Use of a simple titration procedure to determine H_2CO_3* alkalinity and volatile fatty acids for process control in waste-water treatment

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Abstract

A simple 5-pH point titrimetric method developed elsewhere was tested for measurement of bicarbonate (or H_2CO_3*) alkalinity and volatile fatty acids (VFA) in primary sludge, fermented primary sludge or its supernatant, settled sewage and anaerobic digester sludge (under process failure conditions). The titrimetric method incorporates a computer program to calculate the necessary results from a modified Gran titration in the presence of known concentrations of phosphate and ammonia. Comparisons were made between the titrimetric method, a colorimetric method and an HPLC method for VFA determination. The value of the titrimetric H_2CO_3* alkalinity result compared to that of conventional methods for anaerobic digester samples was also investigated. The results indicated good overall agreement between the three methods of VFA determination. From statistical analysis, the titrimetric method was found to over-predict the VFA content of failed anaerobic digester samples by approximately 15%, relative to the colorimetric method. Statistical agreement between the titrimetric and HPLC methods for these samples was good, provided the high frequency of outliers (ca. 20% of the data pairs rejected) was taken into account. No immediate explanation for the deviations between the methods for failed anaerobic digester samples could be found. However, from the point of view of method simplicity, and avoidance of inherent pitfalls in other methods of H_2CO_3* alkalinity estimation, the titrimetric method gave very useful results in process control and chemical dosing during start-up of two full-scale anaerobic digesters. The potential value of the titrimetric method for process control of primary sludge fermentation in biological nutrient removal plants was also highlighted. Although problems were encountered with reaching the lower detection limits of all three methods, the results for settled sewage suggest that the titrimetric method can give a fairly reliable estimate of VFA, even at low concentrations. Using the titrimetric method, good recovery of VFA from spiked samples of settled sewage in the range 40 to 80 mg/ℓ as acetic acid was obtained. Using pure solutions of carbonate and acetate, the detection limits for the titrimetric method were found to be approximately 10 mg/ℓ as $CaCO_3$ and 5 mg/ℓ as acetic acid. Scrupulous attention to pH probe maintenance and calibration was found to be an essential requirement for use of the titrimetric method, particularly at low concentrations when the systematic pH error estimate by the computer program cannot be relied upon.

Introduction

In waste-water treatment, measurement of volatile fatty acid (VFA) concentration and carbonate subsystem alkalinity are important in the control of a number of unit processes. For example, successful operation of anaerobic digesters depends heavily on maintaining a stable neutral pH. Since large masses of organic material are converted to methane, carbon dioxide and water via volatile fatty acid intermediates, for process stability it is recommended that a VFA: Alkalinity ratio of <0.3 be maintained in anaerobic digesters (Ross et al., 1992). On the other hand, biological nutrient removal (BNR) activated sludge plants often require the generation of VFA-rich primary sludge supernatant by fermentation either in the primary settling tanks or in side-stream processes (Barnard, 1984; Osborne et al., 1986; Pitman et al., 1992). The VFA generated may be mixed with the settled sewage or pumped as primary sludge supernatant directly to the activated sludge anaerobic or anoxic reactors to enhance biological P (and/or N) removal. In such cases the higher the VFA conversion from primary sludge, the greater the P and N removal potential (Wentzel et al., 1990). In such cases, process control requires the VFA content of settled sewage and primary sludge (or fermented primary sludge supernatant) to be measured.

The conventional method for measurement of alkalinity involves titration to an endpoint pH of around 4.5 which is usually detected using a mixed methyl red - bromocresol green indicator, and the result is reported as methyl red or total alkalinity (Loewenthal and Marais, 1976). Apart from the carbonate subsystem, total alkalinity includes contributions from phosphates, ammonia, VFA and other weak acid subsystems typically present in waste water. Borates and silicates may be significant in clean water analyses but less so in waste water. Bicarbonate alkalinity may be estimated using nomographs or by difference between the total alkalinity and phenolphthalein (carbonate) alkalinity (*Standard Methods*, 1985). However, in neither of these methods is the carbonate/bicarbonate subsystem alkalinity distinguished from that of the other subsystems which may be very significant in waste water, notably phosphate and VFA. For VFA determination by titration, a preliminary steam distillation step is required (*Standard Methods*, 1985) but this is tedious and poor recovery commonly occurs due to undetected leaks in the distillation system.

DiLallo and Albertson (1961) proposed a titrimetric method for differentiating between carbonate species and "volatile acid alkalinity". The sample is first titrated from its initial pH to pH 4.0 thereby determining the total alkalinity. The pH is then lowered to 3.3 to convert all the carbonate species to carbonic acid and dissolved CO_2. Since the equilibrium is such that dissolved CO_2 predominates, the carbonate species can be very largely expelled by light boiling of the sample for 3 min. The sample is back-titrated with strong base and the amount of base added between pH 4.0 and 7.0 forms an estimate of the "volatile acid alkalinity". In effect, the

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latter lumps together VFA and other weak acid-base subsystems, the VFA being calculated from conversion factors worked out by DiLallo and Albertson (1961) for anaerobic digester supernatant. An even more empirical method for estimating the VFA/carbonate alkalinity ratio was developed by Ripley et al. (1986). It involves titration with standard acid first to an intermediate pH 5.75 and secondly to pH 4.3. On the basis of the various subsystem equivalence points, at the intermediate pH most of the carbonate subsystem alkalinity (principally present as bicarbonate) would have been titrated, whereas the VFA is principally titrated at $5.75 > pH > 4.30$.

The Ripley method and that of DiLallo and Albertson (1961) are popular but have limitations in that they were developed largely empirically for anaerobic digester supernatant liquors. Since these methods do not take other subsystems (e.g. phosphate and ammonia) into account directly, their validity for other samples (e.g. primary sludge supernatant, settled sewage) would need to be carefully examined and modified empirically as required. In reviewing these and other methods, Moosbrugger et al. (1993a) proposed that carbonate (H_2CO_3*) and VFA subsystem total species concentrations (or alkalinities) could be determined from simple pH titration data provided the theory of weak acid-base chemistry was used (made easier with the aid of a personal computer) and total species concentrations of the principal interfering subsystems (phosphate and ammonia for waste water) are known. Moosbrugger et al. (1993b; c) presented a 4 pH point titration method for carbonate subsystem (H_2CO_3*) alkalinity measurement in the presence of known concentrations of total phosphate and ammonia, and a 5 pH point titration method for similar determination of carbonate (H_2CO_3*) alkalinity and VFA (i.e. short-chain fatty acid or SCFA).

The work of Moosbrugger et al. (1993a; b; c) stemmed from research into anaerobic digestion systems. Since the simple pH titration procedures developed could be of value in process control of primary sludge fermentation and sewage characterisation for BNR plant modelling, the aim of this paper was to investigate such applications. Furthermore, access to more expensive equipment (HPLC and spectrophotometers) for direct VFA determination was possible, allowing a comparison of the titrimetric results with those from chromatographic and colorimetric methods to be undertaken. This study ran concurrently with a major process upset of the anaerobic digesters at Darvill Waste-water Works, allowing the methods to be tested on a wide variety of samples not conforming to normal as well as abnormal process operating conditions.

