

## Index - Final Report

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# 1. Final report

## 1.1 Project details

<b>Project title</b>	Optimisation of a titration method for monitoring of VFA - Optimisation and standardisation of a titration method for routine monitoring of VFA in full-scale biogas plants
<b>Project identification</b>	10231
<b>Name of the programme which has funded the project</b>  (ForskVE, ForskNG or ForskEL)	ForskEL
<b>Name and address of the enterprises/institution responsible for the project</b>	Technical University of Denmark Department of Environmental Engineering Miljøvej 113 DK-2800 Kgs. Lyngby <a href="http://www.env.dtu.dk">www.env.dtu.dk</a> Prof. Irini Angelidaki Telephone: +45 4525 1429 Fax: 4593 2850 e-mail: <a href="mailto:iria@env.dtu.dk">iria@env.dtu.dk</a>
<b>CVR</b> (central business register)	30 06 09 46
<b>Date for submission</b>	

## 1.2 Executive summary

The use of biogas as an alternative to fossil fuels is increasing and the Danish Parliament and Government have put focus on utilising biogas in the strategy for Denmark to become independent of fossil fuels within the year 2050 (the Danish Government, 2011). Therefore it is now stressed that the production of biogas should be increased and in order to support the implementation of biogas more widely in the Danish energy sector, it has been decided to increase the financial support to producing and distributing biogas in Denmark (the Danish Parliament, 2012). The production of biogas in Denmark is often based on mixed feedstock containing mainly manure, but also various fractions of other waste, including organic wastes from industries, e.g. from slaughter houses and production of dairy products. From this it follows that the feedstock may vary in biogas potential and a reliable and easy way of monitoring and controlling the production is therefore needed. A good parameter used to monitor the production is to monitor the level of volatile fatty acids (VFAs), e.g. propionate, butyrate and especially acetate. This is often done by titration. Measurement of VFA by titration is simple and cheap, compared to using gas chromatograph. Thus, determination of VFA by titration is widely applied in the Danish biogas plants.

However, the VFA titration procedures currently used in the plants are still not optimised and thus the potential benefit of VFA monitoring is not fully utilised. The current procedures are not validated on manure and lack standardisation which makes the data unable to be interpreted and compared between different plants for process monitoring and for process optimisation. There are several titration procedures suggested in the literature, that have potential to be executed automatically under routine measurements. These procedures have been successfully applied in other anaerobic samples such as samples from sludge digesters or wastewater treatment plants. However, the knowledge about applicability of these procedures for manure based samples is still lacking in the Danish biogas sector, since they have never been reported for measuring VFA in digested manure. The aim of this project is therefore to find a suitable titration procedure for measuring VFAs for digested manure samples.

The literature was reviewed for suitable titration procedures previously applied in waste water treatment applications. In the study, four titration procedures were compared and evaluated for their suitability to be applied to digested manure from biogas plants. Two of them are currently used at the biogas plants and the other two are suggested in the literature and have been applied to other anaerobic sludge samples, though with a less complicated matrix. The two procedures currently used at Danish biogas plants, the simple (Anderson and Yang, 1992) and the 2-step back (Ellegaard, 1990) titration procedures, are relatively simple in the sense that they only require registration of the consumption of acid and base used to titrate the sample to a few pH values. Hereafter the volumes and concentrations of the acid and base is used to calculate the VFA contents using a simple equation.

The two procedures suggested in the literature are however relatively more complex. They require the registration of the consumption of acid and base used to titrate the sample to four, the 4-point (Kapp, 1984) and five, the 5-point (Moosebrugger et al, 1993) titration procedure, pH values, respectively. Within the 4-point procedure the calculations after titration is then based on some empirically defined equations, whereas the 5-point procedure incorporates a range of theoretical considerations with regard to acid/base equilibria, not only for the VFAs, but also for potentially interfering components like the carbonate system as well as for ammonia, dihydrogenphosphate, sulphide and in this case lactate. To use these two procedures at least a more complicated titration instrument (with the option to register more than one equivalence point) has to be obtained and for the 5-point procedure a computer software or at least a spreadsheet with all the equations implemented also need to be obtained.

For the purpose of evaluating these four titration procedures a spreadsheet was set up to handle the calculations solely by entering the obtained titration data like volumes of

acid/base required to titrate to certain pH values, concentration of acid/base, sample volume and start pH. Furthermore, an automatic titration system controlled by a PLC/SCADA which can collect titration data every second was also set up. This made it possible to simultaneously calculate VFA concentrations based on different procedures from one titration, which will eliminate the uncertainty from conducting different titrations for each procedure.

First the procedures were tested and evaluated in terms of basic conditions like aeration, titration volume, dilution, accuracy, reproducibility, and ease of execution and compared with VFAs determined by gas chromatography (GC) as common reference method. It was found that titration of 40 mL of a 4 times diluted manure sample was the most optimal. At an ammonia level of 3.0-3.3 g/L correlations coefficients ( $R^2$ ) > 0.91 were obtained and at a level of 4.6-5.0 g/L  $R^2$  > 0.98 were obtained. It was noted that at the low ammonia level the slope of the correlations were significantly less than 1, whereas at the high ammonia level, the slope was equal to 1.

Interestingly, the simple and the 5-point procedures performed significantly better than the 2-step back and the 4-point procedures.

A range of experiments were set up in order to test the effect of interfering components. The components and their relevant ranges tested were: Ammonia (3.3-6.7 g/L), hydrogencarbonate (15-27 g/L), sulphide (0.0021-0.54 g/L), dihydrogenphosphate (0.26-4.2 g/L) and lactate (0-5.4 g/L). All these components were tested at two VFA levels of 1 and 5 g/L. The mentioned concentrations are concentrations in the manure, taking into consideration the original content of each of the components as well as the spiked.

Generally it is seen that at low VFA levels there is no interference from ammonia and sulphide, but hydrogencarbonate, dihydrogenphosphate and lactate seem to have an influence on the titration results. Whether they have a positive or negative effect is dependent on the titration procedure used.

At high VFA levels there is relatively little interference from hydrogencarbonate, sulphide and hydrogenphosphate, whereas ammonia and lactate have a positive influence on the titration results.

In order to evaluate on an overall basis which procedure is the most suitable to be applied for the purpose of monitoring VFA levels in reactors of biogas plants, a Multi Criteria Assessment (MCA) was performed. The three considered criteria were accuracy, relationship with the individual potentially interfering components as well as how much of the relationship is described by this component.

It turned out that the 2-step back and the 4-point titration procedures are the less suitable procedures. The simple and the 5-point procedures work equally well at a high VFA level. The simple and the 5-point procedure also perform equally well at a low VFA level, but if the pH correction of the 5-point procedure is implemented, the 5-point procedure is the superior at a low VFA level.

It can therefore surprisingly be concluded, that **if no knowledge** of the composition of a certain manure sample is present, the **simple titration** procedure should be the **procedure of choice**, due to its simplicity in both execution and the following calculations of VFA content.

However, when the calculation procedure has been established and if one has access to an autotitrator connected to a computer software, like the one used in this project, the 5-point procedure should be the procedure of choice, as this procedure offers the opportunity to correct for pH and thereby both at the low and high level of VFA obtain good results. The 5-point procedure is also the one that best handles interference by lactate.

Within the project two biogas plants have accepted to test the different titration procedures. One plant was instructed in performing one of the four titration procedures and the other accepted to use the automatic titration instrument coupled to the PLC/SCADA controller. The plants therefore performed the titrations and send titration data as well as a sample vial for GC analysis to the DTU Environment laboratories.

## 1.3 Project results

### 1.3.1 Conditions

The anaerobic digestion of organic waste can treat and recycle nutrient in an environmental friendly way and also give energy with zero CO<sub>2</sub> emission, which supports the commitment in the Kyoto protocol. Biogas is socio-economically the most profitable method to produce renewable energy compared with other bio-fuels. Manure is the largest biomass resource in Denmark and the utilization of manure in biogas plants is well established. With the recent agreement within the energy area, the Danish government had set a strategy resulting in focus on non-fossil fuel energies aiming at becoming non-dependent on fossil fuels in year 2050 (the Danish Government, 2011). Along with this strategy the number of full-scale centralized biogas plants in Denmark is planned to increase from today's 20 to a number around 50 by the year 2020, as well as the recently announced new rules for biogas prices will result in expansion of the biogas sector (the Danish Parliament, 2012).

A better process monitoring can increase both economic benefit in terms of higher biogas yield and less loss from process breakdown and environmental benefit in terms of better CO<sub>2</sub> emissions reduction, pathogen reduction and less smelly manure, for a more neighbour friendly reuse on agricultural farmland. High production efficiency and process stability will make biogas application even more attractive. Expansion of the biogas sector will be one of the most important tools to achieve Denmark's strategic energy plan. The successful and beneficial application of anaerobic digestion for biogas will ensure more application in this area in the future. This will increase the interest for investment in the biogas sector and makes Denmark less dependent on import of fossil fuel from external suppliers. Moreover, improved process efficiency and plant economy can also contribute to reduce the direct and indirect subsidies in the future.

#### 1.3.1.1 Background

Monitoring and control of the biogas process is important in order to keep optimal operating conditions. Many parameters have been used for monitoring the biogas process, such as pH, volatile fatty acids (VFAs) concentration, bicarbonate alkalinity/buffer capacity and gas production. The concentration of VFA is one of the most common parameters used to monitor the biogas process. Several studies from full-scale biogas plants showed that VFAs are a good parameter for monitoring the biogas process (Ahring et al., 1995). It gives a good indication of the balance between different groups of anaerobic microorganisms in the biogas reactors. VFAs are commonly measured by using gas chromatography (GC) with flame ionization detection (FID), or titration of the samples giving the total VFAs, which is cheaper and widely used in commercially operated biogas plants.

