



# Techniques of Water-Resources Investigations of the United States Geological Survey

## Chapter A4

### METHODS FOR COLLECTION AND ANALYSIS OF AQUATIC BIOLOGICAL AND MICROBIOLOGICAL SAMPLES

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Book 5

LABORATORY ANALYSIS

# Oxygen light- and dark-enclosure method for periphyton

(B-8040-85)

## Parameters and Codes:

Productivity, primary, gross [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70960

Productivity, primary, gross [ $\text{mg}(\text{C}/\text{m}^2)/\text{d}$ ]: 70962

Productivity, primary, net [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70964

Productivity, primary, net [ $\text{mg}(\text{C}/\text{m}^2)/\text{d}$ ]: 70966

Respiration [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70968

## 1. Applications

The enclosure method of primary productivity is most suitable for shallow streams and for the littoral zones of lakes where light penetration is sufficient for photosynthesis. Best results are obtained in eutrophic water in which the production rate is about 3 to 200  $\text{mg}(\text{C}/\text{m}^3)/\text{h}$  during the photo-period (Strickland and Parsons, 1968, p. 263; Schindler and others, 1973).

## 2. Summary of method

Known areas of substrates containing living periphyton are isolated in sealed containers and filled with filtered stream or lake water of known dissolved-oxygen concentration. The samples are exposed in the euphotic zone, usually at the original depth, for a known period of time. Changes in the dissolved-oxygen concentrations of the enclosed samples are interpreted in terms of photosynthesis and respiration per unit area of periphyton.

## 3. Interferences

3.1 The method uses isolated periphyton 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 equipment should be made of plastic or glass, and the essential metal parts should be made of stainless steel. Copper, brass, and bronze fittings should not be used. Samples of periphyton should be kept in the shade or in a circulating chamber before incubation to prevent exposure of unadapted algae to full sunlight. Light leaks into the dark chamber must be prevented.

3.2 The formation of bubbles in the experimental containers results in errors in the determination of dissolved-oxygen concentration changes. Air bubbles in circulating chambers result from two causes: (1) Incomplete filling of chambers, or (2) supersaturation. Extra care should be practiced initially to ensure that no trapped air bubbles are present in the chamber at the beginning of the experiment. Supersaturation also may be caused by warming of the sample between collection and filling or by excessive photosynthesis

during the experiment. Supersaturation can be prevented by adjusting the length of the experimental period or by increasing the chamber size for light-bottle and dark-bottle studies.

3.3 Photosynthesis and respiration of phytoplankton in the water used to fill the circulating chambers can affect the results. This is prevented by filtering the water through a glass-fiber or membrane filter.

3.4 Microbial activity and chemical oxygen demand cause losses of dissolved oxygen when incubation times exceed a few hours. Interferences with the chemical determination of dissolved oxygen were described by Skougstad and others (1979) and the 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 used must be free of agents that inhibit photosynthesis and respiration.

4.1 *Artificial substrates*, made of glass slides, Plexiglas or polyethylene strips, tygon tubing, styrofoam, or other materials. See figures 19 and 20 for selected types of artificial substrates.

4.2 *BOD bottles*, numbered, 300 mL, Pyrex or borosilicon glass, that have flared necks and pointed ground-glass stoppers (Note 1).

Note 1: Before use, fill with 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 0.01N sodium thiosulfate solution followed by thorough rinsing using distilled water. Do not use phosphorus-based detergents.

4.3 *Collecting devices*, for the removal of periphyton from natural substrates. Three devices for collecting a known area of periphyton from natural or artificial substrates are shown in figure 18.

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

4.5 *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).

4.6 *Filter flask*, 1 or 2 L. For onsite use, a polypropylene flask is suggested.

4.7 *Filter funnel*, vacuum, 1.2-L capacity, stainless steel.

4.8 *Glass-fiber filters*, 47-mm diameter disks, or membrane filters, white, plain, 0.45- $\mu$ m mean pore size, 47-mm diameter.

4.9 *Light and dark circulating chambers*, of suitable size and shape, made of glass or plastic (McIntire and others, 1964; Wetzel, 1964, 1965; Thomas and O'Connell, 1966; Hansmann and others, 1971; Pfeifer and McDiffett, 1975; Rodgers and others, 1978; Gregory, 1980). Transparent containers can be made opaque by painting them black and covering the paint with overlapping strips of black plastic tape. The exposed parts of stoppers, if present, should be similarly blackened and covered with a hood of several layers of aluminum foil during use.

4.10 *Polyethylene bottles*, 8-L capacity, that has cap and bottom tubulation.

4.11 *Scraping devices*, razor blades, stiff brushes, spatulas, or glass slides, for removing periphyton from artificial substrates. The edge of a glass microscope slide is excellent for scraping periphyton from hard, flat surfaces (Tilley, 1972).

4.12 *Vacuum pump*, water-aspirator pump, or an electric vacuum pump for laboratory use; a hand-operated vacuum pump that has a gauge for onsite use.

4.13 *Water-sampling bottle*, Van-Dorn type. 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.

5.2 *Distilled or deionized water*.

5.3 *Filling water*, for the experimental circulating chambers. Prepare by filtering through a glass-fiber or a 0.45- $\mu$ m membrane filter to remove plankton, unless it is known that plankton metabolism will be insignificant. Filter enough water to rinse and fill the chambers and to determine the initial concentration of dissolved oxygen. The water should be slightly undersaturated with dissolved oxygen. Dissolved oxygen may be decreased to 5 or 6 mg/L by passing the water through a sparging column (Hansmann and others, 1971) or by adding sodium sulfite with cobaltous chloride as a catalyst for the sulfite oxidation reaction (Pfeifer and McDiffett, 1975). For diel studies using large chambers, starting at dusk also will decrease the dissolved-oxygen concentration because periphyton metabolism occurs in the dark. This method requires continuous monitoring for dissolved-oxygen concentration because light and dark measurements are made sequentially in the same chamber.

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

5.5 *Sodium thiosulfate solution*, 0.01N. Dissolve 2.5 g sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in distilled water and dilute to 1 L.

## 6. Analysis

After suitable incubation, remove a sample of water from each circulating chamber and determine the dissolved-oxygen concentration. Average the results from duplicate samples.

## 7. Calculations

Primary productivity is expressed as the quantity of oxygen released or carbon assimilated per unit time. Respiration is expressed as the quantity of dissolved oxygen 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. Average results from duplicate measurements.

7.1 Gross primary productivity [ $\text{mg}(\text{O}_2/\text{m}^2)/t$ ]

$$= \frac{(LC - DC)V}{tA}$$

where

$LC$  = dissolved-oxygen concentration, in milligrams per liter, in the light circulating chamber after incubation;

$DC$  = dissolved-oxygen concentration, in milligrams per liter, in the dark circulating chamber after incubation;

$V$  = volume of water in the circulating chamber, in liters;

$t$  = incubation period, in hours or days; and

$A$  = area of periphyton-covered substrate, in square meters.

7.2 Gross primary productivity [ $\text{mg}(\text{C}/\text{m}^2)/t$ ]

$$= \frac{(LC - DC)V}{tA} \times \frac{12}{32}$$

where

$LC$ ,  $DC$ ,  $V$ ,  $t$ , and  $A$  = as in 7.1;

12 = atomic weight of carbon; and

32 = molecular weight of oxygen.

7.3 Net primary productivity [ $\text{mg}(\text{O}_2/\text{m}^2)/t$ ]

$$= \frac{(LC - IC)V}{tA}$$

where

$LC$ ,  $DC$ ,  $V$ ,  $t$ , and  $A$  = as in 7.1; and

$IC$  = initial dissolved-oxygen concentration, in milligrams per liter, in the light circulating chamber before incubation.

7.4 Net primary productivity [ $\text{mg}(\text{C}/\text{m}^2)/t$ ]

$$= \frac{(LC - IC)V}{tA} \times \frac{12}{32},$$

where

$LC$ ,  $V$ ,  $t$ , and  $A$  = as in 7.1;  
 $IC$  = as in 7.3; and  
 12 and 32 = as in 7.2.

7.5 Respiration [ $\text{mg}(\text{O}_2/\text{m}^2)/t$ ]

$$= \frac{(IC - DC)V}{tA},$$

where

$DC$ ,  $V$ ,  $t$ , and  $A$  = as in 7.1; and  
 $IC$  = as in 7.3.

## 8. Reporting of results

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

## 9. Precision

No numerical precision values are available.

## 10. Sources of information

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# Diel oxygen-curve method for estimating primary productivity and community metabolism in streams

(B-8120-85)

## Parameters and Codes:

Productivity, primary, gross [ $\text{mg}(\text{O}_2/\text{m}^3)/\text{d}$ ]: 70959

Productivity, primary, gross [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70960

Productivity, primary, net [ $\text{mg}(\text{O}_2/\text{m}^3)/\text{d}$ ]: 70963

Productivity, primary, net [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70964

Respiration [ $\text{mg}(\text{O}_2/\text{m}^3)/\text{d}$ ]: 70967

Respiration [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70968

Two analytical approaches are described for evaluating oxygen metabolism in streams. The graphical approach, developed for a hypothetical stream, provides an estimate of gross primary productivity, or the total quantity of oxygen produced during a diel (24-hour) period, and of total community respiration, or the total quantity of oxygen consumed during a diel period. Diel net primary productivity, or the oxygen that was not consumed, is calculated as the difference between gross productivity and total respiration. The graphical approach assumes that daytime respiration is constant or that it varies only linearly with time. This is the major limitation to the graphical approach.

The alternative analytical approach consists of data processing using a Fortran computer program (Program designation: Primary production, J330). A complete description of the program is in the user manual by Stephens and Jennings (1976). The program will calculate daytime net oxygen production and nighttime oxygen respiration for the single-station or the two-station analysis. The arithmetic difference between these is a 24-hour community metabolism that is equivalent to diel net primary productivity and should be entered into the computer using parameter code 70964. Other parameter codes are not compatible for any calculations made by program J330. Gross productivity is not calculated. Program J330 functions by assuming that production occurs only during daylight hours, and any change in dissolved oxygen that occurred during this period, after correcting for diffusion, is due to production. Any change in dissolved oxygen that occurred during hours of darkness, after correcting for diffusion, is due to respiration.

