Standard Operating Procedure for Primary Productivity Using ^{14}C: Field Procedure

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April 13, 1994

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1.0 Scope and Application

This method is used to determine primary productivity and primary productivity parameters from Great Lakes waters.

2.0 Summary of Method

Samples of water, for which the productivity parameters are to be determined, are inoculated with a known quantity of bicarbonate substrate which is labeled with the radiotracer ^{14}C. Samples are incubated at various light intensities for two to four hours, after which the algal cells are separated from the water by filtration. Because the measured radioactivity of each filter will be proportional to the quantity of carbon fixed by the algae into organic material, the radioactivity of the filter containing the algal cells is determined by liquid scintillation counting. Calculation of the productivity parameter also require information about the total inorganic carbon available in the incubation vessel, the length of time of incubation, the chlorophyll content of the incubated sample and specific activity of the radiotracer.

3.0 Safety and Waste Handling

3.1 Safety

3.1.1 ^{14}C is classified as a low-level beta emitter. Wearing personnel protective laboratory gear (rubber apron, protective gloves and glasses) at all times when using with ^{14}C and in the primary productivity lab, can effectively prevent any exposure.

3.1.2 All spills of radioactive or suspected radioactive materials must be immediately reported to the person in charge of radiation safety and decontaminated immediately.

3.1.3 All radioactive samples and standards should be properly labeled with the isotope and activity indicated and properly stored in designated locations.

3.1.4 Use only labeled radioactive items, e.g. glassware, forceps, filtration apparatus. If returned to general use, all equipment must be properly decontaminated.

3.1.5 Use spill trays lined with absorbent paper for all analyses involving ^{14}C.

3.1.6 Since ^{14}C is an inhalation hazard, all innoculations need to be performed under a functional hood.

3.1.7 Under the Atomic Energy Act of 1954, a license is required designating the radioactive source, its use as applicable to the laboratories and conditions by which the licensed material should be used. The current license (#12-10243-01) expires on December 31, 2000.

3.2 Waste Handling

3.2.1 Liquid wastes cannot be poured down the drain in any circumstances. All radioactive liquid waste is contained within 5 gallon cubitainers, and when full, are wrapped up in heavy radioactive waste bags. The following information is clearly marked on the outside using radioactive waste placards: type of radioactive waste, approximate activity (millicuries) and waste volume.

3.2.2 To estimate activity for a complete label, keep accurate records as to the volume contained within each cubie. Multiply the number of milliliters by 0.0167 (assuming the BOD bottles contain 300 mL and the specific activity of 1 mL of ^{14}C is 5µCi: 5μCi/300 = 0.0167µCi/mL) to obtain an estimation of the activity in microcuries.

3.2.3 From each waste cubie take a 1.0 mL sample and put into a liquid scintillation vial. Add 20 mL of Ecoscint. Add 1 mL of phenylethylamine. Clearly label cap to match that of the cubie sampled. Put this vial with sample vials to be analyzed for actual activity at CRL.

3.2.4 The disposal of solid wastes and contaminated articles should be into designated containers and, under no circumstances, into ordinary trash receptacles.

4.0 Apparatus

Two Darkened carboys
Two large insulated coolers
Pipettor (MLA equivalent), 1.0 mL with disposable tip
Pipettor (MLA equivalent), 0.5 mL with disposable tip
Pipettor (MLA equivalent), 0.3 mL with disposable tip
100 mL graduated cylinder
Two incubators capable of achieving temperatures from 0-20°C
Cool white fluorescent lights, six per incubator (General Electric F24T12CWHO 800)
Filtration units, for 47µm, 0.45 diameter filters
Forceps
300 mL BOD bottles
Vacuum system with pressure regulator and waste container system
Brinkman Repippetor with 20 mL capacity
Shallow tray, smooth, non-absorbent surface
Irradiance meter with a remote sensor
Thermometer
Geiger Counter

5.0 Supplies

Liquid scintillation vials, 20 mL capacity
Membrane filters, 47 mm diameter, 0.45 µm pore size Sartorius cellulose acetate
Liquid scintillation cocktail, Ecoscint brand
HCL 0.5 N
Radiotracer-labeled substrate as $NaH^{14}CO_3$; working stock solution of 5µCi/mL

Phenylethylamine, CO_2-free
Absorbent bench paper, plastic backed
Decontaminant surfactant, Radiacwash or equivalent
Kimwipes
Paper towels
Pipette tips, Eppendorf large, medium
Masking tape
Waterproof marker

6.0 Sample Collection and Preparation

6.1 Using a Geiger counter, make an initial check of the laboratory to ensure no residual contamination is present from other assays.

