Acidification protocol

Acidification

1. Fetch the freeze-dried kelp and empty one bag at a time into a large weighing boat, then tare the empty bag on another weighing boat on the 0.1-mg balance
2. Once stable, transfer the kelp into the bag and seal before replacing to weigh
3. Put on a mask to avoid inhaling $CaCO_3$ powder and grind the kelp thoroughly with separate sets of pestle and mortar for each sample until no more flakes are visible
4. Use the Retsch mixer mill with three steel UFO beads at maximum frequency for 1 min for stubborn samples
5. Replace the resulting powder into the original bag using a brush
6. Label and weigh 50-mL tubes on the same balance and note their exact mass
7. Weigh out a ~1-g subsample of each powder into these labelled 50-mL tubes by first taring the empty tube and then adding powder, and note down the exact final mass
8. Put on personal protective equipment: lab coat, nitrile gloves and safety glasses
9. Prepare a bench spot next to the vortex with some paper towels and a tube rack
10. Pipette 20 mL of 1 N HCl into each 50-mL tube
11. HCl reacting with $CaCO_3$ will result in the release of CO_2 so initially close the tubes to avoid foam spillover
12. Slowly release the built-up CO_2 by unscrewing the lids a quarter turn or less and wipe up any spills
13. Vortex thoroughly and turn down the rpm so all particles are suspended in HCl
14. Unscrew the lids a full turn to allow further CO_2 to escape
15. After at least 24 h, centrifuge the tubes at 4500×*g* for 5 min and discard the supernatant using the 10-mL pipette
16. Dispose of HCl waste into a plastic beaker and dilute with water before draining
17. Add 20 mL of MilliQ to each tube, vortex thoroughly, centrifuge at 4500×*g* for 5 min, discard supernatant, and repeat these washing steps twice more
18. This serial elution is required to wash out $CaCl_2$, formed during acidification beside CO_2
19. Freeze the 50-mL tubes in a plastic tube rack and once frozen place in the freeze-dryer, making sure the lids are unscrewed as far as possible without coming off
20. After at least 24 hours, weigh the filled 50-mL tubes on the same balance

Data entry

1. In addition to the re-measuring of total dry mass, there are three new variables being recorded: tube mass, dry mass put into tube and dry mass of tube and contents left after acidification
2. The csv file for mass should look something like this:

ID	Species	Individual	BM	DM	DMi	Tube	DMo
D_1_1	*Laminaria digitata*	1	…	…	…	…	…
D_1_2	*Laminaria digitata*	1	…	…	…	…	…
D_1_3	*Laminaria digitata*	1	…	…	…	…	…

BM is buoyant mass, DM is dry mass, DMi is dry mass of the ~1-g subsample, Tube is labelled 50-mL tube mass with cap, DMo is dry mass of organic remains and tube so that DMo minus Tube is the organic mass