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Standardised methodology – testing effluent samples for NATA labs

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Integrity Ag and Environment
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Executive Summary

Biogas production has increased in Australia substantially in the past 10 years, resulting in a greater need for understanding effluent management. Parameters often used to evaluate the efficiency of biogas are: organic loading rate, the specific gas production (biogas yield) and the degree of degradation, which are all calculated from the volatile solids (VS) that flow into the system. VS represent the organic component of the total solids (TS) and is measured using the residual of TS minus the inorganic content (ash). The international standard method used to determine TS uses oven-drying to evaporate the water component of a sample. However, during this drying process, there is the potential for losses of volatile molecules including: volatile fatty acids (VFAs), sulphurous compounds, phenols, indoles, ammonia, volatile amines and alcohols. The loss of these compounds will result in an underestimation of TS content, and consequently an underestimation of VS content. These volatile compounds, and specifically VFAs, may also be lost during manure handling prior to entry into the biogas system, and diagnosing these losses is important for diagnosing losses and improving biogas yields.

The aim of this project was to determine the evaporation of VFAs in the VS and TS analysis process, to establish if this resulted in a material error in reported VS. Pending the outcome of this, the project proposed developing a method that could be used to compensate for errors obtained in the analysis of the VS and TS content at Australian laboratories, due to the loss of VFAs in the drying stage.

Effluent from five different piggeries and four different analysis methods were included in this study. The methods were: 1. Standard method (SM), 2. Standard-VFA adjusted (SM-VFA), 3. Temperature modified (TM), and 4. Temperature modified-VFA adjusted (TM-VFA). VFA evaporation was determined by comparing the concentration of VFA in the original sample to the concentration in the rehydrated sample, and adjustments were made by correcting VS and TS to account for VFA loss.

The study found that the evaporation of VFAs during the VS/TS-analysis using oven-drying was variable between piggeries and high enough in some samples to be appreciable. The SM approach underestimated VS concentrations by 0-15% in piggery effluents sampled, with a mean error of 6%. A carbon balance was used to determine accuracy of each method and the SM-VFA method that was adjusted VS with VFA concentrations in accordance with Vahlberg et al. (2013) was the most accurate method used to predict VS. The TM and TM-VFA methods were both found to overestimate the VS concentrations of samples. Additionally, there were large temporal variation and stirring effects on the concentrations of VS and VFAs at two piggeries that were sampled on a second occasion to observe the potential impact of temporal and management effects.

Confirming results from previous studies, these results established that in some cases, the SM approach resulted in substantial under-estimates of VS from piggery effluent. However, this result was not consistent, and some evidence of temporal variation was observed. A simple method was examined to adjust VS and improve the accuracy of testing piggery effluent, and this was found to result in up to 15% higher reported VS. Using standard laboratory titration methods to test VFAs and correct VS results provides a relatively low-cost solution for researchers, consultants and producers investigating piggery effluent streams with the aim of optimising biogas production.

It is noted that the study was limited by the small sample size and further research should be conducted to expand the sample size and examine the impact of different pig stages, diets, manure management systems and climate on VFA losses and consequent VS error in other regions of Australia.

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Glossary

CAP	Covered anaerobic pond
COD	Chemical oxygen demand
FCR	Feed conversion ratio
Feedstock	When measuring for biogas production it refers to the mass of volatile solids in the piggery effluent.
MMS	Manure management system
SM	Standard method
SM-VFA	Standard-VFA adjusted
SOP	Standard operating procedure
SS	Suspended solids
TDS	Total dissolved solids
TM	Temperature modified
TM-VFA	Temperature modified-VFA adjusted
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
VS_{VFA}	Volatile solids concentration adjusted with volatile fatty acids concentration

1. Background to Research

Biogas production has increased in Australia substantially in the past 10 years, resulting in greater needs for understanding effluent management. Biogas yield is determined by the mass and characteristics of the volatile solids (VS) that flow into the system. To optimise these systems, it is necessary to be able to accurately measure the "feedstock" for biogas production, which is the mass of VS in piggery effluent. VS are a mixture of many different compounds, some excreted in manure and others contributed by feed waste. Some research such as Vedrenne et al. (2008) has shown that volatile fatty acids (VFAs) can be 25% or more of total VS and are subject to loss from the system during laboratory testing. Birchall (2010) performed calculations at Bear's Lagoon and estimated that 22% of the mean influent chemical oxygen demand (COD) was from VFAs. VFAs can volatilise at low temperatures and can be lost in the effluent system or at the laboratory stage (Figure 1). The loss of VFA in the effluent system may occur before effluent is flushed to a covered anaerobic pond (CAP) or digester, which would result in a lower biogas yield, while losses in the laboratory stage would result in an underprediction of VS, which would make it difficult to accurately identify sources of inefficiency in the process, or to predict methane gas potential in biogas systems.

Based on the research of Vedrenne et al. (2008) it has been postulated that VS analysis of effluent using standard laboratory methods (Standard Method: 2540) at Australian NATA accredited laboratories may result in under prediction of the VFA component of VS (i.e. up to 25% by mass) when the sample is dried (i.e. in moisture and total solid (TS) determination.

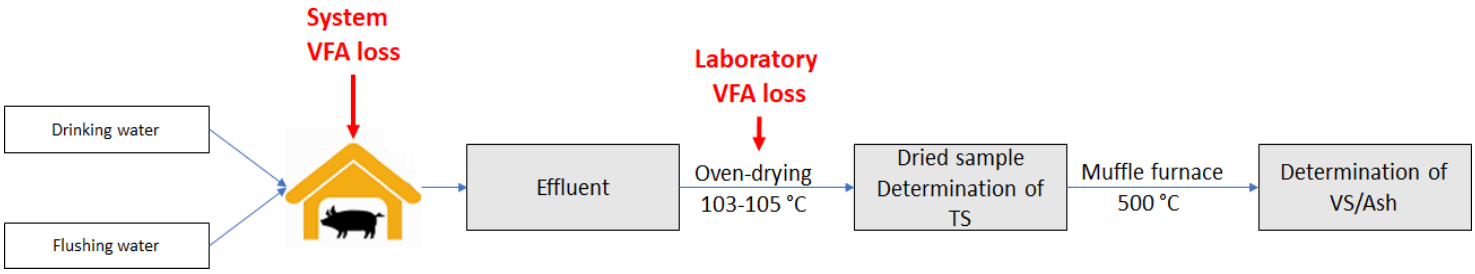


Figure 1 VFA effluent system loss and laboratory stage in piggeries

This problem was first identified as a risk by McGahan et al. (2010) (APL project 4446) based on a review of the relevant literature. However, because standard laboratory methods must be used when conducting research and are typically used by consultants and producers because there is no easy alternative, the potential for this error persists.

1.1 Manure characteristics

The chemical composition of manure is dependent on many factors, including the type, breed and age of the animals, feed availability, feed characteristics (including ash, dry matter digestibility and crude protein), and farming methods (Sánchez and González 2005). Manure is a mixture of urine and faeces. Within a manure management system (MMS) urine and faeces may also be mixed with waste feed and flushing water. Depending on the MMS used, this may be handled in a liquid or a solid form. Liquid (effluent) MMSs use water as a means of transporting and treating manure. Because of the high organic loading associated with manure systems, these systems operate anaerobically. Systems that handle

manure as a solid do not utilise water as a transport mechanism and may use bedding material to absorb excess moisture in urine and manure to maintain a predominantly aerobic environment.

The compounds in effluent and solid manure fractions can be partitioned into different physical components, as described by the following matrix adapted from Taiganides (1977), cited in Birchall (2010):

TS	=	VS	+	Ash
II		II		II
SS	=	VSS	+	FSS
+		+		+
SS	=	VDS	+	FDS

Where;
TS = total solids
VS = (total) volatile solids
Ash = (total) fixed solids
SS = (total) suspended solids
VSS = volatile suspended solids
FSS = fixed suspended solids
TDS = total dissolved solids
VDS = volatile dissolved solids
FDS = fixed dissolved solids.

The characteristics of effluent and manure fractions can be characterised by these components, as explained briefly below.

Total solids (TS):

The total solids content of manure is the mass of solids remaining after a sample has been dried in a 103 C oven for 24 hours ("dry weight") and is comprised of both suspended solids (SS), and total dissolved solids (TDS).

Volatile solids (VS):

The volatile solids component is the biodegradable organic matter or degradable component. It is determined by the quantity of TS burnt or driven off when a material is heated to 550 C for at least 1 hour.

Fixed solids (FS):

The fixed solids constitute the residual inorganic compounds (N, P, K, Ca, Cu, Zn, Fe etc.) in a suspended or dissolved state.

Suspended solids (SS):

Particles that are retained on filters with pore size of 1 µm.

Total dissolved solids (TDS):

All dissolved solids (TDS) are ions. There is a strong correlation between TDS and the electrical conductivity of effluents.

Piggery effluent consists of water, manure, urine and waste feed, and is approximately 5% total solids and 95% water by mass (Figure 1) (Kruger et al. 1995). The TS component consists of organic material (VS) and inorganic material (ash).

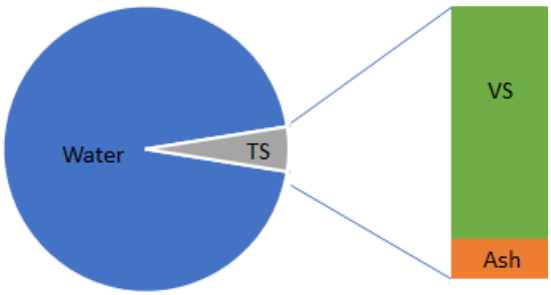


Figure 1 A schematic figure showing the relationship between the TS content, the VS content and the ash content, based on Kruger et al. (1995)

VS can consist of many organic compounds, including: organic matter, volatile fatty acids (VFA), ammonia, sulphurous compounds, phenols and indoles, and volatile amines (Le et al. 2005). The organic carbon component of VS represents the part of the TS which can be converted to methane with anaerobic digestion (Figure 2).

TS and VS measurements are conducted by biogas operations for three main purposes (Vahlberg et al. 2013):

1. Substrate measurements (to monitor the quality of the substrate, organic loading rate and estimation of biogas production).
2. Digestion measurements (to monitor the process stability).
3. Digestate measurements (to evaluate the quality of the bio-fertilizer).