6.2 Using a waterproof marker, numerically label the caps of the scintillation vials.

6.3 Obtain water samples from desired depths using a non-metallic water sampler such as Niskin water bottles. Record the water temperature of the sample using a thermometer.

6.3.1 When lake water temperatures are isothermal, the water sample is a composite or integrated sample, resulting in one set of incubated bottles.

6.3.2 When thermal stratification of lake water occurs in summer, samples are collected from both the hypolimnion and epilimnion. Representative hypolimnion samples are obtained from the M-3 depth. The epilimnion samples are designated from the integrated subsamples. The temperatures used for incubation (to 0.1°C) should be the temperature determined from the M-3 and integrated samples.

6.4 Transfer the water sample from the collection bottle to a 4 L plastic, darkened bottle, marked "sample", taking care to avoid agitation or bubbles that could disrupt cells. A wash bottle labeled "wash" is also filled with any water left over in the rosette.

6.5 Immediately place the darkened bottle into a light-tight, insulated container to maintain constant temperature during transport to the on-board ship laboratory. During the summer, add freezer packs or ice to maintain the hypolimnion temperature.

6.6 Record the following information into field notebook; station number, depth, pH, alkalinity, temperature, date, sampling time and analyst identification.

7.0 Instrument Set-Up Procedure

7.1 Before introducing samples to the incubator, adjust the temperature control to that of the water from which the samples were taken. Confirm temperature setting with thermometer to the nearest 0.1°C.

7.2 Before introducing samples for the first time into the incubator, determine the appropriate locations for two sets of incubation bottles at each of at least five light levels. Each shelf should allow

approximately half the light through as the one above it, e.g. 300, 150, 75, 37, and 17 µE $M^{-2}sec^{-1}$. Perform this procedure with all other bottles in place and filled with water. Mark on the shelf using tape where the bottles should be placed during subsequent incubation. Use grey screening material (e.g., window screen material commonly found in hardware stores) between shelves or bottles if needed to adjust irradiance to those suggested above.

8.0 Analytical Procedures

8.1 Field Operations

8.1.1 Sample collection and initial preparation, see Section 6.0.

8.1.2 In the laboratory, record in the logbook the following data: bottle number, station, depth, date, sampling time.

********All of the Following Procedures Should Be Performed in Green Light****

8.1.3 If possible, each darkened carboy (both rinse and sample water) should be filled to the top with lake water, about 4 L.

8.1.4 In the summer begin with the hypolimnion sample (see Section 6.3.2) first to avoid excess sample warming. Gently mix the water by inversion or rolling. Rinse each incubation bottle with sample lake water and empty into sink.

8.1.5 Place all incubation bottles onto absorbent paper-lined tray and carefully fill each of the 12 incubation bottles with sample water keeping agitation and bubbling to a minimum. Make sure to minimize the air space by filling the bottles up to the top with sample water. Cap each bottle using glass stopper and tilt bottle to remove excess water.

8.1.6 Remove 1.0 mL of $NaH^{14}CO_3$ stock solution using a pipettor. Remove the sample bottle stopper and *gently* inject the stock solution into the bottom half of the bottle. Immediately replace stopper and put a plastic cap over the top of the stopper to eliminate leakage. Only put a plastic cap over those sample bottles which have been inoculated!

8.1.7 Using a new pipette tip for each inoculation, repeat Step 8.1.6 until all 12 bottles have been inoculated. Discard the remaining 1 mL of stock solution into the liquid waste cubie and dispose of the empty vial in the solid waste receptacle.