## 1. Applications

The method is applicable to streams in which the biological productivity is relatively intense. If the incoming water has a metabolic history similar to the outflowing water, the single-station analysis may be made. If the metabolic characteristics of the inflowing water are unknown or are not

similar to the outflowing water, the two-station analysis should be made.

## 2. Summary of method

Dissolved-oxygen concentration and water temperature are determined in the open water continuously or at 1- to 3-hour intervals for at least 24 hours. Community primary productivity and respiration are estimated from rates of oxygen change after correction for the exchange of oxygen between the water and the atmosphere.

## 3. Interferences

3.1 Undetected advection, accrual of surface or ground water, and loss of oxygen from the water in the form of bubbles are possible sources of error. The limited sensitivity of this diel oxygen-curve method precludes its use in unproductive water. Limitations of dissolved-oxygen meters are that oxygen changes can be greater than 0.1 mg/L. Corresponding changes when using the Winkler method require a minimum of 0.02 mg/L. The diel oxygen-curve method should be used in water of comparative homogeneity.

3.2 In shallow, turbulent streams, the rate at which equilibrium is achieved between the water and the atmosphere is too rapid for the diel oxygen-curve method to be used. In these instances, a method based on the equilibrium between carbon dioxide, bicarbonate, and pH has been developed to measure photosynthesis and respiration (Wright and Mills, 1967).

## 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 *Barometer*, for measuring local barometric pressure.

4.2 *Floating-diffusion dome*, clear Plexiglas, approximately 22 cm in diameter, or larger. Suitable domes are available from restaurant equipment suppliers. The device described by Hall (1971) consists of a 40.5-cm-diameter dome sealed

onto a floating collar of 1-cm marine plywood (fig. 60). The oxygen and temperature sensors can be inserted from below into a support inside the dome or through holes in the dome. The dome is painted silver to decrease the greenhouse effect on the inside temperature.

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 *Graph paper*, 1-mm squares.

4.5 *Recorder, portable*, for continuous measurements of dissolved oxygen or for use with oxygen meters.

4.6 *Stirrer, submersible, battery operated*, for use with membrane-electrode oxygen instruments.

4.7 *Thermistor or thermometer*, for determining water temperature and gas temperature in the diffusion dome. Most oxygen meters include thermistors suitable for making these measurements.

4.8 *Water-sampling bottle*, Van-Dorn type. 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 *Reagents required for the azide modification of the Winkler method*, for dissolved oxygen (Skougstad and others, 1979; American Public Health Association and others, 1985).

5.2 *Sodium thiosulfate solution*, 0.01*N*. Dissolve 2.5 g sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in distilled water, and dilute to 1 L.

## 6. Analysis

6.1 *Single-station analysis*. Using the data collected and following the procedures in the "Single-Station Analysis" subsection of the "Primary Productivity" section, tabulate time versus temperature and dissolved-oxygen concentration as listed in table 15, columns 1 through 3, and plot curves as in figure 64A and B. Graph paper that has 1-mm squares is convenient to use for these plots.

6.2 Determine the percentage saturation for each dissolved-oxygen value using tables indicating oxygen solubility at various temperatures, pressures, and salinities (Mortimer, 1981; American Public Health Association and others, 1981). Tabulate the values in table 15, column 6, and plot a curve of time versus measured percentage of dissolved-oxygen saturation as shown in figure 64C.

6.3 Using the measured dissolved-oxygen-concentration data (table 15, col. 3), determine the hourly rate of change in dissolved oxygen (milligrams per liter per hour) by subtracting successive pairs of dissolved-oxygen values. Tabulate the values, and plot the rate curve from the values in table 15, column 4, and as shown in figure 64D (curve labeled "Before correction for diffusion").

6.4 Subtract each percentage-saturation value determined in 6.2 from 100 percent, recording values less than 100 as negative. List these percentage-saturation deficits as in table 15, column 7. Proceed to 6.9 or 6.10 depending on the

method used to determine the diffusion rate. If area-based gas-transfer coefficient,  $K$ , is estimated, proceed to 6.12.

6.5 *Two-station analysis*. Using the data collected and following the procedures in the "Two-Station Analysis" subsection of the "Primary Productivity" section, determine the average dissolved-oxygen concentration and average temperature for the reach between stations for each sample interval. Tabulate time versus average temperature and time versus average dissolved-oxygen concentration as listed in table 15, columns 1 through 3. Plot curves as in figure 64A and B. Graph paper that has 1-mm squares is convenient to use for these plots.

6.6 Determine the average percentage of dissolved-oxygen saturation for each sample interval using tables indicating oxygen solubility at various temperatures, pressures, and salinities (American Public Health Association and others, 1985). Tabulate the values in table 15, column 6, and plot a curve of time versus average percentage of dissolved-oxygen saturation as shown in figure 64C.

6.7 Using the average dissolved-oxygen-concentration data for the reach (table 15, col. 3), determine the average hourly rate of change in dissolved oxygen (milligrams per liter per hour) by subtracting successive pairs of oxygen values. Tabulate the values, and plot the rate curve from the values in table 15, column 4, and as shown in figure 64D (curve labeled "Before correction for diffusion").

6.8 Subtract each average percentage-saturation value determined in 6.6 from 100 percent, recording values less than 100 as negative. List these average percentage-saturation deficits as in table 15, column 7. Proceed to 6.9, 6.10, or 6.13 depending on the method used to determine the diffusion rate. If  $K$  is estimated, proceed to 6.12.

6.9 Determine the volume-based gas-transfer coefficient,  $k$ , for each sample interval from measurements of the hydraulic parameters. The following procedure is adapted from Hall (1971) for  $k$  derived from volume-based gas-transfer coefficient per day,  $k_2$ . Thus, from Churchill and others (1962),

$$k_2 \text{ (at } 20^\circ\text{C)} = 5.026V^{0.969}R^{-1.673},$$

where

$k_2$  = volume-based gas-transfer coefficient per day;  
 $V$  = cross-sectional mean velocity, in feet per second;  
 and

$R$  = hydraulic radius (approximately the depth of flow), in feet.

Using a known dissolved-oxygen-saturation value for a specific time, Hall (1971) obtained the following equation for  $k$  in terms of  $k_2$ :

$$k = \frac{2.3 (k_2 C_s)}{24},$$

where

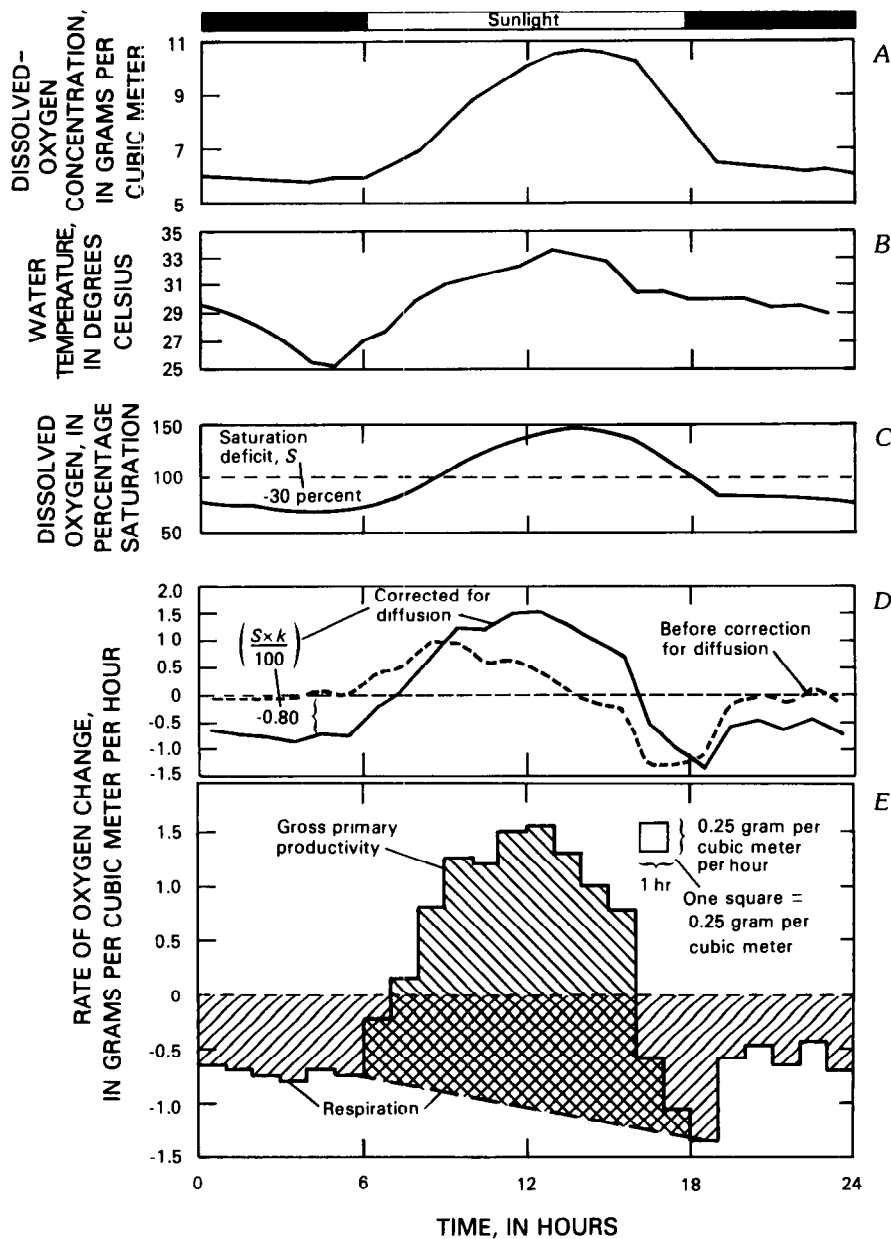
$k$  = volume-based gas-transfer coefficient, in grams per cubic meter per hour, and is for a 100-percent saturated deficit; and

