chemengineering

MDPI

Article

A Simple and Accurate Approach for Determining the VFA Concentration in Anaerobic Digestion Liquors, Relying on Two Titration Points and an External Inorganic Carbon Analysis

Paz Nativ, Yonatan Gräber, Yaron Aviezer and Ori Lahav *

Faculty of Civil and Environmental Engineering, Technion—Israel Institute of Technology, Haifa 32000, Israel; paznativ@campus.technion.ac.il (P.N.); yoni3655@campus.technion.ac.il (Y.G.); aviezery@campus.technion.ac.il (Y.A.)
* Correspondence: agori@technion.ac.il

Abstract: A new analytic approach is presented for determining the total volatile fatty acids (VFA_T) concentration in anaerobic digesters. The approach relies on external determination of the inorganic carbon concentration (C_T) in the analyzed solution, along with two strong-acid titration points. The C_T concentration can be determined by either a direct analysis (e.g., by using a TOC device) or by estimating it from the recorded partial pressure of CO_2(g) in the biogas (often a routine analysis in anaerobic digesters). The titration is carried out to pH 5.25 and then to pH 4.25. The two titration results are plugged into an alkalinity-mass-based equation and then the two terms are subtracted from each other to yield an equation in which VFA_T is the sole unknown (since C_T is known and the effect of the total orthophosphate and ammonia concentrations is shown to be small at this pH range). The development of the algorithm and its verification on four anaerobic reactor liquors is presented, on both the raw water and on acetic acid-spiked samples. The results show the method to be both accurate (up to 2.5% of the expected value for VFA_T/Alkalinity >0.2) and repetitive when the total orthophosphate and ammonia concentrations are known, and fairly accurate (±5% for VFA_T >5 mM) when these are completely neglected. PHREEQC-assisted computation of C_T from the knowledge of the partial pressure of CO_2(g) in the biogas (and pH, EC and temperature in the liquor) resulted in a very good estimation of the C_T value (±3%), indicating that this technique is adequate for the purpose of determining VFA_T for alarming operators in case of process deterioration and imminent failure.

Keywords: two-point titration; VFA analysis; anaerobic digester; total inorganic carbon; alkalinity

Citation: Nativ, P.; Gräber, Y.; Aviezer, Y.; Lahav, O. A Simple and Accurate Approach for Determining the VFA Concentration in Anaerobic Digestion Liquors, Relying on Two Titration Points and an External Inorganic Carbon Analysis. *ChemEngineering* **2021**, *5*, 15. https://doi.org/10.3390/chemengineering5020015

Academic Editor: Jorge Rodríguez-Chueca

Received: 1 March 2021
Accepted: 1 April 2021
Published: 6 April 2021

1. Introduction

The control of anaerobic reactors via titrimetric analysis, applied to determine the sum of the volatile fatty acids concentrations (VFA_T, namely the sum of the acetate, propionate and butyrate systems in solution), has been addressed in many publications [1–14]. More than a few algorithms have been developed in the past to interpret titration results aimed at VFA_T concentration determination [5], ranging from methods relying on two [4,7,14,15] through eight [11] titration points, and almost anything in between [8,9]. Some of the methods entail (external) analytical knowledge of the concentrations of other, dominant, weak-acid systems present in the water, such as orthophosphate (P_T) and ammonia (N_T) [8,11,13], while others [1,2,6,7,10,15] ignore the presence of these species, a practice that often yields only approximate results. The major difficulty in accurately determining the VFA_T concentration through titration lies in the fact that the buffer capacity curves of the carbonate system (pKC_1 = 6.375) and the VFA system (pK_a = 4.75) overlap close to the pH range where the buffering capacity of the VFA system is dominant. This is particularly true under the water composition that develops in intensive anaerobic digesters, which is often characterized by a very high total inorganic carbon concentration (C_T), such as, e.g.,

in thermophilic and mesophilic anaerobic digesters. The fact that the buffering capacity of the carbonate system is often much higher than that of the VFA system at that pH range (4.5 < pH < 6) leads to camouflaging of the titration signal that arises from the VFA species, which can only be overcome by relatively unwieldy titration methods, which often require the execution of multiple titration points. More specifically, most of the methods that have been suggested thus far, e.g., [8,11,16,17] have considered the problem to consist of two unknowns (VFA_T and C_T), which need to be determined simultaneously, while assuming that P_T and N_T had been determined by an external analysis (and thus their effects on the titration interpretation can be calculated and included in the algorithm). Such an approach, although certainly possible and capable of resulting in accurate VFA_T results, invariably leads to cumbersome and lengthy titration procedures. Other approaches exist that attempt to separate C_T from VFA_T by acidification and boiling [1] or by neglecting the effect of C_T in a particular pH range [2,4,15]. Each of these methods has its advantages and disadvantages, elaboration on which can be (partly) found in [12,17].

The current work assumed a different approach, according to which the total inorganic carbon concentration (C_T) is analyzed on the same sample, however separately from the titration method that is performed to determine the VFA_T concentration. Once C_T is determined (e.g., via a TOC analyzer), a very simple and easy to execute 2-point titration procedure can be performed to yield accurate VFA_T concentration results, and, as shown in this paper, this can be done without the need to analyze the P_T and N_T concentrations, as is often required in other titration methods.

Development of the Titration-Data Interpretation Algorithm

Similarly to previous approaches [4,8,11] the fundamental algorithm equation involves equating an equivalent-based term of the overall alkalinity species in solution (relative to the reference species defined below) in terms of the volume of the standard strong acid titrant added (left hand side of Equation (1)) with an equivalent-based term of alkalinity expressed in terms of the concentrations of all proton accepting species likely to be present in the water, i.e., proton accepting species of the carbonate, orthophosphate, ammonia and VFA systems following the addition of a certain equivalent mass, x, of strong acid (right hand side of Equation (1)). The utilized alkalinity equation was formulated against the following reference species: $H_2CO_3^*$, H_3PO_4, NH_4^+ and CH_3COOH (representing all the acidic species of the various VFA weak acid systems).

$$(V_e - V_x)C_a = \left(\begin{array}{l} 2[CO_3^{2-}]_x + [HCO_3^-]_x + 3[PO_4^{3-}]_x + 2[HPO_4^{2-}]_x + [H_2PO_4^-]_x \\ +[NH_3] + [A^-] + [OH^-] - [H^+] \end{array} \right)(V_s + V_x) \quad (1)$$

where: V_e = volume of titrant (strong acid) required to the equivalence point (L); V_x = volume of titrant added to yield pH_x (L); V_s = the volume of the sample (L); C_a = concentration of the titrant (eq/L) and the subscript x represents the concentration of the individual species following the dosage of V_x liter of strong acid to the solution.

Equation (1) can be written explicitly as a function of the total concentration of the various weak acid systems (C_T, P_T, N_T, VFA_T), pH_x and the apparent equilibrium constants:

$$(V_e - V_x)C_a = \left(\begin{array}{l} \frac{C_T(2K'_{C_1}K'_{C_2} + K'_{C_1}10^{-pH_x})}{10^{-2pH_x} + K'_{C_1} \cdot 10^{-pH_x} + K'_{C_1}K'_{C_2}} + \frac{P_T(3K'_{P_1}K'_{P_2}K'_{P_3} + 2K'_{P_1}K'_{P_2}10^{-pH_x} + K'_{P_1}10^{-2pH_x})}{10^{-3pH_x} + K'_{P_1}10^{-2pH_x} + K'_{P_1}K'_{P_2}10^{-pH_x} + K'_{P_1}K'_{P_2}K'_{P_3}} \\ + \frac{N_T K'_N}{K'_N 10^{-pH_x}} + \frac{VFA_T K'_{Ac}}{K'_{Ac}10^{-pH_x}} + \frac{K'_W}{10^{-pH_x}} - 10^{-pH_x} \end{array} \right)(V_s + V_x) \quad (2)$$

where: K'_C, K'_P, K'_N and K'_{Ac} are the thermodynamic equilibrium constants of the carbonate, orthophosphate, ammonia and acetate systems, respectively, adjusted for ionic strength and temperature.

Note that in order to consider the original C_T, VFA_T, P_T and N_T values in the water, they are multiplied in the algorithm by the dilution factor emanating from the titrated volume. For brevity and comprehensibility reasons, this dilution factor is not shown in

Equation (2) through (6), but it is of course included in the computerized algorithm (see code in Appendix B).

