Preparing Biological Samples for Column Chemistry

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Abstract

Protocol for preparing biological samples fro isotopic analysis by column chemistry.

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Protocol

Step 1.

Filtering Cultures

1.1 Oxalate Wash Preparation

Before filtration, make an oxalate wash that will be used in order to remove extracellular iron from diatoms.

Mix consecutively from a to f in 600 ml of ultra pure. Take up to 1 L ultra pure once Ph 8 is reached.

1. 15.3g EDTA
2. 14.7g Sodium Citrate
3. 0.74g Potassium Chloride
4. 5g Sodium Chloride
5. Several drops of Sodium Hydroxide until a Ph of 6-7
6. 12.6g Oxalic Acid

1.2 Harvest Diatoms Once They Have Been Filtered

Once cultures have been grown to their optimal density (approximately 3500 RFU), they need to be harvested. In order to do so, the following items are needed: a pair of tweezers that have been cleaned in 10% HCl overnight, polycarbonate filters (0.1um) that have been washed in 10% HCl overnight and rinsed in UPW, 3 bottles: one to collect waste, one that contains ASW and one that contains UPW, oxalate wash, a 60ml syringe, and syringe filter tip.

Label several 15ml tubes with the designated solution numbers on them. One tube should be labeled for unwashed solutions and a separate tube labeled for washed solutions. For example, the tubes may say, “solution 1#1 unwashed”, “solution 1#2 washed”. Once tubes are labeled, assemble the filtration apparatus. Use polyethylene gloves to handle the tweezers, remove one polycarbonate filter from its container and place on the filter rig. Screw the reservoir onto the filter rig and rinse the filter with UPW, allowing the water to be filtered through. Pour 250ml of culture onto the filter and allow it to drain. Rinse the filter three times with 5ml of ASW. Once filter has completely drained, unscrew the reservoir with polyethylene gloves, remove the filter with tweezers, and place it in the corresponding 15ml tube that is labeled “unwashed”. Syringe filter 60ml of oxalate wash into the remaining 750ml of culture. Invert bottle once and allow to sit for 30min. Invert bottle a second time and place a new polycarbonate filter onto the filter rig, rinse with UPW and pour the remaining culture onto filter. Rinse filter three times with 5ml ASW, drain completely, remove filter, and place in corresponding tube labeled “washed”. Repeat these steps until all of the 1L bottles have been filtered. Rinse tweezers with UPW as well as the filter rig, and put everything back in its place. Rinse the bottles out with UPW and take them to the clean lab to be cleaned.

All cultures, once filtered, need to be run through columns in order to examine iron limitation.

Step 2.

Preparation for Isotopic Analysis

- Transfer filter to a clean 15 mL tube for storage.
- Transfer the filter into a small teflon (PFA) vial.
- Add 1 mL conc. TE clean HCl and 1 mL conc. TE clean HNO3 to each vial. CAREFUL: This reaction will produce a corrosive and foul-smelling orange gas. Place caps loosely on vials and place vials in a fume hood. IF YOU DECIDE TO PUT THEM ON A HOT PLATE, DO NOT TURN ON HEAT. (If hotplate is not available, find another good place to store them overnight in a fume hood.)
- After the samples have reacted overnight, remove the filters from the vials with a pair of teflon tweezers. Try to pick up the filter in a way that most of the remaining diatom shells remain on the filter. The filter will also have a lot of the acid still in it, and this acid contains valuable amounts of Fe.
- Rinse off this acid back into the PFA vial by twice pipetting 1 mL of TE H2O over each filter.
- Evaporate samples to dryness on a hot plate.
- Reconstitute samples in 200 uL of 10N HCl +0.001% H2O2.
- Collect a subsample before samples are run through columns.
- Run samples through ‘mini’ columns (135 uL) according to established procedures.

- While running columns, clean each of your PFA vials by blasting then with TE water from the tap and putting them on the hot plate with 0.1 N HNO3 for an hour or two as columns run.
- Elute from the columns into the same PFA vials your samples came out of.

- Dry down overnight (or for a few hours).
- Re dissolve in 100ul of conc. HNO3
- Put on hot plate in bio lab, capped, for 30min at 350.
- Take off hot plate in clean lab and put on hot plate in biological lab to dry down.
- Reconstitute in 1100ul 0.1N HNO3
- Take off 100ul of sample and dilute to 1ml with .1N HNO3, put in 15 ml vial.
- Give the sample from the step above to Tim to run on element. (This gives the concentration that allows you to calculate step 22).
- When you have calculated the amount of Fe in each sample, add enough double-spike to get a 1:2 sample: spike ratio.
- The sample is ready for isotopic analysis.