Table 15.—Hypothetical data for determining community primary productivity of a stream by the oxygen-curve method

[The mean depth of flow is 1.2 meters; the gas transfer coefficient on a volume basis,  $k$ , is 2.67 grams per cubic meter per hour at 100-percent saturation deficit; h, hours; °C, degrees Celsius; mg/L, milligrams per liter; (mg/L)/h, milligrams per liter per hour; (g/m<sup>3</sup>)/h, grams per cubic meter per hour]

1	2	Dissolved oxygen						
		3	4	5	6	7	8	9
Time (h)	Temperature (°C)	Measured (mg/L) <sup>1</sup>	Rate of change [(mg/L)/h]	Concentrations at saturation (mg/L)	Measured saturation (percent)	Average saturation deficit, $\frac{S}{100}$ (percent)	$\frac{S \times k}{100}$ [(g/m <sup>3</sup> )/h]	Corrected rate of change [(g/m <sup>3</sup> )/h]
0000	29.5	6.00		7.7	78			
0100	29.0	5.95	-0.05	7.8	76	-23.0	-0.614	-0.664
0200	28.0	5.90	-.05	7.9	75	-24.5	-.654	-.704
0300	27.0	5.85	-.05	8.1	72	-26.5	-.708	-.758
0400	25.5	5.80	-.05	8.3	70	-29.0	-.774	-.824
0500	25.0	5.90	+ .10	8.4	70	-30.0	-.801	-.701
0600	27.0	5.90	.00	8.4	70	-28.5	-.761	-.761
0700	28.0	6.30	+ .40	8.1	73	-23.5	-.627	-.227
0800	30.0	6.85	+ .55	7.9	80	-15.0	-.400	+ .150
0900	31.0	7.85	+1.00	7.6	90	-7.5	-.200	+ .800
1000	31.5	8.80	+ .95	7.5	105	+11.5	+ .307	+1.257
1100	32.0	9.40	+ .60	7.4	118	+22.5	+ .601	-1.201
1200	32.5	10.05	+ .65	7.4	127	+32.0	+ .854	-1.504
1300	32.5	10.05	+ .45	7.4	137	+41.0	+1.095	+1.545
1400	33.5	10.50	+ .10	7.2	145	+45.0	+1.202	+1.302
1500	33.0	10.60	-.15	7.3	145	+43.5	+1.161	+1.011
1600	32.5	10.45	-.25	7.4	142	+38.5	+1.028	+ .778
1700	30.5	10.20	-1.30	7.6	135	+26.5	+0.708	-0.592
1800	30.5	8.90	-1.30	7.6	118	+9.0	+ .240	-1.060
1900	30.0	7.60	-1.15	7.6	100	-7.5	-.200	-1.350
2000	30.0	6.45	-.15	7.6	85	-16.0	-.427	-.577
2100	30.0	6.30	.00	7.6	83	-17.5	-.467	-.467
2200	29.5	6.30	-.15	7.7	82	-19.0	-.507	-.657
2300	29.5	6.15	+ .10	7.7	80	-20.0	-.534	-.434
2400	29.0	6.25	-.15	7.8	80	-21.0	-.561	-.711
2400	29.0	6.10		7.8	78			

<sup>1</sup>Milligrams per liter equals grams per cubic meter.



Diffusion correction calculations:

$$K = 3.2 \text{ grams per square meter per hour}$$

$$k = \frac{K}{z} = \frac{3.2 \text{ grams per square meter per hour}}{1.2 \text{ meters}} = 2.67 \text{ grams per cubic meter per hour}$$

$$\frac{S \times k}{100} = \frac{-30 \times 2.67}{100} = -0.80 \text{ gram per cubic meter per hour}$$

Gross productivity =  $\frac{(81.3 \text{ squares})}{\text{day}} (0.25 \text{ gram per cubic meter}) (1.2 \text{ meters}) = 24.4 \text{ grams per square meter per day}$

Community respiration =  $\frac{(84.1 \text{ squares})}{\text{day}} (0.25 \text{ gram per cubic meter}) (1.2 \text{ meters}) = 25.2 \text{ grams per square meter per day}$

Figure 64.—Diel oxygen curve and supported data (from tables 14 and 15) for determining community primary productivity and community respiration of a stream by the oxygen-curve method. The mean depth of flow is 1.2 meters, the gas-transfer coefficient on an area basis, *K*, is 3.2 grams per square meter per hour, and on a volume basis, *k*, is 2.67 grams per cubic meter per hour at 100-percent saturation deficit (modified from Odum and Hoskin, 1958).



$C_s$  = the 100-percent saturation deficit, in grams per cubic meter.

The 2.3 converts the  $k_2$  defined in terms of  $\log_{10}$  to  $k$  defined in terms of  $\log_e$ .

For temperatures other than 20 °C, correct to  $k_2$  at a rate of 2.41-percent increase or decrease per degree above or below 20 °C. Estimate  $k$  for the study period by averaging the  $k$  values determined for each sampling interval (Note 1). Proceed to 6.14.

Note 1: Some situations require use of different gas-transfer coefficients at different times of day as explained in the "Diffusion Rate" subsection.

6.10 Determine the diffusion rate,  $D$ , for each nighttime sample interval from measurements made in the floating-diffusion dome (table 14). Calculate the volume of oxygen in the dome at the beginning and end of the sample interval as follows:

$$V_t = V_d(0.21) \frac{F_t}{100},$$

where

$V_t$  = volume of oxygen, in milliliters, in the dome at a specific time,  $t$ ;

$V_d$  = volume of atmospheric gases, in milliliters, in the dome;

$F_t$  = percentage oxygen saturation in the dome atmosphere at time,  $t$ , when fresh air equals 100-percent oxygen saturation; and

0.21 = fractional volume of oxygen in the air.

Indicate the concentration of oxygen in the floating-diffusion dome in terms of standard temperature and pressure for each sample interval using the equation

$$\Delta V = \frac{273V_0}{273 + T_0} - \frac{273V_1}{273 + T_1},$$

where

$\Delta V$  = change in volume of oxygen, in milliliters, in the dome at standard temperature and pressure;

$V_0$  = volume of oxygen, in milliliters, in the dome at the beginning of the interval;

$T_0$  = temperature, in degrees Celsius, in the dome at the beginning of the interval;

$V_1$  = volume of oxygen, in milliliters, in the dome at the end of the interval;

$T_1$  = temperature, in degrees Celsius, in the dome at the end of the interval; and

273 = factor for converting to absolute temperature.

Oxygen weighs 0.00143 g/mL at standard temperature and pressure. Therefore,  $D$  may be computed from

$$D = \frac{(\Delta V)(0.00143)}{A(\Delta t)},$$

where

$D$  = rate of diffusion of oxygen into the water, in grams per square meter per hour;

$A$  = area of the dome, in square meters, that is in contact with the water surface; and

$\Delta t$  = time interval, in hours, between the two measurements.

6.11 Using the following equation, convert the area-based rate of diffusion for each sampling interval to a value at 0-percent saturation of the water (rate of diffusion if the water contained no oxygen) by dividing  $D$  by the average percentage-saturation deficit during the time of measurement, or

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

where

$K$  = area-based gas-transfer coefficient, in grams per square meter per hour, at 0-percent saturation (100-percent saturation deficit); and

$S$  = average percentage-saturation deficit between the water and the air during the sample interval (derived from 6.4 to 6.8).

6.12 Convert each area value to a volume value by dividing by the mean depth of water, in meters, or

$$k = \frac{K}{z},$$

where

$k$  = volume-based gas-transfer coefficient, in grams per cubic meter per hour, at 0-percent saturation; and

$z$  = mean depth, in meters.

Estimate  $k$  for the study period by averaging the  $k$  values determined for each sampling interval (Note 2). Proceed to 6.14.

Note 2: Some situations require use of different diffusion constants at different times of day.

6.13 Determine the average  $k$  for each sample interval from measurements of the nighttime average rate of oxygen change. This can be estimated by calculating  $k$  values for each nighttime sampling interval using the Odum (1956) method as presented by Eley (1970):

$$k = \frac{q_n - q_{n+1}}{S_n - S_{n+1}},$$

where

$q_n$  = average rate of change in oxygen, in grams per cubic meter, for the reach at nighttime,  $n$ ;

$q_{n+1}$  = average rate of change in oxygen, in grams per cubic meter, for the reach at nighttime,  $n + 1$ ;

$S_n$  = average oxygen-saturation deficit for the reach at nighttime,  $n$ ; and

$S_{n+1}$  = average oxygen-saturation deficit for the reach at nighttime,  $n + 1$ .

Proceed to 6.14.

6.14 Determine the quantity of oxygen (grams per cubic meter) gained or lost by diffusion during each sampling

interval. To adjust for atmospheric reaeration, multiply the average  $k$  (from 6.9, 6.12, or 6.13) by each percentage oxygen-saturation deficit value (from 6.4 or 6.8), and divide by 100 to convert percentage to fractional values. List these values as in table 15, column 8.

6.15 Using figure 64D, the hourly rate-of-change graph plotted as directed in 6.3 or 6.7, prepare a corrected rate-of-change curve by adding or subtracting, graphically, the quantity of oxygen, in grams per cubic meter, gained or lost by diffusion during each sampling interval (from 6.14). Draw the curve as in figure 64D (curve labeled "Corrected for diffusion"). The corrected rate-of-change curve is replotted as a step function to facilitate graphical integration as shown in figure 64E.

6.16 Connect a line between the presunrise and postsunset negative rate-of-change points on the corrected rate-of-change curve as shown in figure 64E (Odum and Wilson, 1962). This line is an estimate of daytime respiration (Note 3).