If C_T is known and two titration points (i.e., two V_x/pH_x pairs) are chosen such that the alkalinity components of the phosphate and ammonia systems hardly change when the two formed equations are subtracted from each other, only one unknown, VFA_T, remains, since V_e is also eliminated. Following [8,11] the two chosen titration points should be located roughly half a pH unit on either side of the pK_{Ac} of acetic acid (i.e., the titration is carried out to pH 5.25 and then to pH 4.25). These areas in the pH scale support stable and accurate pH measurements and the relatively large amount of strong acid that needs to be added between them (when the *VFA* and carbonate concentrations are substantial) allows for high accuracy, particularly when the VFA_T concentration increases, which is exactly the eventuality for which the method is developed. When the VFA_T concentration is very low (i.e., lower than 120 mg/L as CH_3COOH), the accuracy (in % of the true VFA_T concentration) may be lower, but this is perceived inconsequential for anaerobic processes control.

Equations (3)–(5) depict the formation of two equations from Equation (2) following titration to the two pH points (V_{x1}, pH 5.25 and V_{x2}, pH 4.25), and the isolation of VFA_T (Equation (4)) following the subtraction of Equation (3) from Equation (4). For demonstration purposes the values of respective thermodynamic equilibrium constants [16] are shown in these equations, however in the practical algorithm developed in this paper these values are adjusted to reflect the solution's ionic strength and temperature effects [18]. It is noted that in the practical execution of the method there is no need to titrate precisely to pH 5.25 and pH 4.25 but only to their vicinity.

$$(V_e - V_{x1})C_a = \left(\begin{array}{l} \frac{C_T(2K'_{C_1}K'_{C_2} + K'_{C_1}10^{-5.25})}{10^{-10.5} + K'_{C_1} \cdot 10^{-5.25} + K'_{C_1}K'_{C_2}} + \frac{N_T K'_N}{K'_N 10^{-5.25}} + \frac{K'_W}{10^{-5.25}} - 10^{-5.25} \\ + \frac{P_T(3K'_{P_1}K'_{P_2}K'_{P_3} + 2K'_{P_1}K'_{P_2}10^{-5.25} + K'_{P_1}10^{-10.5})}{10^{-15.75} + K'_{P_1}10^{-10.5} + K'_{P_1}K'_{P_2}10^{-5.25} + K'_{P_1}K'_{P_2}K'_{P_3}} + \frac{VFA_T K'_{Ac}}{K'_{Ac}10^{-5.25}} \end{array} \right)(V_s + V_{x1}) \quad (3)$$

$$(V_e - V_{x2})C_a = \left(\begin{array}{l} \frac{C_T(2K'_{C_1}K'_{C_2} + K'_{C_1}10^{-4.25})}{10^{-8.5} + K'_{C_1} \cdot 10^{-4.25} + K'_{C_1}K'_{C_2}} + \frac{N_T K'_N}{K'_N 10^{-4.25}} + \frac{K'_W}{10^{-4.25}} - 10^{-4.25} \\ + \frac{P_T(3K'_{P_1}K'_{P_2}K'_{P_3} + 2K'_{P_1}K'_{P_2}10^{-4.25} + K'_{P_1}10^{-8.5})}{10^{-12.75} + K'_{P_1}10^{-8.5} + K'_{P_1}K'_{P_2}10^{-4.25} + K'_{P_1}K'_{P_2}K'_{P_3}} + \frac{VFA_T K'_{Ac}}{K'_{Ac}10^{-4.25}} \end{array} \right)(V_s + V_{x2}) \quad (4)$$

Subtracting Equation (3) from Equation (4) and isolating VFA_T yields:

$$VFA_T = \frac{\begin{array}{l} (V_{x2} - V_{x1})C_a + (V_s + V_{x2}) \left[\begin{array}{l} \frac{\frac{V_s}{V_s+V_{x2}} C_T(2 \cdot K'_{C_1}K'_{C_2} + K'_{C_1}10^{-4.25})}{10^{-8.5} + K'_{C_1} \cdot 10^{-4.25} + K'_{C_1}K'_{C_2}} + \frac{\frac{V_s}{V_s+V_{x2}} N_T K'_N}{K'_N 10^{-4.25}} \\ + \frac{K'_W}{10^{-4.25}} - 10^{-4.25} + \frac{\frac{V_s}{V_s+V_{x2}} P_T(3 \cdot K'_{P_1}K'_{P_2}K'_{P_3} + 2 \cdot K'_{P_1}K'_{P_2}10^{-4.25} + K'_{P_1}10^{-8.5})}{10^{-12.75} + K'_{P_1}10^{-8.5} + K'_{P_1}K'_{P_2}10^{-4.25} + K'_{P_1}K'_{P_2}K'_{P_3}} \end{array} \right] \\ -(V_s + V_{x1}) \left[\begin{array}{l} \frac{\frac{V_s}{V_s+V_{x1}} C_T(2 \cdot K'_{C_1}K'_{C_2} + K'_{C_1}10^{-5.25})}{10^{-10.5} + K'_{C_1} \cdot 10^{-5.25} + K'_{C_1}K'_{C_2}} + \frac{\frac{V_s}{V_s+V_{x1}} N_T K'_N}{K'_N 10^{-5.25}} + \frac{K'_W}{10^{-5.25}} \\ -10^{-5.25} + \frac{\frac{V_s}{V_s+V_{x1}} P_T(3 \cdot K'_{P_1}K'_{P_2}K'_{P_3} + 2 \cdot K'_{P_1}K'_{P_2}10^{-5.25} + K'_{P_1}10^{-10.5})}{10^{-15.75} + K'_{P_1}10^{-10.5} + K'_{P_1}K'_{P_2}10^{-5.25} + K'_{P_1}K'_{P_2}K'_{P_3}} \end{array} \right] \end{array}}{V_s \cdot \left[\frac{10^{-4.75}}{10^{-4.75} + 10^{-5.25}} - \frac{10^{-4.75}}{10^{-4.75} + 10^{-4.25}} \right]} \quad (5)$$

To substantiate that the P_T and N_T alkalinity-related component concentrations can indeed be neglected in Equation (5) without a significant loss of accuracy, let us consider a fairly typical anaerobic digester water composition, characterized by three typical orthophosphate (P_T) concentrations (100, 150 and 200 mgP/L) and one total ammonia (N_T) concentration of 32.1 mM (450 mgN/L) [19]. Figure 1 shows the buffering capacity curves of the various weak acid systems in solution at the relevant pH range, assuming three VFA_T values and C_T = 51.1 mM. For the derivation of the buffer capacity terms see Lahav and Birnhack (2019) [18]. Since the integration of a buffering capacity curve between two pH values gives, by definition, the overall alkalinity concentration between these values, one can perform a separate integration of each weak acid system and determine from it the

theoretical error in the VFA_T concentration that would arise from ignoring the N_T and P_T values in the calculation represented by Equation (5).

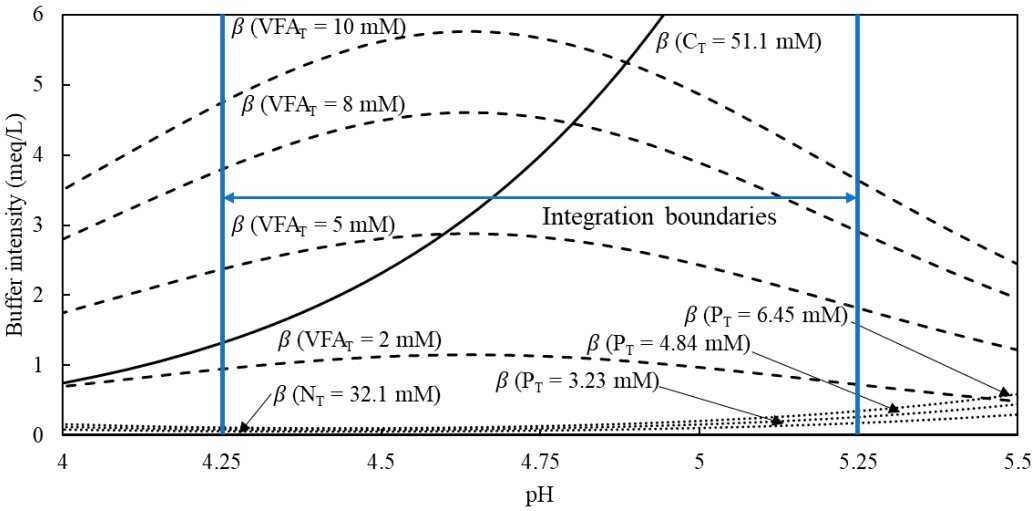

Figure 1. Buffer intensity curves for the various weak acid systems typically present in anaerobic digestion solutions at the pH range relevant for the titration procedure suggested in this work. Three total volatile fatty acids (VFA_T) and P_T values and one C_T (51.1 mM) and N_T (32.1 mM) values were considered (EC = 6 mS/cm, Temp = 25 °C).