Materials and methods

Instrumental

pH meter

The best results were obtained using a new Radiometer pH meter (Model PHM 92) with a combination glass electrode (PHC 2005) and automatic temperature compensation, along with Radiometer buffers pH 4 and 7.

Conductivity meter

Two types were employed, both giving satisfactory performance: WTW (Model LF 196) and Jenway (Model 4020).

Autoanalyser

Two types were employed: a Technicon Model 2 and a Skalar San Plus System. The manufacturers' recommended methods for ammonia and SRP were followed.

Digital burette

Two types were used: Brand Model D-6980 and Merck Model II. Both proved satisfactory.

Magnetic stirrer

An IKA - Combimag Model RCT was used with a 30 mm Teflon-coated stirrer bar.

Spectrophotometer

For the colorimetric determinations, a Beckman Model DU-62 spectrophotometer with a 1 cm flow cell was used.

High Performance Liquid Chromatograph (HPLC)

A Waters HPLC (Model 501 pump, 712 Wisp) with UV detector (Model 486) at 220 nm was used. The column used was a Fast Fruit Juice column (bore 7.3 mm; length 15 cm). A Guard-Pak Fast Fruit Juice precolumn was used.

Procedures

UCT titrimetric method

The 5 pH point titration method described by Moosbrugger et al. (1992) was followed. In summary, this method requires titration with a standard acid solution over the middle pH range in four steps: initial pH to pH 6.7, followed by pH 5.9, 5.2 and 4.3. Standardisation of the acid involves a Gran titration using a solution of known alkalinity, titrated to below the H_2CO_3* equivalence point. If the initial pH is below 6.7, strong base is added initially to reach pH 6.7. Algorithms for calculating H_2CO_3* alkalinity (Alk.) and VFA concentrations have been encoded in a computer program, named TITRA 5 by Moosbrugger et al. (1992). Besides quantifying the carbonate and SCFA subsystems, the computer program allows a check on the pH probe by providing an estimate of systematic pH error, which may be due to poor calibration, residual liquid junction potential or other influences on the glass electrode.

The following exceptions to the method of Moosbrugger et al. (1992) applied:

- The concentration of HCl titrant was reduced from 0.08 mol/ℓ to 0.02 mol/ℓ to facilitate greater accuracy at low alkalinity/VFA concentrations. The lower titre limit shifted down to approximately 1.0 mℓ for an H_2CO_3* Alk. of approximately 10 mg/ℓ as $CaCO_3$ (the detection limit).
- The diluted sample volume (50 mℓ) remained unchanged. However, the volume of sample (before dilution and making up to 50 mℓ with distilled water) was adjusted downwards where necessary in order to keep the total titre below about 20 mℓ.
- For settled sewage samples, no dilution was used (i.e. 50 mℓ sample volume).
- Experiments were carried out with mixed synthetic standard solutions of sodium carbonate and acetic acid such that the requirement (Moosbrugger et al., 1992) that the ratio of total carbonate species (C_T) to total acetate species (A_T) was less than 2:1. The implication of this was that correction for systematic pH error by the TITRA5 computer program could not be carried out. The results were nevertheless examined to test the limits of the procedure.

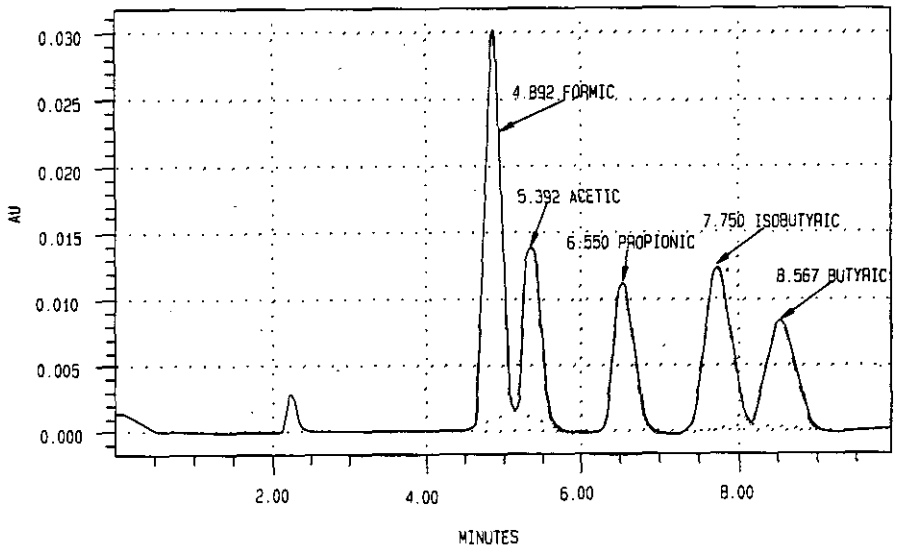

Figure 1
HPLC chromatogram of VFA standards, each at a concentration of 500 mg/ℓ. Isobutyric acid not routinely quantified (See text)

Total alkalinity

Total alkalinity was determined titrimetrically to the methyl red end point (pH approximately 4.5) using a mixed bromocresol green-methyl red indicator (*Standard Methods*, 1985). The titrant (0.01 mol/ℓ H_2SO_4) was standardised against a primary standard solution of sodium carbonate (0.106 g/ℓ anhydrous Na_2CO_3, equivalent to 100 mg/ℓ as $CaCO_3$).

Colorimetric method for VFA

The spectrophotometric method described by the Standing Committee of Analysts (1979) was employed, which is based on the formation of a purple ferric hydroxamate complex. Absorbance was measured with a 1 cm pathlength at 500 nm, giving a range of 20 to 1 000 mg/ℓ as acetic acid with absorbance readings of 0.004 to 0.16 relative to a blank carried through the procedure. The procedure ought to be adjusted to ensure that absorbances fall in the range 0.2 to 0.8 for optimal precision. Furthermore, the method calls for a 4 cm pathlength to give better sensitivity in the low VFA range (<1 000 mg/ℓ). However, since a 4 cm flow cell was not immediately available for the instrument, and since other routine analyses in the laboratory required a 1 cm flow cell, a compromise was necessary. The method was found to be linear in the range 0 to 10 000 mg/ℓ as acetic acid (0.000 to 1.600 absorbance units, with a 1 cm cell).

HPLC method for VFA

Using the HPLC equipment listed above, a mobile phase of 0.5% aqueous phosphoric acid was used at a flow rate of 1.0 mℓ/min, ambient temperature and a pressure of 800 psi. The run time was 10 min and injection volume was 100 mℓ. Peak area was obtained using the integrator connected to the detector. Calibration with five standards (range 50 to 2 000 mg/ℓ) for each of the four SCFA was performed, viz. C1 (formic), C2 (acetic), C3 (propionic) and C4 (butyric). The standards were obtained from Fluka (AR Grade) as concentrated acids. The HPLC result reflected the sum of these four fatty acids, and this was converted to an equivalent result of mg/ℓ as acetic acid. Samples in excess of 2 000 mg/ℓ for any of the fatty acids were re-analysed using a smaller injection volume on the WISP autosampler/injector.

