Cornell University

PRO-DAIRY

Analytical Procedures for Monitoring Farm-based Anaerobic Digestion (AD) Systems: PROTOCOL II

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Preface

We have developed four protocols of analytical procedures for the monitoring of farm-based anaerobic digestion (AD) systems. The use of either protocol will depend on the scopes and lab resources of the facility, and the time availability of the system operator.

Protocol I is the most basic and simple version of the analytical procedures, designed to evaluate the *performance of manure-only AD systems*. This protocol includes the analyses of total solids (TS) and total volatile solids (VS) in the influent and the effluent of the digester, for the evaluation of waste treatment efficiency of the system.

Protocol II is the upper level of protocol I, intended to monitor the stability of on-farm AD systems with a higher level of complexity; for example, systems co-digesting a single, low-strength co-substrate in its operation, such as whey products, in a systematic or intermittent basis. This protocol includes the analyses of total volatile fatty acids (TVFA) and total alkalinity (TA) for the determination of the TVFA:TA (or FOS:TAC) ratio, and includes the measurements of methane content, pH and temperature. Protocol II requires a more complex laboratory setup and analysis time than Protocol I.

Protocol III is a more comprehensive version of protocol II, developed to monitor the stability of on-farm AD systems that co-digest one or more off-farm, high-strength substrates in a continuous basis, particularly for operations receiving protein-rich substrates. Protocol III includes all the analyses of Protocol II in addition to total ammonia-nitrogen (TAN). Protocol III requires virtually the same laboratory setup and analysis time as Protocol II.

Protocol IV is the most advanced version of the protocol, developed for the monitoring of both performance and stability of on-farm anaerobic digesters with the same characteristics as those described in Protocol III. Protocol IV combines all the analyses included in Protocol I and III, and thereby requires a full laboratory setup and a longer analysis time.

This following document describes the materials and analytical procedures of Protocol II.

Table of Contents

1. Total Volatile Fatty Acids (VFA)

1.1. Apparatus

- *pH meter/electrode*
- *Distillation columns (x2)*
- *Hot plates (x2)*
- *Mixing plate (x1)*
- *Graduated cylinders, 250-mL capacity (x2)*
- *Erlenmeyer flasks, 500-mL capacity (x2)*
- *Boiling chips*
- *500-mL glass bottles with screw caps, for storing reagents (x2)*
- *1-mL pipette (x1)*
- *5-mL pipette (x1)*
- *1000-mL plastic bottles with screw caps, for sampling (x2)*
- *200-mL beaker (x2)*

1.2. Reagents

- *Sulfuric acid, H_2SO_4 (1.84 g/L)*
- *Sodium hydroxide, NaOH (0.1 N)*
- *Acetic acid, CH_3COOH (1,020 g/L)*
- *Distilled water, DI*
- *1:1 Sulfuric acid solution (500 mL)*

Using a graduated cylinder, measure 250 mL of distilled water (DI). Pour all DI into a 500-mL screw-cap glass bottle. WITH CAUTION, using the same graduated cylinder, measure 250 mL of sulfuric acid and add it to the same 500-mL screw-cap bottle with DI. (IMPORTANT: ALWAYS ADD ACID INTO WATER, NOT THE OTHER WAY AROUND, BECAUSE IT CAN PRESENT A SAFETY HAZARD). Label this bottle as *1+1 Sulfuric acid + water solution*, and state the date of preparation underneath. This solution will be good for up to a year.

- *2,040 mg/L total volatile fatty acids standard (200 mL)*

Using a 1-mL pipette, add 0.4 mL of acetic acid in a 500-mL Erlenmeyer flask. Using a graduated cylinder, measure 200 mL of DI water and add it to the same Erlenmeyer flask. Using a 5-mL pipette, add 5 mL of sulfuric acid to the same Erlenmeyer flask. Add several boiling chips to the flask, and swirl the solution slowly but thoroughly