Table 1 shows the overall theoretical error incurred from neglecting N_T and P_T. While the effect of N_T on VFA_T is outright negligible (as also demonstrated by the very small the area under the N_T buffer intensity curve), the effect of P_T cannot be considered as such, but it is still small and, as shown in Table 1, it becomes smaller as the total VFA concentration (VFA_T) reaches high (and alarming, from the anaerobic digester operation standpoint) concentrations. For example, the error in determining a VFA_T concentration of 8 mM (480 mg/L as CH_3COOH) was merely 3.3% when N_T and P_T were 450 mgP/L and 200 mgN/L, respectively. Therefore, for the sake of execution simplicity and for anaerobic reactor control purposes both N_T and P_T can be ignored without loss of essential information. This said, if P_T and N_T concentrations are known, even roughly, they could be plugged into the algorithm to attain better accuracy. This is mostly important when P_T reaches very high concentrations, such as 700 mgP/L, which has been reported in some locations [20].

Table 1. The error induced in VFA_T calculation (Equation (5)) by neglecting the alkalinity components related to the ammonia and orthophosphate weak-acid systems.

	N_T = 450 mg/L P_T = 100 mg/L		**N_T = 450 mg/L P_T =150 mg/L**		**N_T = 450 mg/L P_T = 200 mg/L**	
VFA_T (mM)	**VFA_T (mM) Calculated by Neglecting N_T and P_T**	**Error**	**VFA_T (mM) Calculated by Neglecting N_T and P_T**	**Error**	**VFA_T (mM) Calculated by Neglecting N_T and P_T**	**Error**
2	2.13	6.8%	2.2	10.2%	2.27	13.5%
5	5.13	2.7%	5.2	4.0%	5.27	5.4%
8	8.13	1.7%	8.2	2.5%	8. 27	3.3%
10	10.13	1.3%	10.2	2.0%	10. 27	2.7%

2. Materials and Methods

2.1. Titration Procedure

All titrations were performed in a sealed 220-mL glass container, equipped with a cap with perforated holes for placing pH and electrical conductivity (EC) probes, along with a port used for acid dosage. The titration was performed using a digital burette (Dosimat

775, Metrohm) filled with 0.05/0.1/0.25 N hydrochloric acid, under slow magnetic stirring. To obtain good results with the method it is essential to use a high-quality, sensitive pH electrode, which shows a stabilized reading in less than 30 s.

2.1.1. Titration of Synthetic Solutions

To compare the results of the new developed algorithm with the previously published 5- and 8-point methods [8,11], six synthetic solutions with varying VFA_T concentration (mg/L as $CaCO_3$) and carbonate alkalinity concentration (mg/L as $CaCO_3$) ratios were titrated using the 8-point titration procedure [11], which consists in it both the 5-point and the 2-point titration points (note that the VFA unit of concentration is shown here as mg/L as $CaCO_3$, for calculating the VFA to carbonate alkalinity ratio using similar units). The VFA_T concentration was 1 mM in all the synthetic solutions, simulating a dilution factor of 3.3 with deionized water (DIW) for an initial VFA_T concentration of 200 mg/L as HAc. To substantiate the premise that the method is accurate for all VFAs that have a pKa value close to 4.75, a synthetic solution of butyric acid with a VFA_T to alkalinity ratio of 0.31 and a VFA_T concentration of 1 mM was also prepared and tested using the same method.

2.1.2. Titration of Anaerobic Liquor Solutions

The dilution of the sample was done inside the sealed container, using a large as possible solution volume to reduce the head space to a minimum, to restrict the CO_2 mass that could potentially escape from the aqueous phase during the titration. Titrations were performed with an acid concentration of 0.05 N. The initial temperature and EC values are recorded prior to the titration. For detailed guidelines for sample collection and step-by-step execution of the procedure the reader is referred to the first section in the results and to Appendix A.

2.2. Interpretation Program

2.2.1. Synthetic Solutions

The measured parameters (temperature; EC; C_T; dilution factor; initial solution volume and the acid concentration) and the titration results for each synthetic solution were plugged in the program developed by Lahav and Morgan, 2002 [11] and the new excel program developed in the current work. The Excel program can be downloaded from the Supplementary Material.

2.2.2. Anaerobic Liquors

All the measured parameters (temperature; EC; C_T; N_T; P_T; dilution factor; initial solution volume and the acid concentration) and the titration results (two acid volumes and pH values) were plugged into a custom written excel function (see details of the VFA code in Appendix B) to yield the VFA_T concentration (in M).

2.3. Calculating C_T from the Knowledge of the $CO_{2(g)}$ Partial Pressure

To determine the C_T concentration from the biogas-CO_2 partial pressure, the PHREEQC [21] software was used (database = phreeqc.dat). The solution characteristics were plugged into the initial solution sheet and the inorganic carbon (C_T) was set to equilibrate with the measured partial pressure (Pp) of $CO_{2(g)}$ in the biogas. The initial solution C_T was set at equilibrium with a given CO_2 Pp and only the speciation results of the solution were used. The Excel program can be downloaded from the Supplementary Material.

2.4. Sample Preparation and Analyses

2.4.1. Sample Preparation

Synthetic solutions: sodium acetate, butyric acid and sodium bicarbonate were used to simulate the VFA_T and carbonate alkalinity, respectively. The VFA_T concentration was determined prior to addition of the carbonate alkalinity using TOC analyzer and Gran's titration for total alkalinity. C_T was determined using a TOC analyzer. All the chemicals

were of analytical grade, salts were dried at 60 °C overnight and kept in a desiccator prior to use.

Anaerobic liquor: (1) for the VFA_T analysis: samples of anaerobic digester liquor were centrifuged (6000 rpm for 20 min), filtered through a No. 1 Whatman filter paper and refrigerated (4 °C) until analysis and (2) C_T, N_T and P_T determination: samples of the anaerobic digester liquor were immediately centrifuged at 6000 RPM for 20 min after sampling and the clear supernatant was diluted (1:250) and filtered via a 45 µm syringe filter.

2.4.2. Analyses

Analyses: Ammonia was determined using the salicylate method [22]. Phosphate was determined using the metol method [23]. Inorganic carbon concentration (C_T) was determined by a Sievers M5310C (Suez Water Technologies, Boulder Co, USA) TOC Analyzer with a detection range of 0.04–50 mg/L. pH, temperature and electrical conductivity (EC) were measured using a Metrohm 914 pH meter equipped with a pH electrode with a Pt1000 sensor (Unitrode with Pt1000, Metrohm) and an EC electrode (conductivity measuring cell, Metrohm).

Statistical analyses: All the empirical results were analyzed statistically by JMP Pro®, SAS Institute Inc. $\alpha = 0.05$ was used in all calculations.

3. Results and Discussion

3.1. Comparison of the Newly Developed Algorithm to Previous Titration Methods Using Synthetic Solutions

Table 2 presents the titration results and relative error (%) obtained with three titration methods, as compared to a known VFA_T and C_T concentrations, at six VFA_T to alkalinity dimensionless ratios (mg/L as $CaCO_3$ to mg/L as $CaCO_3$).

Table 2. Titration results of six synthetic solutions and the simulated butyric acid solution, showing the calculated VFA concentration and relative error (%), as interpreted by the three titration algorithms.