Note 3: The maximum rate of respiration often occurs immediately after sunset, and the rate declines to a minimum before sunrise. Where presunrise and postsunset respiration differ, connect the line diagonally from the dawn-respiration rate to the sunset-respiration rate on the corrected rate-of-change graph. The values for respiration and gross primary productivity are affected by the placement of the respiration line. The accuracy of the method probably is limited by this step (Odum and Hoskin, 1958, p. 22). Graphs in which the rates of change are very irregular enable more subjectivity of choice of the respiration line than do smooth curves.

## 7. Calculations

The following volume- or concentration-based calculations, in grams per cubic meter per day, can be converted to area-based calculations, in grams per square meter per day, by multiplying by the average water depth of the study area, in meters.

7.1 An estimate of gross primary productivity,  $P_g$ , in grams oxygen per cubic meter per day, is the area above the daytime respiration line and below the daytime rate-of-change line (fig. 64E). The area may be determined from the plot by counting the graph-paper squares and multiplying by the value, in grams per cubic meter, of one square.

7.2 An estimate of community respiration,  $R_t$ , in grams oxygen per cubic meter per day, is the area above the nighttime negative rate-of-change line and the daytime respiration line and below the zero rate-of-change line (fig. 64E). The area may be determined from the plot by counting the graph-paper squares and multiplying by the value, in grams per cubic meter, of one square. The graphical procedure integrates the hourly values during a 24-hour period; hence, the respiration rate is on a per-day basis.

7.3 An estimate of net primary productivity,  $P_n$ , in grams oxygen per cubic meter per day, is the difference between  $P_g$  and  $R_t$ .

7.4 An index of the trophic nature of the community may be calculated as the ratio of photosynthetic productivity to respiration,  $P:R$ . Communities having a  $P:R$  ratio less than 1 have an excess of respiration compared to productivity. They are heterotrophic; that is, they degrade organic compounds through oxygen metabolism at a greater rate than they fix carbon in photosynthesis. Autotrophic communities have a  $P:R$  ratio greater than 1 and release more oxygen through photosynthesis than they consume through respiration.

## 8. Reporting of results

Report community primary productivity and respiration, in milligrams, as follows: less than 10 mg, one decimal; 10 mg or more, two significant figures.

## 9. Precision

No numerical precision data are available.

## 10. Sources of information

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# Diel oxygen-curve method for estimating primary productivity and community metabolism in stratified water

(B-8100-85)

## Parameters and Codes:

Productivity, primary, gross [ $\text{mg}(\text{O}_2/\text{m}^3)/\text{d}$ ]: 70959

Productivity, primary, gross [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70960

Productivity, primary, net [ $\text{mg}(\text{O}_2/\text{m}^3)/\text{d}$ ]: 70963

Productivity, primary, net [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70964

Respiration [ $\text{mg}(\text{O}_2/\text{m}^3)/\text{d}$ ]: 70967

Respiration [ $\text{mg}(\text{O}_2/\text{m}^2)/\text{d}$ ]: 70968

If complete vertical mixing occurs in the water body, a series of single-station analyses may be sufficient to characterize the oxygen regime in the water. However, in many places, the water may be stratified, and a vertical dissolved-oxygen variation from near saturation at the surface to near zero concentration at the bottom may exist. If these conditions do exist, production of oxygen may be limited to the euphotic zone, and an oxygen deficit could exist in the lower or hypolimnetic water.

Two analytical approaches for evaluating oxygen metabolism in stratified water are described and contrasted using synthetic data for a hypothetical lake. The graphical approach provides an estimate of gross primary productivity, or the total quantity of oxygen produced during a diel (24-hour) period, and of total community respiration, or the total quantity of oxygen consumed during a diel period. Diel net primary productivity, or the oxygen that was not consumed, is calculated as the difference between gross productivity and total respiration. The graphical approach assumes that daytime respiration is constant or that it varies only linearly with time. This is the major limitation to the graphical approach.

The alternative analytical approach consists of data processing using a Fortran computer program (Program designation: Primary production, J330). A complete description of the program is in the user manual by Stephens and Jennings (1976). The program will calculate daytime net oxygen production and nighttime oxygen respiration for the single-station or two-station analysis. The arithmetic difference between these is a 24-hour community metabolism that is equivalent to diel net primary productivity and should be entered into the computer using parameter code 70964. Other parameter codes are not compatible for any calculations made by program J330. Gross productivity is not calculated. Program J330 functions by assuming that production occurs only during daylight hours, and any change in dissolved oxygen that occurred during this period, after correcting for diffusion, is due to production. Any change

in dissolved oxygen that occurred during hours of darkness, after correcting for diffusion, is due to respiration. The program also enables exchange between the horizontal segments of a stratified water body using estimated or measured vertical-dispersion coefficients.

## 1. Applications

The method is applicable to eutrophic estuaries, lakes, and other stratified bodies of water in which a vertical variation in dissolved oxygen exists. The lower limit for measurable oxygen production occurs when phytoplankton densities, expressed as chlorophyll *a*, are less than  $1 \text{ mg}/\text{m}^3$  (Talling, 1974).

## 2. Summary of method

From average values for temperature, dissolved oxygen, and, if appropriate, salinity, an average rate of change in dissolved oxygen is calculated for the entire water body. Average dissolved-oxygen values for the surface-water layer are corrected for diffusion. The resulting curve of diel changes in the in-situ concentration of dissolved oxygen, mainly due to photosynthesis and respiration, is used to estimate the primary productivity of the entire aquatic-plant community.

## 3. Interferences

Undetected advection, accrual of surface or ground water, and loss of oxygen from the water in the form of bubbles are possible sources of error. The limited sensitivity of this diel oxygen-curve method precludes its use in unproductive water. Limitations of dissolved-oxygen meters are that oxygen changes can be greater than  $0.1 \text{ mg}/\text{L}$ . Corresponding changes when using the Winkler method require a minimum of  $0.02 \text{ mg}/\text{L}$ . The method should be used in water of comparative horizontal homogeneity.

## 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 *Barometer*, for measuring local barometric pressure.

4.2 *Floating-diffusion dome*, clear Plexiglas, approximately 22 cm in diameter, or larger. Suitable domes are available from restaurant equipment suppliers. The device described by Hall (1971) consists of a 40.5-cm-diameter dome sealed onto a floating collar of 1-cm marine plywood (fig. 60). The oxygen and temperature sensors can be inserted from below into a support inside the dome or through holes in the dome. The dome is painted silver to decrease the greenhouse effect on the inside temperature.

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 *Equipment for determination of salinity*, by titration (Strickland and Parsons, 1968) or by electrical conductivity, if appropriate.

4.5 *Graph paper*, 1-mm squares.

4.6 *Polar planimeter and maps*, appropriate to the study (see 6.1).

4.7 *Thermistor or thermometer*, for determining water temperature and gas temperature in the diffusion dome. Most oxygen meters include thermistors suitable for making these measurements.

4.8 *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.

4.9 *Water-sampling bottle*, Van-Dorn type. 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 *Reagents required for the azide modification of the Winkler method*, for dissolved oxygen (Skougstad and others, 1979; American Public Health Association and others, 1985).

5.2 *Sodium thiosulfate solution*, 0.01*N*. Dissolve 2.5 g sodium thiosulfate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) in distilled water, and dilute to 1 L.

5.3 *Reagents for determination of salinity* (Strickland and Parsons, 1968), if appropriate.

## 6. Analysis

6.1 *Lake morphometry*. The volume of water contained in a lake may be calculated from measurements of each depth contour on a good topographic or bathymetric map. An accurate, scaled map and planimeter are required. Winter (1981) describes errors in bathymetric map drawing. Determine the area enclosed within each contour interval using a planimeter. Typically, the planimeter will indicate area, in square inches (or centimeters), that then must be converted to actual area using the map scale. A small lake (fig. 65) was planimetered to obtain the morphometric data in table 16. Using the map scale of 1:250,000, the actual area represented by 1 in<sup>2</sup> of map was calculated to be  $6.25 \times 10^{10}$

in<sup>2</sup>. This value, when divided by the number of square inches in a square mile ( $4.01 \times 10^9$ ), provides the factor (15.59) used to calculate the actual surface area of each contour. Conversion to metric units is made using the relation 1 mi<sup>2</sup> equals  $2.59 \times 10^6$  m<sup>2</sup> (table 16, col. 3).

The volume of each contour (table 16, col. 4) is calculated as

$$V_{n-m} = \frac{1}{3}(A_m + A_n + A_m A_n)(n-m),$$

where

$V_{n-m}$  = the volume of a given element between contour  $n$  and contour  $m$ , in cubic meters;

$A_m$  = the area at contour  $m$ , in square meters;

$A_n$  = the area at contour  $n$ , in square meters; and

$n-m$  = the interval between contour  $n$  and contour  $m$ , in meters.

Total lake volume is the summation of all element volumes.

6.2 From the data collected, average the temperature, dissolved oxygen, and, if appropriate, salinity values at each depth interval (table 17) for several stations to eliminate the effects of horizontal heat and solute exchange. Tabulate time versus surface dissolved-oxygen concentration and temperature. These surface dissolved-oxygen values are to be corrected for diffusion as described below. Tabulate average dissolved-oxygen values for each remaining depth interval as in table 17, column 3. These values are not corrected for diffusion. Proceed from 6.3 through 6.12 for the graphical-analysis procedure.

6.3 *Graphical analysis*. Determine the percentage saturation for each average surface dissolved-oxygen value using tables indicating oxygen solubility at various temperatures, pressures, and salinities (American Public Health Association and others, 1985). Tabulate the values in table 17, column 6, and plot a curve of time versus measured percentage surface dissolved-oxygen saturation as shown in figure 64C.

6.4 Using the surface dissolved-oxygen-concentration data (table 17) determine the hourly rate of change in dissolved oxygen (milligrams per liter per hour) by subtracting successive pairs of dissolved-oxygen values. Tabulate the values, and plot the rate curve from the values in table 17, column 4, and as shown in figure 64D (curve labeled "Before correction for diffusion").