All samples had been pre-filtered as required for the UCT titrimetric method, using Whatman 2V paper (or equivalent). Samples for HPLC were further filtered (at the same time as sub-sampling for the other methods of VFA determination) through 0.45 mm Millex membrane filters (Millipore) and kept refrigerated at 5°C for <14 d prior to analysis.

A typical HPLC chromatogram for analytical quality control with each fatty acid at 500 mg/ℓ is shown in Fig. 1. In the course of the research, it was occasionally found that a peak eluted between propionic and butyric acid for real samples. This peak was later identified as isobutyric acid but was not quantified, as will be discussed below.

Samples

All samples of sludges and sewage were obtained from Darvill Waste-water Works (Pietermaritzburg) which is owned and operated by Umgeni Water. These samples were kept refrigerated overnight and analysed within 24 h. It should be noted that Darvill Works was undergoing a major capital upgrade programme at the time this work was carried out. The primary sludge pre-thickener was newly commissioned in August 1994. Due to interruptions and delays caused by unforeseen problems with primary sludge thickening, the anaerobic digestion process had become hydraulically overloaded in May-June 1994. This led to failure of methanogenesis and, in effect, complete start-up of the two anaerobic digesters (process volume = 4 500 m^3 each) had to be effected in the period August to December 1994. This provided a unique opportunity to test the validity and usefulness of the UCT titrimetric method for VFA and carbonate alkalinity, especially since $NaHCO_3$ was dosed in large tonnages to aid the digester process start-up.

Results and discussion

Alkalinity

Table 1 gives the total (methyl red) alkalinity and H_2CO_3* Alk. results according to type of sample analysed. Table 2 gives similar results for VFA.

From the results in Table 1 it can be seen that in no case was the H_2CO_3* Alk. found to be more than the total alkalinity, which is correct. There were a limited number of cases where a negative

TABLE 1
RESULTS OF TOTAL (METHYL RED) ALKALINITY AND H_2CO_3* ALKALINITY (5 pH POINT UCT TITRIMETRIC METHOD) ACCORDING TO SAMPLE TYPE ANALYSED. $\bar{x}$ = MEAN; S_x = SAMPLE STANDARD DEVIATION; N = NO. OF OBSERVATIONS. RESULTS IN mg/ℓ AS $CaCO_3$

Sample type		Total alkalinity Methyl red method	H_2CO_3* alkalinity UCT titrimetric method	VFA mg/ℓ acetic acid UCT titrimetric method
Settled sewage	$\bar{x}$ S_x n	154 27 45	120 22 45	50 15 45
Primary sludge	$\bar{x}$ S_x n	330 126 45	56 50 45	671 549 45
Primary thickened sludge	$\bar{x}$ S_x n	660 202 24	78 92 24	2 147 906 36
Primary sludge supernatant	$\bar{x}$ S_x n	287 114 26	45 46 26	472 335 26
Anaerobic digested sludge(Note 1)	$\bar{x}$ S_x n	1 730 702 19 (Note 2)	4 386 965 23 (Note 3)	44 160 19 (Note 2)	1 672 883 23 (Note 3)	5 618 2 400 19 (Note 2)	6 704 1 149 23 (Note 3)

Note 1: Period 29/7/94 to 22/9/94 during process failure conditions.
Note 2: Before $NaHCO_3$ dosing: 27/7 to 25/8
Note 3: After $NaHCO_3$ dosing : 30/8 to 22/9

H_2CO_3* Alk. was recorded by the computer program (usually for samples with low total alkalinity such as the "sour" anaerobic digester before $NaHCO_3$ dosing or fermented primary sludge/supernatant). No simple explanation for this could be found, with no significant deviation from the norm being noted in phosphate or ammonia content of the samples in question. Given the complex nature of the sewage milieu, limitations of the titrimetric procedure are the probable cause.

Comparing the UCT titrimetric H_2CO_3*Alk. result with other methods of bicarbonate alkalinity estimation produced some interesting findings.

- A general rule suggested for anaerobic digestion (Task Force on Anaerobic Sludge Digestion, 1987) is: **BA = Total alkalinity - 0.71 x Volatile Acids**, where BA is bicarbonate alkalinity. Applying this rule to the mean anaerobic digester results in Table 1 for conditions of digester failure gives negative bicarbonate alkalinity. This is illustrated by Figs. 6 and 7. The rule only appears to work reasonably for VFA < 2 000 mg/ℓ acetic acid, and even then, differences in the "BA" result were noted between the two digesters (Nos. 5 and 6) which could not be explained by phosphate or ammonia content. The effect of the ammonium ion weak acid subsystem on H_2CO_3*Alk. is negligible (Moosbrugger et al., 1993c) and the phosphate subsystem influences H_2CO_3*Alk. to the extent of $0.9.P_T$, where P_T is the total phosphate concentration. The latter would not exceed 150 mg/ℓ as $CaCO_3$ (Figs. 6 and 8), which is less than the deviation between the BA and H_2CO_3*Alk. result. Overall, from the point of view of chemical dosing for digester start-up, the UCT titrimetric method gave more accurate and useful information in respect of H_2CO_3*Alk. Considering that $NaHCO_3$ is expensive (R1 200/t, 1994 price) and that the risk exists of sodium ion toxicity at approximately >3 000 mg/ℓ Na (Task Force on Anaerobic Sludge Digestion, 1987), accurate information on H_2CO_3*Alk. was useful in avoiding costly or counter-productive overdosing.

- According to *Standard Methods* (1985), one standard method for H_2CO_3*Alk determination involves performing a phenolphthalein titration as well as a methyl red titration. If the phenolphthalein alkalinity is zero, then the H_2CO_3*Alk. is assumed to equal the total (methyl red) alkalinity. Most biological sludge/sewage samples have a pH < 7.6, especially for primary or fermented sludges and "failed" anaerobic digesters, which are usually in the pH range $4.9 < pH < 6.5$. The phenolphthalein endpoint is 8.3, meaning that the phenolphthalein (or carbonate) alkalinity would be taken as zero for most sewage or sludge samples. From Table 1 it is clear that the rule from *Standard Methods* would therefore over-predict the H_2CO_3*Alk. of such samples by a large margin, mainly because phosphate, ammonia and volatile fatty acid contributions to total alkalinity would be ignored. This would be deceptive in respect of chemical dosing for digester start-up.