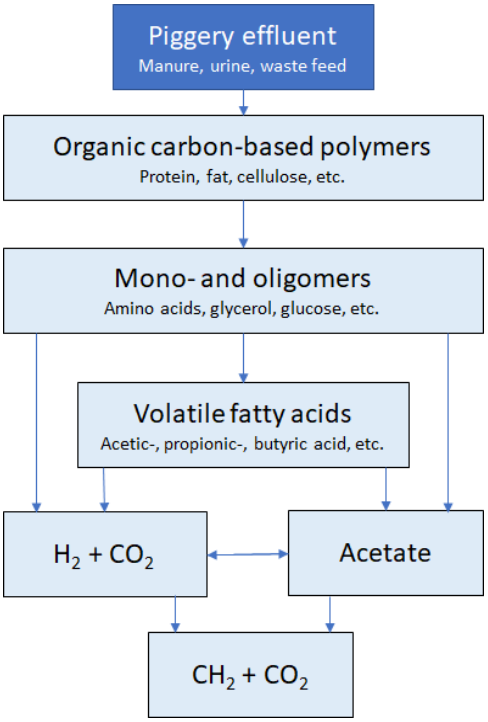


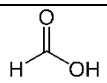
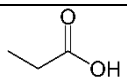
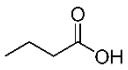
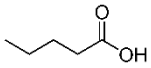
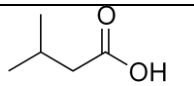
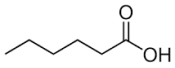
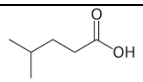
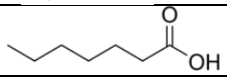
Figure 2 Degradation of organic carbon matter to methane and carbon dioxide under anaerobic conditions (Vahlberg et al. 2013)

Briefly, the use of anaerobic digestion to produce methane is a complex process with a number of biochemical reactions conducted by microorganisms in anaerobic conditions (Khan et al. 2016). The four major stages are: 1. bacterial hydrolysis, 2. acidogenesis, 3. acetogenesis, and 4. methanogenesis. The hydrolysis stage encompasses the enzyme-facilitated conversion from suspended carbohydrates, proteins and fats into soluble amino acids, sugars and fatty acids (Adekunle and Okolie 2015). During the acidogenesis stage, bacteria converts these products into hydrogen, CO_2, acetates and VFAs (Adekunle and Okolie 2015; Khan et al. 2016). The acetogenesis stage involves the conversion of VFAs (acetic, propionic, and butyric acid) and alcohol into acetate, hydrogen gas and carbon dioxide (Sun et al. 2016; Wu et al. 2016). The methanogenesis stage transforms the acetate produced in acetogenesis into methane and carbon dioxide and then converts hydrogen and carbon dioxide into methane (André et al. 2016). VFAs are important intermediate compounds in the production of methane and are of interest because there is the potential for loss during the VS/TS measurement process.

1.2 VFA characteristics

VFAs are carboxylic acids consisting of a hydrocarbon chain and a terminal carboxyl group, usually produced from microbial carbohydrate digestion in ruminants or the anaerobic digestion of manure. Table 1 presents an overview of common VFAs. The longer chain VFAs have a higher boiling point than shorter chain VFAs. The boiling point represents the temperature where most VFA losses occur, however there is a range above and below the boiling point where VFA losses will also occur. Vahlberg et al. (2013) showed that the volatility (percent lost) of several common VFA ranged from 0-33% during drying when analysing for VS. Thus, while the drying temperature of 103-105°C in the Standard Method for TS/VS assessment is lower than the boiling point of most common VFAs listed, there will still be VFA losses.

Table 1 Overview of common volatile fatty acids

Common VFAs	**Structural formula**	**Mass (g/mol)**	**Diagram**	**Boiling point**	**Volatility (%) at 103-105°C, *Vahlberg et al.,* (2013)**
Formic acid	HCOOH	46.03		100°C	Not measured
Acetic acid	CH3COOH	60.05		118°C	0
Propionic acid	CH_3CH_2COOH	74.08		141°C	9
Lactic acid	$CH_3CH(OH)CO_2H$	90.08		122°C	Not measured
Butyric acid	$CH_3(CH_2)_2COOH$	88.11		163°C	15
Iso-butyric acid	$(CH_3)_2CHCOOH$	88.11		155°C	33
Valeric acid	$CH_3(CH_2)_3COOH$	102.13		186°C	0
Iso-valeric acid	$(CH_3)_2CHCH_2COOH$	102.13		176°C	17
Hexanoic acid	$CH_3(CH_2)_4COOH$	116.16		205°C	0
Iso-hexanoic acid	$C_6H_{12}O_2$	116.16		201°C	20
Heptanoic acid.	$C_7H_{14}O_2$	130.19		223°C	17

1.3 Review of current methods for measuring VS, TS and Ash

1.3.1 Standard method 2540 for determining TS (105°C method)

The current method used in Australian laboratories to determine total solids and moisture content is Standard Method 2540. This method is detailed in the "*Standard methods for the examination of water and wastewater* (Vol. 21)" (APHA et al. 2012) (Figure 3). Samples are dried at 103- 105°C to determine the TS of the sample. This is the step where volatile substances, including VFAs, are potentially lost, introducing mass errors. The sample is then incinerated at 550 °C to remove all organic matter to determine the ash content of the sample. The VS portion of the sample is burnt off and only the ash remains. VS is back calculated from TS minus the ash content.

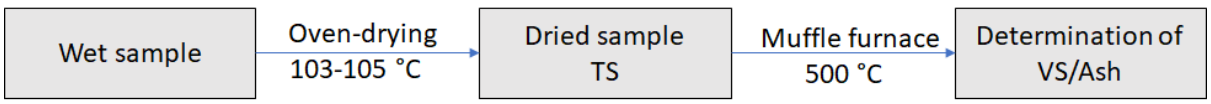


Figure 3 Overview of Standard method 2540 (105°C method)

1.3.2 Temperature modified method (60°C)

The temperature modified method is a modified version of the current standard method for TS determination. The sample is dried at 60°C to determine the TS of the sample (Figure 4), with the aim of reducing the loss of volatiles during drying and improving the accuracy of the TS and VS assessment. After drying at 60°C, samples are transferred to a desiccator with a drying agent under vacuum to ensure that samples are dried, and weight was stable. This modification has been applied by Australian researchers (A. Skerman pers. comm.), however the method has not been published and is not used by NATA accredited laboratories. One unknown aspect of the low temperature method is the accuracy of sample drying and systematic application of this approach would need to ensure there is no potential for error.

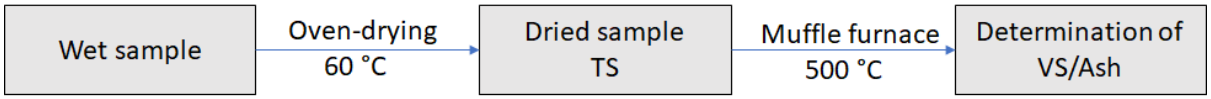


Figure 4 Overview of Temperature modified method (60°C)

1.3.3 Volatile fatty acid (VFA) adjusted method

This method adjusts the current standard method by determining VFA loss and correcting the VS to account for potential losses during drying, based on Vahlberg et al (2013) (Figure 5). Because the loss of VFAs is the primary issue of concern, this method separately analyses VFA concentrations in the wet sample and performs a comparison with the VFA concentration in the dried sample to determine the loss rate during drying. The VFA lost is the difference between the VFA concentration before drying minus the concentration of the rehydrated sample. This value is then used to adjust the VS measured using the Standard method 2540.

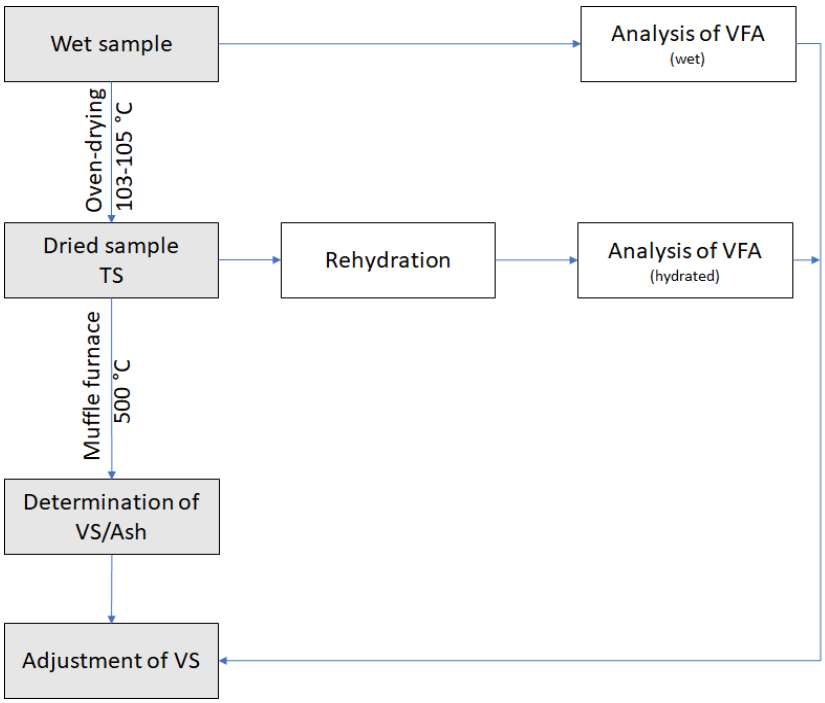


Figure 5 Overview of Volatile fatty acid (VFA) adjusted method, based on Vahlberg et al. (2013)

1.3.4 Standard Method to measure VFA

VFAs can be analysed by titration, distillation, steam distillation, and chromatography, with the latter giving the most precise and accurate results. While chromatography is the most accurate it requires solvent extraction (a complex sample processing) and highly specialised gas chromatography equipment, as well as extensive sample matrix analysis to quantify VFA concentrations, which makes this method expensive. Titration is the most common and cheapest method of VFA measurement in Australian laboratories. Distillation and steam distillation are not common methods in commercial laboratories because they are expensive and labour intensive. It should be noted that gas chromatography will give the concentration of 6-7 specific VFAs, while titration gives the total concentration of VFA. That is, titration would be expected to give a higher concentration than gas chromatography because it is a measure of all VFAs in sample rather than a few specific VFAs (Lützhøft et al. 2014). The specific VFAs measured by gas chromatography is dependent on laboratory, but usually will include some of the following: formic acid, acetic acid, propionic acid, lactic acid, butyric acid, iso-butyric acid, valeric acid, iso-valeric acid, hexanoic acid, iso-hexanoic acid and heptanoic acid. There has been no scientific investigation into the use of titration VFA measurements for VS correction in the literature. However, if found to be suitable, it would provide a method that can be applied at many laboratories at low cost.

