**Yanai Lab – Lab Protocol**

**Created by Dan Hong**

**Grinding**

*Wiley Mill/Small grinder (Marshall B13)*

The key to B13 can be found next to Mary’s desk and it has a key chain that is an 8 ball.

Materials: Wiley Mill, 20ml glass scintillation or scint vials, gloves, and good judgement.

**Prior to grinding,** store your samples in the oven (60°C) for 24 hours to obtain constant mass. Depending on the mass and condition (wet or dry) of your sample, the drying time may vary. Even if your sample had been dried before, put it back in the oven for at least overnight. **Drier samples will go through the grinder easier than wetter samples BUT it could take longer as the finer particles are more prone to adhering to the machinery.** I bring ~10 samples from B9 oven to B13 oven at a time to avoid walking back and forth between every sample. However, do not keep your sample in the B13 oven for too long because it rarely works.

1. Turn on the lights under the hood, open the hood, and turn on the air valve. You should be able to hear the air flowing through the hose if the clip-on is loose enough.
2. Wearing gloves, clean the machine and its parts by blowing air from the hose. Be sure to blow clean the glass plate (both sides), sieve (inside and out), chute, wheel, and funnel. This should be done before you start and in between every sample to prevent cross-contamination.
3. Assemble the machine: Place the metal sieve (engraved 40) right under the wheel with its arm facing right and lock it in place by adjusting the arm-holder and tightening the nut. DO NOT raise the sieve too close to the wheel to a point where it touches the wheel; the machine will make a sharp noise as the two metal parts are abrasing against one another. Place the glass plate over the wheel (it should fit perfectly and rest on two metal pins) and lock it in place by turning the metal arm piece over the glass plate and tightening the nut in the back. If it’s not tight enough, your sample will be lost in the process of grinding (but if too tight, it will break the glass plate).
4. Place your labeled 20ml scint vial with a funnel on top below the sieve.
5. Break up samples manually (wearing gloves obviously) and put them in the chute/hopper at the top of the machine.
6. Turn on the red switch and push down the sample with a wooden stampler. The wooden stampler is not long enough to hit the wheel so you can push it all the way down.
7. You will get some of your ground samples in the scint vial. There is still a lot in the machine (i.e. inside the sieve, on top of the sieve, in and around the wheel, and in the hopper). In order to catch this hidden ground sample (if you have a small sample and want to have as much material recovered), A) turn off the machine, B) carefully detach the sieve from the machine with ground samples sitting on top and dump it into the funnel, C) take the sieve and hit it against the inside of the hopper multiple times so that the finely-ground sample that clings to the sieve will fall back into the hopper, and D) turn on the machine without the sieve. You will see a lot of sample falling into the vial. By doing this, you will reduce the loss of your sample. However, some material may not have been ground finely enough.
8. Cap the scint vial.
9. Detach the glass plate and clean the machine and all of its parts using the air hose.

*Big grinder (Illick sub-basement)*

**Ashing**

We no longer us this method for hot-plate digesting.

**Acid-digesting**

*Acid digestion for plant material (Illick 339)*

Materials: your samples in 20ml glass scint vials, weighing paper, scale (0.0001g ~ 80g), gloves, trace-metal grade nitric acid (in a dispenser), Teflon tubes and caps (two kinds) for the microwave, MARS6 Microwave Digestion System (CEM©), and good judgement.

The microwave can digest up to 40 samples. The microwave needs to be balanced evenly so refer to the tube placement diagram (next to the microwave) if you have less than 40 samples to digest. Each round should have one **BLANK**, two sample standards (**NIST1515**), and one **duplicate** of your sample. With this being said, you can only digest a total of 36 of your samples in a round.

**Prior to weighing,** store your samples in the oven (60°C) for 24 hours to obtain constant mass with the caps slightly unscrewed. Depending on the volume of your sample, the drying time may vary. Even if your sample had been dried before, put it back in the oven for at least overnight. Close the caps once the samples are dry.