Determination of VFAs by titration is based on the equilibrium of VFAs in dissociated and undissociated forms. The concentration of VFA is calculated from the amount of acid/base consumed to change the VFAs between these forms. During titration, other chemical components in the sample will also consume the added acid/base, such as bicarbonate, ammonia, sulphide and phosphate, although bicarbonate contributes to the main part of the sample's alkalinity. When pH changes, the components will change their forms according to pH and their dissociation constants (pKa). The pKa-values are quite similar for all the most common VFAs. This makes determination of individual VFAs by titration difficult and no application of this has been reported. All of the proposed VFA titration techniques in literature were applied for determination of total VFAs in the process sample. Digested manure contains several compounds, such as ammonium, sulphide, phosphate and high concentration of bicarbonate which can interfere with the VFA titration as mentioned above. Presently, most of the full-

scale Danish biogas plants use some titration technique for measuring VFAs for their process monitoring.

The Table 1 below shows the different pKa-values for the most common VFAs as well as other typical constituents of manure.

*Table 1 – pKa values of different components in anaerobic samples at 25°C.*

Component		pKa
HS <sup>-</sup>	HS <sup>-</sup> ↔ S <sup>2-</sup> + H <sup>+</sup>	13.3
HPO <sub>4</sub> <sup>2-</sup>	HPO <sub>4</sub> <sup>2-</sup> ↔ PO <sub>4</sub> <sup>3-</sup> + H <sup>+</sup>	12.3
HCO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup> ↔ CO <sub>3</sub> <sup>2-</sup> + H <sup>+</sup>	10.3
NH <sub>4</sub> <sup>+</sup>	NH <sub>4</sub> <sup>+</sup> ↔ NH <sub>3</sub> + H <sup>+</sup>	9.25
H <sub>2</sub> S	H <sub>2</sub> S ↔ SH <sup>-</sup> + H <sup>+</sup>	7.20
H <sub>2</sub> PO <sub>4</sub> <sup>-</sup>	H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> ↔ HPO <sub>4</sub> <sup>2-</sup> + H <sup>+</sup>	7.20
H <sub>2</sub> CO <sub>3</sub>	H <sub>2</sub> CO <sub>3</sub> ↔ HCO <sub>3</sub> <sup>-</sup> + H <sup>+</sup>	6.35
Citric acid	C <sub>6</sub> H <sub>6</sub> O <sub>7</sub> <sup>2-</sup> ↔ C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> <sup>3-</sup> + H <sup>+</sup>	5.41
Acetic acid	CH <sub>3</sub> COOH ↔ CH <sub>3</sub> COO <sup>-</sup> + H <sup>+</sup>	4.75
Citric acid	C <sub>6</sub> H <sub>7</sub> O <sub>7</sub> <sup>-</sup> ↔ C <sub>6</sub> H <sub>6</sub> O <sub>7</sub> <sup>2-</sup> + H <sup>+</sup>	4.75
Propionic acid	CH <sub>3</sub> CH <sub>2</sub> COOH ↔ CH <sub>3</sub> CH <sub>2</sub> COO <sup>-</sup> + H <sup>+</sup>	4.88
Butyric acid	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> COOH ↔ CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup> + H <sup>+</sup>	4.81
Iso-Butyric acid	(CH <sub>3</sub> ) <sub>2</sub> CHCOOH ↔ (CH <sub>3</sub> ) <sub>2</sub> CHCOO <sup>-</sup> + H <sup>+</sup>	4.84
Valeric acid	CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> COOH ↔ CH <sub>3</sub> CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> COO <sup>-</sup> + H <sup>+</sup>	4.82
Iso-Valeric acid	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> COOH ↔ (CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub> COO <sup>-</sup> + H <sup>+</sup>	4.77
Lactic acid	C <sub>3</sub> H <sub>6</sub> O <sub>3</sub> ↔ C <sub>3</sub> H <sub>5</sub> O <sub>3</sub> <sup>-</sup> + H <sup>+</sup>	3.86
Citric acid	C <sub>6</sub> H <sub>8</sub> O <sub>7</sub> ↔ C <sub>6</sub> H <sub>7</sub> O <sub>7</sub> <sup>-</sup> + H <sup>+</sup>	3.09
H <sub>3</sub> PO <sub>4</sub>	H <sub>3</sub> PO <sub>4</sub> ↔ H <sub>2</sub> PO <sub>4</sub> <sup>-</sup> + H <sup>+</sup>	2.10

### 1.3.1.2 Project activities

#### 1) Theoretical review, computer programming and equipment setup for VFA determination.

The theoretical background of each titration method was reviewed. The boundary assumption of each method was thoroughly analyzed to define application limits related to sample characteristics. From this part, a mathematical model was set up as a computer program for VFA determination based on each method concept. Moreover, the titration equipment was set up connected to the data acquisition system and the computer program for VFA calculation.

#### 2) Titration experiments and data interpretation.

Manure samples from two biogas plants were collected and analyzed for basic components in order to characterise the manure sample. The VFA concentration was measured by titration and compared with the corresponding GC measurement. The samples were amended different concentrations of VFA and various ions (alkalinity) in order to evaluate the effect of VFA, and ions such as ammonia, carbonate, sulphide, phosphate and lactate, on the titration results. The titration methods were compared in terms of accuracy, reproducibility, and ease of execution, in order to evaluate the suitability of application with the digested manure from the Danish biogas plants. Finally, the best titration procedure was suggested for measuring volatile fatty acids (VFA) to use for process monitoring in the Danish biogas plants. A precise correlation between the titration result and the corresponding GC result was also established.

#### 3) Implementation in full-scale biogas plants

The two biogas plants Hashøj and Lemvig were supposed to test two different titration procedures during a period of 3-6 months. Lemvig biogas plant tested the titration equipment connected to a computer for an automated titration procedure with the resulting option to evaluate titration data in all four different titration procedures. Hashøj was supposed to test a simpler autotitrator only allowing the evaluation of titration data for the simple titration procedure. The titration results were compared with the VFA concentrations measured by GC.

#### 4) Summary of the results and reporting

All the scientific achievements will be published in peer reviewed scientific journals and presented at international conferences. Results will also be communicated to plant managers and biogas contractors in Denmark.

##### 1.3.1.3 Project consortium and responsibility

Kanokwan Boe, 4525 1425, [kanb@env.dtu.dk](mailto:kanb@env.dtu.dk),  
Hans-Christian Holten Lützhøft, 4525 1425, [hclu@env.dtu.dk](mailto:hclu@env.dtu.dk),  
Irimi Angelidaki, 4525 1429, [iria@env.dtu.dk](mailto:iria@env.dtu.dk),  
DTU environment,  
Technical University of Denmark  
Miljøvej 113  
2800 Kgs. Lyngby  
CVR: 30060946  
[www.env.dtu.dk](http://www.env.dtu.dk)

Lars Kristensen, 9781 1400/ 2343 0114, [lars@lemvigbiogas.dk](mailto:lars@lemvigbiogas.dk),  
Lemvig Biogas Plant  
Pillevej 12  
7620 Lemvig  
Fax: 9781 1402  
CVR: 28450850  
[www.lemvigbiogas.dk](http://www.lemvigbiogas.dk)

Erik Lundsgaard, 5818 8060/2022 2179, [erik.lundsgaard@adr.dk](mailto:erik.lundsgaard@adr.dk),  
Hashøj Biogas Amba  
Industrivej 17  
4261 Dalmose  
Fax: 5818 8027  
CVR: 17366335  
[www.hashoejbiogas.dk](http://www.hashoejbiogas.dk)

At **DTU-Environment** the biogas activities are under the Bioenergy research group, which is an active and dynamic group with extensive expertise in conversion of biomass to energy by biological methods. The group consists of 18 researchers and a number of students are coupled to the different projects during their master thesis work. The group has extensive expertise within biogas research, ranging from reactor concepts and mathematical models to modern molecular microbiology methods. Additionally, the group has active contact with biogas plants. This project fitted very well into the research strategy of the Bioenergy research group in the area of monitoring and control of the anaerobic process, which is to develop the proper biogas process monitoring system suitable for real application.

At **Lemvig and Hashøj biogas plants** the operators have long experiences in operating the biogas production process. They are very active and are interested in new technology and development for improving their process efficiency. The Lemvig biogas plant is currently running the project for demonstration of serial operation strategy (ForskEL project no.010115), under collaboration with DTU Environment and BWSC A/S.

The current project was executed both at DTU Environment and at Lemvig and Hashøj biogas plants. DTU Environment was the responsible partner for laboratory experiments and method optimization including computer programming. Lemvig and Hashøj biogas plant are the owners of the full-scale biogas process and took part in the validation of the titration method by implementing the methods for their VFA measurements. Apart from the coordination, DTU

Environment also performed validation of the GC method. Both partners at the biogas plants contributed in data acquisition and evaluation of the method for practical use.

The project was managed by Prof. Irini Angelidaki, who has extensive expertise with project management. She has managed several national (Research council (STVF, FMI), EFP, PSO, UVE, Fond supported projects etc.) and international projects (EU under FP6 and FP7, Cost, Interreg). The projects have always been executed with success.

From Lemvig and Hashøj biogas plants, Lars Kristensen and Erik Lundsgaard, respectively, will be the responsible partner for the operation of the biogas plant and the implementation of the titration method for validation.

### 1.3.1.4 Activities, mile stones and time schedule

Due to facing challenges with optimizing the titration procedure, difficulties in implementing the titration procedures at the biogas plants, exchange of scientific staff as well as maternity leave, the original time schedule was extended during the project. All in all, the project has now been fulfilled and the different activities and mile stones were organized as shown in the diagram below.

	År 2009				År 2010				År 2011				År 2012			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1 Theoretical review, computer programming and equipment setup for VFA determination				■												
2 Titration experiment and data interpretation					■	■	■	■	■	■	■	■	■			
3 Optimisation of titration procedure and calculation methods						■	■	■	■	■	■	■	■			
4 Implementation in full-scale biogas plants						■				■	■	■	■			
5 Summary of the results and reporting							■				■			■	■	

### 1.3.1.5 References

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aqueous solution containing also known concentrations of other weak acid/bases. *Water SA*, **19**:29-39.

The Danish Government (2011) Energy Strategy 2050 – from coal, oil and gas to green energy.

The Danish Parliament (2012) Aftale mellem regeringen (Socialdemokraterne, Det Radikale Venstre, Socialistisk Folkeparti) og Venstre, Dansk Folkeparti, Enhedslisten og Det Konservative Folkeparti om den danske energipolitik 2012-2020.