6.5 Subtract each percentage-saturation value determined in 6.3 from 100 percent, recording values less than 100 as negative. List these percentage-saturation deficits as in table 17, column 7. Proceed to 6.6 or 6.7 depending on the method used to determine the diffusion rate. If area-based gas-transfer coefficient,  $K$ , is estimated, proceed to 6.8.

6.6 Determine the diffusion rate,  $D$ , for each nighttime sample interval from measurements made in the floating-diffusion dome (table 14). Calculate the volume of oxygen in the dome at the beginning and end of the sample interval as follows:

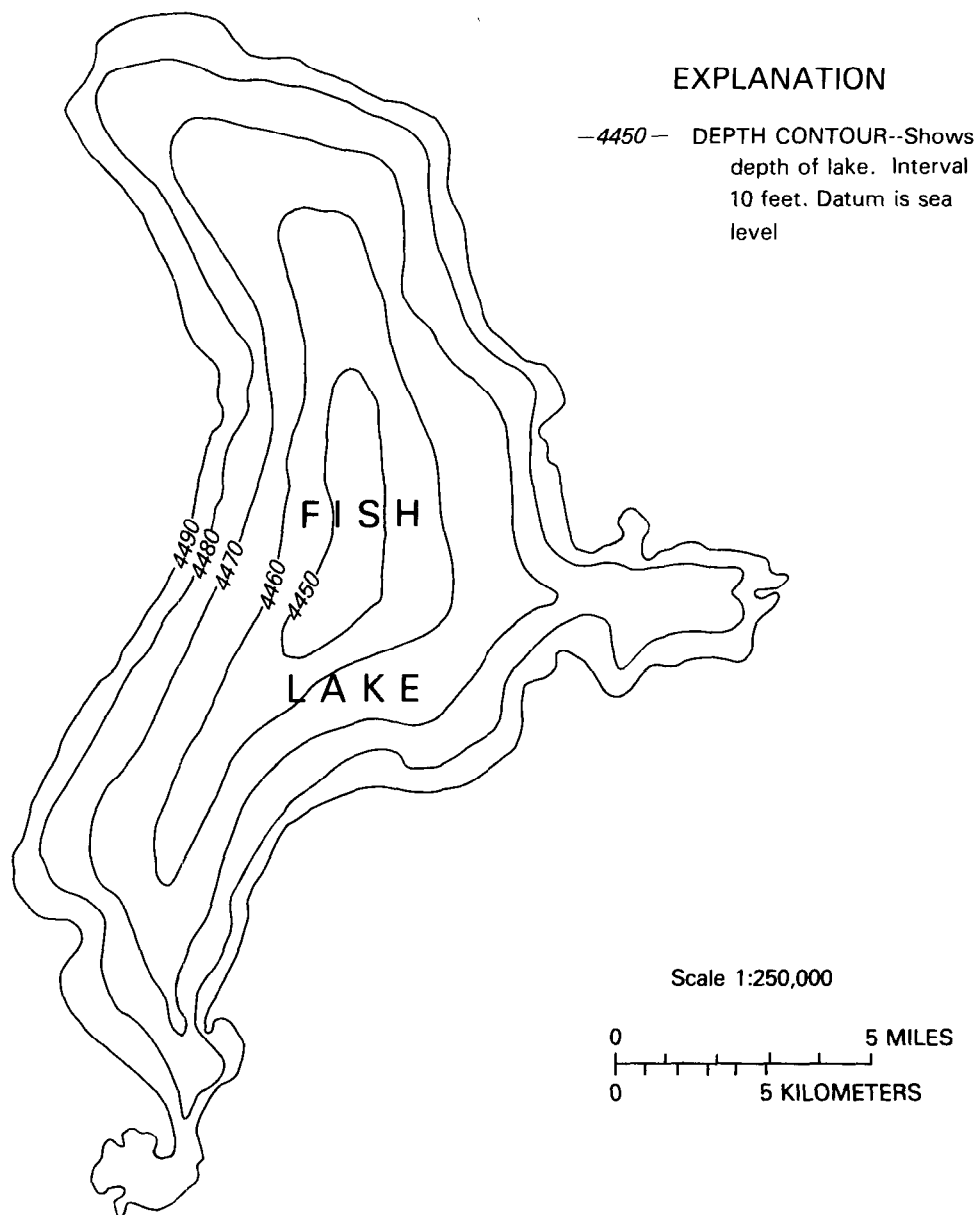


Figure 65.—Fish Lake used in morphometric analysis.

$$V_t = V_d(0.21) \frac{F_t}{100},$$

where

$V_t$  = volume of oxygen, in milliliters, in the dome at a specific time,  $t$ ;

$V_d$  = volume of atmospheric gases, in milliliters, in the dome;

$F_t$  = percentage oxygen saturation in the dome atmosphere at time,  $t$ , when fresh air equals 100-percent oxygen saturation; and

0.21 = fractional volume of oxygen in the air.

Indicate the concentration of oxygen in the floating-diffusion dome in terms of standard temperature and pressure

for each sample interval using the equation

$$\Delta V = \frac{273V_0}{273 + T_0} - \frac{273V_1}{273 + T_1},$$

where

$\Delta V$  = change in volume of oxygen, in milliliters, in the dome at standard temperature and pressure;

$V_0$  = volume of oxygen, in milliliters, in the dome at the beginning of the interval;

$T_0$  = temperature, in degrees Celsius, in the dome at the beginning of the interval;

$V_1$  = volume of oxygen, in milliliters, in the dome at the end of the interval;

Table 16.—Morphometric data and results of graphical analysis of community primary productivity and respiration for Fish Lake

[Area values: gross primary productivity, 73.98 grams per square meter per day; respiration, 81.29 grams per square meter per day; net primary productivity, -2.31 grams per square meter per day; productivity/respiration, 0.972; ----, not applicable]

1	2	3	4	5	6	7	8
Lake slice (depth interval equals 3 meters)	Elevation (feet)	Area ( $\times 10^8$ square meters)	Volume ( $\times 10^8$ cubic meters)	Gross primary productivity (grams per cubic meter per day)	Lake slice gross primary productivity ( $\times 10^8$ grams per cubic meter per day)	Respiration (grams per cubic meter per day)	Lake slice respiration ( $\times 10^8$ grams per cubic meter per day)
Surface	4,490	3.83	----	----	----	----	----
1	4,480	2.81	9.37	20.33	190.49	21.03	197.05
2	4,470	1.82	6.89	9.13	62.91	9.18	63.25
3	4,460	.75	4.24	9.00	38.16	10.05	42.61
4	4,450	.15	1.88	5.30	9.96	4.48	8.42
Total	----	----	----	----	301.5	----	311.33

$T_1$  = temperature, in degrees Celsius, in the dome at the end of the interval; and

273 = factor for converting to absolute temperature.

Oxygen weighs 0.00143 g/mL at standard temperature and pressure. Therefore,  $D$  may be computed from

$$D = \frac{(\Delta V)(0.00143)}{A(\Delta t)},$$

where

$D$  = rate of diffusion of oxygen into the water, in grams per square meter per hour;

$A$  = area of the dome, in square meters, that is in contact with the water surface; and

$\Delta t$  = time interval, in hours, between the two measurements.

Proceed to 6.8.

6.7 Determine the volume-based gas-transfer coefficient,  $k$ , for each sample interval from measurements of the nighttime rate of oxygen change. This can be estimated by calculating  $k$  values for each nighttime surface sampling interval using the Odum (1956) method as presented by Eley (1970):

$$k = \frac{q_n - q_{n+1}}{S_n - S_{n+1}},$$

where

$k$  = volume-based gas-transfer coefficient for oxygen, in grams per cubic meter per hour, at 0-percent saturation;

$q_n$  = rate of change of the surface oxygen, in grams per cubic meter, at nighttime,  $n$ ;

$q_{n+1}$  = rate of change of the surface oxygen, in grams per cubic meter, at nighttime,  $n + 1$ ;

$S_n$  = oxygen-saturation deficit for the surface water at nighttime,  $n$ ; and

$S_{n+1}$  = oxygen-saturation deficit for the surface water at nighttime,  $n + 1$ .

Proceed to 6.9.

6.8 Using the following equation, convert the area-based rate of diffusion for each sampling interval to a value at 0-percent saturation of the water (rate of diffusion if the water contained no oxygen) by dividing  $D$  by the average percentage-saturation deficit during the time of measurement, or

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

where

$K$  = area-based gas-transfer coefficient, in grams per square meter per hour, at 0-percent saturation (100-percent saturation deficit); and

$S$  = average percentage-saturation deficit between the water and the air during the sample interval (derived from 6.5).

6.9 Convert each area value to a volume value by dividing by the depth of water, in meters, for the surface interval, or

$$k = \frac{K}{z},$$

where  $z$  = depth, in meters, of the surface interval.

Estimate  $k$  for the study period by averaging the  $k$  values determined for each sampling interval (Note 1). Proceed to 6.10.

Note 1: Some situations require use of different diffusion constants at different times of day.

6.10 Determine the quantity of oxygen (grams per cubic meter) gained or lost by diffusion at the surface during each sampling interval. To adjust for atmospheric reaeration, multiply the average  $k$  (from 6.9) by each percentage oxygen-saturation deficit value (from 6.5), and divide by 100 to convert percentage to fractional values. List these values as in table 17, column 8.

