BOX, J.D. 1983. Investigation of the Folin-Ciocalteu phenol reagent for the determination of polyphenolic substances in natural waters. *Water Res.* 17:511; discussion by S.J. Randtke & R.A. Larson. 1984. *Water Res.* 18:1597.

# 5560 ORGANIC AND VOLATILE ACIDS*#(136)

## 5560 A. Introduction

The measurement of organic acids, either by adsorption and elution from a chromatographic column or by distillation, can be used as a control test for anaerobic digestion. The chromatographic separation method is presented for organic acids (B), while a method using distillation (C) is presented for volatile acids. Alternative methods using GC or IC are available in the literature and may provide better speciation information for specific situations, but have not yet been recommended as standard methods.

Volatile fatty acids are classified as water-soluble fatty acids that can be distilled at atmospheric pressure. These volatile acids can be removed from aqueous solution by distillation, despite their high boiling points, because of co-distillation with water. This group includes water-soluble fatty acids with up to six carbon atoms.

The distillation method is empirical and gives incomplete and somewhat variable recovery. Factors such as heating rate and proportion of sample recovered as distillate affect the result, requiring the determination of a recovery factor for each apparatus and set of operating conditions. However, it is suitable for routine control purposes. Removing sludge solids from the sample reduces the possibility of hydrolysis of complex materials to volatile acids.

## 5560 B. Chromatographic Separation Method for Organic Acids

### 1. General Discussion

*a. Principle:* An acidified aqueous sample containing organic acids is adsorbed on a column of silicic acid and the acids are eluted with *n*-butanol in chloroform ($CHCl_3$). The eluate is collected and titrated with standard base. All short-chain ($C_1$ to $C_6$) organic acids are eluted by this solvent system and are reported collectively as total organic acids.

*b. Interference:* The $CHCl_3$-butanol solvent system is capable of eluting organic acids other than the volatile acids and also some synthetic detergents. Besides the so-called volatile acids, crotonic, adipic, pyruvic, phthalic, fumaric, lactic, succinic, malonic, gallic, aconitic, and oxalic acids; alkyl sulfates; and alkyl-aryl sulfonates are adsorbed by silicic acid and eluted.

*c. Precautions:* Basic alcohol solutions decrease in strength with time, particularly when exposed repeatedly to the atmosphere. These decreases usually are accompanied by the

appearance of a white precipitate. The magnitude of such changes normally is not significant in process control if tests are made within a few days of standardization. To minimize this effect, store standard sodium hydroxide (NaOH) titrant in a tightly stoppered borosilicate glass bottle and protect from atmospheric carbon dioxide ($CO_2$) by attaching a tube of $CO_2$-absorbing material, as described in the inside front cover. For more precise analyses, standardize titrant or prepare before each analysis.

Although the procedure is adequate for routine analysis of most sludge samples, volatile-acids concentrations above 5000 mg/L may require an increased amount of organic solvent for quantitative recovery. Elute with a second portion of solvent and titrate to reveal possible incomplete recoveries.

## 2. Apparatus

*a. Centrifuge or filtering assembly.*

*b. Crucibles,* Gooch or medium-porosity fritted-glass, with filtering flask and vacuum source. Use crucibles of sufficient size (30 to 35 mL) to hold 12 g silicic acid.

*c. Separatory funnel,* 1000-mL.

## 3. Reagents

*a. Silicic acid,* specially prepared for chromatography, 50 to 200 mesh: Remove fines by slurrying in distilled water and decanting supernatant after settling for 15 min. Repeat several times. Dry washed acid in an oven at 103°C until *absolutely dry,* then store in a desiccator.

*b. Chloroform-butanol reagent:* Mix 300 mL reagent-grade $CHCl_3$, 100 mL *n*-butanol, and 80 mL 0.5*N* $H_2SO_4$ in a separatory funnel. Let water and organic layers separate. Drain off lower organic layer through a fluted filter paper into a dry bottle. CAUTION: *Chloroform has been classified as a cancer suspect agent. Use hood for preparation of reagent and conduct of test.*

*c. Thymol blue indicator solution:* Dissolve 80 mg thymol blue in 100 mL absolute methanol.

*d. Phenolphthalein indicator solution:* Dissolve 80 mg phenolphthalein in 100 mL absolute methanol.

*e. Sulfuric acid,* $H_2SO_4$, conc.

*f. Standard sodium hydroxide,* NaOH, 0.02*N:* Dilute 20 mL 1.0*N* NaOH stock solution to 1 L with absolute methanol. Prepare stock in water and standardize in accordance with the methods outlined in Section 2310B.3*d*.