### **1.3.2 Objectives**

The overall aim of this project was to optimise and implement the most suitable titration method for measuring the VFA concentration in manure in a biogas reactor. This method was to be selected from currently used methods at biogas plants as well as methods published in the scientific literature.

To reach the overall aim, the following individual aims were outlined:

- 1) Review literature to select suitable titration procedures to be tested as candidates to the most suitable titration procedure for measuring the VFA concentration in manure.
- 2) Programme computer and set up equipment for the automated titration for VFA determination.
- 3) Vary different parameters with potential influence on the VFA determination, establish relationship between VFA determined by titration and by GC and perform titrations for studying the parameters influence on the VFA determination by the different titration procedures.
- 4) Interpret titration data and suggest optimal titration procedure.
- 5) Validate titration procedure in full-scale biogas plants.

The optimised procedure for measuring VFA in the biogas reactor will ensure reliability of the monitoring system and also reduce the laboratory workload which will encourage the plant operator to monitor their process for better operation. So far online monitoring of VFA has not been commercially available and alternative offline monitoring, e.g. by titration, has been an inaccurate and time consuming method. In this project the titration method was optimized for accurate measurement of VFA in digested manure. So far, only large-scale biogas plants have paid attention to and put effort in monitoring and optimisation of the process. From the results of this project, the optimised titration method will be reliable, simple, cheap and easy to operate, and will be realistic for application also in the farm-scale biogas plants. The success from this project will encourage many biogas plants and other plants in the anaerobic digestion sector to use this monitoring system in their processes. The results of this project will be exploited at both existing and new biogas plants, making the Danish biogas systems one of the most stable and efficient processes, which will attract a lot of interest internationally. Improvement of operation of Danish biogas plants will contribute to new orders for Danish companies.

## 1.4 Implementation of results

Lemvig biogas plant currently uses manual titration for their VFA measurements with a 2-step back titration method. In general, the results from manual titration of manure have high variation due to uncontrolled gas-liquid exchange, and our previous study (results not shown) showed that the 2-step back titration method had less variation of the titration results compared to simple titration, when doing the titration manually. However, we would recommend Lemvig to change to the simple titration method and use an autotitrator to get more reliable titration results. It has been shown both in our laboratory validation experiments as well as in the real samples from the biogas plant, that the 2-step back titration procedure overestimate the VFA content and that the simple procedure is closer to the GC determined VFA content with the same level of precision.

Hashøj biogas plant currently uses an autotitrator for their VFA measurements with a simple titration method. Even though Hashøj Biogas Plant did not succeed in testing the procedure, we will recommend them to continue using the simple procedure, due to the reasons mentioned above. However, since we found mistakes in the calculation program that Hashøj is currently using, we would recommend them to use the corrected calculation worksheet that was an outcome of this project instead.

## 1.5 Results of the project

### 1.5.1 The investigated titration procedures

In this project, several VFA titration methods will be compared in terms of accuracy, reproducibility, and ease of execution, in order to evaluate the suitability of application with the digested manure from the Danish biogas plants. The methods to be compared will include both titration methods currently used in the plants and suggested methods from the literature. The methods currently used at the plants are the simple titration method (Anderson and Yang, 1992) and the 2-step back titration with air bubbling (Ellegaard, 1990). The methods from the literature are the direct titration methods with the 4-point titration (Kapp, 1984) and the 5-point titration (Moosebrugger et al, 1993).

**In the simple titration method (Anderson and Yang, 1992)**, the sample is weighed and initial pH is measured, then titrated with 0.1 N HCl to pH 5.1 and 3.5 respectively. The VFA concentration is calculated by using the equations:

$$A1 = \{ [\text{HCO}_3^-] * ([\text{H}]_2 - [\text{H}]_1) / ([\text{H}_2] + K_1) \} + \{ [\text{VA}] * ([\text{H}]_2 - [\text{H}]_1) / ([\text{H}_2] + K_2) \} \quad (1)$$

$$A2 = \{ [\text{HCO}_3^-] * ([\text{H}]_3 - [\text{H}]_1) / ([\text{H}_3] + K_1) \} + \{ [\text{VA}] * ([\text{H}]_3 - [\text{H}]_1) / ([\text{H}_3] + K_2) \} \quad (2)$$

Where

- A1 and A2 are the molar equivalents of the standard acid consumed to the first and second endpoints
- $[\text{HCO}_3^-]$  is the bicarbonate concentration
- $[\text{VA}]$  is the VFA ion concentration
- $[\text{H}]_{1,2,3}$  are the hydrogen ion concentrations of the original sample and at the first and the second endpoints
- $K_1$  is a conditional dissociation constant of carbonic acid,  $6.6 * 10^{-7}$
- $K_2$  is a combined dissociation constant of the VFA ( $\text{C}_2$  to  $\text{C}_6$ ),  $2.4 * 10^{-5}$

The two equations are solved to give both the bicarbonate and the VFA concentration.

**In the 2-step back titration with air bubbling (Ellegaard, 1990)**, the sample is first acidified by 1.0 M HCl to pH 3 to change all bicarbonates into the form of  $\text{H}_2\text{CO}_3 \leftrightarrow \text{CO}_2$  and air bubbled through the sample to remove  $\text{CO}_2$ . Then the sample is titrated with NaOH from pH 3.9 to 5.6. The total VFA concentration is calculated by using the equation:

$$\text{Total VFA (mM)} = B \text{ (mL)} * \text{NaOH concentration (M)} * 10 / Z$$

Where

- B is the volume of NaOH used to titrate from pH 3.9-5.6
- factor 10 is a factor based on sample volume of 0.1 L (sample 100 mL = 1/10 L)
- Z is a correction factor (0-1) depending on the actual titration pH range (in case pH 3.9 and 5.6 is not obtained precisely), with a value of 0.7524 for titration from pH 3.9-5.6

To calculate the amount of total VFAs at any titration range, the correction factor is used to account for only a fraction of VFA being titrated.

$$Z = 1 / (10^{(\text{pH}1 - \text{pKa})} + 1) - 1 / (10^{(\text{pH}2 - \text{pKa})} + 1)$$

**In the 4-point titration method (Kapp, 1984)**, the sample is filtered and initial pH is measured, and then titrated with 0.1 N H<sub>2</sub>SO<sub>4</sub> to pH 5.0, 4.3 and 4.0 respectively. The VFA concentration is calculated by using the equation:

$$S_a = 131340 \cdot N \cdot V_{A_{5-4, \text{measured}}} / V_s - 2.99 \cdot \text{Alk}_{\text{measured}} - 10.9$$

Where

- S<sub>a</sub> is the VFA concentration
- N is the acid normality
- V<sub>A<sub>5-4,measured</sub></sub> is the volume of acid used to titrate from pH 5.0-4.0
- V<sub>s</sub> is the sample volume
- Alk<sub>measured</sub> is the bicarbonate alkalinity measured by titration to pH 4.3

**The 5-point titration method (Moosbrugger et al., 1993)** applies a mathematical model based on the theory of weak acid/base chemistry. The model is based on a mass balance of alkalinity in terms of volume of titrant added equating to alkalinity in terms of ion species concentration.

$$V_e \cdot C_a - V_x \cdot C_a = \left\{ \sum (z \cdot [y]_x) \right\} \cdot (V_x + V_s)$$

Where

- V<sub>e</sub> is the volume of acid titrate to the alkalimetric end point
- C<sub>a</sub> is the concentration of the acid titrant
- V<sub>x</sub> is the volume of acid titrate to pH<sub>x</sub>
- [y]<sub>x</sub> is the molar concentration of ion species y after addition of V<sub>x</sub>
- z is the ionic charge of ion species y
- V<sub>s</sub> is the sample volume

The ion species including in the model are HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, VFA<sup>-</sup>, HS<sup>-</sup>, S<sup>2-</sup>, NH<sub>3</sub>, PO<sub>4</sub><sup>3-</sup>, HPO<sub>4</sub><sup>2-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup>, OH<sup>-</sup> and H<sup>+</sup>.

At each point in the titration a linear algebraic equation can be produced. The method requires that the alkalinity concentration from ammonia, sulphide and phosphate are known, and thus, from the titration to pH 6.7, 5.9, 5.2 and 4.4 the concentration of total VFA and total carbonate alkalinity can be calculated.

The two methods from literature have been applied with high accuracy in many anaerobic samples, e.g. raw wastewater, primary sludge, digested sludge and winery waste. However, they have never been tested with digested manure. And since these methods apply direct titration which is easy to implement in the laboratory, the procedure of VFA monitoring can be even more user friendly.

All the procedures mentioned above were used to determine the total VFA concentration in digested manure samples with different VFA and alkalinity concentrations. The VFA results from titration were compared with the VFA measurement by gas chromatography (GC) as the common reference method. Since ammonia and sulphur are present in significant amounts in the biogas digester, the effect of ammonium and sulphate alkalinity was investigated. Moreover, since the biogas plants work by the co-digestion concept where manure is treated together with different organic wastes, the sample matrix was varied and the effect of substrate composition was compared. The effect of these parameters has never been investigated in the Danish biogas plants and needs to be clarified in order to interpret the titration results correctly and be able to compare the data among the plants.

### 1.5.2 Setup of an automatic titration system

A locally-made automatic titration system was constructed as shown in Figure 1 and Figure 2. The system consists of a pH electrode (Radiometer, Denmark) in a titration vessel (Bomex® beaker, low form 150 mL, diameter 60 mm, height 80 mm). During titration, the sample was constantly stirred with a cylinder magnet (diameter 8 mm, length 25 mm) using a magnetic stirrer (stirring speed 200 rpm). The pH meter was manually calibrated daily using buffer solutions of pH 4 and 7, respectively. Addition of acid (0.3 M HCl) and base (0.1 M NaOH) were done by peristaltic pumps with constant flow rates of 1.0 mL/min. For testing the titration procedure with aeration, an aquarium air pump was used with a gas flow rate of 15 mL/min. The titrations were automatically followed by a programmable logic control (PLC) system Proficy 5.5 (GE Fanuc, Luxembourg). The computer interface and data logging on the PLC were using GE Cimplicity HMI 6.1 (GE Fanuc, Luxembourg). The calculation of VFA concentrations from the titration data was managed in a spreadsheet to compare different methods.

