent (Taguchi and Platt, 1977; Gieskes and others, 1979) of the assimilation measured in the light. Therefore, dark-bottle CO_2-uptake rates are subtracted from light-bottle CO_2-uptake rates when calculating productivity.

Sample containment

The ^{14}C method assumes that enclosure of the water sample does not appreciably affect the response of the phytoplankton community to environmental variables, but confinement of the phytoplankton isolates them from many of the physical, chemical, and biological factors they normally encounter and increases their exposure to other variables. The effects of containment have not been investigated thoroughly.

The species composition of a contained population can change markedly during incubation. During incubations of 6 to 24 hours, Venrick and others (1977) noted a decrease in abundance of nearly all components of the phytoplankton and the complete disappearance of some ciliate groups. A tenfold decrease in production by contained samples compared to unenclosed populations is reported by Verduin (1960).

Enclosure in a bottle decreases circulation and turbulent mixing. Sedimentation of heavy cells and flotation of blue-green populations can result, altering the community structure (Goolsby, 1976). Incubation also maintains the organisms at specific depths or light intensity, rather than enabling them to mix vertically through the water column. Estimates of areal photosynthesis have been 19 to 87 percent larger using vertically cycled bottles rather than a series of specific depth samples (Marra, 1978).

Sheldon and others (1973) and Gieskes and others (1979) report that, although bottle volume may cause changes in contained populations, the results are not predictable. Sheldon and others (1973) report a significant increase in particles in small incubation bottles; whereas, no difference could be detected between 4-L bottle populations and the natural community. Gieskes and others (1979) reported little or no production in 30-mL bottles, but more than five times the production in 4-L bottles than that in 300-mL bottles. Although the most prudent approach is to use the largest practical bottle size, the question of optimum incubation bottle size and the effects of sample containment need to be evaluated further.

Respiration

One of the principal limitations of the ^{14}C method is that the respiration rates in phytoplankton cannot be measured directly. Respiration takes place simultaneously with photosynthesis so, in time, some of the ^{14}C photosynthate will be respired back into $^{14}CO_2$ and H_2O. Because a large fraction of many aquatic systems is aphotic, realistic carbon budgets for a system are dependent on accurate estimation of respiration. The rate of heterotrophic ^{14}C fixation in dark bottles is not relevant to this process and, hence, cannot be used to calculate respiration rates (Holm-Hansen, 1974). Measurement of the time required for transfer of carbon through the cellular carbon pool is critical for accurate estimations of net primary productivity. Steemann-Nielsen and Hansen (1959) report respiration rate as the intercept of productivity (in milligrams carbon per hour) at zero irradiance. Until analytical methods are devised, a calculated respiration value rather than a directly measured value will have to suffice when using the ^{14}C method.

Excretion

Estimates of the percent of photosynthate products that are released as extracellular material range from 0 to 75 percent (Sharp, 1977). Refinements in technique (Smith, 1975) have resulted in the conclusion that extracellular products, although a minor component of production [less than 10 percent (Mague and others, 1980)], are real and must be accounted for in accurate estimates of primary productivity. Traditional filtration techniques used in the ^{14}C method hindered the measurement of these substances. Excreted organic material passed through the filter and was discarded with the filtrate. Acidification and bubbling of 0.45-μm filtrate enables measurement of this component of production.

Duration of incubation

The question of the optimal duration for incubation that would result in the most accurate measure of primary productivity is fundamental to the method. The answer depends on many factors and cannot be absolutely prescribed. As evidenced by the preceding discussion, the researcher must decide which is the most suitable incubation period based on the information desired and the limitations with which one is faced. To ensure the standardization and reliability of the data, a 4-hour incubation at midday (1000–1400 hours) is suggested for in-situ light- and dark-bottle methods. The oxygen or ^{14}C method then is chosen on the basis of the limits of measuring oxygen production in the water body in question during that 4-hour incubation.

The most common measures of photosynthesis are gross primary productivity and net primary productivity. The rate of passage of ^{14}C through the carbon cellular pool is of critical importance in determining whether gross or net productivity is being measured. The ^{14}C method cannot measure both types of productivity simultaneously. For short periods, before significant losses by excretion and respiration, gross

rates of production will be measured (Hobson and others, 1976; Savidge, 1978). Incubation periods of at least 24 hours at intense light are required for the ^{14}C method to measure net productivity (Hobson and others, 1976).

