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🌐 Preparing phytoplankton samples for elemental carbon and nitrogen analysis

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Protocol status: Working

We use this protocol and it's working

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Abstract

Protocol for the preparation of phytoplankton samples for elemental carbon and nitrogen analyses.

Attachments



POC PON protocol.doc...

14KB

Materials

MATERIALS

- ✕ Distilled Water
- ✕ Tissue Culture Flask, 50ml, 10/Cs Bio Basic Inc. Catalog #TCF70-50.SIZE.1PK
- ✕ Hydrochloric acid Merck MilliporeSigma (Sigma-Aldrich) Catalog #H1758
- ✕ 6-well plate Corning
- ✕ Vacuum Manifold Filtration Unit Merck Millipore (EMD Millipore) Catalog #MSVMHTS00 or equivalent
- ✕ Whatman GFF filters 25mm Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z242489



Preparing GFF filters

2d

- 1 Wrap up to 20 Whatman GFF filters in aluminium foil and precombust them for 6 hours at 450degrees C, store filters airtight afterwards
- 2 Weigh precombusted filters and store labelled and individually (e.g. in 6-well microtitre plates)

Preparing phytoplankton cultures

5d

- 3 Grow 45 mL phytoplankton cultures in 50 mL flasks until they reach mid-exponential phase, culture are dense enough when some colour is visible

Applying samples onto filters

1d

- 4 1 mL used of each sample used for cell counting (e.g. with fluorescence plate reader/FlowCam/FlowCytometry?Microscopy)
- 5 Use a vacuum filtration unit to apply the phytoplankton samples onto the GFF filters
From the remaining 44 mL per sample, 20 mL each used for 2 replicate filters
Apply the ample onto filter using a pipette, carefully check volume
Wash each the edge of the filter unit and the filter with 10mL dest. water
- 6 Filters with biomass can be stored again in the 6-well plates,
Let filters dry with loose lid overnight

Preparation of filters for elemental analysis

5d

- 7 Dry filters at 40 degrees C for 2-3 days
Weigh all dried filters again and calculate the net weight of the dry biomass for each filter
- 8 For work with an elemental analyzer that measures total carbon content, the inorganic carbon content has to be removed: (this can take place in the 6-well plates
Soak filters with 200uL dest. water, then apply 100uL 2M HCl onto each filter to remove any inorganic carbon from the filters
Let filters dry overnight



9 Filters are now ready for elemental analysis