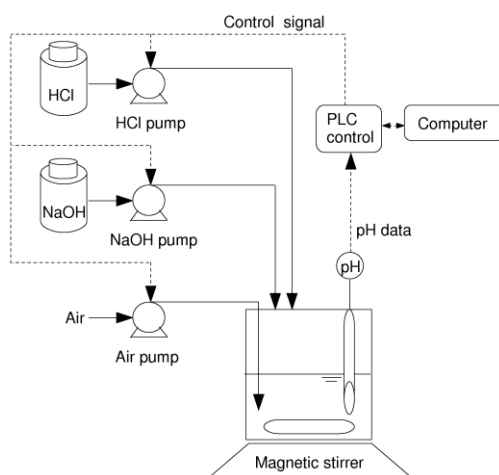


Figure 1 – Schematic diagram of an automatic titration system.

Based on the above mentioned four titration procedures a computer was programmed in order to control the autotitrator in a way that the volume of acid/base added to obtain certain pre-defined pH-values in the manure slurry were recorded. As all the above mentioned four titration procedures use the volume of acid/base added, though at different pH-values, a single titration where volumes to reach different pH-values are recorded, is enough to enable calculations of the VFA concentration for all four methods.

The titration procedure started by measuring the initial pH (pH1) for 20 seconds, where after the addition of acid was initiated. When the sample reached a pH of 3.5, the addition of acid was stopped. The slurry was continuously stirred for 10 seconds (or bubbling for 2 minutes in case of titration with bubbling) and the pH (pH2) was recorded before the addition of base was initiated. The pump added base until the final pH of 5.6. The time used for adding acid and base at each pH was recorded in order to calculate the amount of chemicals used for titration to each specific pH value. The data recorded during acid addition were pH1 and the time used for adding acid to reach pH of 6.7, 5.9, 5.2, 5.1, 5.0, 4.3, 4.0, 3.9, and 3.5, respectively. The data recorded during base addition were pH2 and the time used for adding base to reach pH 3.9 and 5.6, respectively. All titrations were done in triplicates.

The spreadsheet was set up in a way that the determined titrant volumes to reach the different pre-set pH-values, the sample volume and start pH-value are entered and a calculation

of the VFA concentration is then automatically performed. In case of the 5-point titration method the approach is that the observed pH is not exactly the same as the true pH, i.e.  $\Delta\text{pH} = \text{pH}_{\text{true}} - \text{pH}_{\text{obs}}$ . Therefore the calculation procedure has an option for correcting the measured pH-value. This means that the calculation is iterated with the aim of minimising the difference between alkalinity calculated using two sets of pH-values. However, it was found, that this correction only made sense if the VFA content was around or lower than 1 g/L. In case of higher VFA concentrations, correcting the pH-value did not improve the VFA determination, rather the opposite.

*In the following results only VFA contents around or lower than 1 g/L has been optimised by correcting the pH-value. When appropriate corrected is abbreviated with a C, and not corrected is abbreviated with NC.*

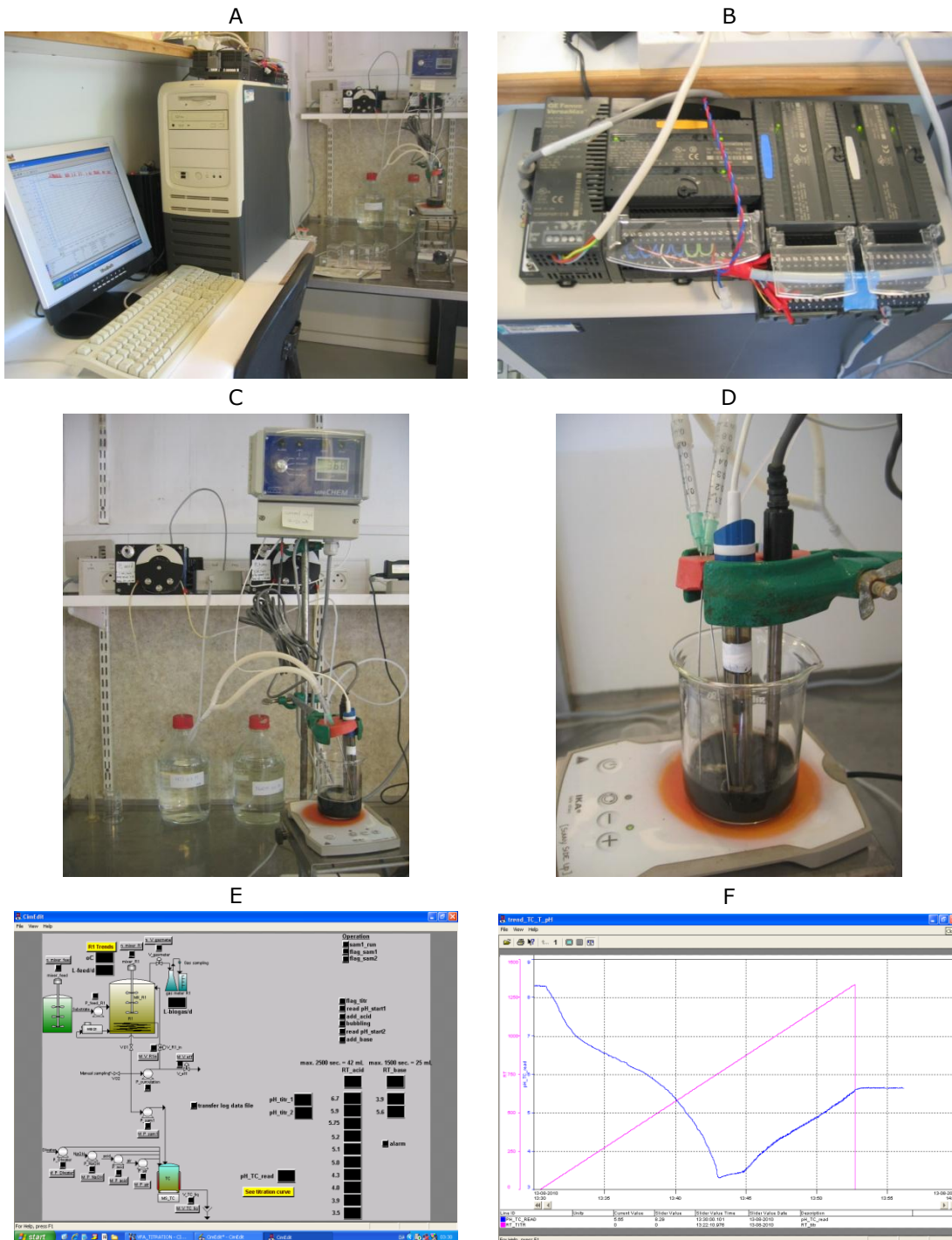


Figure 2 – Photos of the equipment: A: Computer with pumps, bottles with acid and base, pH electrode and sample; B: Unit controlling the titration; C: Pumps, bottles and sample; D: Sample on the magnetic stirring apparatus, pH electrode and tubes and needles to add acid and base; E: Screen dumb of the program interface of the titrator; E: Screen dumb of a titration of a sample with high VFA content.

### 1.5.3 Basic data for manure and the parameters to be varied

The characteristics of the applied test manure as well as the range of the studied parameters are shown below in Table 2.

Table 2 – Contents of different components in manure and at which levels they were varied during the experimental work in this project. nt: not tested.

Component	Value in test manure	Range studied for the influence on titration
Total solids, %	4.9	nt
Volatile solids, %	3.1	nt
Total VFA, g/L as acetate	0.278	1-11
Total N, g-N/L	5.523	nt
NH <sub>4</sub> <sup>+</sup> , g-N/L	3.288	3.3-6.7
HCO <sub>3</sub> <sup>-</sup> , g/L as CaCO <sub>3</sub>	15	15-27
S <sup>2-</sup> , g/L	0.002057	0.0021-0.54
PO <sub>4</sub> <sup>3-</sup> , g/L	0.2575	0.26-4.2
SO <sub>4</sub> <sup>2-</sup> , g/L	0.0900	nt
Lactic acid, g/L	0	0-5.4

## 1.5.4 Different graphs of optimal titration procedure, titration-GC relationship and the influence of the different parameters

### 1.5.4.1 Optimising the titration procedure

In order to find the most suitable titration procedure the following parameters were studied:

1. Aeration or no aeration for the 2-step back titration method
2. Titration volume for all methods
3. Degree of dilution for all methods

#### 1.5.4.1.1 Aeration or no aeration

The method of Ellegaard (1990) used air bubbling to remove surplus CO<sub>2</sub>. But in doing so, there is also a risk of stripping the VFAs off from the manure sample. Therefore it is a balance between overestimating the VFA content by including some of the CO<sub>2</sub> in the titration or underestimating the VFA content if some of the VFAs have been stripped during the aeration procedure to remove CO<sub>2</sub>. The influence of aerating the sample by bubbling with air was therefore tested for this method. The results are shown in Figure 3.

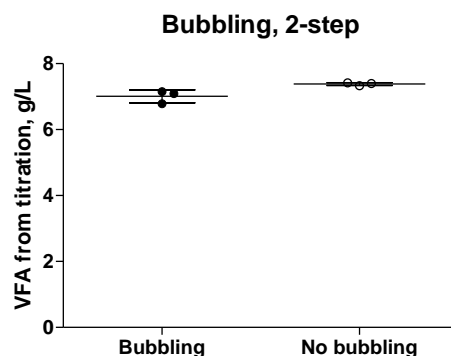


Figure 3 – Effect of aeration on the VFA determination by the 2-step back titration method. The nominal concentration of VFA in the sample was 5 g/L.



It is seen that the mean values are significantly different ( $P=0.0162$ ) from each other and from the nominal concentration of 5 g/L ( $P=0.0031$  and  $0.0001$  for aeration and no aeration, respectively). The variances, however, are not significantly different ( $P=0.1002$ ), but the relative standard deviation (RSD) for the experiment performed with no aeration ( $RSD=0.61$ ) is lower than the RSD performed with aeration ( $RSD=2.79$ ).