VFA

Figures 2, 3, 4 and 5 give a comparison of VFA results between the UCT titrimetric method and colorimetric or HPLC methods. Lines representing a 1:1 agreement between paired results are shown in these figures. Summary data for the comparison of the paired data sets are given in Table 3. For statistical comparison, notched box-and-whisker plots (Figs. 6a through 6l) are presented. In such plots the data are divided into quartiles, with the central box covering the interquartile range (or middle 50% of the data, between the lower and upper quartiles). The central line is the median. A notch is added to each box corresponding to the 95% confidence interval for the median. The confidence level on the notches allows pairwise comparisons to be performed between independent data sets by examining whether the notches overlap. In cases of an abnormally large confidence interval for the median, the notch may extend beyond either or both of the upper and lower quartiles, producing a box which appears to be "curled back". The "whiskers" extend to the extremes of the range (minimum and maximum values). Where unusual values occur, these are plotted as individual points, in which case the whiskers extend only to those points which are within 1.5 times the interquartile range.

TABLE 2
RESULTS FOR VFA DETERMINATIONS ACCORDING TO SAMPLE TYPE AND METHOD USED. REFER TO TABLE 3 FOR FURTHER STATISTICAL COMPARISONS. x = MEAN; S_x = SAMPLE STANDARD DEVIATION; N = NO. OF OBSERVATIONS. RESULTS ARE IN mg/ℓ AS ACETIC ACID.

Sample type		UCT titrimetric VFA	Colorimetric VFA	HPLC VFA
Primary sludge	x	612	635	618
	S_x	442	479	496
	n	42	42	38
Primary thickened sludge	x	2 147	1 921	2 085
	S_x	894	654	501
	n	36	36	34
Primary sludge supernatant	x	468	475	465
	S_x	323	291	328
	n	27	27	21
Anaerobic digested sludge (Note 1)	x	6 254	5 396	5 784
	S_x	1 913	1 688	1 703
	n	47	47	40
Settled sewage	x	50	47	39
	S_x	15	16	43
	n	46	46	41

Note 1: Period 29/7/94 to 22/9/94 during process failure conditions

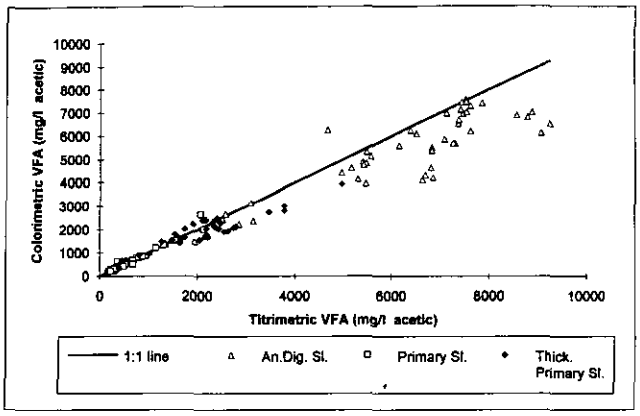

Figure 2
Plot of results for titrimetric vs. colorimetric VFA determinations for primary sludge (unthickened or thickened) and failed anaerobic digester sludge

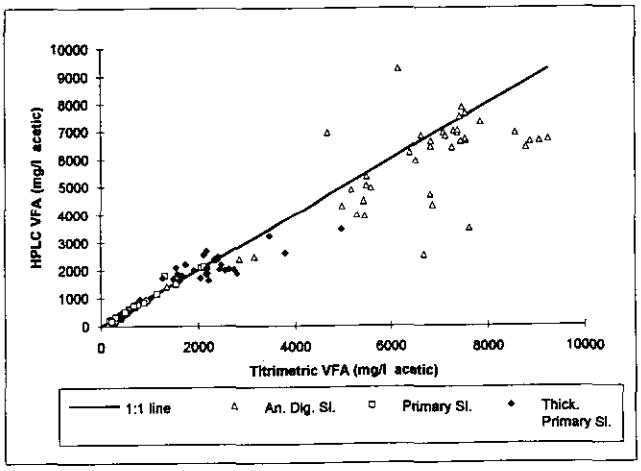

Figure 3
Plot of results for titrimetric vs. HPLC VFA determinations for primary sludge (unthickened and thickened) and failed anaerobic digester sludge

TABLE 3
SUMMARY STATISTICS FOR RESULTS OF PAIRED METHODS (UCT TITRIMETRIC, COLORIMETRIC OR HPLC) FOR VFA ANALYSIS ACCORDING TO SAMPLE TYPE. RESULTS IN mg/ℓ ACETIC ACID WHERE APPLICABLE. SNL = SUPERNATANT LIQUOR. N= No. OF OBSERVATIONS

Sample: Notes	**Anaerobic digester***		**Anaerobic digester***		**Anaerobic digester* 10 outliers rejected**	
Method:	**Titrimetric**	**Colorimetric**	**Titrimetric**	**HPLC**	**Titrimetric**	**HPLC**
n	47	47	40	40	30	30
Mean (x)	6 254	5 396	6 581	5 784	6 280	5 789
Std. dev. (S_x)	1913	1 688	1 639	1 702	1 594	1 638
Notched box-and-whisker plot	Fig. 6a		Fig. 6b		Fig. 6c	

Sample:	**Primary sludge**		**Primary sludge**	
Method:	**Titrimetric**	**Colorimetric**	**Titrimetric**	**HPLC**
n	42	42	38	38
Mean (x)	613	635	645	618
Std. dev. (S_x)	442	479	501	496
Notched box-and-whisker plot	Fig. 6d		Fig. 6e	

Sample:	**Thick. primary sludge**		**Thick. primary sludge**	
Method:	**Titrimetric**	**Colorimetric**	**Titrimetric**	**HPLC**
n	36	36	34	34
Mean (x)	2 147	1 922	2 248	2 086
Std. dev. (S_x)	894	654	813	502
Notched box-and-whisker plot	Fig. 6f		Fig. 6g	

Sample:	**Primary sludge SNL**		**Primary sludge SNL**	
Method:	**Titrimetric**	**Colorimetric**	**Titrimetric**	**HPLC**
n	27	27	21	21
Mean (x)	468	475	485	465
Std. dev. (S_x)	323	291	344	329
Notched box-and-whisker plot	Fig. 6h		Fig. 6i	

Sample: Notes	**Settled sewage**		**Settled sewage**		**Settled sewage 15 outliers rejected**	
Method:	**Titrimetric**	**Colorimetric**	**Titrimetric**	**HPLC**	**Titrimetric**	**HPLC**
n	46	46	41	41	26	26
Mean (x)	50.2	46.8	51.0	39.0	53.3	36.3
Std. dev. (S_x)	15.1	16.4	15.7	42.8	16.9	20.5
Notched box-and-whisker plot	Fig. 6j		Fig. 6k		Fig. 6l	

* Period 29/7/94 to 22/9/94 during process failure conditions

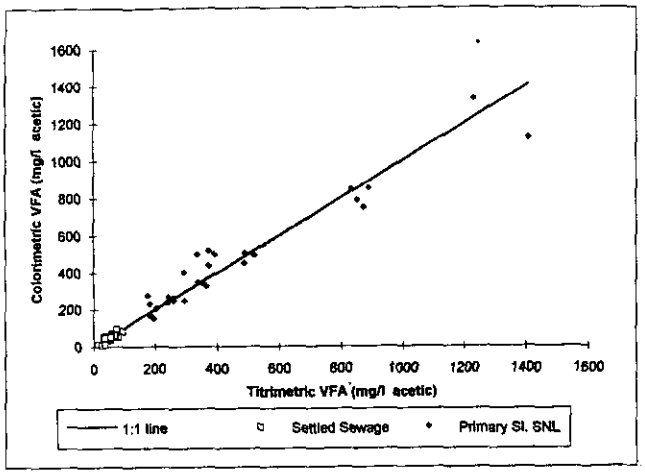

Figure 4
Plot of results for titrimetric vs. colorimetric VFA determinations for settled sewage and primary sludge supernatant