2. Objectives of the Research Project

The objectives of this project were to:

1. Determine the potential error in VS levels by testing VFA levels and VFA losses from samples of effluent collected at Australian piggeries.
2. Identify and test commercial, laboratory methods that can be used to correct or replace current laboratory practices.
3. Develop a standard operating procedure based on the findings.
4. Summarise the technical findings in an industry report.

3. Research Methodology

Four alternative TS/VS methods were tested: 1. Standard method (SM), 2. Standard-VFA adjusted (SM-VFA), 3. Temperature modified (TM), and 4. Temperature modified-VFA adjusted (TM-VFA). Table 2 gives an overview of the VS methods and the abbreviations that will be used in the follow sections.

Table 2 VS method names and abbreviations

Method name	Abbreviation	Description
Standard method	SM	Standard Method 2540 where samples are dried at 103-105°C to determine the TS of the sample. This is the current method used in NATA laboratories.
Standard method - VFA adjusted	SM-VFA	Standard Method 2540 where samples are dried at 103-105°C to determine the TS of the sample. Final VS concentration is adjusted for VFA loss using (Vahlberg et al. 2013).
Temperature modified method	TM	Based on the Standard Method 2540, however samples are dried at 60°C to determine the TS of the sample.
Temperature modified method - VFA adjusted	TM- VFA	Based on the Standard Method 2540, however samples are dried at 60°C to determine the TS of the sample. Final VS concentration is adjusted for VFA loss using (Vahlberg et al. 2013).

Considering the reported losses of VFA when drying occurs at 105°C, it was hypothesised that the Temperature Modified method would reduce VFA losses and result in higher reported VS which would results in similar VS concentrations to the VFA adjusted method (Figure 6).

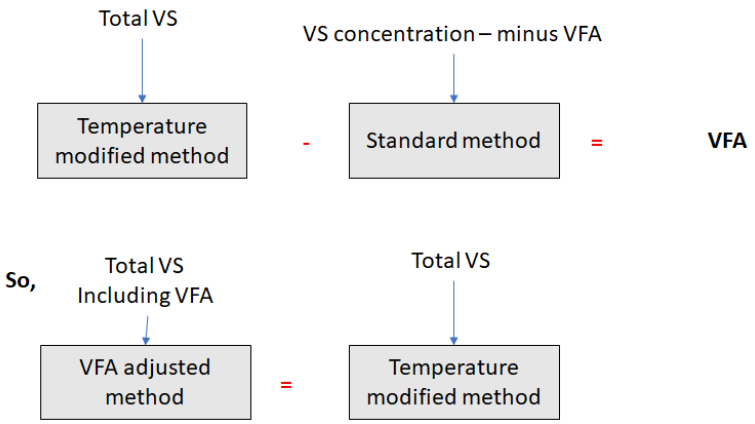


Figure 6 Overview of the Volatile fatty acid (VFA) adjusted method

Other parameters analysed

VFAs were measured with titration using Standard Method 2310B from the Standard Methods for the Examination of Water and Wastewater (Rice et al. 2012). Additionally, alkalinity, pH, sulphur,

phosphorus and ammonia were measured using Method 2320B, 4500, 3125, 4500- P and 4500- NH_3 from the Standard Methods for the Examination of Water and Wastewater(Rice et al. 2012).

3.1 Piggery selection

Differences are known to exist between piggeries with respect to biogas yield (Gopalan et al. 2013), and differences have been observed in VFA levels between different types of piggeries (i.e. breeder, weaner, finisher, see Vedrenne et al. (2008)). The highest VFA levels (and therefore the highest losses) are expected to occur from grower/finisher piggeries. Thus, sampling was conducted in duplicate at five different piggeries in Queensland (A-E), at one stage (grower/finisher stage). Piggeries were screened to check for use of unusual rations (i.e. high proportions of by-products), uncharacteristically poor herd productivity and unusually high feed waste (by checking FCRs). Wheat based diets rather than sorghum based were favoured to minimise differences with southern piggeries. Care was taken to consider the impacts of the sampling strategies, and standard practices were developed to minimise variations caused by different practices such as different volumes of recycled water, different flushing frequency and different effluent system types. All samples were tested in duplicate. Protocols used in previous effluent sampling experiments were used to ensure consistent homogenised samples of effluent were collected (McGahan et al. 2016). Table 3 shows key details for the piggeries sampled.

Table 3 Comparison of piggeries sampled

Grower/finisher piggery	Type	Number of pigs in unit sampled	FCR	Recycled water
A	Static pit	450	2.35	No
B	Pull plug	3,000	2.3-2.45	No
C	Pull plug	5,000	2.2-2.4	Yes
D	Pull plug	1300	2.35-2.45	Yes
E	Static pit	2500	2.3-2.5	No

Two sampling events were conducted. The first event sampled all five piggeries, while the second event sampled two piggeries on a second occasion. The two piggeries that were selected had the highest concentrations of VS and VFA, which allowed the investigation of temporal variation between two months. Additionally, in the first sampling event the mechanical sump stirrer was non-operational at piggery D, while in the second sampling event it was operational. This allowed for the investigation into the effect of stirring on VS and VFA concentrations. Table 4 shows an overview of the duplicate sample collected and the sample name that will be used in the following report.

Table 4 Corresponding sample number for each sampling event and duplicate

Grower/finisher piggery	Sampling event 1	Sampling event 2
	Sample name	Sample name
A	1 and 2	-
B	3 and 4	-
C	5 and 6	11 and 12
D	7 and 8	13 and 14
E	9 and 10	-

3.2 Effluent sampling procedure

Effluent water was sampled from either a flowing effluent stream or a sump. All sampling equipment was cleaned prior to sample collection. The following protocol was used:

1. Effluent to be sampled from a flowing effluent stream, sampled continuously OR a sump that is currently being mixed.
2. For flowing effluent: Determine the total duration of the effluent flow period.
3. Calculate sampling interval to achieve 20L sample with over 20 subsamples
4. Combine all sub-samples into one composite sample in a 20L drum
5. Stir 20L drum to ensure sample is homogenised and collect 3x 1L subsamples.
6. All samples were stored and transported at below 4°C.

3.2.1 Recycled water

In the piggeries that used recycled water for flushing, a sample of recycled water was analysed for VS so that total VS could be adjusted with the residual VS from ponds. Recycled water was sampled from the flushing holding tank in piggery sheds. All sampling equipment was cleaned prior to sample collection. The following protocol was used:

1. Sampling gear was rinsed with the recycling water, which additionally flushed the tap apparatus with fresh sample.
2. One litre of recycling water was collected.
3. All samples were stored and transported at below 4°C.

3.3 Laboratory Analysis

Chemical analysis of VS and VFA was conducted by a commercial laboratory, using adaptions of existing laboratory methods, to ensure the methods were commercially feasible. The methods are described as follows.

1. Method 1: SM (Standard method): This is the current method that is used in Australian laboratories. The sample was dried at 103-105°C to determine the TS of the sample. The sample in then incinerated at 550°C to remove all organic matter to determine the ash content of the sample. VS was determined from the mass difference between the TS sample and the residual ash sample.
2. Method 2: SM-VFA (Standard method adjusted by VFA based on Vahlberg (2013)): VFA concentrations were determined in the wet sample and compared to the VFA concentrations in the dried sample to VFA determine the loss rate during drying. The loss rate was used to adjust the VS measured using the Standard Method (APHA 2540), in accordance with Vahlberg et al. (2013).
3. Method 3: TM (Temperature modified method): This is a modified version of the current standard method. The sample was dried at 60°C to determine TS. The sample was then incinerated at 550°C to remove all organic matter to determine the ash content of the sample. VS was determined from the mass difference between the TS sample and the residual ash sample.
4. Method 4: TM-VFA (Temperature modified method adjusted by VFA based on Vahlberg (2013)): VFA concentrations were determined in the wet sample and compared to the VFA

concentrations in the dried sample to VFA determine the loss rate during drying. The loss rate was used to adjust the VS measured using the TM method, in accordance with Vahlberg et al (2013).

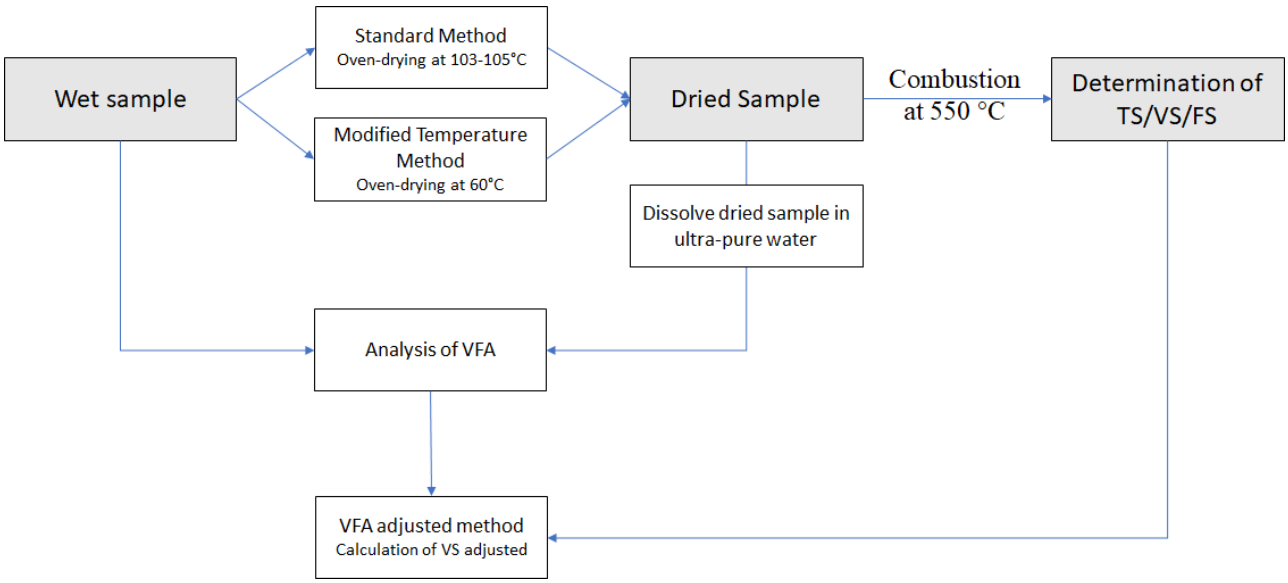


Figure 7 Overview of sample processing with the three methods

4. Results

4.1 Standard method and VFA adjustment

The sample characteristics of raw (original sample, no treatment) and SM re-hydrated samples 11-14 are presented in Table 5. On average, 76% (±6%) of dissolved organic carbon , 88% (±4%) of VFA, 96% (±0.3%) of ammonia and 72% (±1%) of sulphur was lost during the drying of the effluent samples at 105°C (Figure 8). Analysis of the total dissolved organic carbon on wet and dried piggery effluent showed a loss of dissolved organic carbon after drying (Figure 9). The carbon content of the wet effluent samples ranged from 8,440 to 5,730 mg/L, while after drying at 105°C the carbon content decreased to between 1,340 and 1,910 mg/L. The difference in dissolved organic carbon between wet and dried samples allowed the carbon mass loss to be determined. When the VFA loss was used to adjust the dissolved organic carbon concentrations in dried samples to account for this loss in organic matter, which resulted in recovery of 98.5% of the dissolved organic carbon content of the original wet effluent sample was accounted for from the105°C dried samples. This indicates that VFA loss was a significant component of dissolved organic carbon loss (Figure 10).