**Conclusion:**

*It was therefore decided NOT to aerate the samples during the VFA determination also for the 2-step back titration.*

**1.5.4.1.2 Titration volume**

The titration volume suggested in literature (see section 1.5.1 The investigated titration procedures) varies from 5-100 mL. Change of titration volume could also affect the titration results, as large volume would require longer time for equilibration and gas exchange, especially for manure which has high bicarbonate content. For practical operation of the autotitrator, the titration volume should be in the range of 20-50 mL. The test to find the optimal titration volume was set up to find the optimised titration volume using a fixed sample dilution factor of 2. Figure 4 shows the VFA determination by titration relative to the GC determination and Table 3 shows the RSD for the same results. The graphs show how accurate the titration determination is relative to the GC determination.

Generally speaking:

- At low VFA levels, the accuracy depends on the method, but is independent on the titration volume ( $R^2$  values are in the range 0.11-0.42).
- At high levels of VFAs, the titration methods generally overestimate the VFA content as a function of increasing titration volume ( $R^2$  values are in the range 0.74-0.92).
- At both low and high levels of VFAs the highest precision is found at a titration volume of 40 mL.

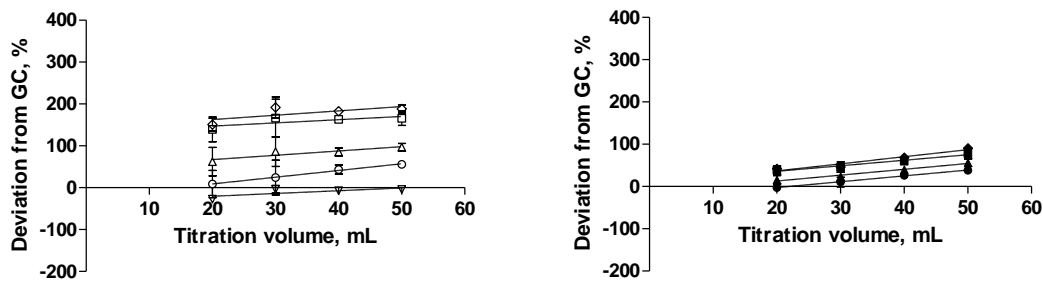


Figure 4 – Graphs showing the effect of titration volume on the VFA determination. The symbols represent the following titration procedures: ○/●: Simple, ◇/◆: 2-step back, □/■: 4-point, △/▲: 5-point not corrected, ▽: 5-point corrected. Open symbols represent low VFA content (1 g/L) and closed symbols represent high VFA content (5 g/L). The unit: deviation from GC, % is calculated by subtracting the GC result from the titration result divided by the GC result and multiplied by 100%.

Table 3 – Relative standard deviation (RSD) in percentage (%) for the different procedures at different VFA levels and titration volumes.

Titration volume, mL	VFA 1 g/L				VFA 5 g/L			
	20	30	40	50	20	30	40	50
Procedure:								
Simple	30	33	7.4	3.8	15	10	4.0	5.1
2-Step	6.0	8.6	1.3	3.4	10	1.6	2.0	1.5
4-Step	12	17	3.2	6.5	12	8.5	2.2	6.3
5-Point NC	21	19	5.2	4.6	12	8.6	2.3	5.8
5-Point C	10	9.4	1.2	8.3	-	-	-	-

**Conclusion:**

40 mL was therefore decided to be the titration volume throughout the experimental work, as well as at the biogas plants.

### 1.5.4.1.3 Degree of dilution

Most of the titration procedures suggested diluting the sample up to 4-times before titration. The main reasons could be to minimise the effect of sample matrix (i.e. minimise ionic interaction, thus, making ion activity close to unity), and also to minimise the use of chemicals. Digested manure contains several ionic compounds that could also interfere with the titration results. Although diluting the sample could minimise other ionic interference, it also decreases the VFA concentration in the solution. It was found in this study that the uncertainty of VFA determination was higher at low VFA levels. Thus, too much dilution will also increase uncertainty of VFA determination. This test was set up to determine the optimised degree of dilution in order to get reliable titration results. Figure 5 shows the VFA determination by titration relative to the GC determination and Table 4 shows the RSD for the same results. The graphs show how accurate the titration determination is relative to the GC determination.

**General discussions:**

- The accuracy depends on the procedure, but generally there is no difference in accuracy from different degrees of dilution within a specific titration procedure. However, the dilution factor affected the variation of the titration results.
- At low VFA levels, there is not much difference in the precision, though it gets better the more diluted the sample is. The variation of the VFA results at low dilution indicated stronger matrix effect (interference from other ions) at low VFA levels.
- At high VFA levels, the sample matrix did not have much influence on the VFA results. The best precision is found at no dilution, but if the sample is diluted, the precision increases with increasing dilution.

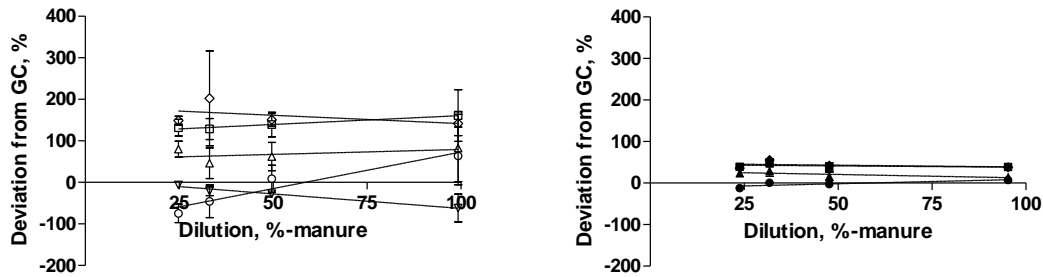


Figure 5 – Effect of dilution on the VFA determination. The graphs at the top show the VFA content determined by titration relative to GC. The graphs at the bottom show the relative standard deviation (RSD) for the different methods at different dilution ratios. The graphs at left show the determination at a low VFA level (1 g/L) and the graphs at right show the determination at a high VFA level (5 g/L). At the top graphs the labels at the x-axis is understood in the following way: first there is a code for the titration method, next follows the VFA content and at last the dilution ratio is given: 4-P L 3 means 4-point titration at low VFA (1 g/L) diluted 3 times.

Table 4 – Relative standard deviation (RSD) in percentage (%) for the different procedures at different VFA levels and degree of dilution.

Dilution, %-manure	VFA 1 g/L				VFA 5 g/L			
	99	50	33	25	95	47	32	24
Procedure:								
Simple	42	30	72	62	2.6	15	7.1	6.3
2-Step	12	6.0	38	4.2	1.7	10	4.1	2.2
4-Step	24	12	11	8.7	1.3	12	6.4	3.1
5-Point NC	44	21	25	11	1.5	12	10	4.4
5-Point C	11	10	14	4.4	-	-	-	-

**Conclusion:**

For samples with high levels of VFAs, no dilution seems to be the most optimal. However, as VFA contents are rarely known in unknown samples, a dilution rate of 4 was chosen to serve both low and high VFA levels in unknown samples.

**1.5.4.2 Relationship between VFA determined by titration and by GC**

In order to establish the relationship between the VFA content determined by titration and by GC, manure was spiked with VFA to make up the concentrations in the range of 1.0-11 g/L as Ac, corresponding to 0.25-2.3 g/L as Ac in the titrated slurry. Ammonia is the main ionic component that can vary significantly in the manure digester due to co-digestion with protein-rich wastes. Two series of titrations were performed, one at a low ammonia concentration of 3.0-3.3 g/L and one at a high ammonia concentration of 4.6-5.0 g/L, see Figure 6.

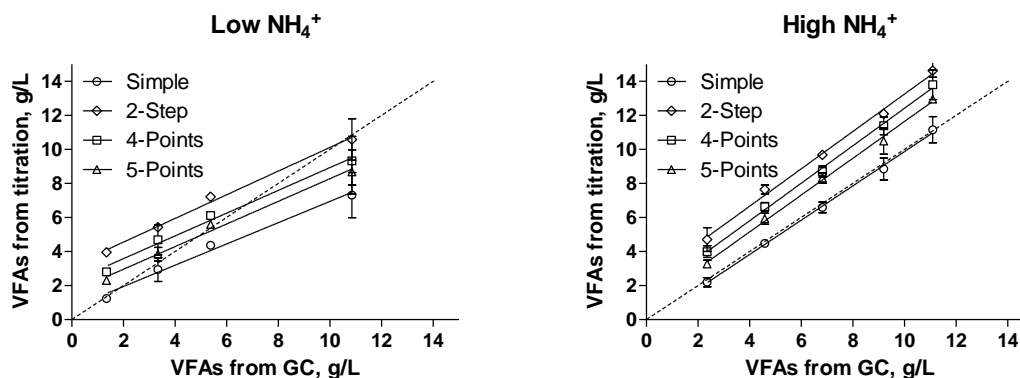


Figure 6 – VFA content determined by titration as a function of VFA determined by GC. Low and high ammonia refer to 3.0-3.3 g/L (i.e. 0.75-0.82 g/L in the slurry) and 4.6-5.0 g/L (i.e. 1.2-1.3 g/L in the slurry), respectively. NB: the 5 points method is in these graphs plotted without correcting data for pH, as the pH-correction only seems to work for VFA-concentrations less than 1 g/L. The dashed line represents a 1:1 relationship.

The regression analyses, as shown in Table 5, show that all titrations show regressions with  $R^2 > 0.91$  with  $R^2$  in the interval 0.91-0.95 at low  $\text{NH}_4^+$  and  $R^2$  in the interval 0.98-0.99 at high  $\text{NH}_4^+$ . All regressions therefore show good linearity. For the regressions at low  $\text{NH}_4^+$  it is shown that the slope of the curve is less than 1, but at the same level. For the regressions at high  $\text{NH}_4^+$  all slopes are almost equal to 1.

All intercepts indicate a background value with lowest to highest impact for the simple, the 5 points, the 4 points and the 2-step, respectively.