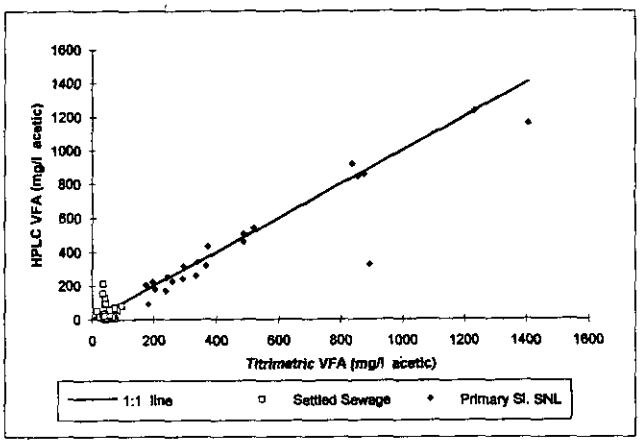

Figure 5
Plot of results for titrimetric vs. HPLC VFA determinations for settled sewage and primary sludge supernatant.

Examining the results in Table 3 and Figs. 6a through 6*l*, it becomes clear that fairly satisfactory agreement between the three methods of VFA determination was found. Comparison of the results tended to be complicated by the variance in the data, which is mainly attributable to the heterogeneity of samples from real sewage treatment processes. Coefficients of variation ($100.S_x/x$ or $100.S_y/y$) tended to be between 25% and 50% for most of the sample types, but as high as 70% to 80% for others, notably primary sludge or its supernatant liquor. The best correlations were found for primary sludge and good correlations were also found for primary sludge supernatant liquor as indicated by the overlap between the 95% confidence intervals of the median (Figs. 6d and 6e; Figs. 6h and 6i). The correlations for thickened primary sludge were slightly less satisfactory (Figs. 6f and 6g), with the titrimetric method tending to predict more VFA than the other two methods. However, the overlap of the 95% confidence intervals of the median again suggests that the difference between the three methods was not significant.

Considering the results for the anaerobic digesters under process failure and start-up conditions, it is clear from Figs. 2, 6a through 6c and Table 3 that the titrimetric method tended to predict higher VFA concentrations relative to the other two methods. However, this difference was only found to be significant at a 95% confidence level for the colorimetric-titrimetric method comparison (Fig. 6a). In the case of the HPLC results, agreement with the titrimetric results was not significantly improved by rejecting 10 data pairs as outliers (Table 3 and Fig. 6c). For two out of the ten outliers, the disagreement between results seemed to stem from low HPLC results. However, no simple explanation for any of the ten outlier data pairs could be inferred from the relevant HPLC chromatograms. In certain cases (for example Fig. 9), co-elution produced a positive baseline shift, but this was not a consistent trend for all samples and was corrected during peak integration in any case. For illustrative purposes, one other chromatogram without co-elution (Fig. 10) is shown. The set of standards including isobutyric acid at 500 mg/ℓ is shown in Fig. 1. Although isobutyric acid was ignored in the quantification of VFA by HPLC, its contribution appeared to be inconsistent. Figure 9 shows a case where the isobutyric contribution would have improved the agreement between the titrimetric and HPLC result, whereas Fig. 10 shows a case where the opposite would have been true.

One hypothetical reason for the titrimetric method tending to predict a higher VFA content for anaerobic digester samples is interference from sulphide ions, which was ignored in this study. However, the sulphide weak acid subsystem has a negligible effect on the VFA result because the relevant pKa values are removed from each other by more than two logs (Moosbrugger et al., 1992). Since certain amino acids have pKa values closer to those of the volatile fatty acids, the expected presence of partially hydrolysed proteins or peptides dissolved in "sour" digester sludge may have produced positive interference in the titrimetric method.

Considering the results for settled sewage (Table 3), satisfactory agreement between the titrimetric and colorimetric method VFA results was obtained. However, an inherent difficulty with all three methods was the low VFA concentration in the sewage. The colorimetric method absorbance (after blank correction) for the sewage samples was generally < 0.020 and the titre in the titrimetric method without dilution was 7 to 10 mℓ (blank titre < 0.4 mℓ). The sewage VFA concentration is therefore relatively close to the detection limit of these two methods (see below). In the HPLC method, the detection limit was also reached with settled sewage samples, which probably explains why the coefficient of variation was very high (110%). At 95% confidence, the titrimetric and HPLC means showed a statistically significant difference for settled sewage (Fig. 6k), even with 15 outliers rejected (Fig. 6*l*). It seems likely, therefore, that both the titrimetric and colorimetric methods significantly over-estimate the settled sewage VFA. In so far as a difference of 20 mg/ℓ VFA in the entire sewage flow is important, particularly in biological phosphate removal with activated sludge systems, it remains essential to measure the sewage readily biodegradable COD (which embraces the VFA component) before attempting to model the process mathematically. Readily biodegradable COD could be measured according to the methods of (Dold et al., 1991; Mamais et al., 1993; or Wentzel et al., 1995). The titrimetric method VFA result could be used with caution, taking due account of the confidence limits. If an independent check with a more sensitive detector on either gas chromatography or HPLC is available, this should be used for confirmation.