VFA_T/Alkalinity	Dilution	VFA_T *	5-Point Method	8-Point Method	2-Point Method
		mg/L as HAc	mg/L as HAc Relative Error	mg/L as HAc Relative Error	mg/L as HAc Relative Error
0.49	3.4	59.3 ± 0.12	59.6 ± 0.7	61.2 ± 1.02	59.1 ± 0.32
			0.5%	3.1%	−0.4%
0.33	3.4	59.3 ± 0.12	59.4 ± 0.8	60.1 ± 0.86	59.9 ± 0.77
			0.2%	1.3%	1.0%
0.26	3.6	55.3 ± 1.21	54.8 ± 0.57	55.7 ± 0.9	56.5 ± 1.78
			−0.8%	0.9%	2.3%
0.20	3.4	59.3 ± 0.12	59.6 ± 1.23	60.5 ± 1.09	59.6 ± 0.96
			0.5%	1.9%	0.4%
0.12	3.8	52.5 ± 0.04	54.6 ± 0.31	55 ± 0.16	54.9 ± 0.51
			4.1%	4.8%	4.5%
0.10	3.4	59.3 ± 0.12	53.9 ± 4.07	53.9 ± 4.2	53.1 ± 3.18
			−9.1%	−9.1%	−10.5%
Butyric acid 0.31	3.4	59.3 ± 1.8	58.7 ± 0.17 −0.9%	60.8 ± 0.7 2.7%	58.8 ± 0.63 −0.8%

* The VFA_T concentration was verified using TOC analysis (no other organic species were present in this solution) and by applying the Gran titration (3 titration points to below pH2.7), which is accurate in the absence of the carbonate system.

Table 2 shows that all the three methods yield accurate results down to VFA_T/alkalinity ratio of 0.12 (relative error of 4.5%) while when the ratio dropped to 0.1, the relative error rose to 10%. The reason for the drop in accuracy at the low ratios stems from the fact that even a small error in the analysis of the (high) C_T concentration yield a high error in the (low) VFA_T concentration. To overcome this inherent drawback, the user is advised to spike the solution with an accurate volume of sodium acetate solution, to increase the ratio to above 0.2. This will ensure the accuracy of the results (in all three methods). At the end of the procedure the known dosed concentration should be subtracted from the result. To make sure that the obtained results are accurate, the user should follow the following guidelines: (1) the VFA_T concentration in the titrated solution should be at least one 1 mM (i.e., 60 mg/L as CH_3COOH); (2) if the VFA_T concentration is lower than 60 mg/L, it is suggested to spike the solution with a known concentration of acetic acid to attain at least 1 mM while also keeping in mind the VFA_T/alkalinity ratio (note that you will need to add the volume of the dosed acetic acid to the total volume, correct the P_T and N_T concentrations, etc.) and (3) an initial titration can be performed to assess the volume of acid required to reach the two pH points. The titration after that can be faster thereby reducing the potential change in C_T due to CO_2 release from the solution to the beaker's headspace; (4) the volume of the titrated acid is crucial to the accurate execution of the method. Choose an acid concentration that will allow for at least 2 mL of titrant between the two pH points. More guidelines and instructions appear in Appendix A.

3.2. Application of the New Method on Four (Raw and Spiked) Anaerobic Digester Liquors

Figure 2 shows the VFA_T concentrations (average of triplicate measurements with lower and upper 95% confidence intervals) obtained by the proposed method upon its execution on four types of both thermophilic (Shafdan, Acre) and mesophilic (Haifa) anaerobic digestion waters treating sludge from a municipal WWTP and another set of measurements performed on a mesophilic anaerobic digester treating winery wastes (Zichron). In all cases the raw water was diluted to obtain a solution with an alkalinity concentration in the range 300–400 mg/L as $CaCO_3$; C_T (and also N_T and P_T) was determined by external analysis and two titration points were executed. The raw results of all the analyses are listed in Tables A1–A4 in Appendix C. As shown in Figure 2, the method was found to be very accurate (an average error lower than 1.5%, relative to the expected, spiked values) in determining the VFA_T concentration of the spiked samples, and also in predicting (via extrapolation of the spiked samples' results) the raw-water VFA_T concentration (compare the free term in the linear regression equations with the raw VFA_T results that appear in Table 3). The slope of all four linear regression equations is very close to 1 (with p value < 0.01), demonstrating the accuracy of the method. The raw VFA_T concentration in two of the solutions (the mesophilic reactors, Haifa and Zichron) was lower, with concentrations of 12 and 28 mg/L as HAc, respectively, while in the thermophilic reactors (Shafdan and Acre) the recorded concentrations were 250 and 580 mg/L as HAc, respectively. The results that appeared in Figure 2 were obtained by plugging the measured C_T, N_T and P_T concentrations into Equation (5), in addition to the results of the two titration points. Table 3 lists the VFA_T results that were returned by Equation (5) when P_T and N_T were neglected (i.e., assuming that both concentrations are zero). As shown, the resulting difference was not larger than 5% when the VFA_T concentration was higher than 5 mM. When the VFA_T concentration was increased to concentrations commonly perceived to be of concern (>8 mM) to the stability of anaerobic digesters, the error induced by neglecting N_T and P_T dropped to 1–5%. Most importantly for VFA_T concentration control purposes, the slope of the increase in the VFA_T concentrations remained the same when P_T and N_T were neglected, which means that, although from a global perspective less accurate VFA_T results were obtained, the method continues to serve its main purpose, which is to alert the operator on a possible decline in the methanogenic bacteria activity.

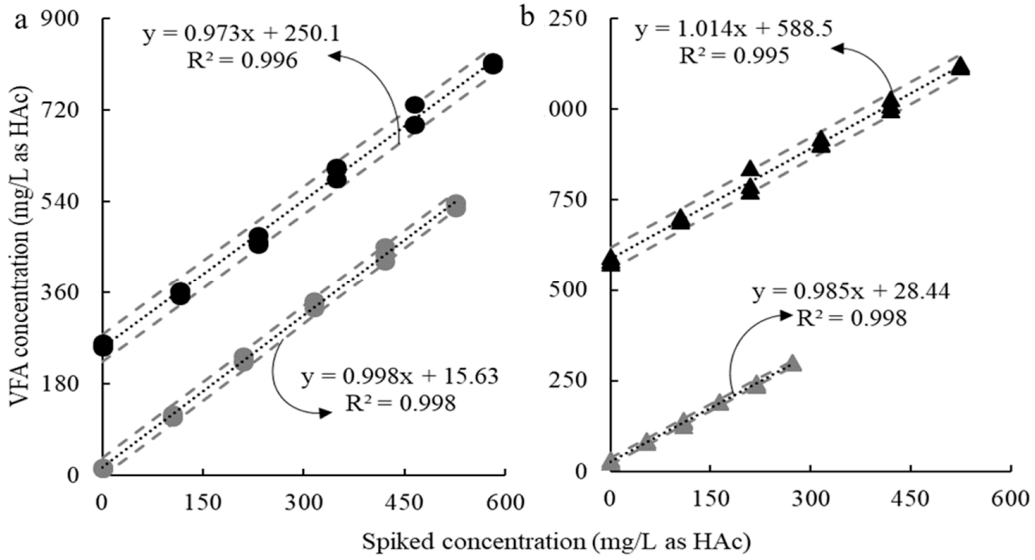

Figure 2. VFA_T results obtained from applying the method to four (spiked) anaerobic digestion liquors ((**a**): ●—Shafdan WWTP; ●—Haifa WWTP; and (**b**): ▲—Acre WWTP; ▲—Zichron winery digester).

Table 3. Estimation of the error obtained in VFA_T's calculation when N_T and P_T are ignored (=0).

