

SUPPORTING INFORMATION

Jayme-Wise Method

Holocellulose Isolation

Step 1. Lipid extraction

- Dry and grind samples, weigh 20 to 150 mg and place in a polyester filter bag (Ankom Technology, Macedon, New York), cut edges for identification and seal. Samples analyzed for ^{14}C should be inspected under a microscope for polyester fibers after the treatment, or wrapped in quartz or borosilicate filters and then placed in polyester bags.
- Place 10 to 20 bags (10-60 samples depending on number of samples per bag) in soxhlet apparatus.
- Fill round bottom flask with 300 ml 2:1 Toluene:Ethanol mixture.
- Assemble soxhlet, flask condenser and run for 24 hours. If solvent in upper soxhlet chamber is still getting dark with each reflux cycle then you must continue for longer (additional 24 to 48 hours).
- Turn off heat and remove samples from solvent. Let dry for ~24 hrs.
- Repeat above two bullets using 300 ml of ethanol only.
- Dry samples for ~ 24 hrs.

Step 2. Bleaching

- Place bags in DDI water and boil the samples. This will extract sugars. Boil until freshly replaced boiling water no longer turns color (~2-4 hours). Boiling times used here were 4 hours (Sample Set 1 and 2) and 2 hours (Sample Set 3). .

1. Sample Set 1:

- In a 600 ml beaker filled with 500 ml DDI add 6.7 g of sodium chlorite and 3 ml glacial acetic acid. Add sample bags. Put container in sonicator with heat on (70°C).
- Add another two sets (three total) of 6.7g sodium chlorite and 3 ml glacial acetic acid to each flask without changing solution at 3 hour intervals. After third addition leave overnight without rinsing.
- Rinse 3 times with DDI the next morning and repeat above two steps a second time.
- Rinse samples by draining acid solution, filling with DDI and putting in sonicator for 30 minutes (repeat for a total of six rinses).

- Place samples into one 1000 ml Erhlnmeyer flask with an outlet arm and set up on continuous DDI rinse for a minimum of 4 hours.

2. Sample Set 2:

- In 1500 ml beaker add sample bags, add 700 ml DDI and add 7 g sodium chlorite and 1 ml glacial acetic acid.
- Heat at 70°C with slow stirring for at least 12 hours
- Add an additional 7.0g sodium chlorite + 1.0 ml glacial acetic acid at 2 hour intervals.
- After fourth addition decant 350 ml of bleach solution and add 350 ml DDI
- Continue heating at 70°C and slow stirring for at least 12 hours
- Decant all solution and soak in 700 ml DDI for 20-30 minutes and repeat twice.
- Decant all solution and put under continuous DDI rinse for at least 3 hours.

2. Sample Set 3:

- In 600 ml beaker add sample bags, fill with 500 ml DDI and add 4-6 g sodium chlorite and 3 ml glacial acetic acid. Heat at 70°C with slow stirring.
- Change the solution every 3-4 hrs. Can take from 1 to 2 weeks to bleach.
- When the tissue is white, rinse samples several times ~500 ml DDI over 3-4 hour period. At the end no sodium chlorite smell should remain.

α -Cellulose Isolation

Step 3.

- Fill 600 ml or greater sized beaker with ~ 500 ml DDI, add ~ 85 g NaOH and stir for 1 hr at room temperature (17% w/v NaOH – hemicellulose is soluble in a strong base).
- Rinse several times: ~500 ml DDI over a 3-4 hr period (Sample Set 3) or place beaker in sonicator for 3 sets of 15 minutes. Add new DDI each time. Do continuous rinse with 2L outlet arm flask for 2 hours (Sample Set 1).
- Fill beaker with ~ 450 ml DDI, add 50 ml glacial acetic acid and stir for 1 hr at room temperature (Sample Set 3) or instead of stirring place beaker in ultrasonic (Sample Set 1).
- Rinse several times: ~ 500 ml DDI over a 3-4 hr period (Sample Set 3) or rinse tissue several times by placing flask in sonicator for 3 sets of 15 minutes (Sample Set 1). Add new DDI each time. Do continuous rinse with 2L outlet arm flask for 4 hours.

- Remove samples from filter bags and dry in oven at 70 °C (Sample Set 3) or 50 °C (Sample Set 1).

Brendel, Modified Brendel and Water-Modified Brendel

Step 1.

- Take 50-100 mg milled wood samples (dried) and place in centrifuge tube
- Add 2 ml 80% acetic acid
- Add 0.2 ml 69% nitric acid
- Cap and mix gently (avoid getting sample stuck high on the glass)
- Boil at 120°C for 20-30 min.

Step 2.

- Allow to cool (~5-10 min)
- Add 2.5 ml 99% ethanol

Step 3.

- Vortex
- Centrifuge for 5 min at highest speed (3500 rpm or higher).
- Decant supernatant

Step 4.

- Add 2x2.5 ml 99% ethanol (a total of 5 ml are added, but in two steps, the first is added and then mixed the second is added to wash down the sides of the glass to force sample back into the solution, also during the last addition try to match the volumes in the tube to balance the centrifuge).
- Repeat step 3

Step 5.

- Add 2x2.5 ml DDI
- Repeat step 3

Step 6. (Modified Brendel and Water-Modified Brendel only)

- Add 2x2.5 ml 17% (w/v) NaOH (17 g NaOH per 100 ml water) use only glass pipettes with this solution.

- Let sit for ~ 10 min
- Repeat step 3

Step 7. (Modified Brendel and Water-Modified Brendel only)

- Add 2x2.5 ml DDI
- Repeat step 3

Step 8. (Modified Brendel and Water -Modified Brendel only)

- Add 2.2 ml DDI + 0.6 ml acetic acid
- Vortex
- Add 2.2 ml DDI only (wash down the sides) and gently mix
- Repeat step 3

Step 9. (Modified Brendel and Water -Modified Brendel only)

- Add 2x2.5 ml DDI
- Repeat step 3

Step 10. (Water -Modified Brendel only)

- Add 2x2.5 ml DDI
- Repeat step 3

Step 11.

- Repeat step 10 an additional 3 times.

Step 12.

- Add 2x2.5 ml 99% ethanol
- Repeat step 3

Step 13.

- Add 2x2.5 ml acetone
- Repeat step 3

Allow sample to dry in the hood or a vacuum evaporator overnight then place in a drying oven at 50 °C.

Acid Base Acid

Step 1.

Place 50-100 mg of sample in polyester bags (Ankom Technology, Macedon, New York) and seal.

Step 2.

Place bags in 600 ml beaker filled with 1M HCl and place in sonicator on heat (70°C) for 15 minutes. Drain liquid.

Step 3.

Add 1 M NaOH to beaker and place in sonicator on heat (70°C) until solution becomes dark. Drain and add fresh NaOH. Repeat until NaOH remains clear. Drain NaOH.

Step 4.

Fill beaker with 1M HCL. Drain HCl, fill with DDI put on sonicator for 15 minutes, drain DDI. Repeat for a total of 3 times.