Primary Production – modified JGOFS ^{14}C method

Reagents and Supplies:

^{14}C working solution – approximately 80μCi/ml
HCl cleaning solution – 0.5N HCl
β-Phenethylamine – prevents radio-labeled inorganic CO_2 from escaping to the atmosphere
Scintillation Cocktail – use Optima Gold XR, same fluor we use for ^{32}Si
250ml polycarbonate bottles for incubation - acid washed / Nano rinsed / taped/labeled / ready for use
Pipetters (1ea 2-20μL, 10-100μL, 100-1000μL), pipette tips, 25mm GF/F filters, forceps
dispensettes for β-Phenethylamine and Optima 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 150μL ^{14}C working solution to each bottle (approximately 10μCi 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:

<u>total radioactivity</u>

1 - dispense 100μL 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 100μL 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)

<u>filtration</u>

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 ^{14}C 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 250μL 0.5N HCl on to filter & let stand for several hours
3 – do 2 small rinses of the sample bottle with Nanopure to remove ^{14}C 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:

Point Sur counter uses USER 9 for quenched 14C counts. Be sure the first rack of samples has the USER 9 card in it. Put all samples in racks and place red HALT rack at end. Press both reset buttons at the same time twice and wait for instrument to respond, press USER NUMBER, press 9, press ENTER 3x and wait for instrument to respond, press AUTOCOUNT and let it run.

Calculations:

DPM values are converted to daily productivity rates as follows:

Production (mg C/m^3/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 ^{14}C DPMs in 0.25ml aliquot
W = DIC concentration in samples (~25000mg C/m^3)
0.25*10^{-3} = conversion of pipette volume to litres
1.05 = correction for the lower uptake of ^{14}C compared to ^{12}C
T = time in days