Sample #	Calculated with Measured N_T, P_T (mg/L as HAc)	Calculated with N_T and P_T = 0 (mg/L as HAc)	Relative Error (%)
Shafdan raw	255.6 ± 3.55	266.5 ± 3.84	4.3%
Shafdan spiked 1	359.6 ± 4.93	370.7 ± 5	3.1%
Shafdan spiked 2	463.8 ± 7.64	475.3 ± 7.4	2.5%
Shafdan spiked 3	598.3 ± 10.34	609.5 ± 10.26	1.9%
Shafdan spiked 4	705 ± 18.65	716 ± 18.51	1.6%
Shafdan spiked 5	811.8 ± 2.43	822.7 ± 2.59	1.3%
Haifa raw	28.5 ± 3.35	43.6 ± 3.38	53%
Haifa spiked 1	83.3 ± 2.34	98.1 ± 2.17	18%
Haifa spiked 2	135 ± 5.74	149.7 ± 5.53	11%
Haifa spiked 3	190.9 ± 1.16	206 ± 1.06	8%
Haifa spiked 4	239.9 ± 3.83	254.7 ± 4.21	6%
Haifa spiked 5	300 ± 1.12	314.8 ± 0.81	5%
Acre raw	584.4 ± 6.95	590.5 ± 6.8	1%
Acre spiked 1	699.4 ± 5.48	705.4 ± 5.57	1%
Acre spiked 2	801.1 ± 27.09	807.3 ± 27.23	1%
Acre spiked 3	910.7 ± 8.15	916.6 ± 8.19	1%
Acre spiked 4	1012.6 ± 11.96	1018.5 ± 12	1%
Acre spiked 5	1119.9 ± 3.34	1126 ± 3.2	1%

3.3. Determining VFA_T Using C_T Values Estimated from $CO_2(g)$ Partial Pressure Values Measured in the Biogas

TOC analyzers may not be available in the (often) rudimentary laboratories that operate within WWTPs. Since the knowledge of C_T is obligatory for executing the presented method, the authors warmly suggest that such device would be available. However, in its absence, C_T can be estimated via a simulation program (PHREEQC or a similar tool) from the knowledge of the $CO_{2(g)}$ partial pressure in the biogas, which is measured routinely in many anaerobic digesters, in addition to the electrical conductivity, pH and temperature prevailing in the anaerobic digester liquor. Such calculation relies on two assumptions: (1) the aqueous phase in the reactor is completely mixed and (2) the aqueous phase tends towards equilibrium with the biogas. The two assumptions, although reasonable, are

clearly not entirely correct, but for the purpose of estimating C_T for use in the suggested method, our hypothesis was that the emanating error would be relatively low, and more importantly, the trend in the increase in the VFA_T concentration can be expected to be obtained accurately regardless of the inaccuracy in the nominal VFA_T concentrations, which is sufficient for raising a red flag in the case of an alarming rise in the VFA_T concentration. To assess whether such estimation is valid, seven anaerobic digesters in the Shafdan WWTP and one in the Acre WWTP were sampled for the percentage of $CO_{2(g)}$ and the overall pressure in the (dry) biogas, along with the required parameters (pH, EC and temperature) in the aqueous phase. PHREEQC was used to calculate the C_T concentrations from these data, and these were compared with measured C_T values obtained from the same samples via a TOC-based analysis. The results are presented in Table 4, which shows that the C_T values obtained from the calculations were very close to the TOC-measured C_T (an average difference of 0.4% and STDV of 2.8%). Such deviations in the C_T concentration can be expected to yield only a small error in the computed VFA_T concentration of several tens of mgHAc/L at the maximum, which translates into inconsequential error in VFA_T when the measured concentration was high (e.g., 8 mM). It can be hence concluded that estimating the C_T value via the $CO_{2(g)}$ partial pressure and its use within Equation (5) is a viable method for determining the trend of the VFA_T concentration, despite the slightly lower accuracy.

Table 4. Comparison of results of TOC-measured vs. estimated C_T values obtained from the knowledge of the CO_2 percentage in the biogas.

Reactor	CO_2 in Dry Biogas	pH	EC	Temp	TOC-Measured C_T	CO_2 Pp-Calculated C_T	Error
	%	-	mS/cm	°C	mM	mM	%
Shafdan 1	38.0	7.28	8.01	52.4	79.3	84.7	+6.8%
Shafdan 2	38.1	7.27	7.99	53.2	82.1	81.6	−0.7%
Shafdan 3	38.0	7.27	7.73	52.4	83.0	82.9	−0.1%
Shafdan 5	38.2	7.26	7.89	53.1	77.6	79.6	+2.5%
Shafdan 6	38.2	7.27	7.89	53.7	80.1	80.1	0.0%
Shafdan 7	17.6	7.70	9.32	53.7	96.7	97.3	+0.6%
Shafdan 8	21.1	7.64	9.40	54.2	101.6	98.9	−2.7%
Acre East	29.3	7.46	9.09	52.1	97.5	101.7	+4.4%

4. Conclusions

A new, titration-based, simple to execute and accurate analytical method for VFA_T analysis in anaerobic digester waters is presented and verified. The method differs from previous techniques by the fact that the total inorganic carbon concentration is analyzed on the same sample, but by an external method, preferably using a TOC analyzer. The titration method consists of only two points, carried out to pH values in the vicinity of pH 5.25 and pH 4.25. The buffer capacity area between these pH values is dominated by the VFA_T and carbonate weak-acid systems, with only a minor effect of the ammonia and orthophosphate systems, hence these weak-acid systems can be neglected in the analysis without significant loss of accuracy, particularly when the VFA_T concentration is high (VFA_T > 8 mM). The method was shown to yield results that are comparable with the 5-point and 8-point methods in terms of accuracy and reproducibility. The method was further tested on four anaerobic liquors and was shown to yield accurate and consistent results, with both raw and spiked samples. The inclusion of the orthophosphate (and to a lesser extent the ammonia) concentration increases the obtained accuracy, however if these are not known, the loss of accuracy is inconsequential (not more than a few percent) when the VFA_T concentration reaches values of concern. A PHREEQC-based procedure was developed for estimating the total inorganic carbon concentration based on the measured partial pressure of CO_2 in the biogas, for possible use in the case that a direct C_T analysis is not available. This technique was shown to yield accurate C_T values with an average error of 0.4% ± 2.8%. The resulting VFA_T computation may only slightly deviate from

the correct value, indicating that this approximated technique is valid for determining the VFA_T trend, and hence adequate as a monitoring tool for the performance of anaerobic digesters. This said, for obtaining the most accurate and reliable VFA_T results the writers recommend using externally measured C_T and P_T values. N_T can be neglected altogether because its effect on the VFA_T value is almost negligible. The presented method is ideal for use by researchers working on anaerobic processes, but it is also appropriate as a routine tool for controlling full-scale anaerobic digesters. The Excel-based interpretation program and the PHRREQC-based procedure for estimating C_T from Pp (CO_2) can be downloaded from the Supplementary Material.

Supplementary Materials: The Excel program and installation instructions are available for download from https://www.mdpi.com/article/10.3390/chemengineering5020015/s1.

Author Contributions: P.N.: Methodology, Data curation, Writing—original draft, Visualization, Validation, Writing—review and editing, Software. Y.G.: Methodology, Data curation, Project administration, Validation, Writing—original draft, Visualization, Writing—review and editing, Software. Y.A.: Validation, Writing—review and editing, Formal analysis. O.L.: Conceptualization, Writing—original draft, Supervision, Writing—review and editing, Visualization. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors wish to acknowledge the great help made available by the operating staff at Shafdan, headed by Eng. Efrat Kohen.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A. Detailed Instructions for Performing the Analytic Procedure

The suggested two-point titration technique for VFA analysis consists of three main steps: sampling, sample preparation and titration.

CO_2 is highly supersaturated in anaerobic digesters. This means that when samples are collected the water has a strong tendency release $CO_{2(g)}$ to the atmosphere. This may cause an error in both the pH measurement (release of CO_2 raises the pH) and in the TOC analysis applied for determining the inorganic carbon concentration (C_T). It is therefore crucial to perform the initial pH measurement at the sampling point and to take measures to collect the sample for analysis with minimal CO_2 loss. After collecting the liquor, the sampling container should be filled to the rim (no headspace) and sealed.

Appendix A.1. pH and Temperature Measurement at the Sampling Point

1. To determine accurately the pH value and the temperature of the digester liquor, pH and temperature measurement should be performed as follows:

2. Fill the measuring beaker continuously from the sampling point, while letting the outlet tube fill the beaker from the bottom.

3. Place the pH electrode and the temperature sensor at the bottom of the beaker, close to the outlet of the water.

Take the value after the electrode has completely stabilized.

Appendix A.2. Sampling and Sample Preparation

The high concentration of suspended solids in the solution influences the measurement of the initial EC and pH values. To avoid this error, the samples for the VFA titration should be centrifuged (6000 rpm for 20 min) prior to performing the method.

To minimize $CO_{2(g)}$ release, the sampling should be performed directly into the test tubes that are inserted into the centrifuge machine. Sampling should be performed as follows:

1. Fill the test tube slowly, by letting the liquor outlet tube fill the test-tube from its bottom.
2. Let the liquor to overflow for at least one volume of the test tube and then seal the tube tightly.
3. After the centrifuge step, the test tubes kept closed until the titration.
4. Only the supernatant part of the centrifuge step is used in the titration.
5. If C_T analysis is carried out using a TOC analyzer, the centrifuge supernatant should be sampled for this purpose.

