

Primary Production – modified JGOFS ¹⁴C method

Reagents and Supplies:

¹⁴C working solution – approximately 80 μ Ci/ml

HCl cleaning solution – 0.5N HCl

β -Phenethylamine – prevents radio-labeled inorganic CO₂ from escaping to the atmosphere

Scintillation Cocktail – use Ultima Gold XR

250ml polycarbonate bottles for incubation - acid washed / Nano rinsed / taped/labeled / ready for use

Pipetters (1ea 2-20 μ L, 10-100 μ L, 100-1000 μ L), pipette tips, 25mm GF/F filters, forceps

dispensettes for β -Phenethylamine and Ultima Gold XR

Sampling:

- light levels for samples as follows: 100%, 54%, 35% 16%, 7%, 3.6%, 1.7% (use par calc program)
- for each depth, rinse 2 250ml PC sample bottles (1 clear, 1 dark) 3x with sample water, dump waste in YELLOW bucket, shake carboy between rinses to re-suspend particulates, and fill PC bottle to brim
- put bottles in bottle carrier, cover with dark plastic bag, take to rad area and add 75 μ L ¹⁴C working solution (~90 μ Ci/mL) to each bottle (approximately 8 μ Ci per bottle), note time on data sheet **!!PIPETTE CAREFULLY!!**
- place the bottles in the corresponding light bag in the incubator (dark bottles can go in the rigged garbage can), incubate for 24hours

Processing:

after 24hours, remove bottles from incubator and process as follows:

total radioactivity

- 1 - dispense 400 μ L of β -phenethylamine into labeled glass 7ml scintillation vials (consecutive sample # plus L or D for lite or dark)
- 2 – with a clean, new pipette tip, withdraw 100 μ L from the sample bottle and add it to the scintillation vial containing the β -phenethylamine – draw and expel the volume twice to rinse tip before drawing the actual sample to go in the scintillation vial (use same tip for L and D bottles) – swirl the sample into the β -phenethylamine
- 3 – add 5ml cocktail to the vial, cap and SHAKE VIGOROUSLY FOR AT LEAST 30sec.
- 4 – place vial into rack to be counted immediately (see counts procedure below)

filtration

- 1 - filter contents of sample bottle on to 25mm GF/F filter, using squirt bottle of filtered seawater (FSW), do 2 small rinses of the sample bottle to remove any trace ¹⁴C from inside, when filter is almost dry, rinse down sides of funnel with small amount of FSW, record time filter dries
- 2 - place filter in labeled glass 20ml scintillation vial (consecutive sample # plus L or D for lite or dark), inside fume hood, pipette 250 μ L 0.5N HCl on to filter & let stand for several hours
- 3 – do 2 small rinses of the sample bottle with Nanopure to remove ¹⁴C from inside – this is liquid waste, do not pour it into the filtration tower
- 4 – add 10ml cocktail, cap and shake vigorously for at least 30seconds (or to 150 count) – BE SURE FILTER IS NOT STUCK TO BOTTOM OF VIAL!
- 5 – count samples on the ship using USER 9 protocol, samples will be re-counted in the lab after a 2-3 week equilibration period.

Counts:

Select the proper user number for quenched ¹⁴C counts on the Beckman LS6500 scintillation counter. Be sure the first rack of samples has the correct user# card in it. Put all samples in racks and place red HALT rack at end. Select autocount and start counter

Calculations:

DPM values are converted to daily productivity rates as follows:

Production (mg C/m³/d) = ((SDPM/V)*(W*0.25*10⁻³)/TDPM)*(1.05/T)

SDPM = DPMs in filtered sample

V = volume of filtered sample in litres

TDPM = total ¹⁴C DPMs in 0.25ml aliquot

W = DIC concentration in samples (~25000mg C/m³)

0.25*10⁻³ = conversion of pipette volume to litres

1.05 = correction for the lower uptake of ¹⁴C compared to ¹²C

T = time in days