Leaf Tissue Digestion Protocol

Purpose

Digest leaf tissue to produce solution with total elements in organic form for analysis.

Preparation:

Sulfuric/salicylic acid solution:

1. Weigh out 34 g of salicylic acid on plastic weigh boat.
2. Gently pour into 1 L flask.
3. Add 18 *M* (concentrated) sulfuric acid to flask (but not totally to 1 L)
4. Add magnetic stir bar and gently stir and heat (on 2) until fully dissolved.
5. Top with stopper and cover in tin foil (to keep it in the dark) to cool overnight or until room temperature.
6. When cool, remove stir bar.
7. Fill to 1 L with sulfuric acid and store in amber bottle in refrigerator.

Weigh leaf tissue

1. Place acid washed 100 mL digestion tubes in metal rack.
2. Use autoclave pencil to write numbers 1-40 on the tubes. You will use this to identify your samples later.
3. Weigh 0.1000 ± 0.0025 g of dried and ground leaf tissue and record weights in data sheet along with tube number.
4. Include 2 blanks, 4 reps, and 2 peach leaf standards (or equivalent).
5. Add three Hengar boiling chips to each tube.
6. If leaving overnight, cover the top with aluminum foil to avoid particles from floating into the tubes.

Digestion:

1. Turn on the digestion block about 30 minutes before the digestion is to begin.
2. Place the rack with weighed plant tissue in the fume hood and on a metal baking sheet.
3. Pipet in 5 mL of H_2SO_4/Salicylic acid to each tube.
4. Pipet 2 mL of H_2O_2 0.5 mL at a time (Fisher H325-500) in 0.5 mL increments.
    a. Add 0.5 mL, vortex (on high), add 0.5 mL, vortex, add 0.5 mL, vortex, add 0.5 mL, vortex
5. Put the tubes on the block when it is under 300 ºC (250 ºC for the first phase, 290 ºC for remaining phases).
6. Set scrubber to high, turn on scrubber. After 10 min, set scrubber to medium and keep it here until step 10.
7. Place the manifold on top of the tubes.
8. The tubes heat on the block 320 ºC for 15 minutes.
9. Remove rack from the block (keep manifold and scrubber on) and place back on the baking sheet.
10. Cool for 20 minutes.
11. Gently remove the manifold and place on a second baking sheet in the hood.

12. Add 1.5 mL H_2O_2 to each tube and vortex.
13. Repeat steps 7-11.
14. Add 1.5 mL H_2O_2 to each tube and vortex.
15. Repeat steps 7-11.
16. Add 1.0 mL of H_2O_2, vortex.
17. Repeat steps 7-11.
18. Add 0.5 mL of H_2O_2, vortex.
19. Repeat steps 7-11.

If the samples are not clear at this point, repeat step 18 and 19, by adding 0.5 mL H_2O_2 and placing back on the block.

20. Turn off block completely
21. Once the tubes have cooled for at least 30 min, remove the manifold and set scrubber to low to suck the remaining acid from the manifold and tubing.
22. Slowly add ~75 mL of DI to each tube.
23. Allow the tubes to cool completely in the hood (overnight or for several hours). Cover with aluminum foil or place stoppers in the tubes to avoid anything from floating in.
24. Top off tubes to 100 mL calibration mark with DI water. Cover with a stopper and invert 5 times.
25. Allow the tubes to cool for at least an hour. Do not invert again!! The digestate should be clear, and there will be white gunk on the bottom (boiling chips).
26. Pour enough digestate to half fill a scintillation vial, close the vial with a cap and shake several times. You are cleaning the scintillation vial with the digestion solution. Pour this into the nalgene waste bucket.
27. Pour enough digestate into a scintillation vial to almost fill it, swirl and pour out. ****Make sure you empty the contents of the nalgene waste bucket into a labeled waste carboy when done.**
28. **Collect your sample:** Fill a scintillation vial with the digestate (third quarter of the sample) and cap.
29. Samples are shelf-stable (do not need to be refrigerated or frozen). Keep in a cool, dry place until ready to analyze.

Waste Tag:

- water = 94%
- sulfuric acid = 4%
- Hengar boiling chips = 1%
- salicylic acid = 1%