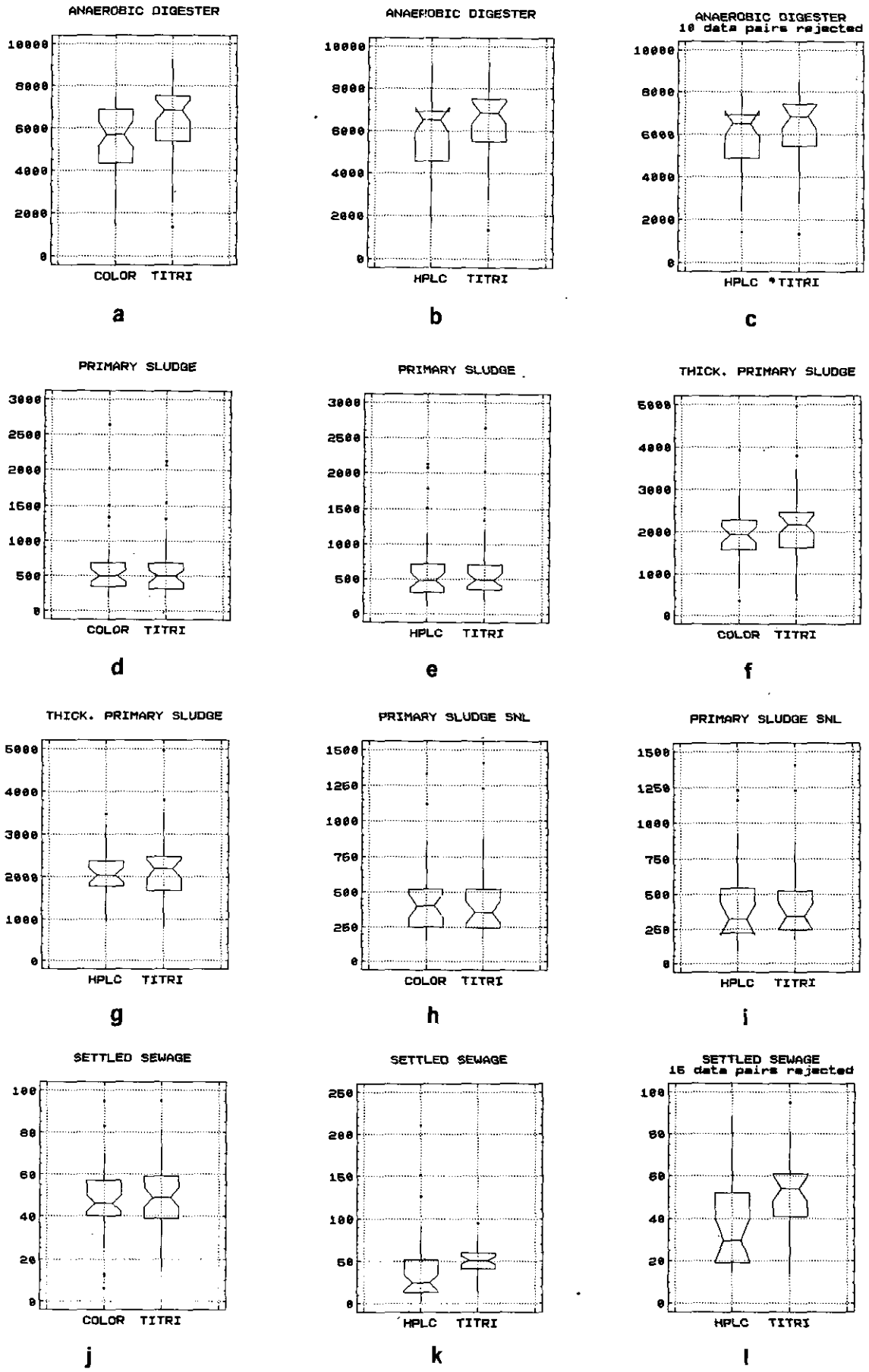

Figure 6a to 6 l

Notched box-and-whisker plots of paired methods for analysis of VFA, grouped according to sample type. Results are in mg/l as acetic acid. COLOR = colorimetric method; TITRI = titrimetric method; HPLC = HPLC method.

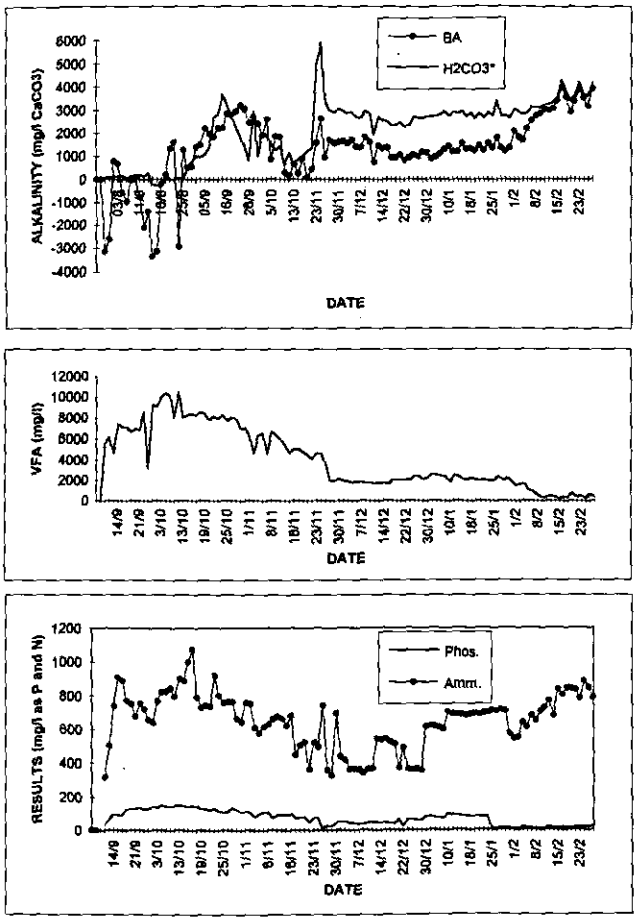

Figure 7

Comparison of bicarbonate alkalinity (from BA = Total Alk. - 0.71.VFA) with H_2CO_3 Alk. result of titrimetric method, VFA, phosphate and ammonia content of filtered samples of Digester 5 (Darvill WWW) under process failure conditions*

Detection limits of titrimetric method

Moosbrugger et al. (1992) recommended that the UCT titrimetric method be used when total carbonate: total acetate species (C_T:A_T) concentrations exceed 2:1, which allows the systematic pH measurement error to be used with confidence. Since it was not always possible to meet this requirement in the present study using primary sludge or fermented sludge samples, detection limits were checked using synthetic solutions with a C_T:A_T ratio of 1:1, as well as >2:1 but at low concentrations. Recoveries from settled sewage and primary sludge were also examined.

Tables 4A and 4B give the results for synthetic solutions. From these results it can be seen that for C_T:A_T=1:1, the practical detection limits can safely be regarded as 10 mg/ℓ as $CaCO_3$ for H_2CO_3* Alk. and 5 mg/ℓ as acetic for VFA. Where C_T:A_T > 2:1 at low concentrations, the VFA detection limit was not adversely affected, but the H_2CO_3* Alk. detection limit was practically ca. 30 mg/ℓ as $CaCO_3$.

A series of ten blanks were also tested for detection limit in the manner described by Peters et al. (1974). The detection limit is defined by Peters et al. (1974) as:

$$\text{Detection limit} > t_{0.005,9} \cdot Sx_b \ (1/n_s + 1/n_b)^{0.5}$$

where Sx_b = sample standard deviation for blank.
$t_{0.005,9}$ = 3.250 for 99% confidence with nine degrees of freedom, from a t-table
n_s = no. of observations of sample = 1
n_b = no. of observations of blank = 1.

The results are given in Table 5.

Recoveries from spiked samples for titrimetric method

Tables 6A and 6B give the results of recoveries of H_2CO_3* Alk. and VFA, from settled sewage and primary sludge respectively. These samples were selected to test the lower working range of the titrimetric method. From the results it is evident that good recoveries were achieved, which confirms the validity of this method in the low range for C_T and A_T (25 to 200 mg/ℓ as $CaCO_3$ and 5 to 200 mg/ℓ as acetic acid, respectively).

