

PERMANGANATE LIGNIN
Updated September 2013

I. References:

Van Soest, P.J. and R.H. Wine. 1968. Journal of A.O.A.C. 51:780.

II. Personal Protective Equipment:

- A. Lab coat
- B. Safety glasses/goggles
- C. Latex gloves

III. Reagents:

A. Saturated Potassium Manganate (KMnO_4)

1. Add 50 g reagent grade KMnO_4 to dH_2O , q.s. to 1 L.
 - a. Keep out of sunlight. Store in amber bottle or clear bottle wrapped with aluminum foil. **Store inside a cabinet.**
2. Lignin Buffer Solution
 - a. Dissolve 6 g $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and 0.15g AgNO_3 in dH_2O , q.s. to 100 mL.
 - b. Combine with 500 ml glacial acetic acid ($\text{C}_2\text{H}_4\text{O}_2$) and 5 g potassium acetate ($\text{C}_2\text{H}_3\text{KO}_2$)
 - c. q.s. to 1L with tertiary butyl alcohol.
 - d. **Mix well**
3. Demineralizing Solution
 - a. Dissolve 50 g Oxalic acid dihydrate in 700 mL 95% EtOH.
 - b. Add 50 mL 12N HCl.
 - c. q.s. to 1 L with dH_2O .
4. 80% Ethanol
 - a. Add 155 mL dH_2O to 845 mL 95% EtOH.
5. KMnO_4 -Buffer Solution
 - a. Combine saturated KMnO_4 solution and lignin buffer solution in a 2:1 ratio.

Caution: Chemicals are extremely corrosive. Handle with care.

IV. Procedure:

- A. Run acid detergent fiber analysis on sample and filter into Gooch crucible.
- B. Place crucibles in shallow Pyrex dish with 2 to 3 cm of dH_2O in it.
- C. Add 25ml of KMnO_4 -Buffer mix to each crucible. Adjust level of water in pan to avoid excess flow of solution out of the crucible.
- D. Place short glass rod in each crucible to stir contents and break up lumps. Leave glass rod in the crucible.
- E. Let crucibles stand 90 ± 10 minutes adding more solution if necessary (solution must be purple at all times).
- F. After 90 minutes, remove crucibles and transfer to filtering apparatus. Suck dry and do not wash.
- G. Place crucibles in clean Pyrex dish and fill crucible $\frac{1}{2}$ full with Demineralizing Solution.

- H. Filter after 15 minutes and refill approximately ½ full with Demineralizing Solution. Rinse sides of crucible with wash bottle containing Demineralizing Solution if necessary. Let stand until fiber is white (20-30 minutes).
- I. Filter and wash crucible and contents 3 times with 80% EtOH. Rinse and remove glass rod so as to lose no dry matter.
- J. Dry at 105 °C overnight and weigh with a desiccator.
- K. Repeat staining if fiber is yellow. This may be required on samples where lignin/ADF fiber is greater than 35%.

V. Calculation:

$$\text{Lignin \%} = \frac{(\text{ADF Residue} - \text{Cellulose Residue})}{(\text{Initial Sample Wt})(\text{DM})}$$