1.3. Procedure

a) *Collect the sample to be measured in a 1000-mL plastic bottle (either from the digester influent or effluent)*

b) *Turn on the analytical balance and tare a 500-mL Erlenmeyer flask*

c) *Directly into the Erlenmeyer flask, weigh approximately 50 g (equivalent to 50 mL) of the collected sample, and record the exact amount weighted on your log sheet/notebook; alternatively, measure exactly 50 mL of the sample in the 250-mL graduated cylinder, and then pour it in the Erlenmeyer flask*

d) *Keep the Erlenmeyer flask on the analytical balance, and weigh the required amount of DI water (approximately 150 mL) to reach 200 g in TOTAL (sample + DI water); alternatively, measure the required amount of DI water to complete 200 mL (g) in the 250-mL graduated cylinder, and then pour it in the Erlenmeyer flask*

e) *Using a 5-mL pipette, add 5-mL of the 1+1 sulfuric acid + water solution*

f) Add several boiling chips and swirl the solution slowly but thoroughly

g) Turn on the hot plate(s) and set it to the MAXIMUM level

h) Turn on the water for the distillation apparatus; you need enough water to keep it flowing around the columns spirals (try to use the same flow of water for every analysis)

i) Place the flask(s) on the hot plate(s) and connect it to the distillation apparatus

j) Using the 250 mL graduated cylinders, collect 150 mL of the distillate, POURING OFF THE FIRST 15 ML COLLECTED (H_2S and CO_2 will be liberated during distillation and will be titrated to give a positive result)

k) Pour the 150 mL of distillate into a 200-mL beaker and place it on the mixing plate

l) Submerge the pH electrode in the beaker and turn on the pH meter

m) Put a magnetic stirrer in the beaker and set the medium mixing, around level 3

n) Carefully, fill the buret to 50 mL with 0.1 N NaOH; read and record the initial mL level (mLi) from the buret

o) Titrate the distillate with 0.1 N NaOH to pH of 8.3 (pH changes really fast after pH 6, so add NaOH very slowly after that)

p) Read and record the mL of NaOH used for titration from the buret

q) Enter the data in the spreadsheet to obtain the results, and keep a hard copy record of these results in your lab notebook

1.4. Calculation

$$VFA\ \left(\frac{mg}{L}\right) = \frac{mL\ NaOH\ \cdot 0.1\frac{mol}{L} \cdot 60{,}000\frac{mg}{mol}}{(g)\ mL\ sample\ \cdot f}$$

f is a correction factor, which is determined for each distillation column as explained below.

NOTE: 1 M NaOH = 1 N NaOH

1.5. Determination of the column correction factor *f*

To determine f for your distillation column, distill the total volatile fatty acid standard (2,040 mg/L) according to the procedure described above from point *g)* onwards, and then calculate using the following formula:

$$f = \frac{VFA\ recovered\ (\frac{mg}{L})}{2{,}040\ (\frac{mg}{L})}$$

2. Alkalinity

2.1. Apparatus

- *pH meter/electrode*
- *Mixing plate (x1)*
- *500-mL glass bottles with screw caps, for storing reagents (x1)*
- *1-mL pipette (x1)*
- *1000-mL plastic bottles with screw caps, for sampling (x2)*
- *200-mL beaker (x2)*

2.2. Reagents

- *Sulfuric acid, H_2SO_4 (1.84 g/L)*
- *Sodium carbonate, Na_2CO_3*
- *Distilled water, DI*
- *Sodium carbonate solution, 0.05 N*

Dry 2 g primary standard Na_2CO_3 at 250°C for 4 h and cool on a desiccator. Weigh 1.25 g on the analytical balance and keep aside. Tare a 500-mL screw-cap glass bottle, add the N_aCO_3, and add DI water until the weight is equal to 500 g, mix thoroughly