Table 5 Comparison of the characteristics of the effluent for SM drying process

Sample	Dissolved organic carbon (mg/L)		VFA (mg/L)		Ammonia (mg/L)		Sulphur (mg/L)	
	Raw	SM	Raw	SM	Raw	SM	Raw	SM
11	6,060	1,780	4,880	975	2350	87	253	72
12	5,730	1,610	5,250	750	2200	87	255	68
13	7,940	1,340	7,130	825	2800	94	461	126
14	8,440	1,910	7,050	1,050	3080	108	470	140

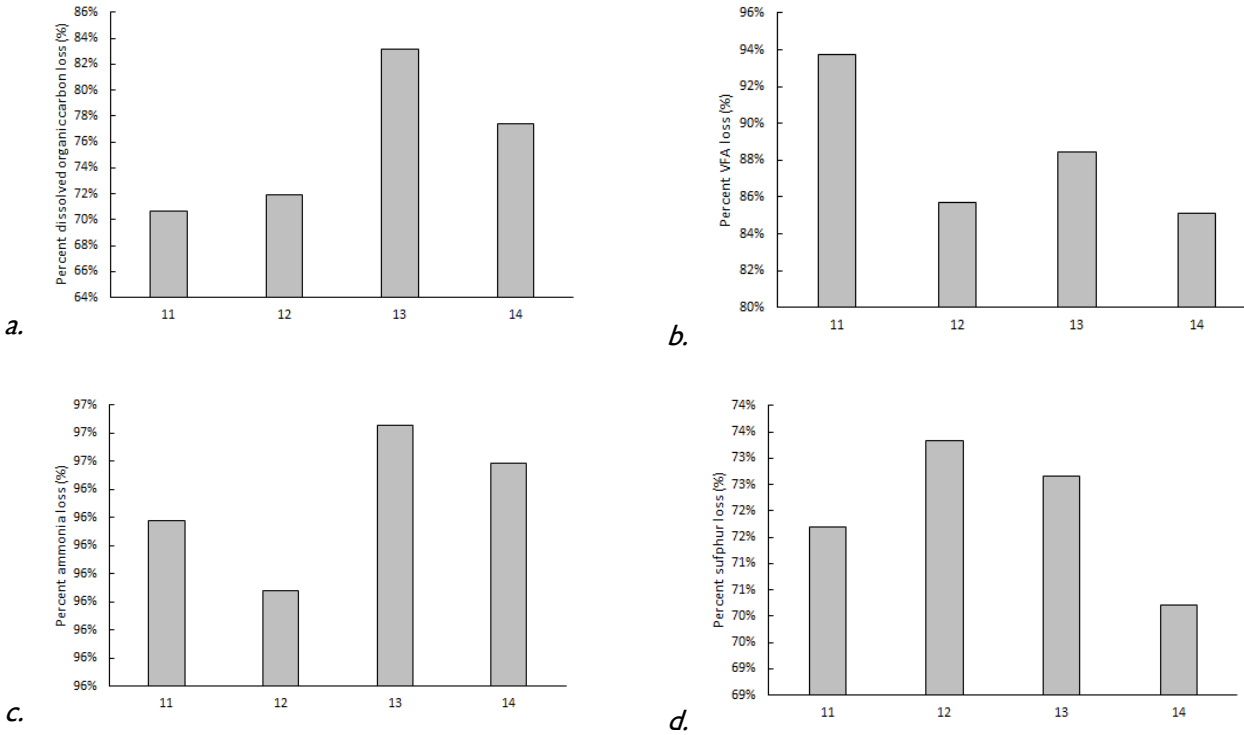


Figure 8 Percent losses of selected volatile components caused by SM drying process: a. dissolved organic carbon, b. VFA, c. ammonia and d. sulphur losses, in samples 11-14

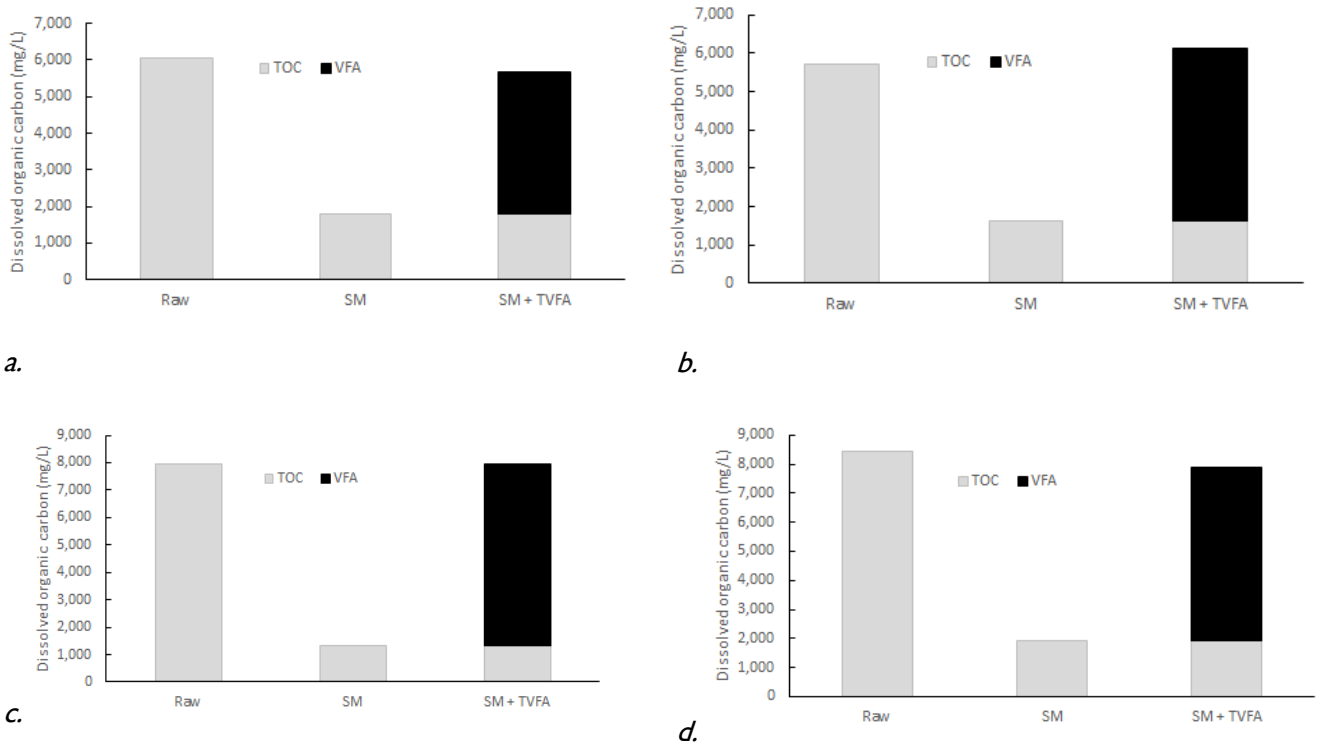


Figure 9 Estimated dissolved organic carbon as VFA compared to dissolved organic carbon content for methods (wet effluent: direct determination on the effluent sample, dried effluent: determination on the solid after drying at 105oC) for a. sample 11, b. sample 12, c. sample 13, and d. sample 14

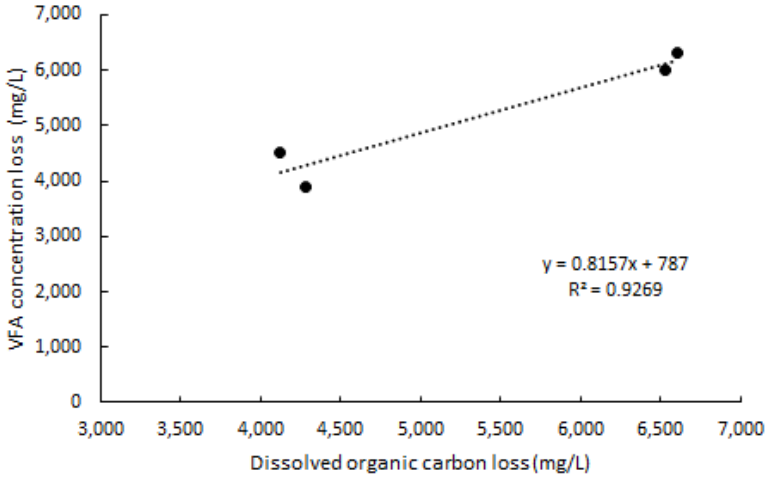


Figure 10 Regression between dissolved organic carbon loss and VFA concentrations loss after drying at as VS at 105oC

Compensation for VFA loss can be made by determining the loss (volatility) of the VFA in effluent samples and adjusting the TS and VS values, resulting in a more accurate estimation of VS. The results of the application of the Vahlberg et al. (2013) method to correct VS concentration with VFA loss of

SM samples are presented in Table 6 and Figure 11. The volatility of VFA ranged from 50-89% (average 65% ±16%).

In the following statistical analysis duplicates have been averaged before analysis, and sample 13 and 14 were excluded to ensure the analysis was not skewed (sample 13 and 14 were effectively a duplicate of sample 7 and 8, while samples 11 and 12 were taken under sufficiently different management conditions (use of mechanical stirring in the effluent system) to warrant inclusion in the dataset. The relative differences between the VS and VS_{VFA} concentrations (i.e. comparing the results of SM and SM-VFA) varied between 0-15% for the piggery effluent analysed. That is, the SM VS concentrations were under-estimated by 0-15%. VS underestimation was higher in piggery effluent with a larger TS/VS and VFA/TS ratios. That is, samples with a relatively high VFA concentration and consequently a high volatile organic content in relation to the TS content had higher error in VS measurements. On average, VS was under-estimated by 6% (±4%), with the standard deviation being 75% of the mean. A paired-samples t-test was conducted to compare the VS concentrations results of SM and SM-VFA and it showed that there were significant differences between SM and SM-VFA methods at the 0.05 level.