6.11 Using figure 64D, the hourly rate-of-change graph plotted as directed in 6.4, prepare a corrected rate-of-change curve by adding or subtracting, graphically, the quantity of oxygen, in grams per cubic meter, gained or lost by diffusion during each sampling interval (from 6.10). Draw the

Table 17.—Hypothetical data for determining community primary productivity for each individual depth in a lake by the oxygen-curve method

[The gas transfer coefficient on an area basis,  $K$ , is 3.2 grams per square meter per hour, and on a volume basis,  $k$ , is 2.67 grams per cubic meter per hour at 100-percent saturation deficit;  $h$ , hours; °C, degrees Celsius; mg/L, milligrams per liter; (mg/L)/h, milligrams per liter per hour; (g/m<sup>3</sup>)/h, grams per cubic meter per hour]

1	2	Dissolved oxygen						
		3	4	5	6	7	8	9
Time (h)	Temperature (°C)	Measured (mg/L) <sup>1</sup>	Rate of change [(mg/L)/h]	Concentrations at saturation (mg/L)	Measured saturation (percent)	Average saturation deficit, $\frac{S}{100}$ (percent)	$\frac{S \times k}{100}$ [(g/m <sup>3</sup> )/h]	Corrected rate of change [(g/m <sup>3</sup> )/h]
0000	29.5	6.00		7.7	78			
0100	29.0	5.95	-0.05	7.8	76	-23.0	-0.614	-0.664
0200	28.0	5.90	-.05	7.9	75	-24.5	-.654	-.704
0300	27.0	5.85	-.05	8.1	72	-26.5	-.708	-.758
0400	25.5	5.80	-.05	8.3	70	-29.0	-.774	-.824
0500	25.0	5.90	+.10	8.4	70	-30.0	-.801	-.701
0600	27.0	5.90	.00	8.1	73	-28.5	-.761	-.761
0700	28.0	6.30	+.40	7.9	80	-23.5	-.627	-.227
0800	30.0	6.85	+.55	7.6	90	-15.0	-.400	+.150
0900	31.0	7.85	+1.00	7.5	105	-7.5	-.200	+.402
1000	31.5	8.80	+.95	7.4	118	+11.5	+.307	+1.257
1100	32.0	9.40	+.60	7.4	127	+22.5	+.601	+1.201
1200	32.5	10.05	+.65	7.4	137	+32.0	+.854	+1.504
1300	33.5	10.50	+.45	7.4	137	+41.0	+1.095	+1.545
1400	33.0	10.60	+.10	7.2	145	+45.0	+1.202	+1.302
1500	33.0	10.60	+.10	7.3	145	+45.0	+1.202	+1.302
1500	32.5	10.45	-.15	7.3	145	+43.5	+1.161	+1.011
1600	32.5	10.45	-.25	7.4	142	+38.5	+1.028	+.778
1600	30.5	10.20	-1.30	7.6	135	+26.5	+0.708	-0.592
1700	30.5	8.90	-1.30	7.6	118	+9.0	+.240	-1.060
1800	30.0	7.60	-1.15	7.6	100	-7.5	-.200	-1.350
1900	30.0	6.45	-.15	7.6	85	-16.0	-.427	-.577
2000	30.0	6.30	.00	7.6	83	-17.5	-.467	-.467
2100	29.5	6.30	-.15	7.7	82	-19.0	-.507	-.657
2200	29.5	6.15	+.10	7.7	80	-20.0	-.534	-.434
2300	29.0	6.25	-.15	7.8	80	-21.0	-.561	-.711
2400	29.0	6.10		7.8	78			

<sup>1</sup>Milligrams per liter equals grams per cubic meter.

curve as in figure 64D (curve labeled "Corrected for diffusion"). The corrected rate-of-change curve is replotted as a step function to facilitate graphical integration as shown in figure 64E. Dissolved-oxygen values for each remaining depth interval are tabulated as in table 17, column 3, but not corrected for diffusion, and their hourly rates of change (col. 4) are plotted as was done for the surface interval in figure 64E.

6.12 Connect a line between the presunrise and postsunset negative rate-of-change points on the corrected rate-of-change curve as shown in figure 64E (Odum and Wilson, 1962). This line is an estimate of daytime respiration (Note 2).

Note 2: The maximum rate of respiration often occurs immediately after sunset, and the rate declines to a minimum before sunrise. Where presunrise and postsunset respiration differ, connect the line diagonally from the dawn-respiration rate to the sunset-respiration rate on the corrected rate-of-change graph. The values for respiration and gross primary productivity are affected by the placement of the respiration line. The accuracy of the method probably is limited by this step (Odum and Hoskin, 1958, p. 22). Graphs in which the rates of change are very irregular enable more subjectivity of choice of the respiration line than do smooth curves.

## 7. Calculations

7.1 An estimate of gross primary productivity, in grams oxygen per cubic meter per day, for each depth increment is the area above the daytime respiration line and below the daytime rate-of-change line (fig. 64E, for the surface interval). The area may be determined from the plot by counting the graph-paper squares and multiplying by the value, in grams per cubic meter, of one square. Total gross productivity of each lake slice, in grams oxygen per cubic meter per day, is obtained by multiplying the lake-slice volumetric-productivity value, in grams oxygen per cubic meter per day, by the total water volume of the lake-slice interval, in cubic meters. Total productivity of the entire water body, in grams oxygen per cubic meter per day, is the summation of all lake-slice-interval productivity values. Total productivity of the water divided by the surface area, in square meters, of the water body will provide an areal value, in grams oxygen per square meter per day, useful when comparing primary-productivity values from diverse water bodies.

7.2 An estimate of community respiration, in grams oxygen per cubic meter per day, for each depth increment is the area above the nighttime negative rate-of-change line and below the zero rate-of-change line (fig. 64E, for the surface interval). The area may be determined from the plot by counting the graph-paper squares and multiplying by the value, in grams per cubic meter, of one square. Total community respiration of each lake slice, in grams oxygen per cubic meter per day, is calculated by multiplying the lake-slice volumetric respiration, in grams oxygen per cubic meter per day, by the total water volume of the lake-slice interval, in cubic meters. Total respiration of the entire water body, in grams oxygen per cubic meter per day, is the summation

of all lake-slice-interval respiration values. Total respiration of the water divided by the surface area, in square meters, of the water body will provide an areal value, in grams oxygen per square meter per day, useful when comparing respiration from diverse water bodies.

7.3 An estimate of primary productivity for each lake-slice interval or the entire water body may be calculated by subtracting the appropriate gross primary-productivity value from the corresponding respiration value.

7.4 An index of the trophic nature of the community may be calculated as the ratio of photosynthetic productivity to respiration,  $P:R$ . Communities having a  $P:R$  ratio less than 1 have an excess of respiration compared to productivity. They are heterotrophic; that is, they degrade organic compounds through oxygen metabolism at a greater rate than they fix carbon in photosynthesis. Autotrophic communities have a  $P:R$  ratio greater than 1 and release more oxygen through photosynthesis than they consume through respiration.

## 8. Reporting of results

Report community primary productivity and respiration, in milligrams, as follows: less than 10 mg, one decimal; 10 mg or more, two significant figures.

## 9. Precision

Mean coefficients of variation among substations within four stations in Keystone Reservoir, Okla., were reported by Eley (1970). The coefficient of variation for gross primary productivity ranged from 2.72 to 9.36 percent, and the coefficient of variation for community respiration ranged from 1.71 to 11.67 percent. Average coefficients of variation among replicate observations in eight laboratory microcosms containing water from Keystone Reservoir were 1.8 percent for gross primary productivity and 5.7 percent for community respiration.

Replications of the diurnal-curve method at three similar stations in the upper Laguna Madre, Tex., were within 20 percent of the mean (Odum and Hoskin, 1958).

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# BIOASSAY

## Introduction

The abundance and composition of algae are related to water quality and are affected by the availability of growth substances, the major components of which are phosphorus and nitrogen. The significance of measuring algal growth potential (AGP) in water samples is that a distinction can be made between the growth substances of a sample determined by chemical analysis and the quantity of algal growth that the water can support. The AGP test that has no spikes does not identify the substances that limit or stimulate growth, nor does it indicate the presence of toxic or inhibitory substances in the water. The test does, however, enable the comparison of growth responses of test water from different sources or from the same source at different times.

Determination of AGP on a sample filtered at the time of collection measures the growth response elicited by dissolved nutrients. Samples that are autoclaved and then filtered measure a growth response that results from nutrients that are present in living organisms and organic matter as well as from dissolved nutrients.

A series of AGP bioassays, using phosphorus and nitrogen spikes, will indicate one of three conditions in a body of water: phosphorus limitation, nitrogen limitation, and the absence of phosphorus or nitrogen limitation. If phosphorus or nitrogen are not limiting—that is, there is no stimulation of growth in the spiked culture flasks—then one of several conditions may exist in the test water: minor element (micronutrient) limitation, limitation by an organic growth factor, or limitation by the presence of a toxic substance. This test will not differentiate between these possibilities; however, autoclaving does remove some biologically produced inhibitors.

In very productive water where the natural concentration of phosphorus and nitrogen exceeds the concentration of phosphorus and nitrogen in the spiked media, the concentration of the spikes may have to be increased. The limiting nutrient in a body of water also may change with time. A system that is phosphorus limited in June may be limited by some other nutrient in August. Consequently, any conclusions based on samples collected at one or two sampling times must be qualified accordingly. In addition, positive results for phosphorus or nitrogen limitation do not imply that those are the only limiting factors. There may be simultaneous micronutrients, light, or other limitations.

The minimum chemical data that must be collected to evaluate the assay response and define nutrient limitation are: initial pH and concentrations of total phosphorus, orthophosphate, nitrite, nitrate, and total ammonia plus organic nitrogen.

## Collection

To ensure maximum correlation of results, water collected for the AGP tests needs to be subsampled for chemical and other biological analyses. The sample-collection method and sample size will be specified by study objectives. Use a nonmetallic sampler. Do not reuse containers when toxic or nutrient contamination is suspected. Collection of samples intended for AGP analysis for dissolved substances only must be filtered at the time of collection.