- *Sulfuric acid solution, 0.1 N*

Pour 500 mL of DI water into a 500-mL screw-cap glass bottle. Using a 1-mL pipette, add 1.4 mL of pure H_2SO_4, mix thoroughly. Standardize H_2SO_4 solution 0.1 N by adding 40 mL Na_2CO_3 solution 0.05 N and about 60 mL DI water to a 200 mL beaker. Add a magnetic stirrer and set to medium mixing speed, around level 3, submerge

the pH electrode and titrate with H_2SO_4 0.1 N to the pH inflection point. Calculate normality as follows:

$$Normality, N = \frac{50}{53 \cdot mL\ H_2SO_4\ used}$$

2.3. Procedure

a) Collect the sample to be measured in a 1000-mL plastic bottle (either from the digester influent or effluent)

b) Tare a 200 mL beaker on the analytical balance and weigh 15 g of sample (record the exact amount).

c) Add a magnetic stirrer and set to medium mixing speed (level 3)

d) Submerge the pH electrode and titrate with H_2SO_4 0.1 N to end point pH = 4.3

e) Read and record the mL of H_2SO_4 used for titration from the buret

f) Enter the data in the spreadsheet to obtain the results, and keep a hard copy record of these results in your lab notebook

2.4. Calculation

$$Alkalinity, mg\ CaCO_3/L = \frac{mL\ H_2SO_4\ used\ \cdot N\ \cdot 50{,}000}{mL\ sample}$$

3. pH

3.1. Apparatus

- *pH meter*
- *pH electrode*
- *Thermocouple (in some pH meters comes as a separate probe)*
- *Beakers*
- *pH standards*

3.2. Reagents

No reagents required

3.3. Procedure

a) Attach the pH electrode AND thermocouple to the pH meter

b) Collect the sample to be measured in a 1000-mL plastic container (either from the digester influent or effluent)

c) Turn the pH meter on and submerge the pH electrode AND thermocouple approximately 1 inch deep in the sample

d) While moving the electrode with a circular motion, check the meter and when the pH reading showing on the display is stable, record the measurement

3.4. Calibration of the pH meter

Calibrate pH meter using the provided standards periodically. Follow the calibration method explained in the pH meter manual, and ALWAYS calibrate in this order: pH 7.00, then 4.01, and lastly pH 10.01.

4. Temperature

4.1. Apparatus

- *pH meter*
- *Thermocouple*

4.2. Reagents

No reagents required

4.3. Procedure

a) Temperature should be measured in the digester influent and effluent

b) Attach the thermocouple to the pH meter

c) Carefully, immerse the thermocouple in the digester influent or effluent and record the temperature from the meter display

5. Methane content

For this method, it is assumed that the biogas is made up of a mixture of ONLY carbon dioxide and methane, thus methane is determined indirectly by measuring the carbon dioxide content in the biogas

5.1. Apparatus

- *Sensidyne pump*
- *Sensidyne carbon dioxide detection tubes*

5.2. Reagents

No reagents required

5.3. Procedure

Follow provided instrument instructions for determining CO_2, then calculate CH_4 by difference as shown below

