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of Volatile Acids*

by

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CHROMATOGRAPHIC DETERMINATION OF VOLATILE ACIDS

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The importance accorded the control of volatile acids in the anaerobic digestion of both domestic and industrial wastes is well known. The method of direct distillation for the determination of volatile acids has been widely used for control purposes in sewage treatment plants. This method is empirical and its accuracy is limited by the hydrolysis of complex compounds with mineral acid, and by variable percentage recovery of each of the volatile acids. These objectionable features have led to the suggestion of steam distillation (1)(2)(3). This technique reduces the possibility of hydrolysis and provides 92 to 98 per cent recovery of the volatile acids. However, the length of time required and the large volume of distillate often necessary for complete recovery of the acids is undesirable.

Chromatography

Recently numerous studies have pointed to the usefulness of chromatographic separation and identification of organic acids from various sources (4) (5) (6) (7) (8). The principle on which chromatography depends is well known and has been described in the earlier colloid literature as selective adsorption.

In the investigation reported here, organic acids are adsorbed on silicic acid from an aqueous solution and subsequently eluted by solutions (eluting agents) of varying concentrations of butyl alcohol in chloroform. The elut-

ing agents force the organic acids to pass through the chromatographic column in such a manner that they are selectively adsorbed and separated into bands of almost pure substances. The collection of fractions permits the identification and quantitative determination of the substances present. Several of these methods employing silicic acid as the adsorbent have proved satisfactory for the fractionation of organic acids from both simple and complex mixtures.

This report specifically concerns an application of the method of Bulen *et al.* (7). The chromatographic column length and solvent system are modified so that members of the lower volatile acids may be completely separated and identified using a solvent system of increasing polarity, or may be completely recovered using a single eluting agent. Recovery studies on mixtures of these commonly occurring acids have indicated the potentialities of this technique for a more accurate and rapid determination of volatile acidity in the control of sludge digestion.

Determination of Volatile Acidity

The chromatographic column is prepared in essentially the same manner as described by Bulen *et al.* (7). Five grams of silicic acid, ‡ washed free of fines, are thoroughly mixed with 3.5 ml. of 0.5 N. sulfuric acid and added to 30 ml. of chloroform, previously equilibrated against 0.5 N. sulfuric acid. The resulting slurry is transferred to the chromatographic tube

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‡ Mallinckrodt's silicic acid (100 Mesh) specially prepared for chromatography.

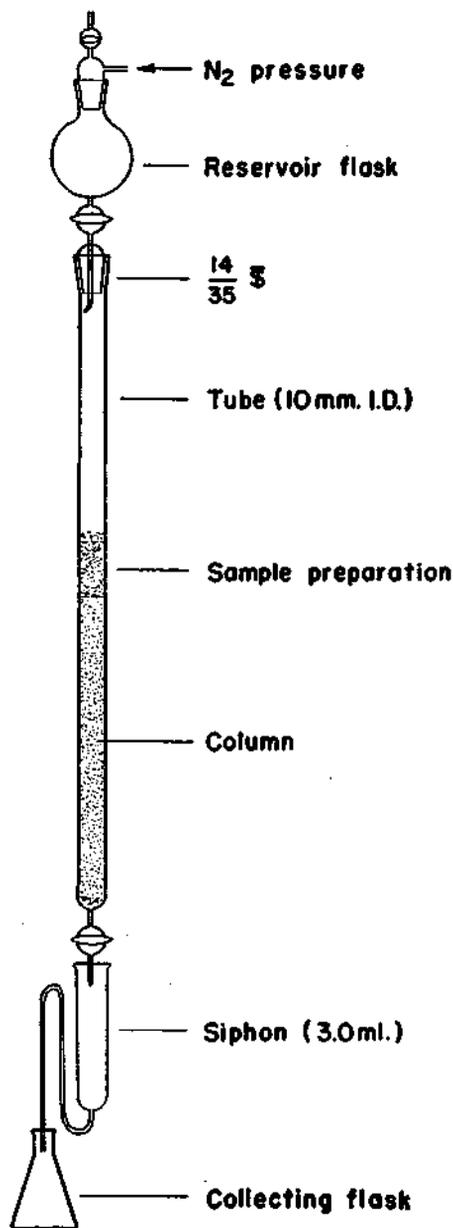


FIGURE 1.—The chromatographic column and related apparatus.

(Figure 1) having a glass wool plug to support the column. The stop cock is opened and a slight gas pressure of nitrogen or air at 10 to 12 cm. of mercury is used to pack the column and hasten the removal of the excess chloroform. Approximately 3 cm. of chloroform is retained above the col-

umn to ensure thorough wetting of the sample to be added.

