Standard Operation Procedure

Crude Fibre Method

1. Scope and Application

The method described is applicable for determination of feeds with a crude fibre content higher than 1%. If sample contains >10% fat, extract fat with petroleum ether prior to beginning analysis.

2. Summary

Crude Fibre includes: most of the cellulose, part of the lignin, no hemicellulose (Novotny 2017)

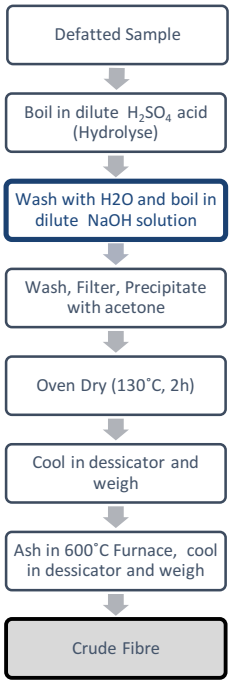


Figure 1: Flow Diagram of the Principles of Determining Crude Fibre Adapted from (Mertens 2003)

3. Apparatus

- 40-100µm pore size sintered glass Gooch crucible, 30mL
- Buchner/Side arm flask for Gooch crucible
- 250mL Beaker
- Volumetric Flask
- Litmus paper (neutral)

- Desiccator
- Analytical balance
- Vacuum pump
- Filtration manifold with variable pressure
- Fume Hood
- Hot plate
- Ventilated oven (103.5 ±2°C)
- Muffled Furnace (550 ±20°C

3. Interferences

Always wash samples after digestion with sulphuric acid until neutral before digestion with pottassium hydroxide to avoid reaction of acid with base. Test neutrality with litmus paper.

4. Safety Precautions

- Handle concentrated sulphuric acid with care (avoid spillage).
- Slowly **add sulphuric acid to water** and not water to acid
- Handle potassium hydroxide with care (avoid spillage – highly hygroscopic)
- Always use safety goggles, gloves and laboratory coat while working in laboratory.
- Wear face shield and protect hands from heat produced when contents of the vessels are heated.
- After the analysis clean bottles and beakers with water then dry.
- Dispose used gloves after completion of analysis.
- Do **not** dispose of sulphuric acid and potassium hydroxide in same container.
- Do **not** dispose of sulphuric acid and petroleum ether in same container.
- Do **not** dispose of potassium hydroxide and petroleum ether in same container.
- Clean hands using antiseptic soap.

- Avoid spillage and contact with skin. In the latter case use copious washings with cold water and call for medical attention.
- Take extra caution when heating acid/ base and always use a fume hood during digestion.
- Inspect glass ware before heating in acid/base, never use cracked glassware.
- Turn on extractor fan when doing moisture analysis on moisture analyser.

5. Sampling

(Samples can be stored at 4°C for up to a week before testing if necessary.)

- *10g wet UD faecal sludge needed per sample (2 replicates)*
- Collect in sealed container.
- Homogenize entire sample.
- Preserve fecal sample (10g) by freeze drying.
- Place in vial and label with name, date and proposed purpose.

6. Sample Preparation –Fecal Sludge

1. Grind dried(in oven for 24hrs at 80 degrees Celsius in oven) UD faeces with a mortar and pestle until fine powder.
2. Test for moisture on a moisture analyser(moisture content should not exceed 10%). Remember to put the extractor on.

7. Reagents

- **Dilute Potassium Hydroxide (0.23M)**
  - Add 12,9042 g KOH pellets to a 1L volumetric flask
  - Fill to the 1L mark with distilled water

- **Dilute Sulphuric Acid (0.15M)**
  - Add some distilled water into a 1L volumetric flask
  - Add 8,1582 mL conc. Sulphuric acid (98%) to the 1L volumetric flask
  - Top up to 1L volumetric flask to the mark with distilled water

- **Petroleum ether (Boiling point: 40-60°C)**

8. Procedure

- Weigh the clean and dry Gooch crucible to 4 decimal places.
- Weigh out 1g dried and ground sample into Gooch crucible – **W1**

Defatting/Pretreatment

(If the UD sample has consistently more than 10% fat continue to use this step)

- Place Gooch crucible on the filtration manifold
- Add 30mL Petroleum ether
- Filter using Vacuum
- Repeat 2 more times
- Dry Residue in air

Digestion & Filtration

Acid digestion and filtration:

- Transfer residue **quantitatively** to 500mL beaker
- Add 150mL 0.15M Sulphuric acid
- Add a glass rod to avoid bumping, see fig 1 below.
- Boil on a heating mantle for 30 ±1 min in a fumehood (maintain volume with hot distilled water)
- The heating mantle should be set on,8 or use a thermometer, boiling point is slightly below boiling point of water
- Leave to cool
- Filter through Gooch crucible in vacuum, refer to fig 2
- Wash the residue 5 times, each time with 10mL hot distilled water(check with litmus paper for neutrality)
- Just cover the residue with acetone, Leave for a few min.
- Apply slight suction to remove the acetone

Base/Alkaline digestion and filtration:

- Transfer residue **quantitatively** to 500mL beaker
- Add 150mL 0.23M Potassium hydroxide
- Add a glass rod to avoid bumping, see fig 1 below
- Boil on a heating mantle for 30 ±1 min in a fumehood (maintain volume with hot distilled water)
- The heating mantle should be set on,8 or use a thermometer, boiling point is slightly below boiling point of water
- Leave to cool
- Filter through Gooch crucible in vacuum, see fig 2
- Wash residue with hot distilled water until rinsing's are neutral (check with litmus paper)
- Wash with 30mL acetone and vacuum filter, repeat 3 times in total.