Extrapolation from short-term incubations to long-term results must include the diel variability in primary productivity by natural populations. Barnett and Hirota (1967) and Malone (1971) reported variability throughout a day in ^{14}C retention by different groups of phytoplankton. Paerl and Mackenzie (1977) report different diurnal patterns of carbon fixation and loss between net phytoplankton and nanoplankton communities; whereas, MacCaull and Platt (1977) were unable to distinguish a diel rhythm in the rate of photosynthesis of coastal marine phytoplankton. The lack of uniformity and predictability in ^{14}C assimilation during short-term incubations limits the suitability of assessing long-term trends based on short-term incubations. MacCaull and Platt (1977) report that differences in estimates of daily productivity based on early morning or midday productivities were as much as four times. However, Schindler and Holmgren (1971) reported midday incubations to be satisfactory.

If short-term incubations are necessary, a correction similar to that proposed by Vollenweider (1965) should be applied to decrease the magnitude of the error. He reported that if one divided the light day (sunrise to sunset) into 5 equal periods (I to V), then 10, 31, 30, 22, and 7 percent of daily productivity occurred during light periods I through V, respectively. Estimation of total daily productivity from partial-day incubations can be made using the graph shown in figure 63.

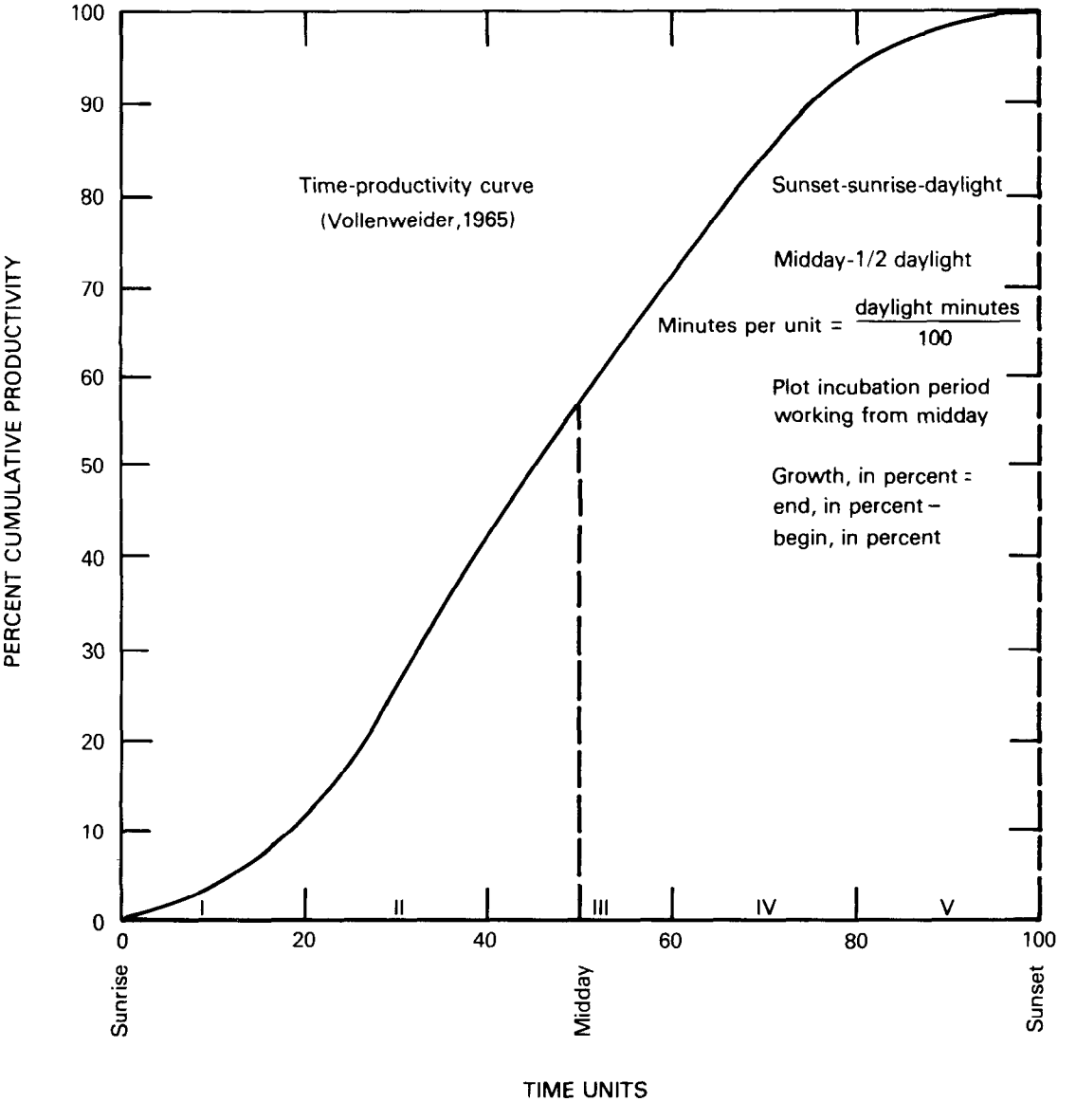

Figure 63.—Cumulative percentages for Vollenweider's five-period light day (modified from Janzer and others, 1973).

Example calculation:

Daylight period (sunrise to sunset):

0600 - 1800 hours = 12 hours = 720 minutes;

$$\text{minutes per unit} = \frac{720}{100} = 7.2 \text{ minutes/time unit.}$$

Incubation period, 1027 to 1427 hours:

0600 - 1027 hours = 4 hours 27 minutes = 267 minutes ÷ 7.2 = 37 time units;

0600 - 1427 hours = 8 hours 27 minutes = 507 minutes ÷ 7.2 = 70 time units;

37 time units = 38 percent cumulative productivity (from fig. 63); and

70 time units = 85 percent cumulative productivity.

Growth, in percent = 85 percent − 38 percent = 47 percent. Alternatively, the correction proposed by Schindler and Holmgren (1971) that uses the ratio of solar radiation for the day to solar radiation during the incubation period is suggested.

Handling and disposal of radioactive wastes

