Tips for SIA processing

**Freeze drying process**

* **Freeze dryer Model at BAS Cambridge:** LABOGENE Scanvac
* Freeze samples at -80oC without lids and pre- parafilmed \* (if necessary) before freeze trying.
* Start to cool freeze drier to below -40 (minimum operating temperature) before transferring samples.
* Transfer samples immediately from -80 to freeze dryer.
* Store lids in a separate clean box lined with layers of tin foil.
* When samples are dried, lay lids on tinfoil in order and sort sample vials in order to increase efficiency of putting lids back on vials.

\*For volatile samples that may contain high moisture, parafilm top of vial

**Freeze drier times**

* Sediment dried for 48 hours.
* Invertebrates dried for 24 hours.

**Acidification**

1. Grind samples using pestle and mortar by hand.
2. Wash pestle and mortar using water and then ethanol to remove all traces.
3. Use ‘kimtech’ wipes (lint free) and wipe only ONCE to reduce lint shedding.
4. Transfer an aliquot of sample of a 7 ml glass vial (specifically with a PTF lid) suitable for chloromethanol extractions.
5. Transfer samples from pestle and mortar into vials whilst connecting to a static matt. Static matt is particularly bad for ground algae and especially bad if using plastic Eppendorf’s. I choose to use glass but this is a more expensive option.
6. Grind sample then add variable acid concentrations.

For inverts I used 1 N which = 1 M which equates to 8.2% HLC solution.

For macroalgae I used 0.1N.

1. Add acid until stop fizzing using pipette.
2. Leave to fizz.
3. Add MILIQ using glass pasture pipettes (150mm pipettes, 15 cm long).
4. Vortex (‘Genie 2’, G-560E) sample for ~10 seconds.
5. Put samples into ‘eppendorf’ centrifuge 5702 for 10 mins at 4.4 RMP max setting (if using glass 7 ml vials).

If conducting acidification in eppendorfs using smaller Centrifuge (‘eppendorf’ 5414) then setting is 12,000 RMP.

1. Pipette off residual liquid.
2. Add another round of MILIQ for the final rinse. Vortex + centrifuge as above.
3. Pipette supernatant
4. Put in oven at 50 oC for 24 hours. Or longer if sample has not dried.

**Lipid extraction / delipidification**

1. Work with either an aliquot or the remaining samples that have been acidified.
2. 2ml of 2:1 chloromethanol (Chloroform and Methanol) was added to each acidified sample.
3. Samples were put into a sonic bath for 30 mins (‘Decon’, FS300).
4. Samples were then vortexed (Genie 2, G-560E) for ~10 seconds before being placed into the centrifuge.
5. Samples were centrifuged for 10 mins at 3.4 RMP in Centrifuge 5720**.**
6. This step was repeated twice more to equal a cumulative total of 6 ml of chloromethanol being added to the samples.
7. Samples were then rinsed with 2 ml of MILIQ water, sonicated for 30 mins and centrifuged for 10 mins at 3.4 RMP.
8. MILIQ rinse was performed once mor with a cumulative total of 4ml of MILIQ per sample.
9. Samples were put in the oven for 24 hours at 50 oC, or longer if bigger samples had not dried.

**Tin capsuels, packing**

**Center for Ecology and Hydrology -** Capsules are 6 x 4 mm product code D1006

**SUERC, East Kilbride-** Tin capsules 5 x 3.5 mm

Packing conducted on microbalance scale Mettler MT5 (precision 0.001 mg)

* Grab tin capsule from with hooked tweezers from capsule pot
* Tare scale
* Work on a glass slate that has been cleaned with ethanol and wiped with ‘Kimtech’ lint free tissues
* Put sample material into the capsule, 0.6-0.8 mg for inverts and macroalgae and >10mg for sediment
* Close capsules using flat tweezers and fold

**Packing filters: TBC**

* Filters must have been pre-weighed if the total carbon content wants to be calculated.
* Punch a hole in the centre of the filter using something akin to cork borer sets
* Multiple filter discs can be packed together
* Conduct preliminary trials to assess how much filter material is needed to get meaningful peaks of C and N

**Data interpretation: TBC**

* Gabi Stowasser says: C/N ratios must be above 3.5
* Jason Newton says: Macroalgae will have high C:N ratios; they are lower than terrestrial plants but will still be I guess >10. This will differ by species and I suspect also where you take the sample (this also applies to a small extent in d15N and d13C – though I don’t recall the publication).