Fig 1
ACID/BASE DIGESTION SET UP

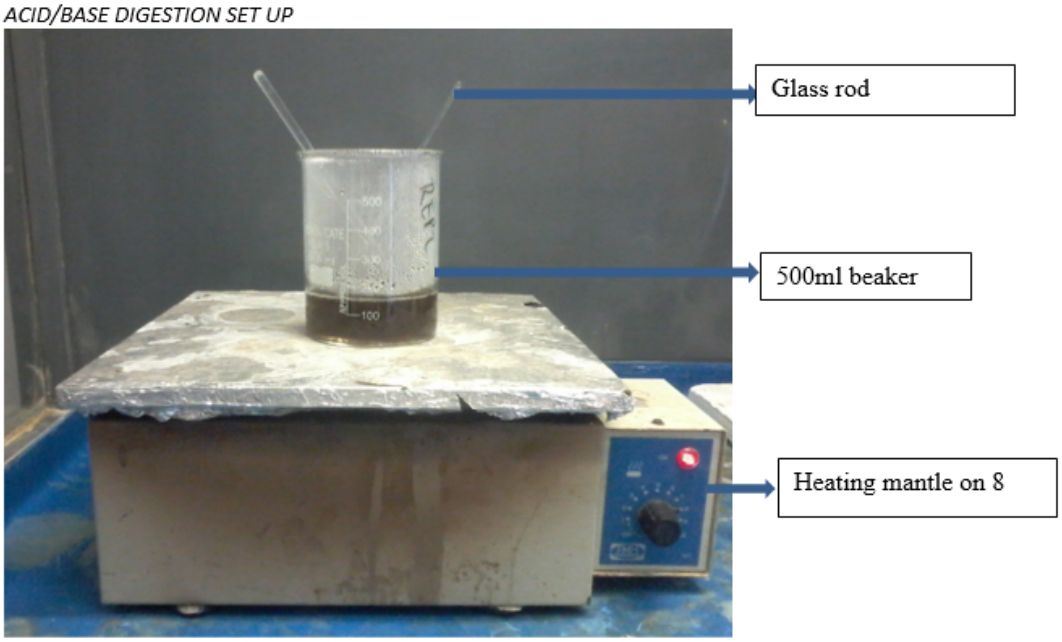
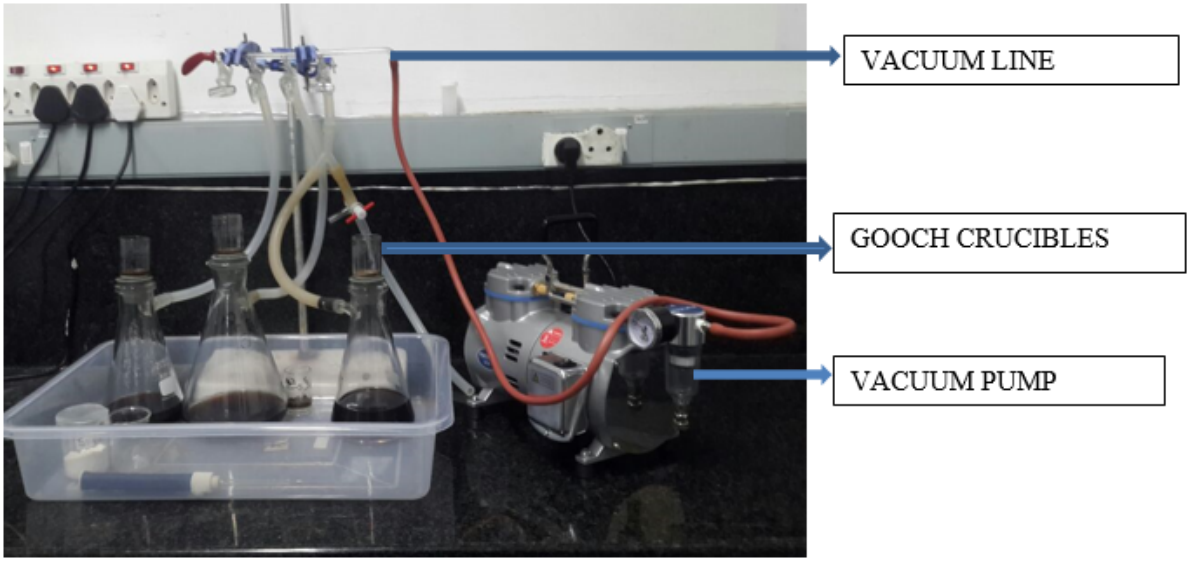


Fig 2
VACUUM FILTRATION SET UP



Oven Drying

- Place Gooch crucible in oven at 103.5 ±2°C for 4h
- Place in desiccator to cool
- Weigh Gooch crucible straight out of the desiccator – **W2**

Muffle furnace

- Place Gooch crucible in muffle furnace at 550 ± 2°C for 2h
- Place in desiccator to cool
- Weigh Gooch crucible straight out of the desiccator – **W3**

Calculation

$$\%\ Crude\ Fibre = \frac{W2 - W3 \times 100}{W1}$$

Where:

W1 = weight of the sample (g),
W2 = weight Gooch crucible and residue after drying (g)
W3 = weight Gooch crucible and residue after incineration (g).

9. References

Balthrop, J., B. Brand, R. Cowie, J. Danier, J. de Boever, L. de Jonge, F. Jackson, H. Makkar and C. Piotrowski (2011). Quality assurance for animal feed analysis laboratories, FAO.

Mertens, D. (2003). "Challenges in measuring insoluble dietary fiber." Journal of Animal Science **81**(12): 3233-3249.

Novotny, L. F., G; Layton, B; Walter, M (2017). Critical Factors in Determining Fiber in Feeds and Forages A. s. L. M. a. S. Committee, Fiber Best Practices Working Group (Revision 1).

APPROVAL OF STANDARD OPERATING PROCEDURE

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