Generally, the simple and the 5 points are better than the 2-step and the 4 points, and in many cases the simple could be first choice.

Table 5 – Regression analyses of the various titration procedures to determine VFA content. The VFA content was determined at both a low and a high ammonia content of 3.0-3.3 g/L and 4.6-5.0 g/L, respectively.

Procedure	Low $\text{NH}_4^+$				High $\text{NH}_4^+$			
	Simple	2-step	4-point	5-point	Simple	2-step	4-point	5-point
Intercept	0.6981	3.163	2.244	1.629	-0.2031	2.270	1.402	0.8306
Slope	0.6251	0.6954	0.6687	0.6651	1.007	1.098	1.102	1.081
$R^2$	0.9186	0.9547	0.9120	0.9380	0.9823	0.9875	0.9834	0.9875

### 1.5.4.3 Influence of different parameters

As mentioned above, five typical manure components were selected to be studied for their influence on the VFA determination by four different titration procedures. The effects on VFA determination by titration of spiking manure with four levels of each of the five components are shown in Figure 7. Linear regression was performed on the deviation from the GC result as a function of the concentration of the interfering component. Graphs in the left column show the effects at a low level of VFA, i.e. 1 g/L, and graphs in the right column show the effects at a high level of VFA, i.e. 5 g/L. It is generally seen that the low level of VFA is more affected compared with the high level of VFA. Also generally, the simple as well as the 5-point procedures perform better than the 2-step back and the 4-point procedures. It is worth to mention, that especially the 2-step back and to some extent also the 4-point procedures have deviations ranging up to 400%! Table 6 shows the details of the linear regressions performed for each of the titration procedures and each of the components at the two levels of VFAs.

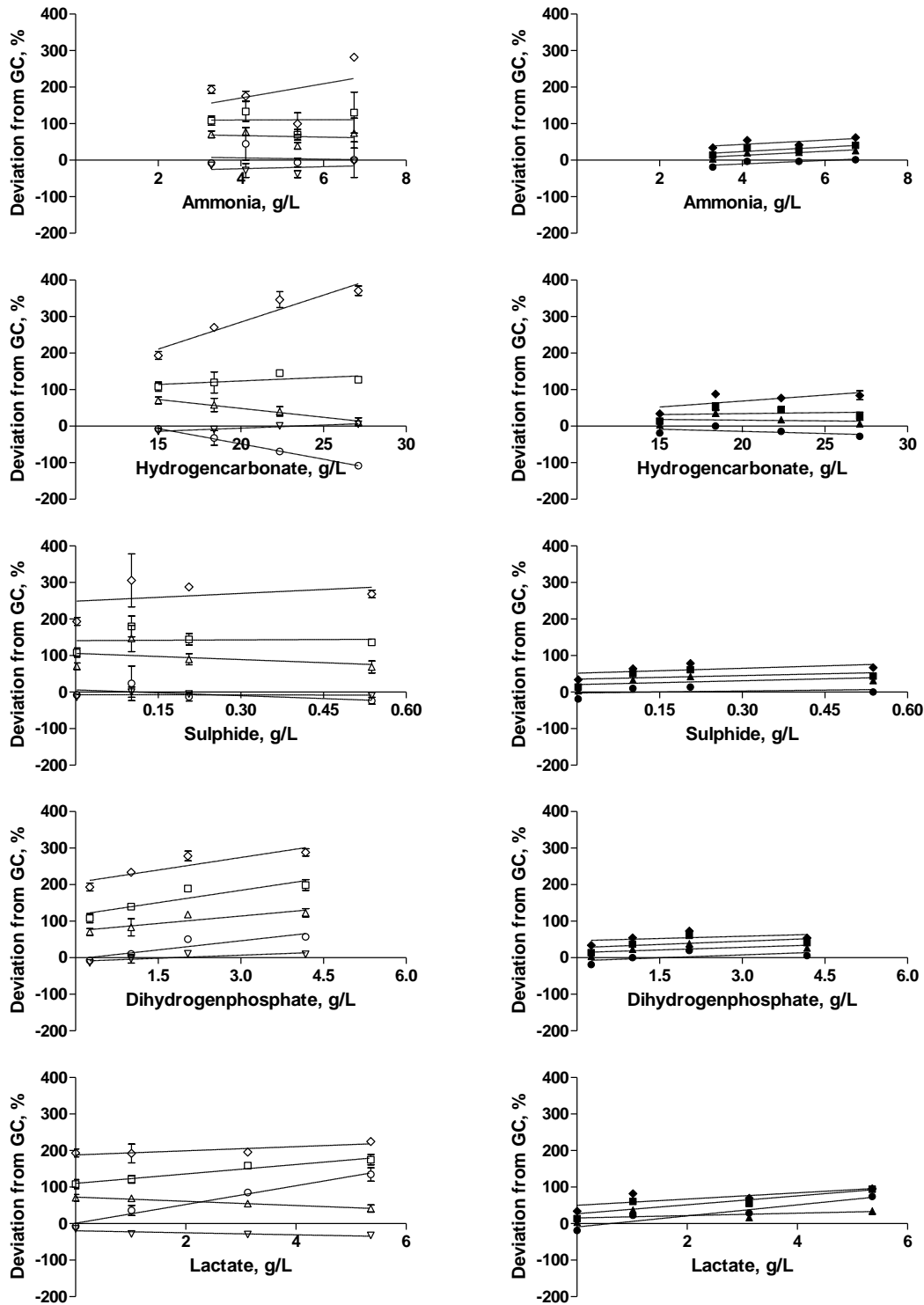


Figure 7 – Graphs showing the influence of ammonia, hydrogencarbonate, sulphide, dihydrogenphosphate and lactate on the VFA content determined by titration. The symbols represent the following titration procedures: O/●: Simple, ◇/◆: 2-step back, □/■: 4-point, △/▲: 5-point not corrected, ▽/▼: 5-point corrected. Open symbols represent low VFA content (1 g/L) and closed symbols represent high VFA content (5 g/L). The unit: deviation from GC, % is calculated by subtracting the GC result from the titration result divided by the GC result and multiplied by 100%.

Table 6 – Statistical data for the linear regression analyses of the influence of ammonia, hydrogencarbonate, sulphide, dihydrogenphosphate and lactate on the determination of VFA by different titration procedures. The “deviation from zero” is a measure to see if the VFA content increases or decreases proportionally with the component. It is tested on a 5% significance level.  $R^2$  is the correlation coefficient.  $\alpha$  is the slope of the regression. -: no data.

Component/procedure	VFA 1 g/L			VFA 5 g/L		
	Deviation from zero	$R^2$	$\alpha$	Deviation from zero	$R^2$	$\alpha$
<b>NH<sub>4</sub><sup>+</sup></b>						
Simple	0.8290	0.005464	-1.757	0.0024	0.6179	4.848
2-Step	0.2500	0.1438	19.50	0.0112	0.4905	5.893
4-Point	0.9960	0.0001586	0.3542	0.0042	0.5767	6.134
5-Point not corrected	0.7245	0.01298	-2.077	0.0018	0.6374	5.592
5-Point corrected	0.4857	0.0498	2.736	-	-	-
<b>HCO<sub>3</sub><sup>-</sup></b>						
Simple	<0.0001	0.9369	-8.409	0.1061	0.2398	-1.169
2-Step	<0.0001	0.8983	14.78	0.0185	0.4409	3.276
4-Point	0.1372	0.2071	1.910	0.6603	0.02009	0.5329
5-Point not corrected	<0.0001	0.7973	-4.877	0.6712	0.01876	-0.3966
5-Point corrected	0.0002	0.7587	1.700	-	-	-
<b>S<sup>2-</sup></b>						
Simple	0.2065	0.1543	-52.73	0.3920	0.07412	17.29
2-Step	0.3916	0.07422	70.87	0.0695	0.2923	44.59
4-Point	0.8883	0.002073	6.630	0.2370	0.1366	32.70
5-Point not corrected	0.3199	0.09872	-56.60	0.1152	0.2293	34.79
5-Point corrected	0.8617	0.003187	-2.310	-	-	-
<b>H<sub>2</sub>PO<sub>4</sub><sup>-</sup></b>						
Simple	0.0002	0.7683	16.65	0.0611	0.3079	5.361
2-Step	0.0002	0.7559	22.86	0.1676	0.1812	4.038
4-Point	0.0002	0.7646	22.54	0.0950	0.2537	5.826
5-Point not corrected	0.0021	0.6300	13.56	0.0697	0.2920	4.796
5-Point corrected	0.0071	0.5326	5.600	-	-	-
<b>Lactate</b>						
Simple	<0.0001	0.9495	25.68	<0.0001	0.8768	15.12
2-Step	0.0226	0.4201	5.670	0.0036	0.5881	8.540
4-Point	<0.0001	0.8611	12.90	0.0002	0.7681	12.22
5-Point not corrected	0.0002	0.7721	-5.770	0.1272	0.2167	3.288
5-Point corrected	0.0069	0.5344	-2.769	-	-	-

### 1.5.4.3.1 Visual and general comments

#### 1.5.4.3.1.1 Ammonia

- In most cases the simple method gives the best results
- For the low VFA level, the 5 point pH corrected method also gives good results

#### 1.5.4.3.1.2 Hydrogen carbonate

- In most cases the simple method gives the best results
- For the low VFA level, the 5 point pH corrected method also gives good results
- For the high VFA level, the simple method is a bit more off the targeted value, than for  $\text{NH}_4^+$

#### 1.5.4.3.1.3 Sulphide

- In most cases the simple method gives the best results
- For the low VFA level, the 5 point pH corrected method also gives good results

#### 1.5.4.3.1.4 Phosphate

- In most cases the simple method gives the best results
- For the low VFA level, the 5 point pH corrected method gives better results than the simple
- For the high VFA level, the simple method is a bit more off the targeted value, than for  $\text{NH}_4^+$

#### 1.5.4.3.1.5 Lactate

- At the low VFA level and at low lactic acid levels, the simple method is best
- Overall, at the low VFA level, the 5 point methods are the best
- At the high VFA level, only the 5 point method is the best

#### 1.5.4.3.2 Multi Criteria Assessment

In order to compare the different methods a simple Multi Criteria Assessment (MCA) was set up. The criteria used for the MCA were:

1. the relative deviation from the VFA content determined by GC ( $\Delta \text{GC}$ )
2. the slope's, obtained from linear regression, deviation from zero ( $\Delta 0$ )
3. the correlation coefficient obtained from linear regression ( $R^2$ )

Re. 1. this is used to evaluate how precise the titration procedure is; the less deviation the greater score.