The VS concentrations for SM and SM-VFA were converted to their corresponding estimated dissolved organic carbon concentrations. By analysing both VS (converted to dissolved organic carbon) and dissolved organic carbon, the mass balance of dissolved organic carbon within the samples was calculated. Specifically, adjusting the dissolved organic carbon content with a sample corresponding SM VS, dissolved organic carbon corresponds to 95% (±6%) (ranging from 88-102%) of the wet sample dissolved organic carbon concentration. Adjusting the dissolved organic carbon content with a sample corresponding SM-VFA, VS dissolved organic carbon corresponds to 98% (±5%) (ranging from 91-106%) of the wet sample dissolved organic carbon concentration. This confirms that the underestimation of VS measurement SM when compared to the SM-VFA values is a real phenomenon and the adjustment of VS with VFA concentrations with the SM-VFA method can increase the accuracy of VS estimation.

Table 6 Calculated TS-adj and VS-adj values after compensation for the VFA evaporation in the SM. The errors in the TS and VS values are also shown

Day	Sample	VFA (mg/L)	VS (mg/L)	TS (%)	VS (% of TS)	VFA-vol (%)	TS-adj (%)	VS-adj (% of DM)	TS/VS ratio	TS-error (%)	VS-error (%)	VS_{VFA} (mg/L of TS)
1	1	2,850	32,140	4.01	80.13	56.49	4.17	80.90	1.38	3.86%	4.77%	33,673
	2	2,850	32,440	4.04	80.34	56.49	4.20	81.09	1.35	3.83%	4.73%	33,974
	3	2,630	37,420	4.48	83.45	51.52	4.62	83.94	1.25	2.93%	3.49%	38,728
	4	2,700	38,270	4.59	83.32	50.00	4.73	83.80	1.28	2.86%	3.41%	39,574
	5	4,350	28,020	3.80	73.68	53.33	4.04	75.19	1.24	5.75%	7.65%	30,163
	6	4,430	27,950	3.79	73.77	54.18	4.03	75.33	1.23	5.96%	7.91%	30,160
	7	5,290	46,090	5.80	79.40	57.47	6.11	80.42	1.19	4.98%	6.19%	48,942
	8	5,630	46,810	5.92	79.03	60.04	6.26	80.16	1.19	5.40%	6.73%	49,962
	9 [a]	175	5,460	0.80	66.50	0.00	0.80	66.50	1.33	0.00%	0.00%	5,460
	10 [a]	175	5,420	0.78	68.21	0.00	0.78	68.21	1.33	0.00%	0.00%	5,420
2	11 [b]	4,880.00	27,000	3.89	69.50	80.02	4.28	72.28	1.24	9.12%	12.62%	30,408
	12 [b]	5,250.00	28,200	3.98	70.90	85.71	4.43	73.86	1.25	10.16%	13.75%	32,079
	13 [c]	7,130.00	35,200	4.58	76.90	88.43	5.21	79.70	1.50	12.10%	15.18%	40,545
	14 [c]	7,050.00	37,000	4.79	77.20	85.11	5.51	78.03	1.47	13.04%	13.96%	42,165

VFA-vol: VFA volatility
TS-adj: TS VFA adjusted percent
VS-adj: VS VFA adjusted percent
TS-error: TS error in original TS measurement
VS-error: VS error in original VS measurement
VS_{VFA} : VS concentration adjusted with VFA concentrations
a: [a] the VFA results for this piggery are likely to be close to the detection limit of this method and would need further testing to determine accuracy
b: replicate of samples 5 and 6 two months apart
c: replicate of samples 7 and 8 two months apart with the mechanical stirrer operational

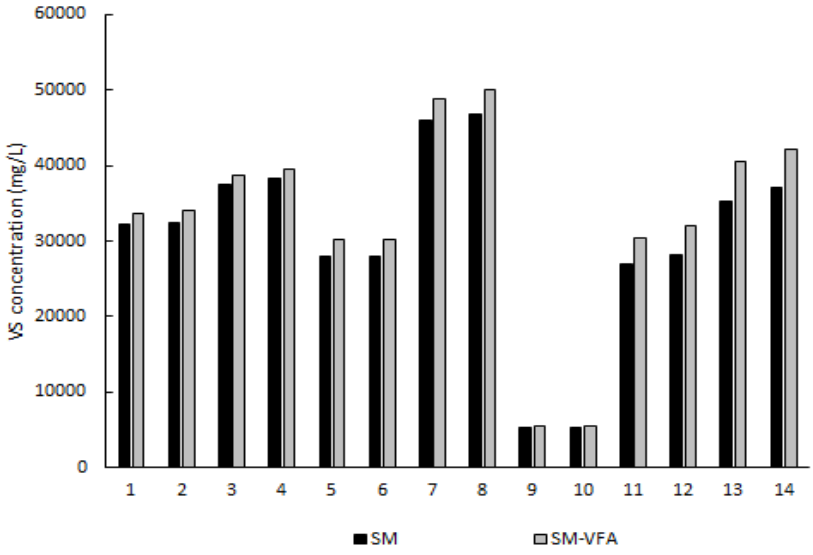


Figure 11 Comparison of VS concentrations from SM and SM-VFA

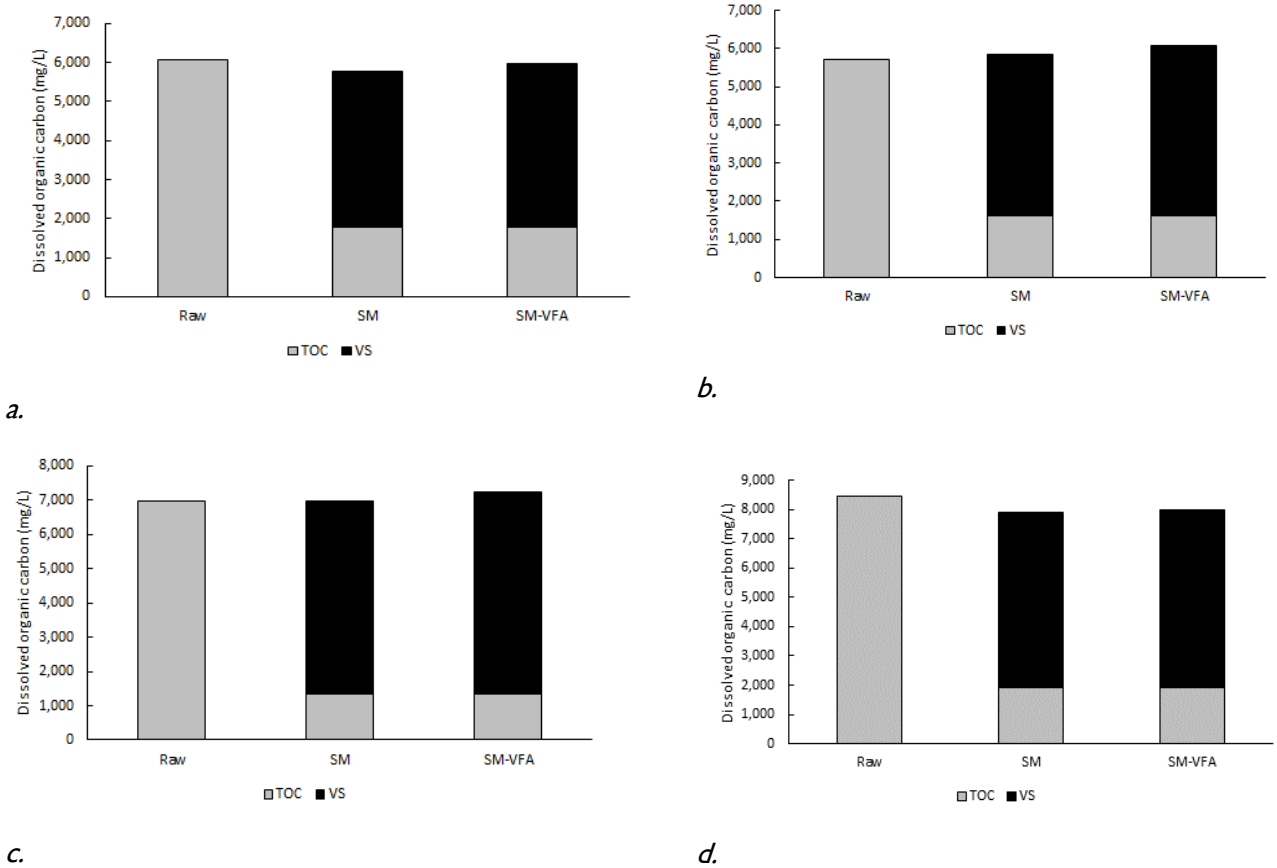


Figure 12 Estimated dissolved organic carbon as VS compared to dissolved organic carbon content for methods (raw: direct determination on the effluent sample, SM and SM-VFA: determination on the solid after drying at 105oC) for a. sample 11, b. sample 12, c. sample 13, and d. sample 14.

4.2 Temperature modified method and VFA adjustment

Considering the reported losses of VFA when drying occurs at 105°C, it was hypothesised that the TM (drying at 60°C) would reduce VFA losses and result in higher reported VS, which would result in similar VS concentrations to the SM-VFA adjusted method. This would negate the need for VFA analysis, potentially reducing analysis costs and complexity. Figure 13 shows the differences in VFA percent losses between the SM (drying at 105◦C) and TM (drying at 60 ◦C) method. The TM method was found to result in slightly lower VFA losses than the SM approach (6% lower losses) which was insufficient to substantially reduce overall error from the drying process.