5.4. Calculations

$$Methane\ (\%) = 100 - Carbon\ dioxide\ (\%)$$

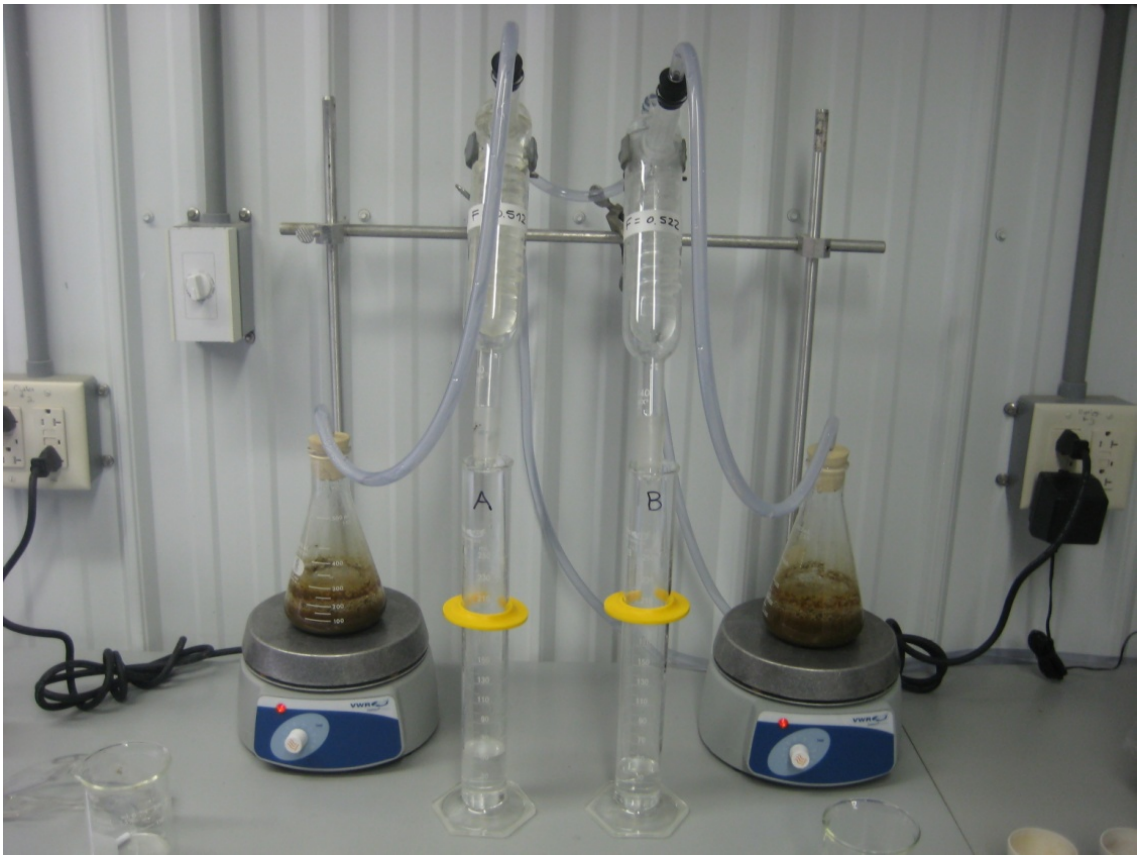
6. Safety

Hard copies of the Material safety data sheets (MSDS) are inside a binder in all labs. MSDSs explain safety protocols in detail, please, make sure to have the bider in a visible and fixed location of the lab