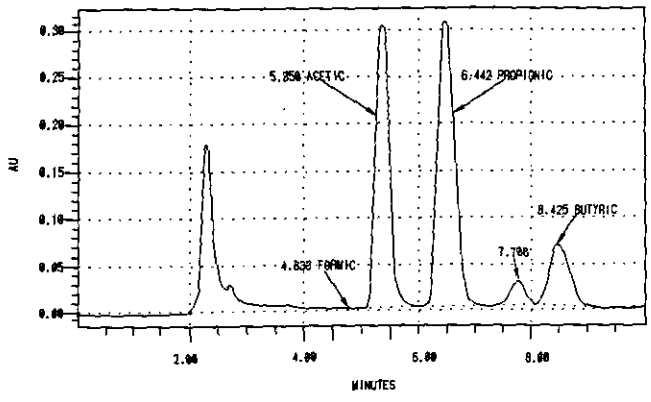

Figure 8

Comparison of bicarbonate alkalinity (from BA = Total Alk. - 0.71.VFA) with H_2CO_3 Alk. result of titrimetric method, VFA, phosphate and ammonia content of filtered samples of Digester 6 (Darvill WWW) under process failure conditions*

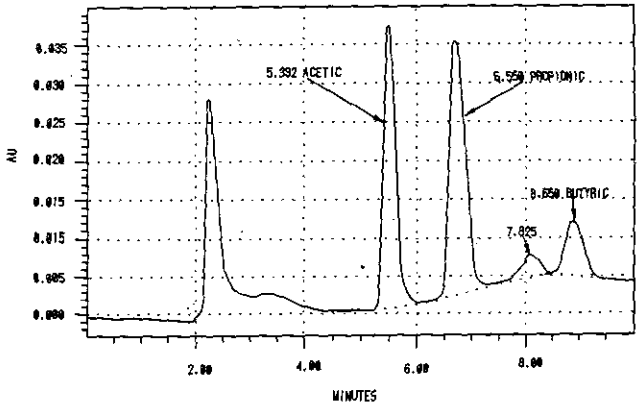

Figure 9

Chromatogram of Digester 6 filtered sludge (HPLC = 6 647 mg/ℓ VFA; titrimetric = 9 061 mg/l VFA) showing co-elution and presence of isobutyric acid

TABLE 4A
DETECTION LIMIT RESULTS FOR TITRIMETRIC METHOD USING SYNTHETIC SOLUTIONS OF $NaHCO_3$ (FOR H_2CO_3* ALK.) AND ACETIC ACID (FOR VFA), WHERE $C_T = A_T$.

H_2CO_3* Alk. (mg/ℓ as $CaCO_3$)						VFA (mg/ℓ acetic)					
True value	Replicates			Average +	% Relative	True value	Replicates			Average +	% Relative error
	(1)	(2)	(3)				(1)	(2)	(3)		
Blank	4.5	3.9	2.6	3.67	N/A	Blank	0	0	0	0	N/A
200	204.0	204.7	202.8	200.2	0.1	200	201.1	199.5	197.8	199.5	-0.3
100	105.9	107.4	106.0	102.8	2.8	100	97.9	98.4	100.5	99.9	-1.1
75.2	80.1	81.0	81.5	77.2	2.7	75.2	73.4	72.5	73.6	73.2	-2.7
50	52.0	52.6	52.0	48.5	-3.0	50	48.3	47.3	48.6	48.1	-3.9
25	27.0	28.0	29.1	24.4	-2.4	25	27.0	24.4	24.4	25.3	1.1
10	12.7	12.7	12.3	8.9	-11.0	10	10.0	9.9	10.1	10.1	0.7
8	10.8	10.2	11.8	7.3	-8.8	8	8.2	9.0	7.0	8.1	0.8
6	8.4	8.4	8.3	4.7	-21.7	6	7.4	5.0	6.6	6.3	5.6
4	6.4	6.9	6.9	3.1	-22.5	4	1.4	2.8	2.1	2.1	-47.5
2	5.4	4.6	5.6	1.5	-25.0	2	0.2	3.6	0.1	1.3	-35.0
1	5.4	4.4	3.8	0.5	-50.0	1	0	1.3	1.7	1.0	0

+ Corrected for blank, where applicable
% Relative Error = (Average-TV)/TV x 100 where TV is the true value

TABLE 4B
DETECTION LIMIT RESULTS FOR TITRIMETRIC METHOD USING SYNTHETIC SOLUTIONS OF $NaHCO_3$ AND ACETIC ACID, WHERE $C_T > 2A_T$ AT LOW CONCENTRATIONS

H_2CO_3* Alk. (mg/ℓ as $CaCO_3$)						VFA (mg/ℓ acetic)					
True value	Replicates			Average +	% Relative	True value	Replicates			Average +	% Relative error
	(1)	(2)	(3)				(1)	(2)	(3)		
Blank Table5	-	-	-	3.2	N/A	Blank	-	-	-	0	N/A
28.3	24.0	26.2	27.4	22.7	-19.8	10	10.5	9.8	9.8	10.03	0.3
22.6	22.6	23.7	23.1	19.9	-11.9	8	8	7	7.4	7.5	-6.7
17.0	18.3	17.3	17.9	14.6	-13.9	6	5.2	5.8	5.7	5.6	-7.2
11.3	13.5	12.4	2.4	9.6	-15.3	4	3.1	3.2	3.1	3.1	-22.5
5.66	6.6	7.1	7.0	3.7	-34.6	2	1.5	1.9	0.8	1.4	-30.0
2.83	4.5	4.4	4.8	1.4	-51.7	1	0	0.2	0	0.07	-93.0

+ Corrected for blank, where applicable
% Relative Error = (Average-TV)/TV x 100 where TV is the true value

Conclusions

A new 5 pH point titrimetric procedure developed by Moosbrugger et al. (1992) for carbonate subsystem (H_2CO_3*) alkalinity and volatile fatty acid (VFA) measurement in the presence of known concentrations of phosphate and ammonia was tested and proved reliable.

Provided scrupulous attention is given to pH probe maintenance and calibration, the titrimetric procedure can be used on samples in which the total carbonate to total acetate species ratio (C_T : A_T) is < 2:1 meaning that the carbonate subsystem does not dominate. The systematic pH error correction incorporated in the method (Moosbrugger et al., 1992) does not work under such conditions, but good recoveries of both H_2CO_3* Alk. and VFA from settled sewage or primary sludge were nevertheless found, down to approximately 7.5 mg/ℓ as $CaCO_3$ and 10 mg/ℓ as acetic acid.

The detection limits for the titrimetric procedure using deionised water blanks were found to be 3.2 mg/ℓ as $CaCO_3$ and 0 mg/ℓ as acetic acid for H_2CO_3* Alk. and VFA, respectively, based on the results of TITRA 5 computer program (Moosbrugger et al., 1992).