1. Using the scale and weighing paper, weigh out ~0.25g of your ground sample (I just shake the vial to mix the material and pour little by little onto the weighing paper, but a spatula can be used). Record this number on a datasheet with your sample ID.
2. Pour this ~0.25g of sample into the Teflon tube and resume with the rest of your samples.
3. Don’t forget to include a BLANK, two sample standards, and one duplicate. BLANK would have 0 for mass whereas the other three will be ~0.25g each.
4. Bring those weighed samples in Teflon tubes in a Teflon rack under the hood with a dispenser of trace-metal nitric acid. The dispenser should be calibrated to 10ml per pump.
5. Add 10ml of trace-metal nitric acid into each of the Teflon tubes.
6. Put the two caps onto the Teflon tubes. There is a blocker and a cap; put the blocker onto the tube first and then put the cap on. Use the little block (should be on the shelf by the sink) to close the caps tightly.
7. Place the capped Teflon tubes into the microwave wheel (refer to the tube placement diagram for how to place the tubes) and put the wheel into the microwave. Close the door.
8. Turn on the microwave (right side), press “One Touch” on the screen, and scroll down to find “Plant Material”. Click on this method and and press “Start”. The machine will initialize and check if the samples are balanced. Once done, it will run for about 1.5~2 hours (including cooling time).
9. Stay with the microwave when it’s running.
10. Once the microwave is finished running, take out the wheel and samples on to the Teflon rack and bring that under the hood. Turn off the microwave.
11. Uncap the tubes slowly and carefully, letting out the fumes from the tubes. Set the dirty caps aside for washing later.
12. Add distilled deionized water (DDW) into the Teflon tube using a squeeze bottle. Transfer this solution into a prelabeled and pre-weighed 50ml centrifuge tube.
13. Rinse the inside of the Teflon tube with more DDW and pour that into the same 50ml centrifuge tube. Do this three times or until the solution in the centrifuge tube reaches 50ml.
14. Cap the tubes and record their final masses (50ml of digested and diluted solution + centrifuge tube).

**Acid-washing**

We no longer acid wash used centrifuge tubes. Get new ones from the chemical stock room (Marlene is awesome!).

**ICP**

*Inductively-Coupled Plasma (Optical Emission Spectrometer) is located in Baker 126.*

Materials: your sample digests, ICP-OES, internal standard (Yttrium; Deb has it), ICP calibration standards including a blank, and good judgement.

**Prior to analyzing your samples**, you need to provide Deb with a few of your samples so that she can run them to come up with the right matrix of your calibration standards (usually 4~6 standards). You also need to tell her which elements you are interested in analyzing. You can decide whether to include Yttrium (an internal standard) to your sample. Inquire Deb about this approach. For more detailed protocol on using the machine, talk to Deb! She will train you.

1. Start with a “calib blank” and calibrate the machine with the standards that Deb provides (she needs to make these standards so it may take some time).
2. Run the QC and make sure all the elements are passing (there are some elements that constantly behave worse than others and this is where your good judgement comes in).
3. If your elements of interest don’t pass, recalibrate the machine or consult with Deb.
4. Run a blank as a sample.
5. Run the rest of your samples. I like to run QC every 5 samples. And a blank is run after every QC. If it’s a good ICP day and the QC seems to pass consistently, then I run QC every 10 samples.

**Now what do I do?**

Assuming you saved and exported your ICP outputs and sent them to yourself, it’s time to make a sense out of what all these numbers mean! You will need to know your Sample ID, Analyte Name, Conc (Calib), and sometimes Calib Units.

The outputs that ICP provides are in mass/volume unit, more specifically ug/L or mg/L. These outputs and/or numbers are based on the 50ml diluted digests. So in order to represent the concentration of an element per mass of ground sample material, you need to do some conversion. You can either copy and paste (transpose) the ICP output or use “Lookup” command in Excel to transfer the ICP outputs onto your dataseet.

For example, if you have a phosphorus ICP output of 3.80275 mg/L:

8.957 mg/L \* (50.2353 ml of diluted digest) \* (1L/1000ml) / (0.2487g of ground sample) = 1.8092 mg of P/g of sample

I find the format of the datasheet below most helpful in keeping track of all the numbers.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Teflon tube # | Sample ID | Ground sample (g) | Centrifuge tare (g) | Sample + tube (g) | Sample mass or volume (g or ml) | P 213.617 (ICP) | P 214.914 (ICP) | Converted P (mg/g) | Converted P (mg/g) |
| 1 | C1-N-BE435 | 0.2487 | 14.1047 | 64.3400 | 50.2353 | 8.957 | 8.787 | 1.8092 | 1.7748 |
| 2 | … | … | … | … | … |  |  |  |  |