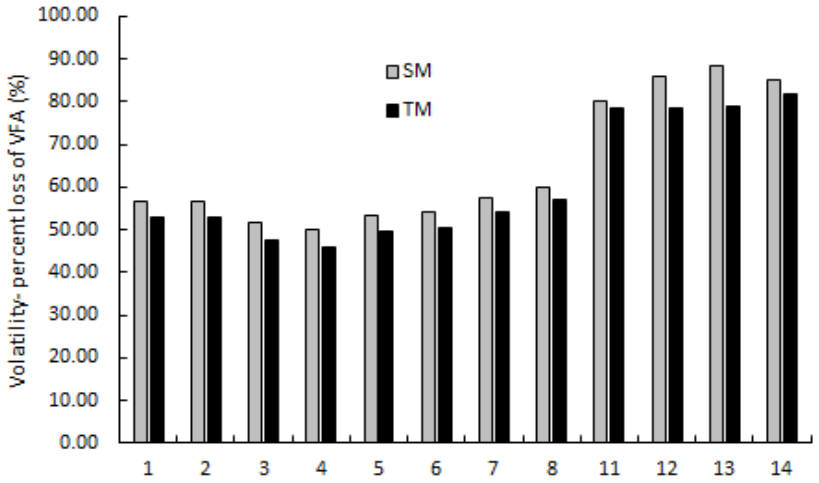


Figure 13 Comparison of the volatility (percent loss) of VFA in the SM (drying at 105oC) and TM (drying at 60oC)

The sample characteristics of raw (original sample, no treatment) and TM re-hydrated samples (11-14) are presented in Table 7. On average, 69% (±7%) of dissolved organic carbon , 83% (±6%) of VFA, 93% (±2%) of ammonia and 74% (±5%) of sulphur was lost during the drying of the effluent samples at 60◦C (Figure 14). Analysis of the total dissolved organic carbon on wet and dried piggery effluent showed a loss of dissolved organic carbon after drying (Figure 15). The carbon content of the wet effluent samples ranged from 8,440 to 5,730 mg/L, while after drying at 60◦C the carbon content decreased to 1,910 to 2,340 mg/L. The difference in dissolved organic carbon between wet and dried samples allowed the carbon mass loss to be determined. When the VFA loss was used to adjust the dissolved organic carbon concentrations in dried samples, to account for this loss in organic matter, 99.5% (±7) of the dissolved organic carbon content of the original wet effluent sample was accounted for from the105◦C dried samples. This indicates that VFA loss was a significant component of dissolved organic carbon loss (Figure 15).

Compensation for VFA loss can be made by determining the loss (volatility) of the VFA in effluent samples and adjusting the TS and VS values, resulting in a more accurate estimation of VS. The results of the application of Vahlberg et al. (2013) method to correct VS concentration with VFA loss of TM samples is presented in Table 1 and Figure 17a. The volatility of VFA ranged from 50-82% (average 60% ±14%).

In the following statistical analysis, duplicates have been averaged before analysis as described previously. The relative differences between the VS and VS_{VFA} concentrations (i.e. comparing the results of TM and TM-VFA) varied between 0-13% for the piggery effluent analysed. That is, the SM VS concentrations were 0-13% underestimated. On average, VS was 5% (±4%) underestimated, with the standard deviation being 90% of the mean. A paired-samples t-test was conducted to compare the VS concentrations results of the TM and TM-VFA and it showed that there were significant differences between the TM and TM-VFA methods at the 0.05 level.

The VS concentrations for TM and TM-VFA were converted to their corresponding estimated dissolved organic carbon concentrations. By analysing both VS (converted to dissolved organic carbon) and dissolved organic carbon, the mass balance of dissolved organic carbon within the samples was calculated. Specifically, adjusting the dissolved organic carbon content with a sample corresponding TM VS dissolved organic carbon corresponds to 103% (±4%) (ranging from 97-107%) of the wet sample dissolved organic carbon concentration. Adjusting the dissolved organic carbon content with a sample corresponding TM-VFA VS dissolved organic carbon corresponds to 106% (±5%) (ranging from 100-113%) of the wet sample dissolved organic carbon concentration. The overestimation of the average carbon content in the temperature modified methods, indicates that non-carbon components (for example, ammonia and sulphurous compounds) could be contributing to the VS weight.

Comparison of TM and TM-VFA to SM-VFA VS concentrations showed that these methods overestimated VS concentrations by 24% and 26% respectively, in all samples but one (Figure 17b). The differences between VFA losses in the SM/SM-VFA and TM/TM-VFA methods did not reflect the differences in their VS concentrations. That is, the differences between SM and TM are not solely due to the differences in VFA losses. The carbon balance indicates this difference may be due to non-carbon components. Overall, both the TM and TM-VFA were found to overestimate the VS concentrations in the present experiment, and further research would be required to correct for these errors if this method was to be applied for effluent testing.

Table 7 Comparison of the characteristics of the effluent for TM drying process

Sample	**Dissolved organic carbon (mg/L)**		**VFA (mg/L)**		**Ammonia (mg/L)**		**Sulphur (mg/L)**	
	Raw	**TM**	**Raw**	**TM**	**Raw**	**TM**	**Raw**	**TM**
11	6,060	3,840	4,880	3830	2350	2,215	253	170
12	5,730	3,730	5,250	4120	2200	2,069	255	189
13	7,940	5,600	7,130	5630	2800	2,549	461	345
14	8,440	6,580	7,050	5,770	3080	2,901	470	368

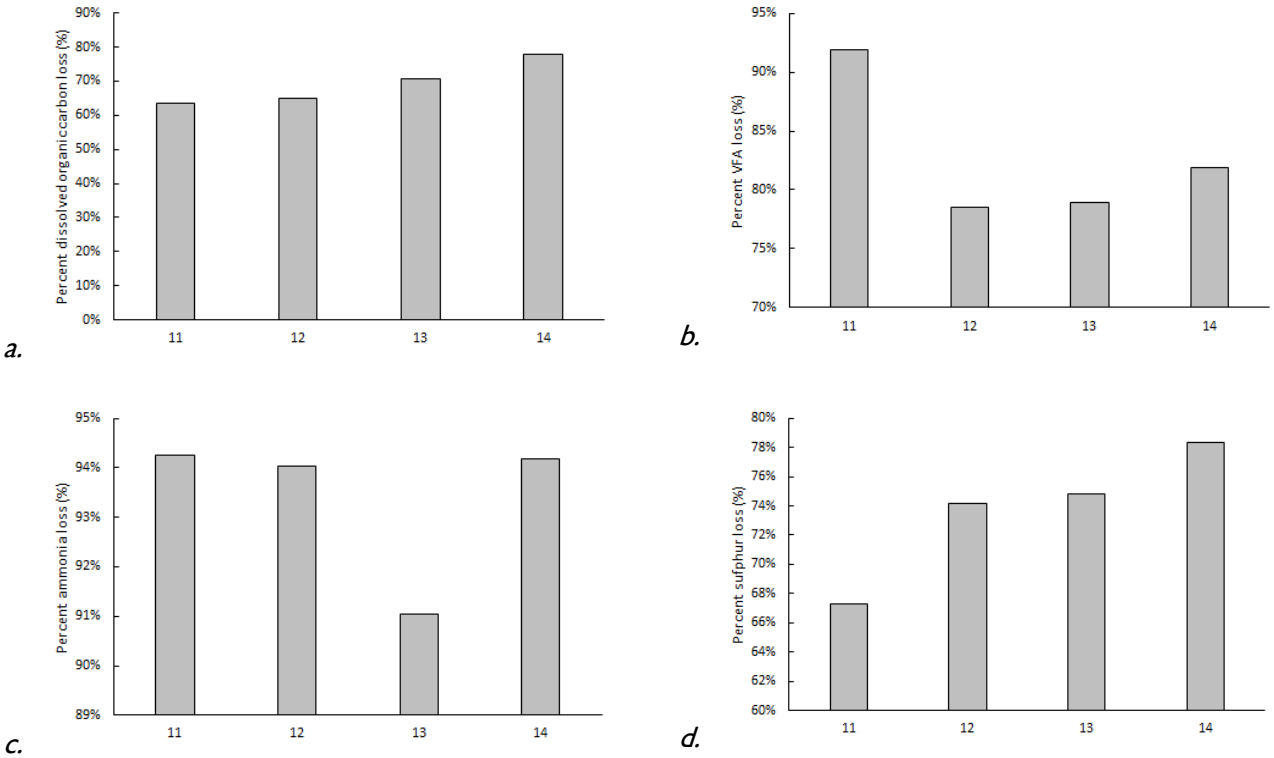


Figure 14 Percent losses of selected volatile components caused by TM drying process: a. dissolved organic carbon, b. VFA, c. ammonia and d. sulphur losses, in samples 11-14

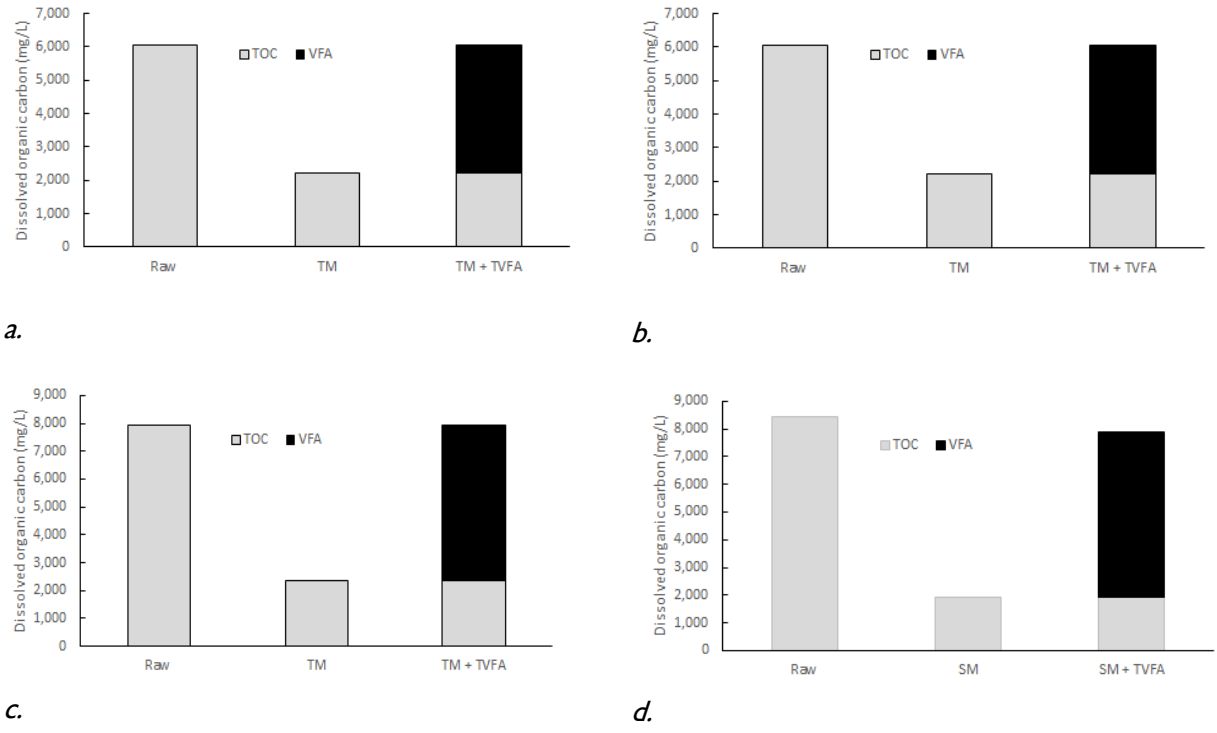


Figure 15 Estimated dissolved organic carbon as VS compared to dissolved organic carbon content for methods (raw: direct determination on the effluent sample, SM and SM-VFA: determination on the solid after drying at 60oC) for a. sample 11, b. sample 12, c. sample 13, and d. sample 14