Prepare the sample for analysis by autoclaving or filtering (0.22- $\mu\text{m}$  pore-size membrane, low-water extractable, membrane filter), or both. Autoclaving will solubilize additional nutrients, including many of those contained in filterable organisms. If a sample is collected during an algal bloom, it especially may be important to autoclave the sample. The autoclaving will oxidize algal excretions that would inhibit algal growth and result in erroneous data (Boyd, 1973). If autoclaving is desired, the length of time at 121 °C and 1.1 kg/cm<sup>2</sup> should be 10 to 30 minutes per liter. After autoclaving, the sample needs to be cooled to room temperature and then bubbled with a mixture of 1-percent carbon dioxide in air until the original pH is restored, or bubbled for about 5 minutes. The bubbling will minimize loss by resolubilizing some precipitates that might have formed during autoclaving. In very hard water or water containing large concentrations of suspended particulate matter, autoclaving may cause irreversible precipitation of certain constituents in the sample; therefore, the pH before and after autoclaving and carbon dioxide equilibration should be reported. Allow the sample to equilibrate in air at 24 °C. Shaking will speed the equilibration.

Changes can occur in a sample during storage regardless of conditions, so keep the storage time to a minimum. Store the sample in the dark at 0 to 4 °C and have a minimum of air space over the sample. If storage for more than 1 week is necessary, autoclave or filter, or both, the sample before storage.

# Algal growth potential (AGP), spikes for nutrient limitation

(B-8502-85)

## Parameters and Codes:

Algal growth potential, filtered (mg/L): 85209

Algal growth potential, filtered and spiked with 0.05 mg/L P

Algal growth potential, filtered and spiked with 1.0 mg/L N

Algal growth potential, filtered and spiked with 1.0 mg/L N and 0.05 mg/L P

Algal growth potential, unfiltered (mg/L): 70988

Algal growth potential, unfiltered and spiked with 0.05 mg/L P

Algal growth potential, unfiltered and spiked with 1.0 mg/L N

Algal growth potential, unfiltered and spiked with 1.0 mg/L N and 0.05 mg/L P

## 1. Applications

The method is suitable for all freshwater and is similar to the original method developed by Oswald and Golveke (1966) and the method developed by the U.S. Environmental Protection Agency (1978).

## 2. Summary of method

2.1 A water sample is autoclaved or filtered, or both, and placed in a covered Erlenmeyer flask. This sample is inoculated with the test algal species and incubated under constant temperature and light intensity until the rate of growth is less than 5 percent per day. The number of algal cells and the mean cell volume are determined using an electronic particle counter (fig. 66), and these values are used to determine the maximum standing crop.

2.2 The electronic particle counter has been used for counting and sizing nonfilamentous unialgal species (Hastings and others, 1962; El-Sayed and Lee, 1963). Operation of the counter is as follows: The algal cells, which are relatively poor electrical conductors, are suspended in an electrolyte solution, and as they pass through a small aperture, each cell causes a voltage drop that is recorded as a count. The height of the pulse resulting from the voltage drop is proportional to cell volume. The knowledge of the cell number per unit volume of sample and the change in mean cell volume enable standing crop to be measured reproducibly and accurately.

## 3. Interferences

3.1 Particles in the counting medium (for example, dust or lint) may block the aperture of the electronic particle counter or may cause false counts. These interferences are eliminated by passing all media and water samples through a 0.22- $\mu$ m pore-size, low-water extractable, membrane filter. Samples for the analysis should be collected in a nonmetallic sampler because certain metals in a metallic sampler may affect results.

3.2 Autoclaving may cause precipitation of certain constituents in the sample and increase the pH. These precipitates

may not be irreversible. The sample often may be clarified by exposing it to 1 percent carbon dioxide plus air until the original pH is restored.

## 4. Apparatus

Most of the materials and apparatus listed in this section are available from scientific supply companies.

4.1 *Centrifuge*, either swing-out or fixed-head cup-type, 3,000 to 4,000 r/min, 15- to 50-mL conical or 100-mL pear-shaped *centrifuge tubes*, and simple *siphoning* or *suction device* to remove excess fluid after centrifugation.

4.2 *Electronic particle counter and mean cell volume accessory*, that has 100- $\mu$ m aperture tube and a 500- $\mu$ L manometer.

4.3 *Environmental chamber* (walk-in), that has temperature control ( $24 \pm 2$  °C) and illumination (cool, white fluorescent that provides 4,300 lumens/m<sup>2</sup>).

4.4 *Onsite filtration apparatus*, nonmetallic, and vacuum apparatus.

4.5 *Flasks*, Erlenmeyer, 250 mL, covered with 50-mL beakers, both glass, and prepared as follows. Wash using detergent and rinse thoroughly using tap water. Rinse using a 10-percent hydrochloric acid (HCl) solution by swirling the HCl solution so the entire inner surface of the flask is coated. The flasks then are rinsed thoroughly using particle-free distilled or deionized water (filtered through a 0.22- $\mu$ m membrane filter) and covered with the 50-mL beakers. Autoclave at 1.05 kg/cm<sup>2</sup> (15 psi) for 20 minutes, and dry in an oven at 50 °C. Sterilized flasks and beakers must be stored in closed cabinets until used.

4.6 *Laboratory filtration apparatus*, sterile, disposable.

4.7 *Membrane filters*, 0.22- $\mu$ m pore size, 47-mm diameter, low-water extractable.

4.8 *Oven*, for use at 50 °C.

4.9 *pH meter*.

4.10 *Pipets and disposable tips*, 0.1- and 1-mL capacities.

4.11 *Refrigerator(s)*, without circulation blower.



Figure 66.--Electronic particle counter. (Photograph courtesy of Coulter Electronics, Inc., Hialeah, Fla.)

4.12 *Sample container*, linear polyethylene bottles, 1 L.  
 4.13 *Shaker*, rotatory, capable of 120 oscillations per minute.

4.14 *Sterilizer*, horizontal steam autoclave, or vertical steam autoclave.

**CAUTION.**—If vertical autoclaves or pressure cookers are used, they need to be equipped with an accurate pressure gauge, a thermometer with the bulb 2.5 cm above the water level, automatic thermostatic control, metal air-release tubing for quick exhaust of air in the sterilizer, metal-to-metal-seal eliminating gaskets, automatic pressure-release valve, and clamping locks preventing removal of lid while pressure exists. These features are necessary in maintaining sterilization conditions and decreasing safety hazards.

To obtain adequate sterilization, do not overload sterilizer. Use a sterilization indicator to ensure that the correct combination of time, temperature, and saturated steam has been obtained.

4.15 *Vacuum pump*.

4.16 *Water-sampling bottle*, Van-Dorn type. 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 *Aperture cleaner*. Bleach or nitric acid may be used, but aperture tube should be removed when these are used.

5.2 *Calcium chloride solution*. Dissolve 2.205 g calcium chloride ( $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ ) in 500 mL distilled water.

5.3 *Cultures of test alga*, *Selenastrum capricornutum* *Printz*. The culture medium is prepared in the following manner. Add 1 mL each of sodium nitrate ( $\text{NaNO}_3$ ), magnesium sulfate ( $\text{MgSO}_4$ ), magnesium chloride ( $\text{MgCl}_2$ ), sodium bicarbonate ( $\text{NaHCO}_3$ ), calcium chloride ( $\text{CaCl}_2$ ), micronutrient, and potassium phosphate ( $\text{K}_2\text{HPO}_4$ ) solutions, in the order listed, to 900 mL distilled water, and then dilute to 1 L. Filter the medium through a membrane filter (0.22- $\mu\text{m}$  mean pore size) at 25 cm mercury. Place about 100 mL in 250-mL Erlenmeyer flasks rinsed with filtered culture medium and cover with a 50-mL beaker. Autoclave the prepared flasks at 121 °C at 1.05 kg/cm<sup>2</sup> (15 psi) for 30 minutes and allow to equilibrate for 12 hours in the environmental chamber. Store extra culture medium at 0 to 5 °C until used.

The cultures used for inoculum are maintained by weekly transferring an aliquot of a 7- to 10-day-old culture to new media. The quantity of culture maintained depends on the conditions necessary to provide an adequate supply of algal cells at the proper growth stage for the AGP test. Extreme care must be used to prevent contamination of stock cultures.

Media that contain 1-percent agar are used to maintain stock cultures for a long period of time. Cultures on agar should be prepared every 6 to 8 weeks. The algal transfer should be streaked on the agar to isolate colonies. A clean colony should be transferred every 6 weeks to culture

medium that is five times the strength 1-percent agar, and this (5 $\times$ ) culture should be transferred to the (1 $\times$ ) medium in about 2 weeks to reestablish fresh inoculum. Seven- to ten-day-old liquid cultures always should be used to provide inoculum for the AGP test.

5.4 *Distilled or deionized water*. Filter if in doubt about the water being particle free.

5.5 *Hydrochloric acid (HCl)*, 10 percent.

5.6 *Magnesium chloride solution*. Dissolve 6.082 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  in 500 mL distilled water.

5.7 *Magnesium sulfate solution*. Dissolve 3.593 g  $\text{MgSO}_4$  in 500 mL distilled water.

5.8 *Micronutrient solution*. Dissolve 92.76 mg  $\text{H}_3\text{BO}_4$ , 207.69 mg  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 1.64 mg  $\text{ZnCl}_2$ , 79.88 mg  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ , 150 mg  $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$  (ethylenediaminetetraacetate) 0.39 mg  $\text{CoCl}_2$ , 3.63 mg  $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$ , and 5.7  $\mu\text{g}$   $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  in 500 mL distilled water.

5.9 *Potassium phosphate solution* (particle free). Dissolve 0.522 g  $\text{K}_2\text{HPO}_4$  in 500 mL distilled water. Filter the solution.

5.10 *Potassium phosphate solution*. Dissolve 143 mg  $\text{K}_2\text{HPO}_4$  in 500 mL distilled water (for spike).

5.11 *Saline solution* (diluent), particle free.

5.12 *Sodium bicarbonate solution*. Dissolve 7.5 g  $\text{NaHCO}_3$  in 500 mL distilled water.

5.13 *Sodium nitrate solution* (particle free). Dissolve 12.75 g  $\text{NaNO}_3$  in 500 mL distilled water.