## 4. Procedure

*a. Pretreatment of sample:* Centrifuge or vacuum-filter enough sludge to obtain 10 to 15 mL clear sample in a small test tube or beaker. Add a few drops of thymol blue indicator solution, then conc $H_2SO_4$ dropwise, until definitely red to thymol blue (pH = 1.0 to 1.2).

*b. Column chromatography:* Place 12 g silicic acid in a Gooch or fritted-glass crucible and

apply suction to pack column. Tamp column while applying suction to reduce channeling when the sample is applied. With a pipet, distribute 5.0 mL acidified sample as uniformly as possible over column surface. Apply suction momentarily to draw sample into silicic acid. Release vacuum as soon as last portion of sample has entered column. Quickly add 65 mL $CHCl_3$-butanol reagent and apply suction. Discontinue suction just before the last of reagent enters column. Do not reuse columns.

*c. Titration:* Remove filter flask and purge eluted sample with $N_2$ gas or $CO_2$-free air immediately before titrating. (Obtain $CO_2$-free air by passing air through a $CO_2$ absorbant.*#(137))

Titrate sample with standard 0.02*N* NaOH to phenolphthalein end point, using a fine-tip buret and taking care to avoid aeration. The fine-tip buret aids in improving accuracy and precision of the titration. Use $N_2$ gas or $CO_2$-free air delivered through a small glass tube to purge and mix sample and to prevent contact with atmospheric $CO_2$ during titration.

*d. Blank:* Carry a distilled water blank through steps ¶s 4*a* through 4*c*.

## 5. Calculation

$$\text{Total organic acids (mg as acetic acid/L)} = \frac{(a - b) \times N \times 60\,000}{\text{mL sample}}$$

where:

*a* = mL NaOH used for sample,
*b* = mL NaOH used for blank, and
*N* = normality of NaOH.

## 6. Precision

Average recoveries of about 95% are obtained for organic acid concentrations above 200 mg as acetic acid/L. Individual tests generally vary from the average by approximately 3%. A greater variation results when lower concentrations of organic acids are present. Titration precision expressed as the standard deviation is about ±0.1 mL (approximately ±24 mg as acetic acid/L).

## 7. Bibliography

MUELLER, H.F., A.M. BUSWELL & T.E. LARSON. 1956. Chromatographic determination of volatile acids. *Sewage Ind. Wastes* 28:255.

MUELLER, H.F., T.E. LARSON & M. FERRETTI. 1960. Chromatographic separation and identification of organic acids. *Anal. Chem.* 32:687.

WESTERHOLD, A.F. 1963. Organic acids in digester liquor by chromatography. *J. Water Pollut. Control Fed.* 35:1431.

HATTINGH, W.H.J. & F.V. HAYWARD. 1964. An improved chromatographic method for the determination of total volatile fatty acid content in anaerobic digester liquors. *Int. J. Air Water Pollut.* 8:411.

POHLAND, F.G. & B.H. DICKSON, JR. 1964. Organic acids by column chromatography. *Water Works Wastes Eng.* 1:54.

## 5560 C. Distillation Method

### 1. General Discussion

*a. Principle:* This technique recovers acids containing up to six carbon atoms. Fractional recovery of each acid increases with increasing molecular weight. Calculations and reporting are on the basis of acetic acid. The method often is applicable for control purposes. Because it is empirical, carry it out exactly as described. Because the still-heating rate, presence of sludge solids, and final distillate volume affect recovery, determine a recovery factor.

*b. Interference:* Hydrogen sulfide ($H_2S$) and $CO_2$ are liberated during distillation and will be titrated to give a positive error. Eliminate this error by discarding the first 15 mL of distillate and account for this in the recovery factor. Resides on glassware from some synthetic detergents have been reported to interfere; use water and dilute acid rinse cycles to prevent this problem.

### 2. Apparatus

*a. Centrifuge,* with head to carry four 50-mL tubes or 250-mL bottles.

*b. Distillation flask,* 500-mL capacity.

*c. Condenser,* about 76 cm long.

*d. Adapter tube.*

*e. pH meter or recording titrator:* See Section 2310B.2*a*.

*f. Distillation assembly:* Use a conventional distilling apparatus. To minimize fluctuations in distillation rate, supply heat with a variable-wattage electrical heater.

### 3. Reagents

*a. Sulfuric acid,* $H_2SO_4$, 1 + 1.

*b. Standard sodium hydroxide titrant,* 0.1*N:* See Section 2310B.3*c*.

*c. Phenolphthalein indicator solution.*

*d. Acetic acid stock solution,* 2000 mg/L: Dilute 1.9 mL conc $CH_3COOH$ to 1000 mL with deionized water. Standardize against 0.1*N* NaOH.