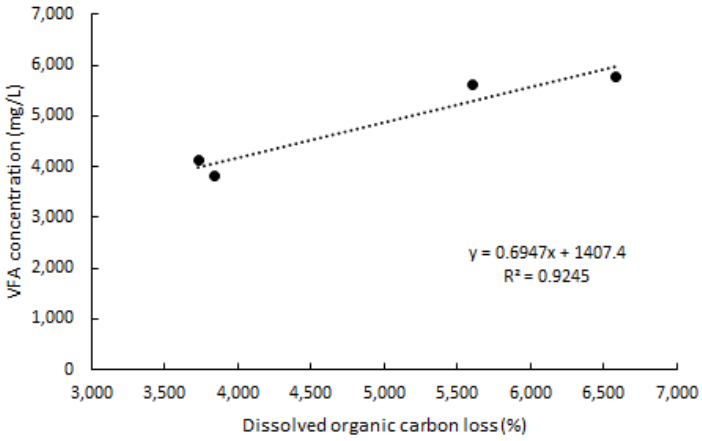


Figure 16 Regression between dissolved organic carbon loss and VFA concentrations loss after drying at as VS at 60oC

Table 8 Calculated TS-adj and VS-adj values after compensation for the VFA evaporation in the TM. The errors in the TS and VS values are also shown

		VFA	VS	TS	VS	VFA-vol	TS-adj	VS-adj	TS/VS	TS-error	VS-error	VS_{VFA}
Day	**Sample**	**(mg/L)**	**(mg/L)**	**(%)**	**(% of TS)**	**(%)**	**(%)**	**(% of DM)**	**ratio**	**(%)**	**(%)**	**(mg/L of TS)**
1	1	2,850	47,840	5.59	85.63	53.01	5.74	86.01	1.40	2.63%	3.06%	49,305
	2	2,850	44,010	5.20	84.65	53.01	5.35	85.08	1.38	2.82%	3.32%	45,471
	3	2,630	41,050	4.87	84.36	47.64	4.99	84.75	1.24	2.51%	2.96%	42,266
	4	2,700	43,950	5.14	85.44	46.00	5.27	85.78	1.24	2.36%	2.75%	45,158
	5	4,350	29,390	3.93	74.76	49.60	4.15	76.08	1.16	5.20%	6.84%	31,400
	6	4,430	31,150	4.13	75.52	50.51	4.35	76.77	1.18	5.15%	6.70%	33,238
	7	5,290	92,140	10.42	88.43	54.06	10.71	88.74	1.18	2.67%	3.01%	94,914
	8	5,630	83,680	9.60	87.19	56.84	9.92	87.61	1.17	3.23%	3.68%	86,762
	9 [a]	175	5,460	0.81	67.08	0.00	0.81	67.08	1.31	0.00%	0.00%	5,460
	10 [a]	175	5,420	0.81	66.83	0.00	0.81	66.83	1.30	0.00%	0.00%	5,420
2	11 [b]	4,880	25,600	3.73	68.60	78.48	4.11	71.52	1.13	9.31%	13.02%	28,933
	12 [b]	5,250	26,800	3.84	69.80	78.48	4.25	72.73	1.14	9.69%	13.32%	30,371
	13 [c]	7,130	38,100	4.83	78.70	78.96	5.39	80.92	1.49	10.44%	12.90%	43,015
	14 [c]	7,050	40,100	5.12	78.40	81.84	5.70	80.59	1.50	10.13%	12.57%	45,140

VFA-vol: VFA volatility
TS-adj: TS VFA adjusted percent
VS-adj: VS VFA adjusted percent
TS-error: TS error in original TS measurement
VS-error: VS error in original VS measurement
VS_{VFA} : VS concentration adjusted with VFA concentrations
a: [a] the VFA results for this piggery are likely to be close to the detection limit of this method and would need further testing to determine accuracy
b: replicate of samples 5 and 6 two months apart
c: replicate of samples 7 and 8 two months apart with the mechanical stirrer operational

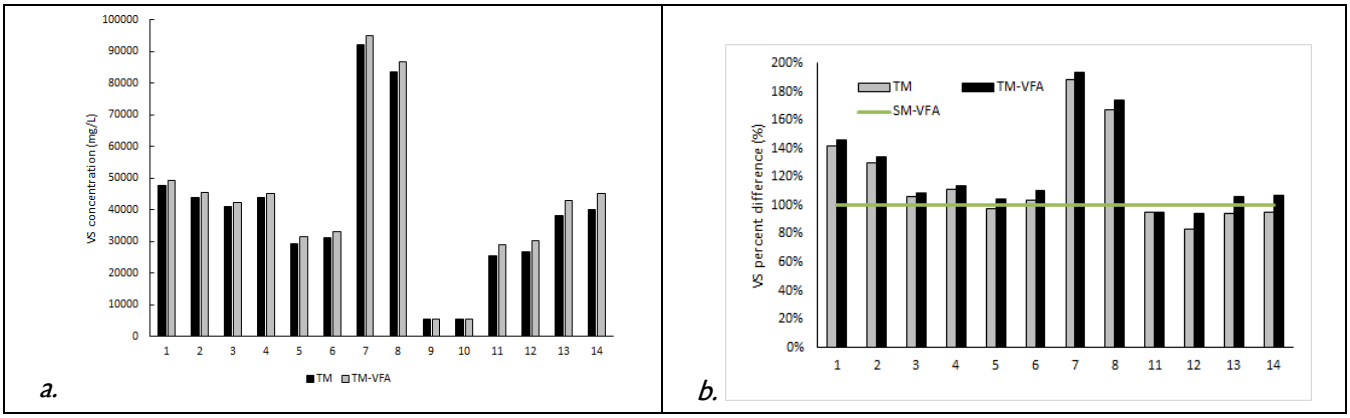


Figure 17 Comparison of VS from TM and TM-VFA: a. VS concentrations and b. VS concentrations of TM and TM-VFA relative to VS concentrations of SM-VFA

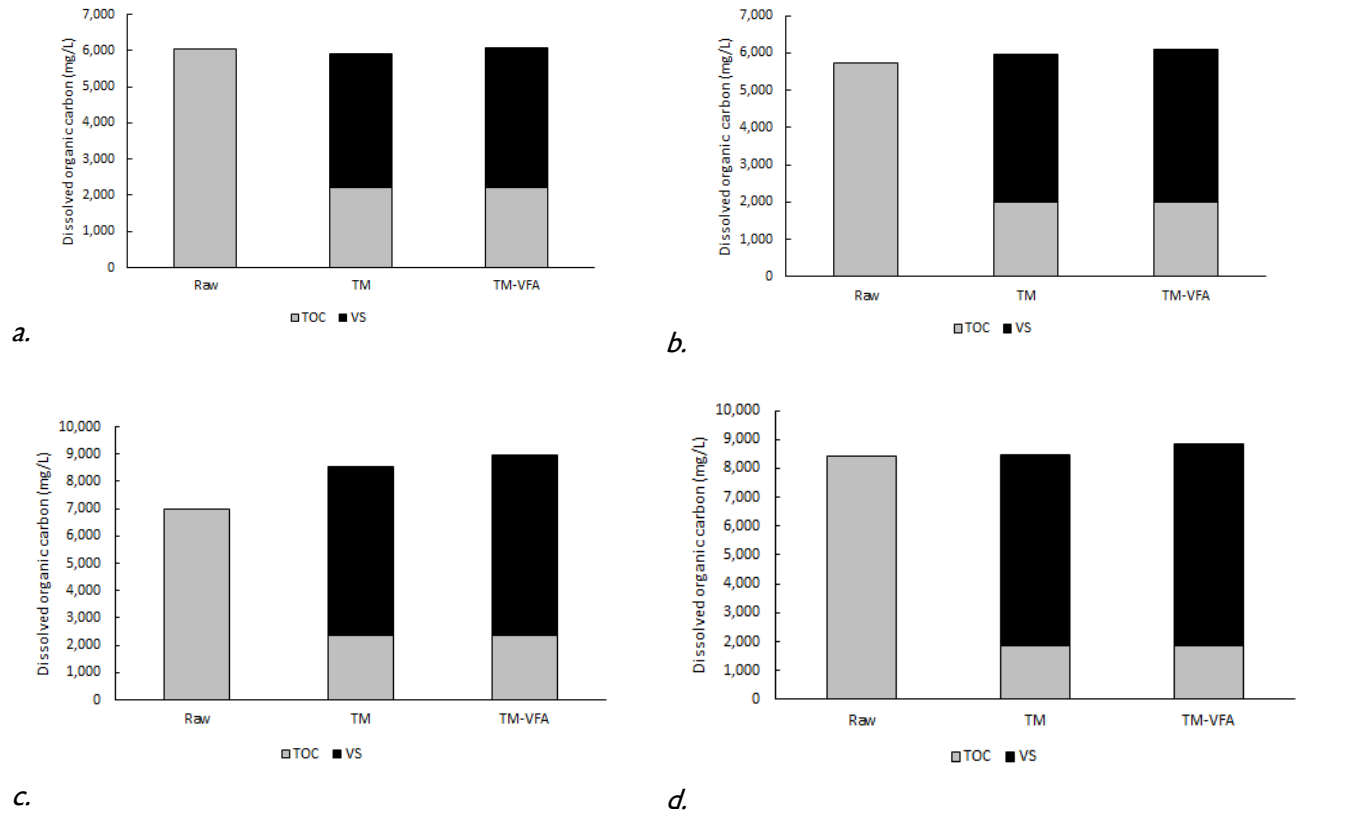


Figure 18 Estimated dissolved organic carbon as VS compared to dissolved organic carbon content for methods (wet effluent: direct determination on the effluent sample, dried effluent: determination on the solid after drying at 60oC) for a. sample 11, b. sample 12, c. sample 13, and d. sample 14

5. Discussion

In this study, the loss of VFAs from different piggery effluent during VS/TS analysis using oven-drying was investigated. Results showed that significant concentrations of the VFAs in samples were lost during the TS-analysis using the oven-drying step in the SM (80-88%) and TM (78-82%) methods. The results showed that the loss of VFAs during the VS/TS analysis using oven-drying could result in significant underestimation of effluents with a relatively high concentration of VFAs in relation to the TS content, in agreement with Pind et al. (2003), Kreuger et al. (2011), Vahlberg et al. (2013) and Vedrenne et al. (2008).