Re. 2. this is used to evaluate if there is a relationship with this component; the less the P-value is the better is the relationship and the lower is the score.

Re. 3. this is used to evaluate how much of the relationship is described by this component; the higher the  $R^2$  value is the more of the variance is described by this component and the lower is the score.

The intervals used for the different scorings are shown in Table 7. It appears that the maximum score for each procedure at each VFA level for one component is 9 and that the total maximum for all components is 45. Also, the minima are 3 and 15, respectively. The overall evaluation will therefore range in the interval of 15-45 for each titration procedure at each VFA level.

Table 7 – Intervals used to score the different criteria.

Criteria	Score		
	3	2	1
$\Delta \text{GC}$	$\leq \pm 20$	$> \pm 20$ and $\leq \pm 100$	$> \pm 100$
$\Delta 0$	$> 0.05$	$> 0.0001$ and $\leq 0.05$	$\leq 0.0001$
$R^2$	$\leq 0.2$	$> 0.2$ and $\leq 0.7$	$> 0.7$

Combining the values for the deviation from the GC measurement as shown in Figure 7 and the values for the slope's deviation from zero and the correlation coefficient as shown in Ta-

ble 6 with the scoring as shown in Table 7 reveals the total comparison as presented in Table 8.

*Table 8 – Multi Criteria Analysis of the influence of different components on the determination of the VFA level by different titration procedures. Minimum/maximum scores (subtotal) at the individual level of VFA and for the single component is 3/9. Total scores consequently range from 15 to 45, where scores from 15 to 25 inclusive are coloured in red, scores from 26 to 35 inclusive are coloured in yellow and scores from 36 to 45 inclusive are coloured in green.*

VFA level	Simple		2-Step		4-Point		5-Point not corrected		5-Point corrected
	Low	High	Low	High	Low	High	Low	High	Low
<b>NH<sub>4</sub><sup>+</sup></b>									
Δ GC	3	3	1	2	3	2	2	3	2
Δ 0	3	2	3	2	1	2	3	2	3
R <sup>2</sup>	3	2	3	2	1	2	3	2	3
Sub total	9	7	7	6	5	6	8	7	8
<b>HCO<sub>3</sub><sup>-</sup></b>									
Δ GC	2	3	1	2	1	2	2	3	3
Δ 0	1	3	1	2	3	3	1	3	2
R <sup>2</sup>	1	2	1	2	2	3	1	3	1
Sub total	4	8	3	6	6	8	4	9	6
<b>S<sup>2-</sup></b>									
Δ GC	3	3	1	2	1	2	2	2	3
Δ 0	3	3	3	3	3	3	3	3	3
R <sup>2</sup>	3	3	3	2	3	3	3	2	3
Sub total	9	9	7	7	7	8	8	7	9
<b>H<sub>2</sub>PO<sub>4</sub><sup>-</sup></b>									
Δ GC	2	3	1	2	1	2	2	2	3
Δ 0	2	3	2	3	2	3	2	3	2
R <sup>2</sup>	1	2	1	3	1	2	2	2	2
Sub total	5	8	4	8	4	7	6	7	7
<b>Lactate</b>									
Δ GC	2	2	1	2	1	2	2	2	2
Δ 0	1	1	2	2	1	2	2	3	2
R <sup>2</sup>	1	1	2	2	1	1	1	2	2
Sub total	4	4	5	6	3	5	5	7	6
<b>Total</b>	<b>31</b>	<b>36</b>	<b>26</b>	<b>33</b>	<b>25</b>	<b>34</b>	<b>31</b>	<b>37</b>	<b>36</b>

In conclusion:

- The simple and the 5-point procedures are always better than the 2-step and the 4-point procedures
- Except for lactate, the simple procedure are equal to or performs better than the 5 point procedure
- No procedure obtains an overall top evaluation, i.e. 45 points in the MCA.
- At a high level of VFA the simple and the 5-point not corrected procedures obtain 36 and 37 points, respectively.



- At a low level of VFA the simple procedure performs equally well as the 5-point procedure, if no pH correction is used, i.e. 31 points. However, if pH correction is used, the 5-point procedure obtains 36 points.

### 1.5.5 Data from biogas plants

Two biogas plants, Hashøj and Lemvig biogas plants, have been associated with this project. The aim was to have them to test our suggested procedures for VFA titration in a real biogas plant. Although both plants were sincerely engaged in the project, both plants have faced some challenges during the project.

Hashøj has had a series of problems and have therefore been forced to prioritise otherwise during the timeframe of the project, than performing the titrations needed to validate the titration procedures in real biogas plants.

Lemvig has on the other hand been performing the titration, but have been facing problems on the experimental side, but managed in the end to undertake weekly titrations for several months. However, it was later discovered that the samples for GC measurement had not been properly preserved at the biogas plant. Thus, the VFA data from the GC measurements were believed to be unreliable and we decided not to use them for comparison with titration results.

#### 1.5.5.1 Problems observed by Lemvig biogas plants

First of all there were some challenges with keeping the pH electrode clean enough for performing proper titrations. Secondly, in order for the electrode to work, there need to be free access from the electrode to the surrounding aqueous phase for registration of the content of the hydrogen ions. When the manure sample contains varying amounts of more or less degraded biological material, there is a risk for the electrode to be blocked, resulting in error associated measurements. One of the typical experiences were therefore that the autotitrator, as programmed, continued to add acid at the preset rate, while the electrode did not register the added volume of acid, resulting in no pH change. At a certain point in time, the slurry became so diluted that the electrode got proper access to the surrounding solution and started to register the pH change. But at that point in time, it was too late, as the addition of acid had resulted in the pH to be lowered to for instance pH 3 or less. Sometimes it was not this extreme, but often the pH electrode could not follow the added volume of acid.

And when the entire titration procedure was planned to register the added volume of acid to reach certain pH values, the acquired data were seriously affected with errors.

Figure 8 shows titration data from Lemvig biogas plant during a period from June 2011 to January 2012, where some of the data from July and August had been omitted due to technical problems with the pH meter.

From Figure 8, it is noticed that there is a significant difference between the results obtained from the different titration procedures in the full-scale test. Based on our old record of VFA concentrations measured by GC for samples from Lemvig biogas plant, the VFA concentrations in reactor R1, R2 and R4 are in the range of 0.5-2 g/L measured as acetate at normal operation, while reactor R3, which served as second stage, had VFA contents lower than 1.5 g/L. Since the operating conditions had been relatively stable, it is assumed that the VFA ranges did not significantly change and this implied that the 2-step back titration method gave quite overestimated VFA results. This had also been observed from the laboratory titration test (Figure 6) where the 2-step back titration gave the most overestimated results compared with the other procedures. Both Figure 6 and Figure 8 showed that the simple titration procedure gave lowest VFA estimation, followed by the 5-point, the 4-point, and the

2-step back titration procedures, respectively. And based on the normal VFA concentration range at Lemvig biogas plant, the simple titration and the 5-point titration procedure should give the most reliable results.

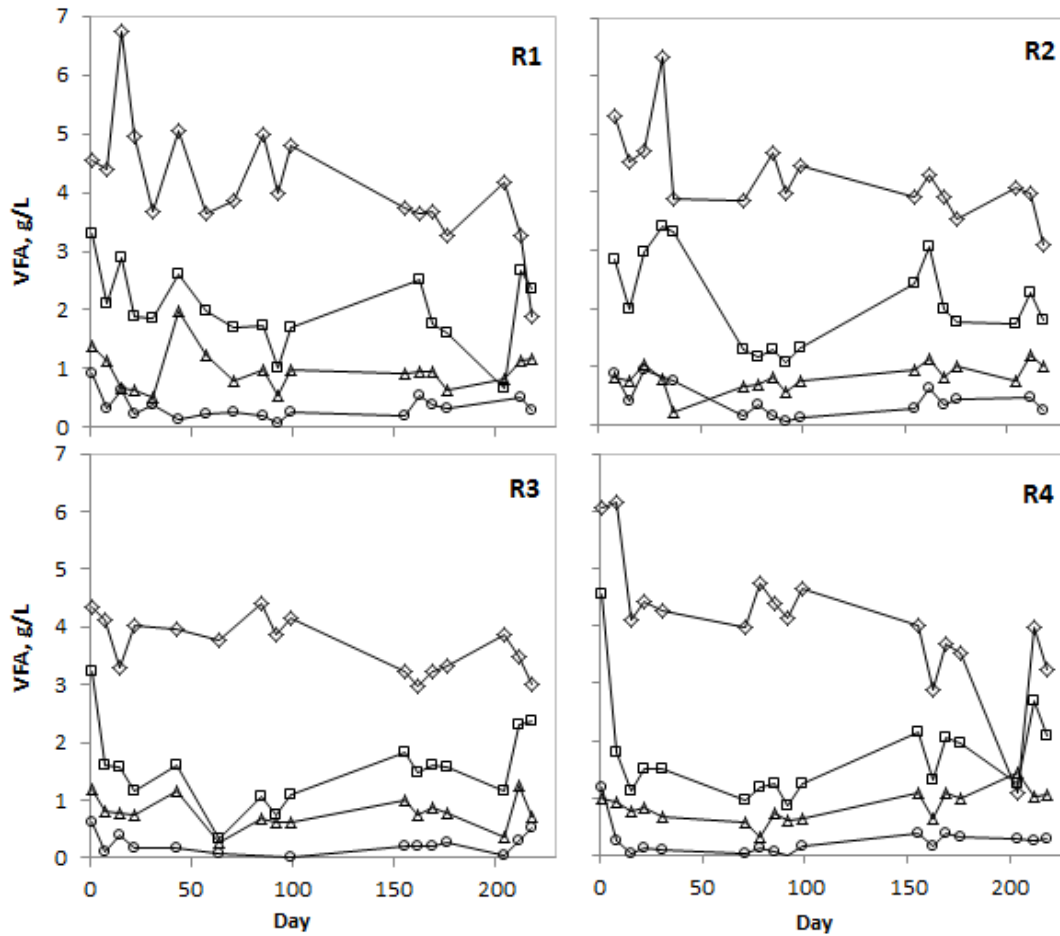


Figure 8 – Results from testing the titration procedures in Lemvig biogas plant. The symbols represent the following titration procedures: ○: Simple, ◇: 2-step back, □: 4-point, △: 5-point corrected.

