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Bacterioplankton Leucine Incorporation Rate, Field Procedures
Uses larger volume for oligotrophic systems

Modified from
Kirchman, D. et al. 1985. Leucine incorporation and its potential as a measure of protein synthesis by bacteria in natural aquatic systems. Appl. Environ. Microbiol. 49:599-607

Equipment:
Designated 'Radioactive' Cooler
Field notebook (e.g. "Rite in the Rain")
Sharpie pen
Pencil
1 gallon "rad waste" ziplock bag taped to inside of Rad. cooler
Small round styrofoam cooler (~10" tall and 5" in diameter, the kind used for shipping glass bottles of chemicals)
Blue-ice bag that will fit in cooler (frozen)
4 1L plastic sample bottles (acid washed and sterile)
4 Thermoses (1 quart, stainless steel, wide mouth)
Incubation vials: each consists of
- 20 ml scintillation vial (HDPE) pre-sterilized, uncapped (e.g. Fisher #03-337-23B)
- Black open top cap (Fisher #NC9652480)
- 22 mm Teflon/Silicon septa (sterile) (Fisher #NC9706925)

4x10 rack for vials
25 ml plastic pipette and pipette bulb
20-200 ul pipette
1 Rack of 20-200 ul pipette tips (sterile)
5 cc syringe with needle
0.22 um pore-size 25 mm Isopore filters (Millipore #GTBP02500)
filter forceps
Vacuum filtration rig for 25 mm diameter filters designated 'radioactive'
7 ml scintillation vials (e.g., Fisher #03-337-1)
squirt bottle for 5% trichloroacetic acid
gloves

Solutions
14C-leucine stock solution (~300 Ci/mol)
10 uM 14C-leucine working solution diluted in sterile water
100% trichloroacetic acid solution (100% TCA e.g., Sigma 490-10)
5% trichloroacetic acid solution (5% TCA)
Ethylene glycol monoethyl ether ("Methyl Cellusolve", Fisher #E1801)
Scintisafe 30% liquid scintillation cocktail (Fisher #SX23-5)

Procedure

Preparation
Assemble and number the incubation vials (cap, septa with teflon side down, vial)
Put 14C-Leucine working solution in sealed plastic container in small styrofoam cooler with a frozen blue-ice bag.

In the field
Collect sample in 1L bottle and in thermos.
Do all sample manipulation in the 'Radioactive' cooler.
Load 5 cc syringe with 100% TCA.
Open 4 incubation vials per sample.
Pipette 5 ml of sample (from bottle) into each vial.
Add 0.25 ml 100% TCA to the first "T0" vial with syringe and swirl.
Put on gloves.
Add 25 ul 14C-leucine to each vial with new sterile tip (final concentration 50 nM Leucine, or approximately 25 uCi per vial)
Eject tip into Rad solid waste bag
Close vials carefully, and swirl to mix.
Submerge vials in thermos and close thermos.
Incubate 1 hour.
Remove vials from thermos (its OK to pour the liquid out of the thermos)
Add 0.25 ml 100% TCA to the three "T1" vials through the septa with the syringe and needle, and shake.
Return vials to the rack.
Cool and store in refrigerator

In the lab
Filter TCA-fixed samples through Isopore filters
Rinse vials with ice-cold 5% TCA and pour through filters
Turn off vacuum and cover filters with 5 ml ice-cold 5% TCA
Incubate for 5 minutes
Rinse filter 2 times with 5 ml ice-cold 5% TCA
Transfer filters to 7 ml scintillation vials
Add 1 ml methyl cellusolve and incubate until filters are transparent
Add 6 ml Scintisafe 30% scintillation cocktail
Count radioactivity in scintillation counter