The loss of VFAs during the drying stage lead to an underestimation of the VS concentrations by 0-15% using the SM. Similarly, Vahlberg et al. (2013) showed a 0-14% underestimation of biogas reactor digestate VS concentration. It is possible that this error could explain some of the error between actual measured and PigBal predicted VS mass in the studies conducted by Skerman et al. (2016) and McGahan et al. (2016). The adjustment of VS with VFA concentrations with the SM-VFA method can correct for VFA loss and address consequential VS underestimation, increasing the accuracy of VS measurement.

On the other hand, the TM and TM-VFA methods both overestimated the VS concentrations of samples (by 24% and 26% respectively). The results indicate that the differences between the SM and TM methods were not solely due to the differences in VFA losses. Furthermore, the carbon balance indicates this difference may be due to non-carbon components. Overall, both the TM and TM-VFA methods overestimated the VS concentrations in piggery effluent sampled and thus these methods are not recommended for the estimation of VS in piggery effluent without further research to mitigate the sources of error found in this study.

There was a large temporal variation in VFA and VS concentrations between sampling events. Similarly, McGahan et al. (2016) found temporal variation between summer and winter VS concentrations of an Australian piggery. This indicates that for optimisation of biogas systems, regular monitoring of VS to assess temporal variation may be needed.

Additionally, there was a large difference in VS and VFA concentrations between the duplicate samples depending on whether the mechanical stirrer was non-operational or operational at the time of sampling. The lower concentrations when the mechanical stirrer became operational, may be due to increased volatilisation caused by stirring or better diffusion of VFA throughout the effluent. While the conclusions that can be drawn from one piggery are limited, it does indicate that stirring could have a significant impact on VS and VFA concentrations and removing this process could be used to optimise biogas systems. Several studies, including: Vedrenne et al. (2008), Vavilin and Angelidaki (2005) and Karim et al. (2005) have reported variable effects from mixing, depending on the organic load and the mode of mixing used.

As the calculations of central process parameters of biogas production and manure management are based upon the TS and VS concentrations, it is essential that they are accurate. Thus, it is recommended that SM-VFA method is used to estimate VS in Australian piggery effluent. Additionally, VS concentrations should be sampled regularly to assess temporal variation. Further research is required to assess the effect of stirring on VS and VFA concentrations. For companies that would like to adopt the findings in this study, the methods suggested for compensation when analysing TS using oven-drying, are presented in Appendix 1-3..

6. Implications & Recommendations

The current standard method used by commercial Nata laboratories (SM) underestimated the VS concentrations by 0-15% in the piggery effluent sampled. The standard method adjusted with VFA in accordance with Vahlberg et al. (2013) was the most accurate method used to predict VS.

Because of the importance of VS measurement when it is being used to evaluate the efficiency and optimisation of biogas production process (including: organic loading rate, the specific gas production (biogas yield) and the degree of degradation), it could be beneficial for industry to include VFA adjustment in VS analysis. Additionally, the use of VFA adjustment in VS for piggeries that are measuring VS for manure management purposes would increase the accuracy and potentially help with the optimisation of management practices. Measuring VFA using titration requires two titrations to be run (on the wet sample and again on the re-hydrated sample after drying). At the commercial laboratory used in the experiment, this costed an additional $88 per effluent sample. If conducting a targeted analysis to optimise biogas performance (testing focused on TS and VS only), this resulted in an overall analysis cost of approximately $130 per sample at the partner laboratory, though laboratory costs can vary widely between providers. This was deemed to provide a relatively low-cost solution to the error.

The project was limited to 5 piggeries in Queensland and only one stage of the piggery system due to budget restraints. The research should be expanded to different pig stages, diets, manure management systems, organic loading rates, flushing and regions across Australia to ensure the methods are scientifically rigorous. Additionally, it would be beneficial to investigate the effect of VSs adjusted with VFA concentration on the prediction of methane potentials.

For industry, it is recommended that:

- For best practice VS measurement: SM-VFA is used to assess VS in Australian piggeries.
- Regular VS and VFA sampling should be conducted to assess temporal variations, ideally every 3 months or every month seasonally during optimisation of a biogas system.

Research recommendations are as follows:

- Research is expanded to a larger sample of Australian piggeries, pig stages and diets.
- Research is expanded to investigate different manure management systems, organic loading, flushing and stirring rates.
- Research is expanded to confirm higher methane potentials and laboratory scale methane yield where VFA loss is minimised.

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8. Appendix I – Volatile Solids-VFA Adjusted Standard Operating Procedure (SOP)

General

The VFA concentration of a homogenised wet sample is analysed. A homogenised sample is evaporated in a weighed dish and dried to a constant weight in a 103-105°C oven. A proportion of this dried sample is rehydrated with ultra-pure water and the VFA are measured again to determine the VFA loss during the during stage. The dish and sample are ignited at 550°C for 30 minutes. The total, fixed, and volatile solids are determined by comparing the mass of the sample before and after each drying step. The loss of sample mass upon ignition represents the volatile solids. The remaining sample after ignition represents the fixed solids. The lost VFA concentration is used to correct the volatile solids concentration.

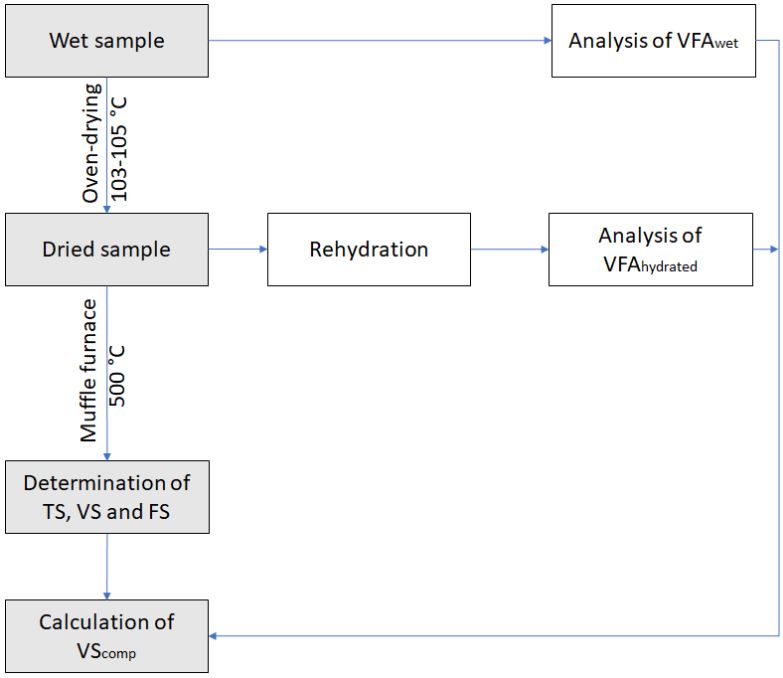

Figure 190 Overview of the analysis procedure used in the VFA adjusted standard operating procedure

VFA adjustment procedure

The follow procedure describes the VFA adjustment to the current standard method for VS analysis. Note: All equipment and supplies, reagents and standards, sample collection, preservation and storage. quality control, and calibration and standardisation drawn from the Standard method 2540 (APHA et al. 2012) should be followed. The procedure is as follows:

1. Analysis VFA concentration of homogenise wet sample. Record this mg value as VFA_{wet}.
2. Use an analytical balance to weigh the clean aluminium dish to the nearest 0.1 mg (0.0001 g). Record this mg value as "C".
3. Homogenise wet sample and place a known (record this mg value as wet weight) amount into in clean aluminium dish. (the dried amount weight needs to be enough for both VS and VFA_{dry} measurements).

4. Place the sample in a preheated oven and evaporate at 103-105 °C until sample is dry (weight is steady).
5. Remove the dish from the oven. Let the dish temperature decrease to room temperature in a desiccator.
6. Use an analytical balance to weigh the dish to the nearest 0.1 mg (0.0001 g). Record this mg value as "A".
7. A known amount (Record this mg value as m_{dry}) of dried sample is rehydrated with ultra-pure water in a known amount (Record this mg value as m_{water}) and homogenised.
8. Analysis VFA concentration of rehydrated dry sample. Record this mg value as $VFA_{hydrated}$
9. Put the aluminium dish into a pre-heated muffle furnace at 550 °C for 30 minutes.
10. Remove the dish from the muffle furnace. Let the dish temperature decrease to room temperature in a desiccator.
11. Use an analytical balance to weigh the dish to the nearest 0.1 mg (0.0001 g). Do steps 5-6 again, until the difference between two successive sample weighings is not more than 4% or 0.5 mg, whichever is less. Record this mg value as "B".
12. Calculations: The loss of weight is total volatile solids. Weighed residue is total fixed solids. Calculate the test results:

$$Total\ Solids\ (TS)\ mg = \ (A - C)$$

$$Volatile\ Solids\ (VS)\ mg/L\ = (A - B)$$

$$Fixed\ Solids\ (FS)\ mg/L\ = (B - C)$$

$$TS\ (\%) = \frac{TS}{wet\ weight} * 100$$

$$VS\ (\%\ of\ TS) = (1 - \frac{FS}{TS}) * 100$$

$$VFA_{dry} = VFA_{hydrated} \left(\frac{(m_{dry} + m_{water})}{wet\ weight}\right)$$

$$VFA_{lost}\ (\%) = \left(1 - \frac{VFA_{dry}}{VFA_{wet}}\right) * 100$$

$$TS_{adj}\ (\%) = \ TS\ (\%) + \left(\frac{VFA_{lost}}{100} * VFA_{wet}\right) * 10^{-4}$$

$$VS_{adj}\left(\%\ of\ TS_{comp}\right) = \frac{(\ TS\ (\%) * \ VS(\%\ of\ TS)) + (10^{-4} * VFA_{lost} * VFA_{wet})}{TS_{comp}}$$

Thus, VFA adjusted VS concentration:

$$[VS]_{adj}\left(mg/L\ of\ TS_{comp}\right) = VS\ (mg/L) * \frac{VS_{adj}\ (\%)}{VS\ (\%)}$$