The digester liquor sample, clarified by centrifuging and acidified to a pH value of approximately 2, is thoroughly mixed with two grams of silicic acid, and the preparation is quantitatively added to the top of the column. The sample container is rinsed with 1.0 ml. of chloroform which in turn is used to wash down any particles of the sample preparation adhering to the inside of the tube. A glass wool plug is forced down firmly on the surface of the sample preparation in the column using a glass rod. This packing action thoroughly wets the sample with the chloroform retained above the initial column preparation.

The chromatographic column now may be used for either the separation and identification of individual organic acids, or for the determination of the total volatile acidity attributed to butyric, propionic and acetic acids. The technique differs only in the eluting systems used.

Separation of Volatile Acids

The individual volatile acids may be separated and identified by the addition of several solvent systems, each of increasing polarity. The solvent systems are equilibrated against 0.5 N. sulfuric acid and passed through dry filter paper prior to use. The selected solvent schedule for the separation of the acids from the sludge consists of successive applications of 40 ml. of chloroform, 75 ml. of 2 per cent, 40 ml. of 5 per cent, and 75 ml. of 15 per cent n-butanol in chloroform. The solvents are added to the top of the column through the reservoir shown in Figure 1 and are forced to travel through the column under a gas pressure (nitrogen or air) of 10 to 12 cm. of mercury which is applied to the top of the reservoir. This pressure is maintained throughout the elution of the acids. Each subsequent solvent is added as

the previous solvent just enters the glass wool capping. The applied pressure provides a flow rate of approximately 3.0 ml. per minute. Using an intermittent siphon, fractions 3.0 ml. in volume are collected. To each fraction an equal volume of ethanol and one drop of 0.1 per cent phenol red indicator is added. The mixture is titrated with 0.02 N. sodium hydroxide to an end-point color which remains stable for 30 sec. The use of a magnetic stirrer insures contact between the two phases.

A determination is made on a blank from each of the solvent systems and these values are subtracted from the respective effluent titration values. The volume held up in the column (approximately 14.0 ml.) is compensated for by subtracting each new blank value from the fifth 3 ml. fraction subsequent to the addition of each solvent system.

Each acid has a characteristic peak effluent volume which is that fraction having the highest titration value. This volume is determined by the column dimensions and solvent systems employed, and is independent of the

concentration of the acids present within the range of the column. These values can be determined for each acid using known acids of high purity, both singly and in mixtures. The method permits the detection of amounts as small as 0.0025 milliequivalents of the individual acids, however, this column is best suited for separations of the volatile acids in the range of 0.5 to 5.0 mg. of each acid (calculated as acetic acid).

Figure 2 illustrates the separations of the organic acid mixture by the technique described. The titration values clearly show the peak effluent volumes and the order of elution of the individual acids. Table I shows recovery data on mixtures of the lower volatile acids with the method described. The accuracy is seen to be exceedingly good.

The recovery of formic acid is not quantitative if fumaric or glutaric acids are present. These acids eluted with formic acid, may be separated if desired, by the use of a longer column and the solvent changes suggested by Bulen *et al* (7). Lactic and succinic

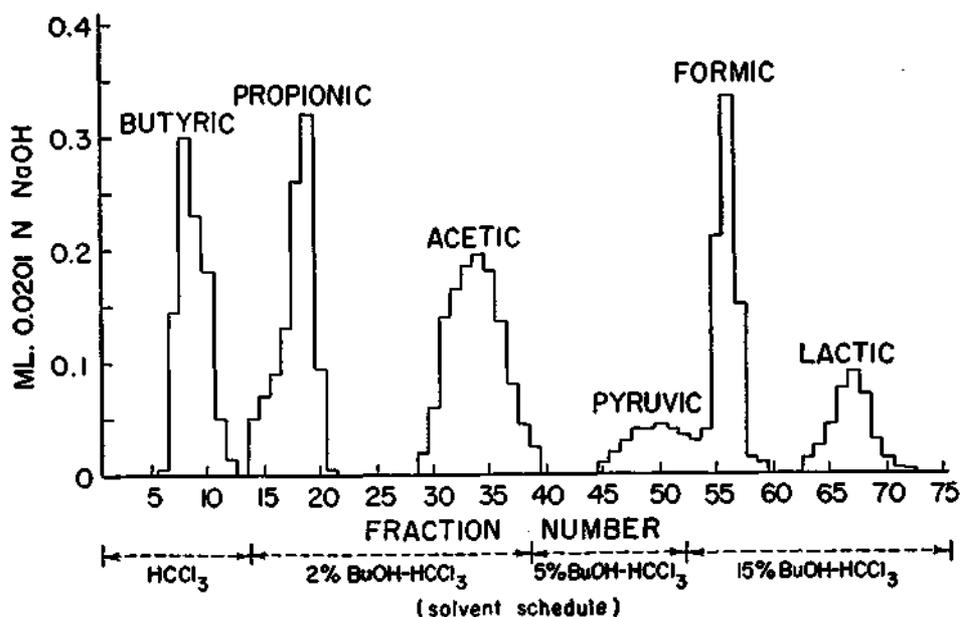


FIGURE 2.—The chromatographic separation of organic acid mixtures.