7. Equipment and materials glossary

7.1. Analytical balance

7.2. Distillation apparatus



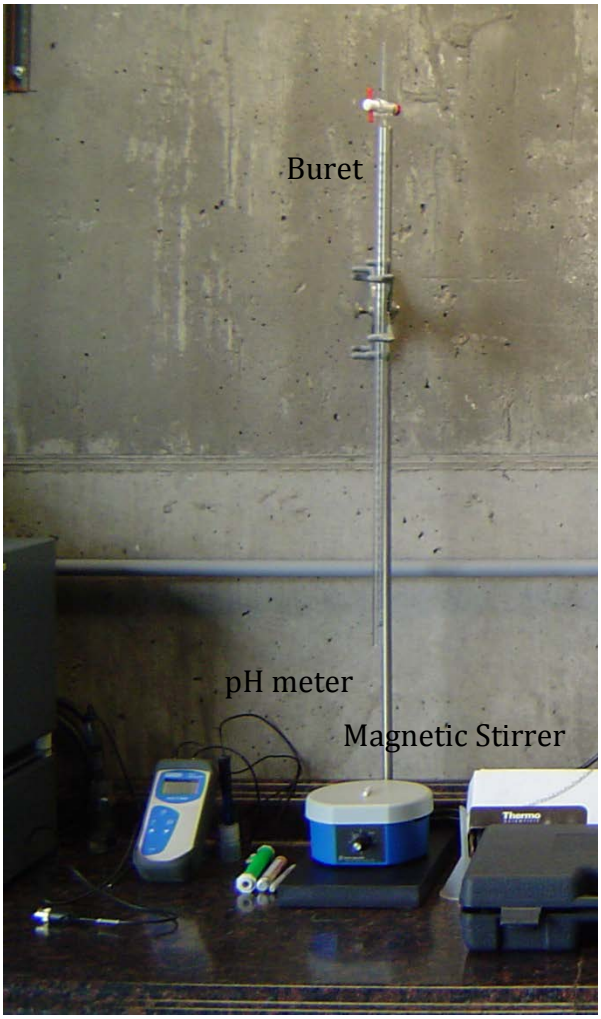
Graduated cylinder

Distillation columns

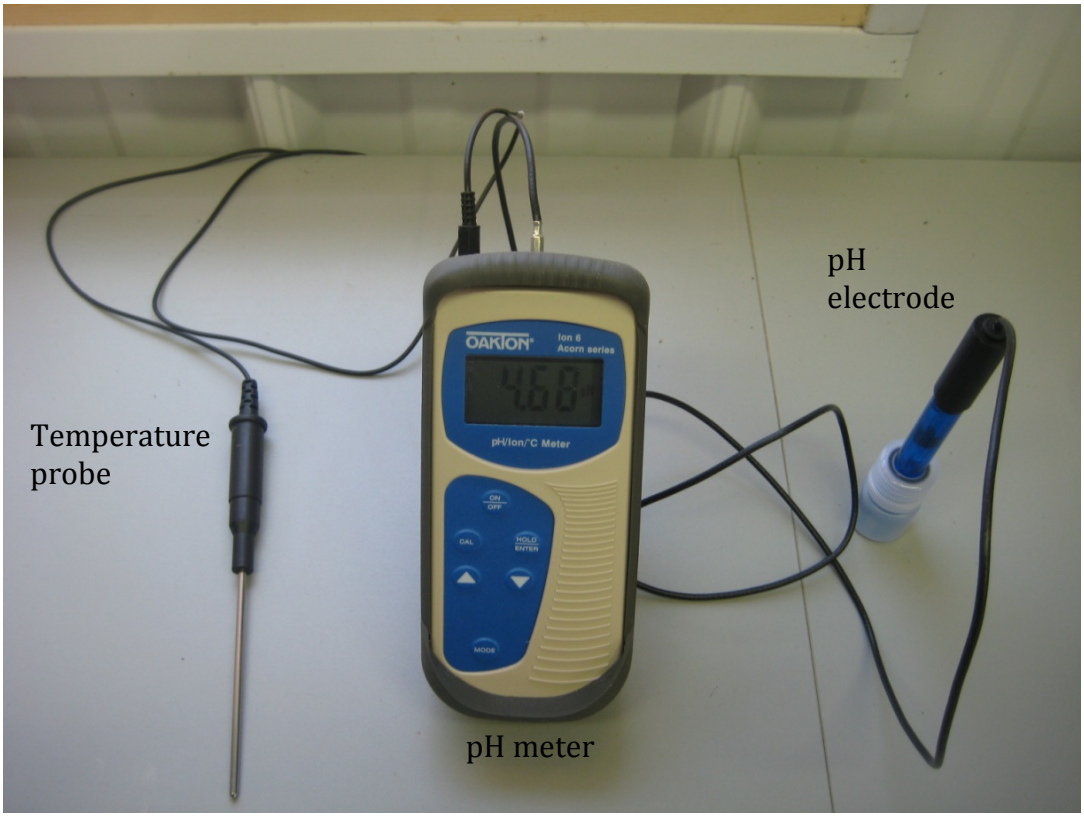
Hot plate

Erlenmeyer flask

7.3. Titration system



7.4. pH meter



8. List of supplies

The following is a list of the lab supplies that will need to be purchased by the farms when the initial supplies are exhausted. The table includes the suppliers' information and the product number.

	Brand/model	Provider	Characteristics	Product number
Sulfuric acid	Sulfuric Acid, BAKER ANALYZED Reagent. ACS Grade	VWR	Case of 6 x 2.5-L (95-98% purity)	JT9681-3
Sodium hydroxide	Sodium Hydroxide, Volumetric Solution, BAKER ANALYZED* Reagent. 10N	VWR	Case of 6 x 1-L 10 N (9.95-10.05 N)	JT5674-2
Acetic acid	Acetic Acid, BAKER ANALYZED* Reagent. ACS Grade	VWR	500 mL bottle (99.7% purity)	JT9508-2

