STANDARD OPERATION PROCEDURE
Faculty of Biosciences, NMBU

Method name: ADL (Acid Detergent Lignin)
BIOVIT No .: Arb1035

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1. Introduction

ADL (Acid Detergent Lignin) is defined as the residual material after extraction with a boiling acidic acetyltrimethyl ammonium bromide (CTAB) solution, followed by extraction with 72% sulfuric acid. In practice, this is done by first having the samples undergo an ADF procedure (Acid Detergent Fiber-ARB 1036). The samples are then extracted into H_2SO_4 for 3 hours with regular stirring, before finally rinsing the samples well and ashing them in order to correct for inorganic material. The content of ADL is determined gravimetrically.

2. Reagents and control sample

- 24 M H_2SO_4 (1634 g / L)
    - Add 350 mL of distilled H_2O in a 1 L volumetric flask and cool down
    - Add 1200 g concentrated H_2SO_4 (while cooling !!)

- Acetone
- Control test: LabTek-kontrollen (same as NDF/ADF)

3. Risk assessment

- H_2SO_4: Wear thick gloves and work in fume hood. Always add the acid to water (NOT the other way around). In case of acid spillage on skin; wash with large amounts of water.

- Acetone: Highly flammable. Work in fume hood. Avoid inhalation and skin contact. Make sure that all acetone has evaporated before placing the samples in the oven.

4. Equipment

- Ankom 200 Fiber Analyzer
- Heat sealer
- Filter bags (F57)
- Scale (accuracy: 0.1 mg)
- Drying cabinet (103 ± 2 °C)
- Desiccator pouch
- Marker (permanent marker)
- 2L and 3L beakers

- Muffle furnace (550 °C)
- Small glasses (which can tolerate over 550 °C)

5. Sample material

The method can be used on most sample types, but the particle size should not be smaller than 1 mm. Smaller particles will increase the probability of errors in the analysis results.

6. Work description

1. Label the filter bags with the sample number
2. Weigh the filter bag and note the weight **(W_0)**
3. Tare the scale with the bag on top
4. Weigh 0.5 g sample directly into the filter bag and note the weight **(W_1)**
5. Heat seal the filter bag approx. 0.5 cm from the opening
6. Shake the bag so that the sample material is evenly distributed in the bag
7. Repeat these points with all the samples and an empty filter bag

ADF procedure

8. Do the ADF determination on the samples (see Arb 1036 ADF)

Extraction with H_2SO_4

9. After ADF: place the dried bags with sample in a 3 L beaker
10. Cover the bags completely with 72% H_2SO_4 (approx. 250 mL)

    NB: The bags MUST be completely dry and at room temperature before point 10 is carried out !! Otherwise the result will be incorrect due to heat generation.

11. Place a 2 L beaker inside the 3 L beaker to hold the bags down (***wear thick gloves!***)**.**
12. Gently push the 2 L beaker up and down 30 times (to stir)
13. Repeat the stirring at 30-minute intervals (30/60/90/120/150/180 min)
14. After 3 hours: pour off H_2SO_4 (carefully !!)

Rinsing

15. Rinse with tap water to remove all acid

NB: If there are acid residues on the bags, the samples will burn, and the ADL results will be too high.

16. Repeat rinsing until pH paper shows neutral color when placed on the bags (same pH as tap water).
17. Let the samples airdry over night

Drying

18. Dry the bags at 105 °C for 2-4 hours

IMPORTANT INFO: DO NOT dry the bags overnight in the oven! Longer drying time then 2-4 hours (or higher temperature) can damage the filtration material in the bags.

19. Take the bags out of the oven and place directly in the desiccant pouch. Flatten out to remove air.
20. Cool to room temperature and weigh the bags **(W_2)**

Ashing

21. Mark a glass with the sample number
22. Weigh the glass and note the weight (**W_3**)
23. Place the bag (with sample) in the glass and place the sample(s) in the tray.
24. The marking on the glasses will disappear during ashing so note the location of the glasses on a sheet.
25. Fill up the tray with empty glasses standing upside down. This is done to keep the glasses in place.
26. Place the samples in the furnace when it is cold
27. Close the door and press start (The program is set to heat up to 550 °C)
28. Leave the samples overnight (minimum four hours)

29. When the ashing is complete (and the temperature in the furnace is below 200 °C), the samples are removed using a metal rod (the tray holds between 400 and 500 °C and padded gloves do NOT withstand such temperatures)
30. The samples are put in a desiccator (using a small metal clip) so that they can be cooled to room temperature without attracting moisture (about 45 min.)
31. Weigh one of the samples to check if the weight is stable
    (*If the weight is not stable the sample must be cooled longer*)
32. Weigh the glasses with the ash
33. Note the weight by four decimal places (**W_4**)

7. Calculation of the analysis result

$$\frac{(W_2 - W_0 \times F) - (W_4 - W_3)}{W_1} \times 1000 = amount\ of\ ADL\ in\ the\ sample\ \left(\frac{g}{kg}\right)$$

W_0 = weight of bag

W_1 = weight of sample

W_2 = weight of extracted sample + bag

W_3 = weight of glass

W_4 = weight of glass + ash

F = bag correction factor