I. References:


II. Personal Protective Equipment:
A. Lab coat
B. Safety glasses/goggles
C. Latex gloves

III. Reagents:
A. Saturated Potassium Manganate (KMnO₄)
   1. Add 50 g reagent grade KMnO₄ to dH₂O, 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 Fe(NO₃)₃·9H₂O and 0.15g AgNO₃ in dH₂O, q.s. to 100 mL.
      b. Combine with 500 ml glacial acetic acid (C₂H₄O₂) and 5 g potassium acetate (C₂H₃KO₂)
      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 dH₂O.
   4. 80% Ethanol
      a. Add 155 mL dH₂O to 845 mL 95% EtOH.
   5. KMnO₄-Buffer Solution
      a. Combine saturated KMnO₄ 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 dH₂O in it.
C. Add 25ml of KMnO₄-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 ½ 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:

\[
\text{Lignin} \% = \frac{(ADF \text{ Residue} – Cellulose \text{ Residue})}{(\text{Initial Sample Wt})(\text{DM})}
\]