Applied Microbiology
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Biotechnology Laboratory

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Standard Operating Procedure

AMBL-101-A

Prepared:	April 12, 2006
Revised:	July 16, 2014
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Approximate Volatile Acids by Titration

METHOD SUMMARY

This method describes a laboratory titration procedure for determining the approximate concentration of volatile acids (as acetic acid) in anaerobic digester sludge. The procedures herein are based on the original procedure described by DiLallo and Albertson (1961), further outlined by Dupont (1982), and further modified by Baxter (1988). A sample of digested sludge is centrifuged to obtain the supernatant liquid. The sample is titrated as part of the alkalinity method, boiled or placed under vacuum to remove carbon dioxide, and then titrated back to pH 7.0. The volatile acids acidity between pH 4.0 to 7.0 is used to calculate the approximate concentration of volatile acids expressed as acetic acid.

ENVIRONMENTAL HEALTH AND SAFETY

Hazards Assessment: This method uses the following equipment, samples or chemicals that present a hazard to health and safety. Where specified and before conducting this method, consult the appropriate MSDS for the specific hazards.

1. Sodium hydroxide: Chemical pellets and concentrated and dilute solutions (see MSDS)
2. Sulfuric acid: Concentrated and dilute solutions (see MSDS).
3. Heat: Hot plate, beaker and watch glass cover when boiling sample, and heat of reaction when preparing concentrated sodium hydroxide solutions.

Safety Equipment and Engineering Controls: Knowing the location and being able to use the eye wash and shower station, the fire extinguisher and the first aid kit are required. The following equipment is also required or recommended.

- Fume hood with protective sash (required).
- Heat resistant gloves required when attempting to move the hot beaker or watch glass cover
- Tongs capable of securely holding the hot beaker.

Personal Protective Equipment (PPE): When conducting this method, handling samples, working with chemicals or preparing solutions that are associated with conducting this method the following PPE are required.

- Lab coat, eye protection, and gloves (Nitrile or latex).

Review and follow the additional procedures regarding PPE in NAU's Environmental Health and Safety's Chemical Safety Standard Operating Procedure, P16-Personal Protective Equipment (2008) and P25-Safety Glasses (2012).

Analysis-derived Wastes and Disposal: Wastes that are generated by this procedure and the appropriate method to be used for their disposal are summarized in the following table.

Waste Generated	Hazardous (Y / N)	Disposal
Liquid sample consumed by test with final pH = 7.0.	N	May be rinsed down sink.

METHOD DESCRIPTION

1.0 Application

The volatile acid concentration in anaerobic digester sludge may be approximated during the titration of a sample for alkalinity followed by a back-titration step with sodium hydroxide to determine the acidity due to the volatile acids. Carbon dioxide acidity is removed between the titration steps by either boiling the sample or by the placing the sample under a vacuum for a specified amount of time. Volatile acids are calculated from the acidity valued determined and expressed as acetic acid.

This procedure is for use with samples obtained from an anaerobic digester and are intended for monitoring digester operations.

2.0 Apparatus

a. pH meter and electrode
b. 150-mL or 200-mL beaker, borosilicate glass
c. small vacuum flask, vacuum source and tubing
d. 50-mL buret for H_2SO_4 titrant
e. 50-mL buret for NaOH titrant
f. magnetic stir bar and stirring apparatus
g. Stirring hot plate
h. Watch glass covers for beaker

i. Low-form rectangular glass water bath or casserole dish

3.0 Reagents

a. 0.05 N H_2SO_4 standardized according to procedures given in Method 2320 Alkalinity (Standard Methods).

b. 0.05 N NaOH standardized according to procedures given in Method 2310 Acidity (Standard Methods).

c. pH buffers

4.0 Pretreatment of Sample

a. Excessive solids can interfere with the titration of the sample.

b. Settle digester sludge samples and decant the lighter fraction of liquid.

c. Transfer this decanted liquid into 50 mL centrifuge tubes and centrifuge at 2500 rpm for 15 minutes. Prepare enough to perform this procedure.

d. Do not use a vacuum filtration step with the digester sludge, the decanted liquid or the liquid after centrifugation to remove solids further. This practice may result in the loss of unionized volatile acids species.

5.0 Procedure

a. Standardized the pH meter according to manufacturer's instructions.

b. Place sample volume into the beaker and make certain that the starting volume is enough to immerse a pH probe. Add distilled water if needed. Typical volume of sample may range from 10 mL to 50 mL, where the smaller volumes will require the addition of distilled water.

c. Add the magnetic stir bar and turn on the stirring plate.

d. Note the beginning buret reading and then titrate the sample with an appropriate normality H_2SO_4 to pH 4.0. Take a second buret reading and record the volume used.

e. Continue titrating until the pH is between 3.3 and 3.5. Turn off the stirring plate.

f. Lift the pH probe out of and above the sample and rinse the pH probe into the beaker.

g. Remove the beaker.

h. Remove the remaining carbon dioxide using one of the following methods.

1. *Boil sample.* Place beaker with sample on a hot plate and place a watch glass cover on top. Add a small amount of cold water on the watch glass to condense vapors. While slowly stirring, bring the sample to a gentle boil for 3 to 5 minutes. The sample may turn a light yellow or straw color. When done, remove the beaker and place it in a shallow, cold water bath and allow the sample to cool to room temperature. Tip the watch glass cover to drain off the water into the bath and rinse the condensate on the underside into the beaker.
2. *Vacuum extraction.* Transfer the sample and magnetic stir bar into a 250-mL vacuum flask. Rinse the remaining beaker contents into the flask. Place the sample under approximately 15 psi vacuum for approximately 6 minutes while stirring. Transfer the sample and magnetic stir bar back into the beaker. Rinse the flask into the beaker.

i. Back titrate the sample with an appropriate normality NaOH to pH 4.0 and note the buret reading. Complete the titration by continuing to add NaOH up to pH 7.0. Note this second buret reading and record the volume of titrant used.

6.0 Calculation and Reporting

a. Alkalinity (Alk) to pH 4.0 as mg/L $CaCO_3$

$$\text{Alkalinity (Alk), as mg/L } CaCO_3 = \frac{A \times N \times 50{,}000}{S}$$

where A = mL of sulfuric acid titrant used

N = normality of the standardized acid titrant

S = mL of sample volume

b. Acidity due to volatile acids (VA) to pH 7.0 as mg/L $CaCO_3$

$$\text{Volatile Acids (VA) acidity, as mg/L } CaCO_3 = \frac{B \times N \times 50{,}000}{S}$$

where B = mL of sodium hydroxide base titrant used

N = normality of the standardized base titrant

S = mL of sample volume

c. Approximate volatile acids concentration (HAc) as mg/L acetic acid

Case 1: If the volatile acids acidity is less than 180 mg/L

$$\text{Volatile Acids, as mg/L Acetic Acid (HAc)} = \text{VA}$$

Case 2: If the volatile acids acidity is greater than 180 mg/L

$$\text{Volatile Acids, as mg/L Acetic Acid (HAc)} = 1.5 \times \text{VA}$$

7.0 Quality Control

The precision and bias for this procedure has not been determined.

8.0 Bibliography

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