Practical detection limits using synthetic solutions of carbonate and acetic acid were found to be approximately 10 mg/ℓ as $CaCO_3$ for H_2CO_3* Alk. and 5 mg/ℓ as acetic acid for VFA where $C_T \approx A_T$. However, as may be expected, at low concentrations ($C_T < 30$; $A_T < 10$) the method is unreliable.

For samples of primary sludge (thickened or unthickened) and primary sludge supernatant, good statistical agreement was found between the VFA result by the titrimetric method and a colorimetric or HPLC method. Variance in the data was probably mainly attributable to real process fluctuation, and this tended to weaken regression analysis. However, good correlation was obtained in most cases for these samples, implying that the titrimetric method can be reliably used to monitor the performance of VFA generation processes on BNR plants.

For samples from a failed anaerobic digestion process (pH < 6.0; VFA >1 500 mg/ℓ as acetic acid), the titrimetric method tended to over-predict the VFA concentration by approximately 15% relative to the colorimetric method. No simple explanation for this deviation was found, but dissolved peptides or amino acids in the "sour" digester liquor may have produced interference.

The titrimetric method is more useful than conventional methods for estimating H_2CO_3*Alk. and VFA in anaerobic digester samples, since more accurate results are obtained and the pitfalls of inherent assumptions in other methods are avoided. This proved to be especially important during digester start-up when chemical dosing was required to maintain a suitable bicarbonate alkalinity. However, for H_2CO_3* Alk. determination, the titrimetric method does require a reasonably accurate estimate of particularly the dissolved phosphate (and preferably ammonia) content of the samples. Careful attention to pH probe calibration and maintenance are also essential.

Comparing the titrimetric method and HPLC results for failed anaerobic digester samples, it was found that outliers occurred for approximately 20% of the data pairs in which mainly the titrimetric method appeared to over-predict VFA by >2 000 mg/ℓ as acetic acid. No simple explanation for this observation could be found. However, statistical comparison at a 95% confidence level suggested that, given the large variances in the data for samples from the failed digesters, the titrimetric and HPLC methods did not give significantly different VFA results.

For settled sewage, the low VFA concentrations tended to produce large coefficients of variation for all the methods. Although the titrimetric method and colorimetric method tended to correlate, a statistically significant difference in the means for the titrimetric vs. HPLC method was observed. with the HPLC results tending to be lower. Outliers in the titrimetric-HPLC data pairs were again a problem. Since the lower detection limit of the HPLC with UV-detector (as well as the other methods) was approached, it is recommended that the VFA content of sewage be measured by a more sensitive method, or following a pre-concentration step. Satisfactory recovery of VFA in spiked samples of settled sewage was obtained in the range of approximately 40 to 80 mg/ℓ as acetic acid.

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The help of Dr Mark Wentzel (University of Cape Town) in the early stages of this project is gratefully acknowledged.

This paper is published with the approval of Mr W N Richards (Director of Scientific Services, Umgeni Water).

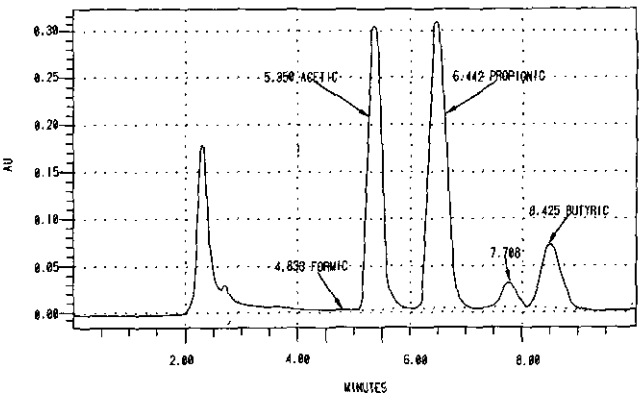

Figure 10
Chromatogram of Digester 6 filtered sludge (HPLC = 9 279 mg/ VFA; titrimetric = 6 163 mg/ℓ FA) without co-elution. Isobutyric acid present

TABLE 5
TEN BLANKS (DEIONISED WATER) ANALYSED AS SAMPLES. DETECTION LIMIT GIVEN FOR ONE SAMPLE AND ONE BLANK ANALYSIS

Blank number	H_2CO_3* Alk. mg/ℓ as $CaCO_3$	VFA mg/ℓ acetic
1	3.3	0
2	3.3	0
3	3.6	0
4	3.1	0
5	3.1	0
6	2.7	0
7	3.5	0
8	3.0	0
9	3.6	0
10	2.7	0
Mean	3.19	0
Std. dev., Sx	0.33	0
Detection limit +	1.52	0

+ Peters et al. (1974)

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TABLE 6A

RECOVERY OF MIXED SPIKE OF $NaHCO_3$ AND ACETIC ACID SOLUTION WITH SETTLED SEWAGE. VOLUME PROPORTIONS OF SEWAGE SAMPLE : SPIKE SOLUTION WERE 3:1, 7:1 AND 19:1 RESPECTIVELY

H_2CO_3* Alk. (mg/ℓ as $CaCO_3$)								VFA (mg/ℓ acetic)							
Sample only	Spike	True value	Replicates (1) +	(2) +	(3) +	Ave. +	% Recovery	Sample only	Spike	True value	Replicates (1) +	(2) +	(3) +	Ave. +	% Recovery
-	0	N/A	125.2	125.2	124.8	125.1	N/A	-	N/A	N/A	35.9	37.3	36.2	36.5	N/A
93.8	141.6	235.4	239.8	238.3	238.7	238.7	101.5	27.4	50	77.4	72.6	75.0	74.0	73.9	95.4
109.5	70.8	180.3	183.2	184.3	186.0	184.5	102.3	31.9	25	56.9	54.3	52.9	53.0	52.4	93.8
118.8	28.3	147.1	151.9	152.9	152.5	147.1	103.6	34.7	10	44.7	38.1	38.4	38.6	38.4	85.8

+ Corrected for blank (From Table 5)

TABLE 6B

RECOVERY OF MIXED SPIKE OF $NaHCO_3$ AND ACETIC ACID SOLUTION WITH DILUTED PRIMARY SLUDGE. VOLUME PROPORTIONS OF SEWAGE SAMPLE : SPIKE SOLUTION WERE 3:1, 7:1 AND 19:1 RESPECTIVELY.

H_2CO_3* Alk. (mg/ℓ as $CaCO_3$)								VFA (mg/ℓ acetic)							
Sample only	Spike	True value	Replicates (1) +	(2) +	(3) +	Ave. +	% Recovery	Sample only	Spike	True value	Replicates (1) +	(2) +	(3) +	Ave. +	% Recovery
-	0	N/A	31.8	30.5	31.4	31.2	N/A	-	0	N/A	27.4	27.8	26.6	27.3	N/A
15.6	283.2	298.8	298.4	302.2	299.1	299.99	100.4	13.6	100	113.6	115.0	114.6	117.5	113.6	101.8
27.3	70.8	98.1	98.2	98.9	98.1	8.4	100.3	23.9	25	48.9	45.0	43.7	46.9	45.2	92.4

+ Corrected for blank (From Table 5)

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