Appendix A.3. Titration

The following set of conditions must be met for attaining stable and accurate results:

- The titration must be performed in a sealed, gently magnetically stirred beaker (see Figure A1).
- The inlet holes for the pH and EC electrodes and for the acid tube or burette should be sealed as tightly as possible.
- Only analytical grade acid (HCl) ampules of a known concentration (diluted to a concentration of 0.05–0.2 eq/L) should be used.
- The pH and EC electrodes must be calibrated and be in a good working condition.
- From experience, to obtain accurate results, the alkalinity of the titrated solution (after dilution) should be around 300–400 mg/L as $CaCO_3$. The sample should be diluted to meet this condition.
- A minimal head space should be left in the sealed beaker for allowing the addition of acid with a small safety margin.

The titration procedure:

1. Place the required volume of deionized water for the required dilution in the beaker.
2. Close the beaker cover. Insert the EC and pH electrodes in their designated inlet holes.
3. Gently open the centrifuged test tube and take the required volume of the supernatant by using a pipette.
4. Insert the volume of sample below the water level (to minimize CO_2 losses) through the inlet hole of the acid tube or burette in the cap of the sealed beaker.
5. Place the acid tube or burette in place.
6. Wait for the pH reading to stabilize.
7. After stabilization, register the EC and temperature values (the temperature can be measured via the EC or pH meters).
8. Start titrating the strong acid to pH = 5.25. Register the exact pH that was reached and the volume of strong acid that was titrated. These measurements are marked pH_{X1} and V_{X1}, respectively.
9. Continue titrating the strong acid to pH = 4.25. Register the exact pH reached and the cumulative volume of strong acid up to that pH point. These measurements are marked pH_{X2} and V_{X2}, respectively.

Figure A1. A sealed beaker with inlet holes for electrodes and for the acid titration tubing.

Appendix B. The Code Used for Calculating VFA_T from the Acquired Data

The following algorithm was written in VBA, to create an Excel function:

```
Function VFA (pHx1, pHx2, Vx1, Vx2, Temp, EC, CT, NT, PT, Dilution, Vs, Ca)
Dim gamma_m, gamma_d, gamma_t, kc1, kc2, kN, kp1, kp2, kp3, kA, Hx1, Hx2 As Double
Hx1 = 10 ^ (−1 * pHx1)
Hx2 = 10 ^ (−1 * pHx2)
'literature data for equilibrium constants'
Hcarbon1 = 7700
Hcarbon2 = 14,900
Hammonia = 52,210
Hphosphate1 = −8000
Hphosphate2 = 4200
Hphosphate3 = 14,700
Hacetic = −200
Hwater = 55,830
Rgas = 8.314
pkc1 = 6.375
pkc2 = 10.335
pkN = 9.25
pkp1 = 2.12
pkp2 = 7.2
pkp3 = 12.7
pkA = 4.75
pkw = 14
'Transforming the equilibrium constants to observed'
I = EC * (2.5 * 10 ^ −5) * 670
gamma_m = 10 ^ (−1.82 * (10 ^ 6) * ((78.3 * (Temp + 273.15)) ^ (−1.5)) * 1 * (((I ^ 0.5)/(1 + (I ^ 0.5))) − 0.2 * I))
gamma_d = 10 ^ (−1.82 * (10 ^ 6) * ((78.3 * (Temp + 273.15)) ^ (−1.5)) * 4 * (((I ^ 0.5)/(1 + (I ^ 0.5))) − 0.2 * I))
gamma_t = 10 ^ (−1.82 * (10 ^ 6) * ((78.3 * (Temp + 273.15)) ^ (−1.5)) * 9 * (((I ^ 0.5)/(1 + (I ^ 0.5))) − 0.2 * I))
```

```
kc1 = ((10 ^ (−1 * pkc1)) * Exp (−1 * Hcarbon1 * ((1/(273.15 + Temp)) − (1/298))/Rgas))/
gamma_m
kc2 = ((10 ^ (−1 * pkc2)) * Exp (−1 * Hcarbon2 * ((1/(273.15 + Temp)) − (1/298))/Rgas))
* gamma_m/gamma_d
kN = ((10 ^ (−1 * pkN)) * Exp (−1 * Hammonia * ((1/(273.15 + Temp)) − (1/298))/Rgas))
* gamma_m
kp1 = ((10 ^ (−1 * pkp1)) * Exp (−1 * Hphosphate1 * ((1/(273.15 + Temp)) − (1/298))/
Rgas))/gamma_m
kp2 = ((10 ^ (−1 * pkp2)) * Exp (−1 * Hphosphate2 * ((1/(273.15 + Temp)) − (1/298))/
Rgas)) * gamma_m/gamma_d
kp3 = ((10 ^ (−1 * pkp3)) * Exp (−1 * Hphosphate3 * ((1/(273.15 + Temp)) − (1/298))/
Rgas)) * gamma_d/gamma_t
kA = ((10 ^ (−1 * pkA)) * Exp(−1 * Hacetic * ((1/(273.15 + Temp)) − (1/298))/Rgas))/
gamma_m
kw = ((10 ^ (−1 * pkw)) * Exp(−1 * Hwater * ((1/(273.15 + Temp)) − (1/298))/Rgas))/
gamma_m
'species for alklalnity x1'
HCO3_x1 = (Hx1 * kc1 * CT * (Vs/(Vs + Vx1)))/(Dilution * (Hx1 * Hx1 + Hx1 * kc1 +
kc1 * kc2))
CO3_x1 = (kc1 * kc2 * CT * (Vs/(Vs + Vx1)))/(Dilution * (Hx1 * Hx1 + Hx1 * kc1 + kc1
* kc2))
NH3_x1 = kN * NT * (Vs/(Vs + Vx1))/(Dilution * (kN + Hx1))
H2PO4_x1 = (Hx1 * Hx1 * kp1 * PT * (Vs/(Vs + Vx1)))/(Dilution * (Hx1 * Hx1 * Hx1 +
Hx1 * Hx1 * kp1 + Hx1 * kp1 * kp2 + kp1 * kp2 * kp3))
HPO4_x1 = (Hx1 * kp1 * kp2 * PT * (Vs/(Vs + Vx1)))/(Dilution * (Hx1 * Hx1 * Hx1 +
Hx1 * Hx1 * kp1 + Hx1 * kp1 * kp2 + kp1 * kp2 * kp3))
PO4_x1 = (kp1 * kp2 * kp3 * PT * (Vs/(Vs + Vx1)))/(Dilution * (Hx1 * Hx1 * Hx1 + Hx1
* Hx1 * kp1 + Hx1 * kp1 * kp2 + kp1 * kp2 * kp3))
OH_x1 = kw/Hx1
Alk_x1 = 2 * CO3_x1 + HCO3_x1 + 3 * PO4_x1 + 2 * HPO4_x1 + H2PO4_x1 + NH3_x1
+ OH_x1 − Hx1
'species for alklalnity x2'
HCO3_x2 = (Hx2 * kc1 * CT * (Vs/(Vs + Vx2)))/(Dilution * (Hx2 * Hx2 + Hx2 * kc1 +
kc1 * kc2))
CO3_x2 = (kc1 * kc2 * CT * (Vs/(Vs + Vx2)))/(Dilution * (Hx2 * Hx2 + Hx2 * kc1 + kc1
* kc2))
NH3_x2 = kN * NT * (Vs/(Vs + Vx2))/(Dilution * (kN + Hx2))
H2PO4_x2 = (Hx2 * Hx2 * kp1 * PT * (Vs/(Vs + Vx2)))/(Dilution * (Hx2 * Hx2 * Hx2 +
Hx2 * Hx2 * kp1 + Hx2 * kp1 * kp2 + kp1 * kp2 * kp3))
HPO4_x2 = (Hx2 * kp1 * kp2 * PT * (Vs/(Vs + Vx2)))/(Dilution * (Hx2 * Hx2 * Hx2 +
Hx2 * Hx2 * kp1 + Hx2 * kp1 * kp2 + kp1 * kp2 * kp3))
PO4_x2 = (kp1 * kp2 * kp3 * PT * (Vs/(Vs + Vx2)))/(Dilution * (Hx2 * Hx2 * Hx2 + Hx2
* Hx2 * kp1 + Hx2 * kp1 * kp2 + kp1 * kp2 * kp3))
OH_x2 = kw/Hx2
Alk_x2 = 2 * CO3_x2 + HCO3_x2 + 3 * PO4_x2 + 2 * HPO4_x2 + H2PO4_x2 + NH3_x2
+ OH_x2 − Hx2
'calculation of the VFA concentration–results in molar'
AT = (Dilution * ((Vx2 − Vx1) * Ca + (Vs + Vx2) * Alk_x2 − (Vs + Vx1) * Alk_x1))/(((Vs
* kA)/(kA + Hx1)) − ((Vs * kA)/(kA + Hx2)))
'output of the total VFA concentration in molar'
VFA = A_T
End Function
```

Appendix C. Raw Data Showing the Analytical Results Obtained with the Four Anaerobic Digester Liquors + Computational Results

Table A1. Results of Shafdan VFA_T + spiking.