### 4. Procedure

*a. Recovery factor:* To determine the recovery factor, *f*, for a given apparatus, dilute an appropriate volume of acetic acid stock solution to 250 mL in a volumetric flask to approximate

the expected sample concentration and distill as for a sample. Calculate the recovery factor

$$f = \frac{a}{b}$$

where:

$a$ = volatile acid concentration recovered in distillate, mg/L, and
$b$ = volatile acid concentration in standard solution used, mg/L.

*b. Sample analysis:* Centrifuge 200 mL sample for 5 min. Pour off and combine supernatant liquors. Place 100 mL supernatant liquor, or smaller portion diluted to 100 mL, in a 500-mL distillation flask. Add 100 mL distilled water, four to five clay chips or similar material to prevent bumping, and 5 mL $H_2SO_4$. Mix so that acid does not remain on bottom of flask. Connect flask to a condenser and adapter tube and distill at the rate of about 5 mL/ min. Discard the first 15 mL and collect exactly 150 mL distillate in a 250-mL graduated cylinder. Titrate with 0.1*N* NaOH, using phenolphthalein indicator, a pH meter, or an automatic titrator. The end points of these three methods are, respectively, the first pink coloration that persists on standing a short time, pH 8.3, and the inflection point of the titration curve (see Section 2310). Titration at 95°C produces a stable end point.

Distill and analyze a blank and reference standard with each sample batch to insure system performance.

## 5. Calculation

$$\text{mg volatile acids as acetic acid/L} = \frac{\text{mL NaOH} \times N \times 60\,000}{\text{mL sample} \times f}$$

where:

$N$ = normality of NaOH, and
$f$ = recovery factor.

## 6. Bibliography

OLMSTEAD, W.H., W.M. WHITAKER & C.W. DUDEN. 1929–1930. Steam distillation of the lower volatile fatty acids from a saturated salt solution. *J. Biol. Chem.* 85:109.

OLMSTEAD, W.H., C.W. DUDEN, W.M. WHITAKER & R.F. PARKER. 1929– 1930. A method for the rapid distillation of the lower volatile fatty acids from stools. *J. Biol. Chem.* 85:115.

BUSWELL, A.M. & S.L. Neave. 1930. Laboratory studies of sludge digestion. *Ill. State Water Surv. Bull.* 30:76.

HEUKELEKIAN, H. & A.J. KAPLOVSKY. 1949. Improved method of volatile-acid recovery from

sewage sludges. *Sewage Works J.* 21:974.

KAPLOVSKY, A.J. 1951. Volatile-acid production during the digestion of seeded, unseeded, and limed fresh solids. *Sewage Ind. Wastes* 23: 713.

# 5710 FORMATION OF TRIHALOMETHANES AND OTHER DISINFECTION BY-PRODUCTS*#(138)

## 5710 A. Introduction

Trihalomethanes (THMs) are produced during chlorination of water. Only four THM compounds normally are found: chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$), and bromoform ($CHBr_3$). Additional chlorination by-products can be formed (including haloacetic acids and halonitriles; for example, see 5710D) during the relatively slow organic reactions that occur between free chlorine and naturally occurring organic precursors such as humic and fulvic acids. The formation potentials of these additional by-products also can be determined, but different quenching agents and different analytical procedures may be needed. Predictive models for estimating/calculating THM formation exist, but because eventual THM concentrations cannot be calculated precisely from conventional analyses, methods to determine the potential for forming THMs are useful in evaluating water treatment processes or water sources or for predicting THM concentrations in a distribution system.

To obtain reproducible and meaningful results, control such variables as temperature, reaction time, chlorine dose and residual, and pH. THM formation is enhanced by elevated temperatures and alkaline pH and by increasing concentrations of free chlorine residuals, although THM formation tends to level off at free chlorine residuals of 3 mg/L and above; a longer reaction time generally increases THM formation.[1,2]

Low concentrations of bromide exist in most natural waters and are responsible for the formation of brominated organic compounds. Figure 5710:1 shows that an oxidant ratio of about 40 times more chlorine than bromine (on a molar basis, = 40 on the *x* axis) is required to form equimolar amounts of substituted organic chloride and bromide (= 1 on the *y* axis); small amounts of bromide also can increase the molar yield of THMs.[3]

The possible addition of organic precursors contained in reagent solutions cannot be accounted for accurately without a great deal of extra work; therefore, sample dilutions resulting from reagent additions (approximately 2%) are ignored in the final calculations. However, sample dilution may need to be taken into account if other volumes are used. Sample dilution also changes the concentrations of bromide and organic matter, potentially leading to speciation changes.

### 1. Definition of Terms