## 1.6 Utilization of project results

The results obtained in this project will be disseminated among biogas plants practitioners, both the two plants involved, as well as among the biogas plant association. The dissemination will take place in form of oral presentations and an article in the magazine "FiB – Forskning i Bioenergi, Brint & Brændselsceller". Furthermore, an article in the scientific journal "Bioresource and Technology" is about to be submitted.

Also within the EU funded InterReg project: "Biorefinery Øresund" there have been ongoing presentations about the project's results and progress. The final results will also within this framework be presented at the final conference of that project, see <http://www.oresund.org/biorefinery>.

DTU ENV, as the responsible institution for the project, will use the results in their continued work in delivering tools to be used within the bioenergy sector, in this case tools to monitor and control the biogas reaction in order to optimise the yield of biogas.

## 1.7 Project conclusion and perspective

### 1.7.1 Conclusions

From the performed laboratory validation of the four different titration procedures it can surprisingly be concluded, that **if no knowledge** of the composition of a certain manure sample is present, the **simple titration** procedure should be the **procedure of choice**, due to its simplicity in both execution and the following calculations of VFA content.

However, when the calculation procedure has been established and if one has access to an autotitrator connected to a computer software, like the one used in this project, the 5-point procedure should be the procedure of choice, as this procedure offers the opportunity to correct for pH and thereby both at the low and high level of VFA obtain good results. The 5-point procedure is also the one that best handles interference by lactate.

The validation performed *in situ* on real biogas plant samples showed first of all lower VFA levels than expected. But for these samples also the two above mentioned procedures, the simple and the 5-point procedures, perform closest to the GC derived VFA levels, though in most cases with no real dependency on the VFA level.

### 1.7.2 Perspectives

Manure contains compounds that during titration can evaporate into the gas phase, such as hydrogencarbonate (in form of  $\text{CO}_2$ ), undissociated VFA,  $\text{H}_2\text{S}$ , and some  $\text{NH}_3$  (during high pH). Thus, the rate of acid addition (mL/min) will also affect the titration results as slow acid addition rate will allow longer time for liquid-gas exchange, resulting in potential evaporation of some compounds. However, an increased rate of acid addition will have a contradictory effect on how precise the electrode registers the hydrogen ion concentration, as pH changes very high rates may not be registered in due time, whereby a slower rate of addition will contribute to a more precise pH-added titrant relationship. The acid addition rate of 1 mL/min has been optimised based on the specific titrator setup used in this project. The titration rate was optimised to minimise the whole titration time, but still slow enough to correctly register the pH value and distinguish the amount of acid used at different titration points. The use of an autotitrator will give a more precise rate of acid addition than manual titration. Another factor that can influence the titration results is the stirring intensity. A fast stirring will increase the accessibility of the electrode to the surrounding hydrogen ion concentration (i.e. the pH value), however, it will also increase the liquid-gas exchange rate. The optimum stirring rate used in this titration setup was 200 rpm using a magnet size of 8 mm diameter and 25 mm length (see details in section 1.5.2 Setup of an automatic titration system). Thus, in order to obtain the titration results that are comparable between different biogas plants, both the titration procedure and the setup of the autotitrator should be standardised. The suggested titration procedure obtained in this project is described in Appendix A. Moreover, as part of the project outcome, the calculation worksheet in MS Excel format is also provided, so the biogas plant operators can use this file for VFA calculation from titration data.

It would also be of interest to study the different components' influence in the VFA range of 0.2 to 2.0 g/L, to better understand possible effects on current real biogas plant samples. Additionally it would also be interesting to consider how the different components affect the VFA level under the influence of other components, and not only under variation of a single component.

## **1.8 Annual export of electricity (only ForskVE)**

Not required for this project.

## **1.9 Updating Financial Appendix and submitting the final report**

The final accounting has been uploaded to the website.

## Appendix A

### Method description for VFA measurement in digested manure samples

#### Reagent

1. 0.3 M HCl

#### Apparatus

1. 140 mL beaker (d:60mm, h:80mm)
2. Magnetic stirrer (dia:8mm, l:25mm)
3. Scale
4. pH meter

Note: It is suggested to use an autotitrator for more reliable titration data.

#### Procedure

1. Take a sample of 10 mL (approx. 10 g) in the 140-mL beaker. Record the exact sample weight. Add 30 mL distilled water. Record the pH start.
2. Titrate with 0.3 M HCl to reach different pH values as shown in the table (depending on the calculation method). Record the volume of acid used at each pH.
3. Key the data in the calculation worksheet to calculate total VFA and alkalinity.

#### Data required for the calculations

Simple titration	5-points titration
Sample weight (g)	Sample weight (g)
pH start	pH start
Volume of acid used to titrate from pH: - start to 5.1 - 5.1 to 3.5	Volume of acid used to titrate from pH: - start to 6.7 - 6.7 to 5.9 - 5.9 to 5.2 - 5.2 to 4.3
pKa-values of the: - carbonate species and - VFAs (average of the VFAs in focus)	pKa-values of the: - interfering components, e.g. carbonate, ammonia, lactate, etc. and - VFAs (average of the VFAs in focus)

The data below are already provided in the calculation worksheet. However, if the biogas plants use a setup different from the procedure described above, these data must be corrected in the calculation worksheet.

Simple titration	5-points titration
Acid titrant concentration = 0.3 M	Acid titrant concentration = 0.3 M

## Appendix B

### Minor modifications to the calculations for the simple titration procedure

The simple titration procedure is based on a mass balance of VFA and the bicarbonate component of  $[\text{HCO}_3^-]$  and  $[\text{H}_2\text{CO}_3]$ . From the calculation program provided by Anderson and Yang (1992), the authors multiplied the volume of acid used for the titration from pH 5.1 to 3.5 with 75% (a factor of 0.25). This factor was obtained by a blank titration with distilled water to calibrate the acid consumption of the second stage titration, in order to obtain accurate results at low VFA concentrations.

From experiments performed within this project, the factor of 0.25 did not seem to be enough to correct the VFA calculation in manure samples. Figure 9 shows the %-deviation of VFA results obtained with the simple titration procedure using different empirically derived correction factors compared with the GC obtained VFA contents.

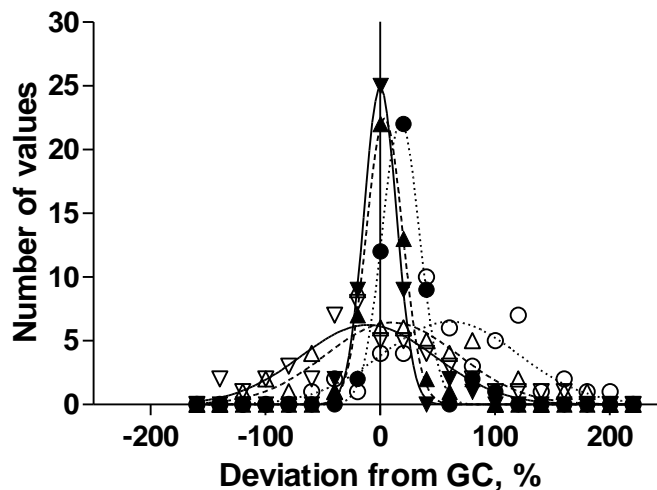


Figure 9 – VFA titration results obtained with the simple titration procedure using different correction factors;  $\circ/\bullet$ : without correction factor,  $\triangle/\blacktriangle$ : 0.25 as suggested in literature and  $\nabla/\blacktriangledown$ : 0.35 as suggested in this work. Open symbols represent low VFA content (1 g/L) and closed symbols represent high VFA content (5 g/L). The data have been grouped in intervals of 20, this means for instance that all data with a deviation in the interval -20 to 0 have been grouped and so on.

The loss of the carbonate component in form of  $\text{CO}_2$  during the second stage of the titration, i.e. from pH 5.1 to 3.5, increased pH and resulted in an over consumption of acid and thus, an over estimation of the VFA content from this procedure. Implementation of a higher factor than 0.25 will also compensate this error. The mean values' deviation from zero have been tested in a t-test. For the low VFA, a correction factor of 0 results in a difference from zero, but not 0.25 and 0.35. However, only 0.35 results in no difference from zero at the high VFA-level, see Table 9. The experimental distribution data have also been fitted to the Gaussian distribution and the results are shown in Table 9. All fit show a nice correlation with the Gaussian distribution with  $R^2$  in the range 0.72-0.84 at low VFA and  $R^2 > 0.99$  at high VFA. Thus, the calculation worksheet provided from this project will use the correction factor of 0.35, instead of 0.25 as originally provided in the literature.



Table 9 - t-test of the means' deviation from zero and the distributions' fitting to the Gaussian distribution. Note: The two mean values for the same conditions don not have to be identical, as the means in the Gaussian distribution test are a product of the pooling into groups, and they may therefore differ from the mean values for the whole data population.

Correction factor	Low			High		
	0	0.25	0.35	0	0.25	0.35
<b>t-test</b>						
Mean	64.58	14.13	-6.054	20.54	6.820	1.334
P-value delta 0	<0.0001	0.1343	0.5345	<0.0001	0.0467	0.6914
Different from 0?	Yes	No	No	Yes	Yes	No
<b>Gaussian</b>						
Amplitude	6.497	6.432	6.244	22.15	22.49	24.94
Mean	60.95	10.13	-11.00	17.92	3.738	-0.1044
SD	59.28	57.62	58.26	16.34	15.74	14.10
R <sup>2</sup>	0.7159	0.7236	0.8375	0.9903	0.9908	0.9888