VFA_T	pH_{x1}	V_{x1}	pH_{x2}	V_{x2}	Temp	EC	Dilution	[HCl]	Spike Volume	TIC	P_T	N_T
mg/L as HAc		mL		mL	°C	mS/cm		N	mL	ppm	mg/L as P	mg/L as N
251.6	5.220	8.550	4.288	9.440	22.1	0.352	38.00	0.05	0.00	985	149	1038
260.2	5.278	8.606	4.272	9.608	22.6	0.352	38.00	0.05	0.00	985	149	1038
255.0	5.276	8.532	4.259	9.534	22.0	0.352	38.00	0.05	0.00	985	149	1038
352.7	5.260	8.410	4.260	9.470	21.9	0.352	38.01	0.05	0.04	985	149	1038
362.8	5.269	8.248	4.254	9.360	24.5	0.358	38.01	0.05	0.04	1000	149	1038
363.4	5.247	8.420	4.270	9.462	22.4	0.352	38.08	0.05	0.40	985	149	1038
455.8	5.307	8.122	4.231	9.388	23.0	0.352	38.02	0.05	0.08	985	149	1038
461.5	5.302	8.130	4.240	9.386	23.5	0.352	38.02	0.05	0.08	985	149	1038
474.1	5.269	8.354	4.290	9.510	22.9	0.352	38.16	0.05	0.80	985	149	1038
607.5	5.242	7.906	4.240	9.200	24.8	0.358	38.02	0.05	0.12	1000	149	1038
583.9	5.285	8.120	4.259	9.430	22.5	0.352	38.24	0.05	1.20	985	149	1038
603.5	5.270	8.160	4.247	9.480	23.7	0.352	38.24	0.05	1.20	985	149	1038
692.6	5.272	7.878	4.266	9.250	22.5	0.352	38.03	0.05	0.16	985	149	1038
690.9	5.271	8.000	4.264	9.372	22.4	0.352	38.32	0.05	1.60	985	149	1038
731.3	5.232	8.006	4.242	9.374	23.7	0.352	38.32	0.05	1.60	985	149	1038
814.9	5.267	7.800	4.274	9.260	22.5	0.352	38.40	0.05	2.00	985	149	1038
811.6	5.239	7.898	4.275	9.302	22.8	0.352	38.40	0.05	2.00	985	149	1038
809.0	5.230	7.800	4.263	9.200	22.8	0.352	38.40	0.05	2.00	985	149	1038

Table A2. Results of Haifa VFA_T + spiking.

VFA_T	pH_{x1}	V_{x1}	pH_{x2}	V_{x2}	Temp	EC	Dilution	[HCl]	Spike Volume	TIC	P_T	N_T
mg/L as HAc		mL		mL	°C	mS/cm		*N*	mL	ppm	mg/L as P	mg/L as N
25.59	5.2	9.9	4.2	10.9	23.4	0.5	19.0	0.1	0.0	2.4	207.2	697.9
26.76	5.2	10.0	4.2	10.9	23.5	0.5	19.0	0.1	0.0	2.4	207.2	697.9
33.22	5.3	10.0	4.2	10.9	22.8	0.5	19.0	0.1	0.0	2.4	207.2	697.9
85.3	5.2	9.8	4.2	10.8	22.1	0.5	19.0	0.1	0.4	2.4	207.2	697.9
80.0	5.3	9.8	4.3	10.8	22.1	0.5	19.0	0.1	0.4	2.4	207.2	697.9
84.6	5.2	9.8	4.2	10.9	22.0	0.5	19.0	0.1	0.4	2.4	207.2	697.9
140.8	5.2	9.6	4.3	10.7	21.4	0.5	19.1	0.1	0.8	2.4	207.2	697.9
137.0	5.2	9.6	4.2	10.7	21.3	0.5	19.1	0.1	0.8	2.4	207.2	697.9
127.2	5.2	9.6	4.3	10.7	23.6	0.5	19.1	0.1	0.8	2.4	207.2	697.9
192.5	5.2	9.4	4.3	10.6	23.5	0.5	19.1	0.1	1.2	2.4	207.2	697.9
190.2	5.3	9.4	4.3	10.6	23.4	0.5	19.1	0.1	1.2	2.4	207.2	697.9
189.9	5.3	9.4	4.3	10.6	24.4	0.5	19.1	0.1	1.2	2.4	207.2	697.9
236.0	5.2	9.0	4.3	10.2	21.2	0.5	19.2	0.1	1.6	2.4	207.2	697.9
245.1	5.3	9.0	4.2	10.4	21.1	0.5	19.2	0.1	1.6	2.4	207.2	697.9
238.6	5.3	9.1	4.2	10.4	21.7	0.5	19.2	0.1	1.6	2.4	207.2	697.9
298.7	5.3	8.9	4.2	10.4	21.3	0.5	19.2	0.1	2.0	2.4	207.2	697.9
301.4	5.2	8.9	4.2	10.3	21.5	0.5	19.2	0.1	2.0	2.4	207.2	697.9
299.7	5.3	8.9	4.2	10.4	20.9	0.5	19.2	0.1	2.0	2.4	207.2	697.9

Table A3. Results of Zichron VFA_T + spiking.

VFA_T	pH_{x1}	V_{x1}	pH_{x2}	V_{x2}	Temp	EC	Dilution	[HCl]	Spike Volume	TIC	P_T	N_T
mg/L as HAc		mL		mL	°C	mS/cm		*N*	mL	ppm	mg/L as P	mg/L as N
12.2	5.142	7.604	4.194	8.286	23.0	0.2744	19.0	0.05	0.0	511.05		
17.4	5.161	7.692	4.181	8.412	22.3	0.2744	19.0	0.05	0.0	511.05		
12.7	5.236	7.612	4.224	8.398	24.2	0.2744	19.0	0.05	0.0	511.05		
113.3	5.252	7.278	4.237	8.254	24.2	0.2744	19.1	0.05	0.7	511.05		
114.4	5.230	7.306	4.204	8.272	22.9	0.2744	19.1	0.05	0.7	511.05		
121.9	5.238	7.290	4.244	8.258	25.1	0.2744	19.1	0.05	0.7	511.05		
223.1	5.233	7.078	4.242	8.198	21.8	0.2744	19.1	0.05	1.4	511.05		
225.9	5.189	7.082	4.219	8.160	21.9	0.2744	19.1	0.05	1.4	511.05		
234.3	5.232	7.048	4.248	8.180	21.9	0.2744	19.1	0.05	1.4	511.05		
344.4	5.240	6.720	4.228	8.088	23.7	0.2744	19.2	0.05	2.1	511.05		
339.2	5.238	6.736	4.243	8.070	23.0	0.2744	19.2	0.05	2.1	511.05		
329.1	5.236	6.730	4.228	8.070	24.9	0.2744	19.2	0.05	2.1	511.05		
451.7	5.255	6.418	4.233	7.998	23.8	0.2744	19.3	0.05	2.8	511.05		
435.3	5.242	6.474	4.247	7.978	23.2	0.2744	19.3	0.05	2.8	511.05		
421.8	5.241	6.444	4.177	8.026	25.6	0.2744	19.3	0.05	2.8	511.05		
536.3	5.257	6.120	4.244	7.842	24.6	0.2744	19.4	0.05	3.5	511.05		
538.6	5.246	6.172	4.245	7.868	23.6	0.2744	19.4	0.05	3.5	511.05		
527.5	5.242	6.140	4.223	7.850	25.5	0.2744	19.4	0.05	3.5	511.05		

Table A4. Results of Acre VFA_T + spiking.

