JOB DESCRIPTION
Department of Animal and Aquaculture Sciences, NMBU

JOB DESCRIPTION Method name: Buffer capacity
BIOVIT No: Arb1069

1. Introduction / purpose

The method is mainly intended for analyzing buffer capacity in grass.
The purpose of the analysis is to investigate whether the grass material is easy or difficult to ensile, i.e. how much acid is needed to lower the pH of the grass material. Even though the conditions in small test tubes do not simulate large farm silos exactly, studies have shown that chemical and bacteriological changes follow the same pattern in both test tubes and large metal silos (capacity 1000 kg).

A buffer solution is a solution where the pH is almost constant when smaller amounts of acid or base are added. The buffer capacity of grass is mainly determined by plant acids and amino acids. The most quantitatively important acids are malic acid (2-Hydroxybutanedioic acid) and citric acid (2-hydroxy-1,2,3-propanetricarboxylic acid).

2. Reagents

A. 0.2 M hydrochloric acid (HCl)
B. 0.2 M sodium hydroxide (NaOH)
Distilled water

A. 0.2 M hydrochloric acid (HCl):

1. Fill the volumetric bottle to about half with distilled water.
2. Add slowly concentrated hydrochloric acid (37%) - **work in a fume hood.**
3. When the acid is added to water and the solution temperature is approx. 25 °C, refill with distilled water.

Number of samples	Volumetric bottle (mL)	Hydrochloric acid, 37% (mL)
1-10	100	1,7
11-25	250	4,2
26-50	500	8,3
50-75	750	12,5
76-100	1000	16,6

B. 0.2 M sodium hydroxide (NaOH):

1. Weigh in the correct amount of sodium hydroxide in a volumetric bottle.
2. Add enough distilled water to get pellets to dissolve NOTE - heat development!
3. Add about ¾ of all the distillated water.
4. When the acid is added to the water and the solution temperature is approx. 25 °C, refill with distilled water.
5. Calculate the exact sodium hydroxide concentration using the formula below:

$$C_{NaOH} = \frac{m_{NaOH}}{40{,}01\ g/mol} \times \frac{1}{V_{tot}} \times \frac{1}{1000\ \frac{mL}{L}}$$

Where:
C_{NaOH} = calculated sodium hydroxide concentration
m_{NaOH} = weight of sodium hydroxide pellets
V_{tot} = volume to the volumetric bottle

Number of samples	Volumetric bottle (mL)	NaOH (g)
1-10	100	0,80
11-25	250	2,00
26-50	500	4,00
50-75	750	6,00
76-100	1000	8,00

3. Risk assessment

Wear a lab coat, goggles, and acid resistant gloves (not regular dishwashing gloves) when making hydrochloric acid and sodium hydroxide. Concentrated hydrochloric acid evaporates! Therefore make 0.2 M hydrochloric acid in the **fume hood!** Pure sodium hydroxide is in pellets. Wear gloves when handling the pellets, moisture on hands may result in a formation of a very alkaline solution.

If concentrated hydrochloric acid or sodium hydroxide comes in contact with your skin, flush immediately with plenty of water for at least 15 minutes and remove any contaminated clothing.

In case of serious skin contact, use water, a disinfectant soap, and anti-bacterial cream. Seek immediate medical attention.

4. Equipment

- ➢ Scale (precision 0.0001 g)
- ➢ pH meter
- ➢ Blender
- ➢ Burette
- ➢ Beaker
- ➢ Magnetic stirrer
- ➢ Magnet

5. Sample material

Raw or dried grass or silage. The sample must be homogenous and uniformly chopped.
The sample can be frozen at -20 ºC.
Sample amount: 20 g homogeneous sample.

6. Job description

1. Calibrate the pH meter according to the calibration procedure.

For correction of blank sample:

2. Add 0.2 M HCl to the 250 mL of distillated water until the pH reaches 3.00.
3. Add 0.2 M NaOH until the pH reaches 4.00.
4. Read how much NaOH is needed to titrate this blank sample from pH 4.00 to pH 6.00 (approx. 0.1 mL).

To determine the buffer capacity of the samples:

5. Weigh in 20 g of raw sample directly and put it into the blender (10 g if the sample is pre-dried).
6. Add 250 mL of distillated water and run the blender on power 1 for 1 min. (NOTE! The blender cannot be run for more than 2 minutes at a time!).
7. Pour the grass moss into a beaker and remove foam from the surface.
8. Put a magnet into the beaker and stir.
9. Measure the pH of the solution.
10. Add 0.2 M HCl until the pH is 3.00.
11. Add 0.2 M NaOH until the pH is 4.00.
12. Read off the consumption (Vx) of NaOH by titration from pH 4.00 to pH 6.00.
13. Read off the pH immediately when it has stabilized at pH = 6.00.

7. Reporting and calculation of the analysis results

$$\frac{C_{NaOH} \times (V_x - V_{blank}) \times 1000 g/kg}{m_{sample}} = \text{mekv. NaOH / kg } \underline{\text{sample received}}$$

C_{NaOH} = exact NaOH concentration in molar, mol / L
V_x = consumption NaOH (mL) to adjust the pH from 4.00 to 6.00 in the sample,
V_{blank} = consumption NaOH (mL) to adjust pH from 4.00 to 6.00 in blank sample,
m_{sample} = mass (g) of weighed sample