α cellulose extraction from wood (KJA-1)
modified from *Brendel et al.* (2000)

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Materials

1. wood samples, size: 400-1500μg
2. 1.5mL polypropylene tubes
3. 80% acetic acid, reagent grade (can be made from glacial acetic acid by adding 4 parts acid to 1 part DDW).
4. 69% nitric acid, reagent grade
5. micropipettes: 20, 200, and 1000μL capable; disposable pipette tips
6. centrifuge
7. hot plate and aluminum heating blocks
8. 100% ethanol
9. distilled deionized water (DDW)
10. acetone
11. drying oven at 45-50^{o}C
12. vacuum evaporator

Procedure

1. Prepare wood samples in labelled polypropylene tubes.
2. Using 200μL pipette, add 120μL acetic acid into sample tubes.
3. Using 20μL pipette, add 12μL nitric acid into sample tubes.
4. Cap securely; insert tubes into heating blocks and extract for 30 minutes at 120^{o}C. Be careful that temperature does not exceed 130^{o}C. The optimal temperature range is between 119^{o}C and 124^{o}C.
5. Tap the tubes approximately every 5 minutes to improve the reaction and mix the acids and sample in tube.
6. Remove tubes from heat; allow to cool (3-5 minutes). Uncap slowly in case of overpressure.
7. Using 1000μL pipette, add 400μL ethanol to tubes; cap; invert and shake; centrifuge for 5 minutes at 10,000 rpm. Using the 1000μL pipette, carefully remove as much supernatant as possible from samples into waste beaker. Discard pipette tip.
8. Add 300μL DDW to tubes; cap; invert and shake; centrifuge for 5 minutes at 10,000 rpm. Using the 1000μL pipette, carefully remove as much supernatant as possible from samples into waste beaker. Discard pipette tip.

9. Add 150μL Ethanol to tubes using 200μL pipette; cap; tap firmly 2-3 times, but don't invert; centrifuge for 5 minutes at 10,000 rpm. Using the same 200μL pipette, carefully remove as much supernatant as possible from samples into waste beaker. Discard pipette tip.

10. Add 150μL Acetone to tubes. Do not invert nor shake. Centrifuge for 2 minutes at 10,000 rpm.Using the same 200μL pipette, carefully remove as much supernatant as possible from samples into waste beaker. Discard pipette tip.

11. Place samples in warm oven (45-50^{o}C) for about 1/2 hour or until they appear dry.

12. Place samples in vacuum evaporator and cover with aluminum foil. Be sure lid is well-seated on base of unit. With vent valve closed, slowly open vacuum line valve. Allow to evaporate overnight; clean tube surfaces with a damp towel, and cap when complete.

13. Rinse tube caps 3 times in DI water, then with a little ethanol. Place in hood overnight to dry.

14. Cleanup: Turn off hot plate and unplug. Empty waste beaker into 3 gallon waste pail in satellite waste accumulation area; rinse waste beakers with detergent and water, then let sit under running DI water for 5-10 minutes. Wipe up any acid or reagents splashed in hood using a little ethanol. Put reagents away, keeping acids apart. Refill reagent bottles if necessary. Inventory reagents and other supplies, and thinking ahead a week or more, ask Mike or Kevin or John to reorder if necessary.

Notes

1. Wood sample should be finely chopped or ground to improve acid extraction. This is very important: we've found that chunks of raw wood do not process to clean cellulose very well.

2. Batches of 20 or 40 are convenient to process at a time. (20x1, 20x2 ...)

3. Heating blocks require about about 1 hour to reach temperature. Start heating, then use the time to cut the wood samples or cap samples from the previous day.

4. Pipette supernatant slowly to avoid sucking sample up the pipette.

5. The final product should appear white, fine, papery or cottony.

Safety

1. Know where rinse faucets, fire extinguishers, exits and telephones are before beginning chemistry.

2. Wear gloves and safety glasses during procedure.

3. Perform all chemical transfers under fume hood, with the splash shield lowered.

4. Pipette slowly, and against tube walls, to avoid splashing acid or reagent on yourself.

5. Do not eat or drink while performing protocol.

References

Brendel, O., P. P. M. Iannetta, and D. Stewart (2000), A rapid and simple method to isolate pure α-cellulose, *Phytochemical Analysis*, *11*, 7–10.

Evans, M. N., and D. P. Schrag (2004), A stable isotope-based approach to tropical dendroclimatology, *Geochim. Cosmochim. Acta*, doi:10.1016/j.gca.2004.01.006.