VFA_T	pH_{x1}	V_{x1}	pH_{x2}	V_{x2}	Temp	EC	Dilution	[HCl]	Spike Volume	TIC	P_T	N_T
mg/L as HAc		mL		mL	°C	mS/cm		*N*	mL	ppm	mg/L as P	mg/L as N
583.1	5.255	9.576	4.227	10.974	20.3	0.777	38.00	0.05	0.00	1170.75	61.9	2927.2
593.5	5.252	9.542	4.207	10.970	20.9	0.777	38.00	0.05	0.00	1170.75	61.9	2927.2
576.6	5.268	9.622	4.230	11.054	22.9	0.777	38.00	0.05	0.00	1170.75	61.9	2927.2
699.3	5.227	9.496	4.236	10.938	21.9	0.777	38.07	0.05	0.35	1170.75	61.9	2927.2
706.1	5.256	9.434	4.243	10.936	22.3	0.777	38.07	0.05	0.35	1170.75	61.9	2927.2
692.7	5.222	9.558	4.259	10.959	22.4	0.777	38.07	0.05	0.35	1170.75	61.9	2927.2
789.4	5.250	9.374	4.244	10.926	20.9	0.777	38.14	0.05	0.70	1170.75	61.9	2927.2
775.4	5.226	9.344	4.188	10.920	23.3	0.777	38.14	0.05	0.70	1170.75	61.9	2927.2
838.6	5.260	9.352	4.287	10.924	22.9	0.777	38.14	0.05	0.70	1170.75	61.9	2927.2
904.1	5.238	9.214	4.221	10.870	20.7	0.777	38.21	0.05	1.05	1170.75	61.9	2927.2
922.1	5.229	9.218	4.250	10.838	22.1	0.777	38.21	0.05	1.05	1170.75	61.9	2927.2
905.8	5.230	9.242	4.257	10.840	22.0	0.777	38.21	0.05	1.05	1170.75	61.9	2927.2
999.2	5.234	9.126	4.248	10.826	22.1	0.777	38.28	0.05	1.40	1170.75	61.9	2927.2
1028.2	5.227	9.118	4.246	10.834	22.8	0.777	38.28	0.05	1.40	1170.75	61.9	2927.2
1010.3	5.180	9.126	4.180	10.818	22.6	0.777	38.28	0.05	1.40	1170.75	61.9	2927.2
1119.0	5.212	8.960	4.259	10.698	22.7	0.777	38.35	0.05	1.75	1170.75	61.9	2927.2
1116.4	5.253	8.930	4.266	10.750	22.5	0.777	38.35	0.05	1.75	1170.75	61.9	2927.2
1124.4	5.224	8.944	4.244	10.730	21.4	0.777	38.35	0.05	1.75	1170.75	61.9	2927.2

References

1. Dilallo, R.; Albertson, O.E. Volatile Acids by Direct Titration. *J. Water Pollut. Control Fed.* **1961**, *33*, 356–365. Available online: http://www.jstor.org/stable/25034391 (accessed on 28 February 2021).
2. McGhee, T.J. A method for approximation of the volatile acid concentrations in anaerobic digesters. *Water Sew. Work.* **1968**, *115*, 162–166.
3. Chatterjee, B.; Radhakrishnan, L.; Mazumder, D. New approach for determination of volatile fatty acid in anaerobic digester sample. *Environ. Eng. Sci.* **2018**, *35*, 333–351. [CrossRef]
4. Mu, Z.-X.; He, C.-S.; Jiang, J.-K.; Zhang, J.; Yang, H.-Y.; Mu, J. A modified two-point titration method for the determination of volatile fatty acids in anaerobic systems. *Chemosphere* **2018**, *204*, 251–256. [CrossRef]
5. Sun, H.; Wu, S.; Dong, R. Monitoring Volatile Fatty Acids and Carbonate Alkalinity in Anaerobic Digestion: Titration Methodologies. *Chem. Eng. Technol.* **2016**, *39*, 599–610. [CrossRef]
6. Zan, F.; Zeng, Q.; Chi, K.; Hao, T.; Ekama, G.A. A novel approach for rapidly measuring volatile fatty acids in anaerobic process. *Water Res.* **2020**, *182*, 115960. [CrossRef] [PubMed]
7. Jenkins, S.; Morgan, J.; Sawyer, C. Measuring Anaerobic Sludge Digestion and Growth by a Simple Alkalimetric Titration. *J. Water Pollut. Control Fed.* **1983**, *55*, 448–453. Available online: http://www.jstor.org/stable/25041903 (accessed on 28 February 2021).
8. Moosbrugger, R.E.; Wentzel, M.C.; Ekama, G.A.; Marais, G.V.R. A 5 pH Point Titration Method for Determining the Carbonate and SCFA Weak Acid/Bases in Anaerobic Systems. *Water Sci. Technol.* **1993**, *28*, 237–245. [CrossRef]
9. Buchauer, K. A comparison of two simple titration procedures to determine volatile fatty acids in influents to waste-water and sludge treatment processes. *Water SA* **1998**, *24*, 49–56.
10. Münch, E.v.; Greenfield, P.F. Estimating VFA concentrations in prefermenters by measuring pH. *Water Res.* **1998**, *32*, 2431–2441. [CrossRef]
11. Lahav, O.; Morgan, B.E.; Loewenthal, R.E. Rapid Simple and Accurate Method for Measurement of VFA and Carbonate Alkalinity in Anaerobic Reactors. *Environ. Sci. Technol.* **2002**, *36*, 2736–2741. [CrossRef] [PubMed]
12. Fernández, R.; Dinsdale, R.M.; Guwy, A.J.; Premier, G.C. Critical analysis of methods for the measurement of volatile fatty acids. *Crit. Rev. Environ. Sci. Technol.* **2016**, *46*, 209–234. [CrossRef]
13. Charnier, C.; Latrille, E.; Lardon, L.; Miroux, J.; Steyer, J.P. Combining pH and electrical conductivity measurements to improve titrimetric methods to determine ammonia nitrogen, volatile fatty acids and inorganic carbon concentrations. *Water Res.* **2016**, *95*, 268–279. [CrossRef] [PubMed]
14. Sun, H.; Guo, J.; Wu, S.; Liu, F.; Dong, R. Development and validation of a simplified titration method for monitoring volatile fatty acids in anaerobic digestion. *Waste Manag.* **2017**, *67*, 43–50. [CrossRef] [PubMed]
15. Ripley, L.E.; Boyle, W.C.; Converse, J.C. Improved Alkalimetric Monitoring for Anaerobic Digestion of High-Strength Wastes. *J. Water Pollut. Control Fed.* **1986**, *58*, 406–411. Available online: http://www.jstor.org/stable/25042933 (accessed on 28 February 2021).
16. Benjamin, M.M. *Water Chemistry*, 2nd ed.; McGraw-Hill: New York, NY, USA, 2002.
17. Lahav, O.; Morgan, B.E. Titration methodologies for monitoring of anaerobic digestion in developing countries—A review. *J. Chem. Technol. Biotechnol.* **2004**, *79*, 1331–1341. [CrossRef]
18. Lahav, O.; Birnhack, L. *Aquatic Chemistry*, 1st ed.; De Gruyter: Berlin, Germany, 2019. [CrossRef]
19. Moragaspitiya, C.; Rajapakse, J.; Millar, G.J.; Ali, I. Optimization of mesophilic anaerobic digestion of a conventional activated sludge plant for sustainability. *Alex. Eng. J.* **2019**, *58*, 977–987. [CrossRef]
20. Mehta, C.M.; Latif, M.A.; Bastrone, D.J. Improved phosphorus solubility during acidic anaerobic digestion. In Proceedings of the AWA Biosolids Conference, Melbourne, Australia, 25–27 June 2014; p. 60.
21. Charlton, S.R.; Parkhurst, D.L. Modules based on the geochemical model PHREEQC for use in scripting and programming languages. *Comput. Geosci.* **2011**, *37*, 1653–1663. [CrossRef]
22. Willis, R.B.; Montgomery, M.E.; Allen, P.R. Improved Method for Manual, Colorimetric Determination of Total Kjeldahl Nitrogen Using Salicylate. *J. Agric. Food Chem.* **1996**, *44*, 1804–1807. [CrossRef]
23. McGregor, A.J. A colorimetric modification of McLean's method for the determination of phosphorus in soils. *J. Sci. Food Agric.* **1950**, *1*, 105–107. [CrossRef]