8.1.8 Place each sample bottle on tape-marked areas (see Section 7.2) in the incubator. Use a spherical irradiance sensor, and measure the light intensity at each bottle location *with all other bottles in place*. Record readings into logbook**.**

8.1.9 Incubate the samples for two hours, recording the incubation starting time into the logbook. If conditions dictate, the incubation period can persist for up to four hours. However, to maintain consistency, attempt to keep the incubation period as close to two hours as possible.

8.1.10 Remove the top two bottles receiving the highest light intensity (top shelf, nearest the lights). *Record the Time* when the samples were removed and incubator temperature into the logbook.

8.1.11 After gently mixing the sample by inversion, remove the cap, measure 100 mL in a graduated cylinder and filter through 47 mm Sartorius .45 µm pore cellulose acetate filter under 8 PSI (equal to 2.3 inches in mercury) vacuum. Sample volume to be filtered may be adjusted to conditions, i.e. reduce volume if high density of algae causes clogging of the filter.

8.1.12 Rinse the filter funnel thoroughly with distilled water.

8.1.13 Remove the filter from the funnel base by grasping the edge with forceps and rolling it into a loose cylinder, algae side inward. Set into a clean liquid scintillation vial and loosely cap.

8.1.14 Repeat Steps 8.1.10 through 8.1.13 until a sample from each incubation bottle has been filtered.

8.1.15 From one of the incubation bottles exposed to one of the three highest irradiances, filter a *second* duplicate sample. Record the bottle number of the sample and the duplicate onto the logbook.

8.1.16 Filter 100 mL deionized water through a filter and place it into a clean liquid scintillation vial to serve as a blank.

8.1.17 Into each liquid scintillation vial that contains a filter, inject 0.3 mL of 0.5 N HCL into the bottom. *Loosely* cap, and let sit for at least one hour.

8.1.18 After one hour, add 20 mL of liquid scintillation cocktail, Ecoscint brand or equivalent, into each vial that has received the acid treatment.

8.1.19 Cap each vial and gently shake it until all of the filter has been covered with cocktail and has sunk to the bottom of the vial.

8.1.20 Into two clean liquid scintillation vials, add 20 mL liquid scintillation cocktail plus 1 mL phenoethylamine (a CO_2 absorber).

8.1.21 Choose, at random, two incubation vessels and transfer 1.0 mL of each into a corresponding vial containing the cocktail and phenolethylamine (Section 8.1.20). These subsamples will be used to confirm the actual specific activity of the isotope in the incubation vessels. Record the bottle numbers into the logbook.

8.1.22 Make sure each vial cap is *tightly* secured and properly labeled. Store the vials for transport to CRL for scintillation counting.

8.2 Clean-up Procedures

8.2.1 Dispose of remaining sample in the sample bottles in the liquid waste cubie.

8.2.2 Soak incubation bottles in decontaminant surfactant for at least one hour. Rinse at least three times with tap water, until *absolutely no suds remain*. Using deionized water, rinse all bottles a final time and allow to air dry.

8.2.3 As needed, or once per day, wipe working areas with decontaminant wash. Wipe dry with paper towels.

8.2.4 Change absorbent bench paper if it becomes contaminated or ineffective because spills.

8.2.5 Dispose of potentially radioactive solid waste in specified receptacle.

8.2.6 At the end of the survey, after the lab has been completely cleaned, use filters moistened with distilled water to wipe 4 inch smears of all working surfaces. Put into a clean scintillation vial with 20 mL of Ecoscint and 1 mL of phenethylamine. Label cap with smear number and record location information into the logbook.

8.2.7 All solid and liquid waste is required to be labeled with estimated activity, volume and radioactive source. Prepare for transport to CRL by putting parafilm around the lid and covering liquid waste containers in radioactive waste bags. Tape solid waste boxes completely shut. The waste activity must be clearly seen from the outside of transport material and be accompanied by a Bill of Lading and a ^{14}C waste form (see Appendix 1).

9.0 Quality Control

Although a blank, duplicate and two total activity samples are completed for each depth, there is no on board analysis capabilities for reanalysis.

Appendix 1:
Radioactive Waste Form