5.14 *Sodium nitrate solution*. Dissolve 303.4 mg  $\text{NaNO}_3$  in 500 mL distilled water (for spike). Filter the solution.

## 6. Analysis

6.1 Depending on type of analysis requested, AGP for dissolved substances with or without spikes or AGP for digested sample (autoclaved) with or without spikes, filter 100-mL aliquots of sample to provide each test, 6.3 to 6.6, with three replicate flasks. (Prepare filter by filtering 100 mL through each filter to saturate filter; use filtrate to wash replicate flasks. Filter vacuum should not exceed 25 cm mercury.)

6.2 Prepare one replicate for each sample to be used as an uninoculated batch control to determine particle background of sample.

6.3 Prepare three flasks to be used as controls for the following spikes or to provide the basic AGP test.

6.4 Add 1 mL potassium phosphate solution to three of the flasks.

6.5 Add 1 mL sodium nitrate solution to three more of the flasks.

6.6 Add 1 mL sodium nitrate solution and 1 mL potassium phosphate solution to each of three more flasks.

6.7 Place the covered flasks in the environmental chamber for temperature equilibration at 24 °C for at least 12 hours.

6.8 Rinse algal inoculum (see 5.3) free of culture medium using the following procedure: Place 30 mL in two 50-mL centrifuge tubes, cover, and centrifuge at 5,000 r/min for

5 minutes. Decant the supernatant and add 30 mL of filtered distilled water and resuspend the cells. Repeat the centrifugation and decantation step. Add 10 mL filtered distilled water and resuspend the cells. Combine tube contents. Mix.

6.9 Determine the concentration of the algal particles using the electronic particle counter. (Final concentration should be about  $10 \times 10^6$  cells/mL.)

6.10 Pipet a volume of the cell suspension into each of the sets of test samples in the flasks to make a final concentration in the test water of about 10,000 particles (cells) per milliliter.

6.11 Place the flasks (inoculated replicates plus uninoculated control) in the environmental chamber on a rotatory shaker at 120 oscillations per minute and expose to constant illumination of 4,300 lumens/m<sup>2</sup> produced by cool, white fluorescent tubes.

6.12 Incubate 3 to 4 days, counting the number of cells in the flasks each day; thereafter, count until the growth rate is less than or equal to 5 percent per day.

### 7. Calculations

Maximum standing crop is determined when the increase in algal density (cells per unit volume) is less than 5 percent per day and is defined as milligram(s) dry weight algae per liter by the following equation:

$$\begin{array}{rcl} \text{cells/mL} & & \text{micrograms} \\ \times MCV & & \text{dry weight} \\ \times 2.5 \times 10^{-7} & = & \frac{\text{per liter}}{1,000} = \text{milligrams} \\ \times \text{dilution factor} & & \text{dry weight} \\ & & \text{per liter,} \end{array}$$

where

cells/mL = coincident corrected cell count per milliliter (determined by the electronic particle counter);

MCV = mean cell volume (determined by mean cell volume accessory), in cubic micrometers;

$2.5 \times 10^{-7}$  = factor to convert maximum standing crop to dry weight of algal biomass (determined gravimetrically). The  $2.5 \times 10^{-7}$  conversion factor was determined by dividing the known total cell volume of *Selenastrum capricornutum* Printz culture in artificial media into the gravimetric dry weight measured from the corresponding cell suspension. The factors should be determined for each laboratory performing the analysis. As a maintenance function, recompute these factors every 6 months. Question calculations and experimental procedure if the new factor is not within  $\pm 2$  to  $3 \times 10^{-7}$ ; and

Dilution factor = dilution of algal cells from pure culture using particle-free saline solution for proper counting.

This equation is valid only when MCV has been determined using an electronic particle counter calibrated using an appropriate reference particle.

### 8. Reporting of results

Report maximum standing crop, in milligram(s) dry weight algae per liter, as follows: two significant figures.

### 9. Precision

The precision is dependent on the biomass of *Selenastrum capricornutum* produced. For typical samples, the precision is approximately  $\pm 10$  percent.

Examples of growth responses of *Selenastrum capricornutum* and chemical analyses in nitrogen- and phosphorus-limited water are listed in tables 18 to 21 in the "Supplemental Information" subsection at the back of this section.

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## Supplemental information

The kind of responses that can be expected when phosphorus and nitrogen are limiting are listed in tables 18 and 20. There is no significant increase in maximum standing crop (MSC) when nitrogen is added alone; however, the phosphorus spike produced more than double the MSC of the control. The combined spike of phosphorus and nitrogen increased growth even more, indicating that the phosphorus spike was large enough that, when added alone, it caused nitrogen to become the limiting nutrient in the medium.

The yield coefficients 430 and 38 listed in table 19 to predict the MSC were developed by the U.S. Environmental Protection Agency (1978). The ratio of these factors is about 11:1 and is considered to be the optimum N:P ratio. A ratio of greater than 11:1 indicates probable phosphorus limitation, and a ratio of less than 11:1 indicates probable nitrogen limitation. The ratio of total soluble inorganic nitrogen to orthophosphorus is 27:1 (table 19), a strong indication of phosphorus limitation. The assay response confirms this prediction.

Table 18.—Growth responses representative of phosphorus limitation

[Adapted from the U.S. Environmental Protection Agency, 1978]

Sample treatment	Maximum standing crop (milligrams dry weight per liter)
Control	2.16
Control + 0.05 milligrams per liter phosphorus	5.81
Control + 1.0 milligrams per liter nitrogen	2.30
Control + 0.05 milligrams per liter phosphorus and 1.0 milligrams per liter nitrogen	23.69

Table 19.—Chemical analysis of phosphorus-limited control test water and predicted phosphorus and nitrogen yields of *Selenastrum capricornutum*

[Adapted from the U.S. Environmental Protection Agency, 1978]

Nutrient	Predicted yield <sup>1</sup> (milligrams per liter)
0.021 milligrams per liter total phosphorus	
.006 milligrams per liter orthophosphorus	} = 0.006 × <sup>2</sup> 430 = 2.58 ±20 percent
.368 milligrams per liter total nitrogen	
.120 milligrams per liter nitrate plus nitrite as nitrogen	
.040 milligrams per liter ammonia as nitrogen	
.160 milligrams per liter nitrite plus nitrate plus ammonia as nitrogen	} = 0.160 × <sup>2</sup> 38 = 6.10 ±20 percent
27:1 N:P ratio	

<sup>1</sup>Predicted yield of *Selenastrum capricornutum* based on soluble inorganic phosphorus or nitrogen concentrations in the test water if all other essential nutrients are present in excess.

<sup>2</sup>Yield coefficients of 430 and 38 determined experimentally by Miller and others (1978) and the U.S. Environmental Protection Agency (1978).

Comparing the predicted results in table 19 and the growth response in table 18 indicates that the growth of the control (2.16 MSC) corresponds with (within the stipulated confidence limits) the predicted result of 2.58 MSC. The control and phosphorus spikes (5.81 MSC) correspond with the predicted results based on total soluble inorganic nitrogen (6.10 MSC), again clearly indicating phosphorus limitation and indicating that by adding 0.05 mg/L of phosphorus, the system was nitrogen limited.

A representative growth response and chemical analysis for a system that is nitrogen limited is listed in tables 20 and 21. The N:P ratio is less than 11:1 (2.5:1). The predicted

yield based on the orthophosphorus concentration is 12.90 MSC and 2.85 MSC based on the total soluble inorganic nitrogen. No significant increase occurs in the sample when a phosphorus spike is added. The nitrogen spike produces an MSC that corresponds with that predicted by the phosphorus concentration, and the combined spike produces a threefold increase in the MSC, indicating that, by adding the nitrogen spike, the system has been changed to one that is phosphorus limited.

When a test water does not attain the predicted yields and nutrient spikes do not cause an increase in MSC, one of the following causes should be investigated: (1) Some other

Table 20.—Growth responses representative of nitrogen limitation

[Adapted from the U.S. Environmental Protection Agency, 1978]

Sample treatment	Maximum standing crop (milligrams dry weight per liter)
Control	4.06
Control + 0.05 milligrams per liter phosphorus	4.21
Control + 1.0 milligrams per liter nitrogen	12.68
Control + 1.0 milligrams per liter phosphorus and 1.0 milligrams per liter nitrogen	34.52

Table 21.—Chemical analysis of nitrogen-limited control test water and predicted phosphorus and nitrogen yields of *Selenastrum capricornutum*

[Adapted from the U.S. Environmental Protection Agency, 1978]

Nutrient	Predicted yield <sup>1</sup> (milligrams per liter)
0.072 milligrams per liter total phosphorus	
.030 milligrams per liter orthophosphorus	} = 0.030 × <sup>2</sup> 430 = 12.90 ±20 percent
.160 milligrams per liter total nitrogen	
.055 milligrams per liter nitrate plus nitrite as nitrogen	
.020 milligrams per liter ammonia as nitrogen	
.075 milligrams per liter nitrite plus nitrate plus ammonia as nitrogen	} = 0.075 × <sup>2</sup> 38 = 2.85 ±20 percent
2.5:1 N:P ratio	

<sup>1</sup>Predicted yield of *Selenastrum capricornutum* based on soluble inorganic phosphorus or nitrogen concentrations in the test water if all other essential nutrients are present in excess.

<sup>2</sup>Yield coefficients of 430 and 38 determined experimentally by Miller and others (1978) and the U.S. Environmental Protection Agency (1978).

nutrient instead of phosphorus or nitrogen was limiting; (2) chemical analysis for orthophosphorus and total soluble nitrogen was inaccurate; or (3) toxicants were present.

Phosphorus limitation is the most usual case. Nitrogen limitation is not as common. Trace-element limitation is rare but has been documented (Goldman, 1972). The U.S. Environmental Protection Agency (1978) indicates that less than 2 percent of all water is trace-element limited. This method does not describe trace-element limitation, nor does it describe toxicity. With modification, this method can be used to detect trace-element limitation and the presence of toxic substances.

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