Radioactive ^{14}C (half-life 5,730 years) may be used in quantities as much as 100 μCi (1×10^{-6} Ci) specified by the license exempt provisions of Title 10, Part 30, Section 30.71 Schedule B, October 15, 1971, revision, "Rules of General Applicability to Licensing of Byproduct Materials," U.S. Atomic Energy Commission. Although the quantities used may be license exempt, all efforts should be made to minimize the release of ^{14}C to the environment and to avoid contamination of onsite and laboratory equipment.

The $^{14}CO_3$ and dissolved carbonate species remaining in solution after the phytoplankton have been removed by filtration are precipitated from the water as barium carbonate ($BaCO_3$) by mixing the filtrate with a solution of ammoniacal barium chloride ($BaCl_2 \cdot 2H_2O$) solution in a 20-L polyethylene waste carboy. After the waste solution has been added to the carboy, add 1*N* sodium carbonate (Na_2CO_3) solution to the waste to further scavenge $^{14}CO_3$ from solution. Calculate the maximum volume of 1*N* Na_2CO_3 needed using the following equation:

$$\text{Volume of } 1N\ Na_2CO_3 = 10.1\ [40.4 - (A_s \times V_w \times 0.00197)],$$

where

10 mL 1*N* Na_2CO_3 = 1 g $BaCO_3$;

40.4 g $BaCO_3$ = 50 g $BaCl_2 \cdot 2H_2O$ in polyethylene waste carboy;

A_s = sample alkalinity as calcium carbonate ($CaCO_3$), in milligrams per liter;

V_w = volume of waste in the carboy; and

0.00197 = factor to convert weight of $CaCO_3$, in milligrams, to grams $BaCO_3$.

Example: If a carboy contained 10 L of liquid waste that had an alkalinity of 85 mg/L, the volume, in milliliters of 1*N* Na_2CO_3 required to completely react with the 50 g $BaCl_2 \cdot 2H_2O$ added to the carboy, would be

$$\text{Volume} = 10.1\ [40.4 - (85 \times 10 \times 0.00197)] = 391 \text{ mL required for total precipitation.}$$

Scavenging of the ^{14}C from solution is more complete if the Na_2CO_3 solution is added in four or five volumes. The resulting $BaCO_3$ precipitate is allowed to settle before making the next addition of Na_2CO_3.

After settling, the $BaCO_3$ is separated by decantation of the supernatant. Add plaster of paris to the $BaCO_3$ slurry to form a solid block that is sent to the counting laboratory for disposal as radioactive waste. Retain the supernatant until a laboratory check of an aliquot by liquid-scintillation counting has indicated that the ^{14}C scavenge essentially was complete. The supernatant then may be discarded.

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