TABLE I.—Recovery Data on Individual Volatile Acids Separated from Mixtures

Acid	Acid Added ¹ (meq.)	Acid Recovered ¹ (meq.)	Recovery (%)
Butyric	0.0173	0.0171	99
	0.0140	0.0133	95
	0.0140	0.0141	101
Propionic	0.0204	0.0199	98
	0.0163	0.0158	97
	0.0163	0.0170	104
Acetic	0.0263	0.0271	103
	0.0383	0.0390	102
	0.0763	0.0785	102

¹ Calculated as milliequivalents.

acids also require a second treatment for complete separation.

Determination of Total Volatile Acids

In digestion control the procedure is quite simple for the determination of the total volatile acidity due to butyric, propionic and acetic acids, but it is limited to samples where interfering acids are known to be absent. Tests by the authors revealed that organic acids, such as pyruvic, lactic, etc., which would interfere usually do not appear or are present in insignificant amounts.

The solvent system employed for the total volatile acid determination consists of 15 per cent n-butanol in chloroform. This system has been experimentally shown to elute the desired acids in a 30-ml. volume of effluent.

The chromatographic column and sample are prepared and treated as previously indicated; The solvent is then added to the reservoir above the sample preparation and pressure applied as previously described to elute a 30 ml. fraction. Twenty milliliters of ethanol and five drops of 0.1 per cent phenol red indicator are added to this fraction. The mixture is titrated with 0.02 N. sodium hydroxide. A determination is made on a blank from each solvent mixture preparation and this value is subtracted from the

titration value. The total volatile acidity is calculated as acetic acid. Table II shows recovery data on known mixtures of the pure volatile acids present in a 30-ml. volume of effluent.

Technique Application

The application of the techniques described is further illustrated by the comparative data in Table III. These data were obtained on samples of overflow liquor from two anaerobic digesters, one treating wastes from an industrial yeast plant, and the other, wastes of domestic origin. The samples were clarified by centrifugation prior to use (time and speed not controlled). Qualitative analysis indicated the presence of only butyric, propionic and acetic acids. Pyruvic, formic, lactic and succinic acids were not present in either sample.

As can be seen from the results tabulated for each sample, fairly close agreement for total volatile acidity was obtained by the two chromatographic procedures described. However, the values obtained for the total volatile acidity by the direct distillation method are not consistent with these values in either case. This discrepancy is due to the fact that in the direct distillation method, a factor of 100/62.5 is used when calculating total volatile acidity as acetic acid. This factor, based on the distillation rate of acetic acid is in error since experimentation has shown 89 per cent of the propionic acid and 97 per cent of the butyric acid to be recovered by the distillation technique. Corrected values for total volatile acidity ob-

TABLE II.—Recovery of Butyric, Propionic, and Acetic Acids by Elution in Toto Using a Single Solvent System

Acid	Acid Added ¹ (meq.)	Acid Recovered ¹ (meq.)	Recovery (%)
Butyric	0.1282	0.1252	98
Propionic	0.0641	0.0625	98
Acetic	0.1282	0.1242	97

¹ Calculated as milliequivalents.

TABLE III—Volatile Acidity of Sewage Effluent Samples as Determined by Direct Distillation and Chromatographic Procedures

Sample	Distillation		Chromatographic Separation				Chromatographic Total
	Total ¹	Total ¹ (Corrected)	C-4	C-3	C-2	Total ¹	Total ¹
Industrial waste	1,891	1,640	227	496	991	1,548	1,808
	1,673	1,423	223	529	1,075	1,656	1,734
	1,689	1,439					1,772
	1,703	1,453					
	1,927	1,677	225	512	1,033	1,602	1,610
	1,815	1,560					
Domestic ² waste	6,221	4,920	1,423	1,869	2,890	5,377	5,104
	6,161	4,860	1,481	1,960	3,147	5,632	5,495
	6,080	4,779					
			1,452	1,914	3,018	5,504	

¹ Calculated as acetic acid.

² Sample from sour digester.

tained by the distillation method, as determined by the ratio of the acids separated by the multiple solvent technique, approach the values obtained by the chromatographic techniques.

Even the poorest checks (Line 1, Table III) are within the interpretative limits for treatment plant operation.

Summary

The suitability of a chromatographic technique for the separation and

identification of volatile acids was tested on pure compounds as well as on samples of domestic and industrial origin. Comparative data on total volatile acidity determinations with the direct distillation method, commonly used for control purposes, indicate the potential use of this technique for the controlled loading of anaerobic digesters. The determination of metabolic intermediates, butyric, propionic and acetic acids offers possibilities as a research method in this field.

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