TAMK University of Applied Sciences
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Atte Penttilä

Final Thesis

Initialization of the Oxitop® system for biogas production tests

Supervisor Senior Lecturer Eeva-Liisa Viskari
Commissioned by TAMK University of Applied Sciences
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Abstract

The Oxitop® measuring system provides a mechanism to understand biogas production, and offers a possibility to test the biodegradability of different organic substances in small scale. The purpose of this thesis was to take into use and create simple instructions on how to measure biogas production with the Oxitop® measuring system.

The main components of biogas are methane (CH_4) and carbon dioxide (CO_2). As these substances are produced in sample bottles from horse manure and biowaste, the pressure inside the bottle increases. The Oxitop®-C measuring head reads this change in pressure inside the sample bottle and saves the reading. From these pressure readings the rate of biogas production can be seen and eventually the total amount of carbon present in the sample bottle can be calculated, from which the amounts of methane and carbon dioxide can then be derived.

The study was done in two parts, first in the spring of 2009 and the latter in the fall of 2009. During the first trial with the Oxitop® measuring system the purpose was to find out how much biological material should be used in what kind of combinations in order to achieve the most stable results of biological degradation. The second trial was then conducted with the results from the first trial in order to achieve steady biogas production and separate carbon dioxide from methane to find out the amounts of each substance.

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Tiivistelmä

Oxitop® mittauslaitteiston avulla voidaan tutkia biokaasun tuotantoa ja se mahdollistaa pienen mittakaavan kokeiden tekemisen erilaisten biologisesti hajoavien aineiden biohajoavuuden osalta. Tämän opinnäytetyön tarkoitus oli ottaa käyttöön Oxitop® laitteisto ja tehdä yksinkertaiset ohjeet biokaasun tuoton mittaamiseksi.

Biokaasun pääaineet ovat metaani (CH_4) ja hiilidioksidi (CO_2). Näitä yhdisteitä muodostuu näytepullojen sisällä hevosen lannasta ja biojätteestä, jolloin pullojen sisäinen paine kasvaa. Oxitop®-C mittauspää lukee ja tallentaa tämän paineen muutoksen, josta voidaan selvittää biokaasun tuoton aste ja laskea kokonaishiilen määrä sekä erottaa metaani ja hiilidioksidi.

Työ suoritettiin kahdessa osassa, ensimmäinen keväällä 2009, toinen syksyllä 2009. Ensimmäisessä vaiheessa selvitettiin biologisten materiaalien optimaaliset määrät ja yhdistelmät, jotta saataisiin aikaan mahdollisimman tasainen biokaasun tuotanto. Toisessa osassa, ensimmäisen kokeen tuloksia hyväksi käyttäen, tavoitteena oli saada aikaan tasainen biokaasun tuotanto sekä erottaa hiilidioksidi metaanista, jotta molempien yhdisteiden määrät pystyttiin mittaamaan.

Foreword

Times of peak oil and evident global warming have brought about discussion, and even some action, on using non-fossil fuels to power our daily lives. One of these renewable resources is biogas, a by-product of anaerobic bacterial activity in the past, but now a prominent source for energy.

I wish to express my gratitude for certain people for the past four and so years and the completion of this final thesis. Eeva-Liisa Viskari for not only supervising my thesis, but also for giving me the possibility to initialize equipment that would later be used in educational purposes for future environmental engineers. The classmates that did not finish the program and the ones that did, I thank you for your company and comradeship. Family and Heidi for your support at all times.

Tampere, November 2009

Atte Penttilä

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1. Introduction

Due to the rising awareness of environmental issues with using fossil fuels, such as contaminated soils and water basins from crude oil excavation, air pollution from coal burning power plants and global warming, the public have started to pay more attention on renewable energy sources. Solar power plants are being planned and built in several nations, one example being the solar tower in Seville, Spain. This power plant generates 11 MW of electricity without any environmental effects in addition to the original setup. /1/

It is obvious that the investments for renewable energy resources are, at the moment, minute when compared to the influence of big oil to the climate and the environment as a whole. Many times, not every time, the clean energy initiatives of large companies are not much more than greenwashing attempts to win the unthinking majority into believing that these big enterprises would be interested in anything else except increasing income. Nevertheless, new and old ideas of renewable energy resources are being revised in order to find suitable, environmentally friendly, alternatives to fossil fuels for different regions e.g. solar power in dry areas, wind power in windy areas, tidal power in coastal areas and biogas everywhere. /2/, /3/, /4/

Biogas is a carbon neutral energy resource, meaning that burning biogas releases only the amount of CO_2 that it took up when the biomass, from which the gas is created, was growing. Biogas is available wherever there is biological activity, it is going to be formed whether we, as humans, like it or not. Formed mainly of methane and carbon dioxide, biogas is produced when biological material biodegrades in anaerobic conditions. As it is known methane is a very potent greenhouse gas, 21 times more than carbon dioxide, so it is unwise to let that methane vanish to the atmosphere, but to gather it and burn it in order to create energy. Using biogas is not a new invention, already in 1897 in Exeter, United Kingdom, street lamps were lit with gas from waste water. /5/

This study will show how biogas is formed from horse manure and biowaste with the help of Oxitop® equipment from TAMK University of Applied Sciences. The second chapter deals with the theory behind biogas formation and the formulas for the calculations for the amounts of total

carbon, carbon dioxide and methane, third the tests done in the laboratory, including calculations and results, then conclusions and recommendations for future tests are given.

2. Theory

2.1 Basics

Biogas is mostly consisted of methane (CH_4) and carbon dioxide (CO_2), the general composition being 71 percent CH_4 and 29 percent CO_2, nitrogen (N) and sulfur (S) are present in small amounts and do not affect the ratio. /5/

Organic materials, carbohydrates, are produced by plants with the help of the sun, from carbon dioxide and water by photosynthesis following the reaction:

$$CO_2 + H_2O \rightarrow CH_2O + O_2$$

This organic material can then be degraded into biogas by anaerobic fermentation by the following reaction:

$$CH_2O \rightarrow 0.5CH_4 + 0.5CO_2$$

When the resulting methane is combusted, carbon dioxide and water are produced, from which the photosynthesis can be fed again.

$$0.5CH_4 + O_2 \rightarrow 0.5CO_2 + H_2O$$

Burning of fossil fuels, such as coal or crude oil, add carbon into the earth's atmosphere which offsets the natural balance. As seen from these reactions biogas production is a carbon neutral way of producing energy. The carbon that is left from the burning of methane is, theoretically, used again in photosynthesis and does not add carbon to the natural cycle. /5/, /6/

The fermentation of methane is not as simple as that, though it requires complex processes of degradation, hydrolysis, acidogenesis, acetogenesis and methanation. /5/, /6/

2.2 Hydrolysis

In hydrolysis complex organic compounds (undissolved compounds) e.g. lipids, cellulose, carbohydrates and proteins are altered into monomers, simple soluble products, such as sugars, amino acids and glycerin by extracellular enzymes of the anaerobic bacteria. This step, hydrolysis, is also known as liquefaction because the covalent bonds in the compounds are broken down by water in a chemical reaction. /5/, /6/

Hydrolysis of different compounds happens in different time frames. The hydrolysis of carbohydrates takes place within a few hours, of lipids and proteins in a few days and for cellulose and lignin are broken down only at a slow rate and incompletely. /5/

2.3 Acidogenesis

In acidogenesis, or acidogenic phase, the simple soluble compounds, monomers, from hydrolysis are fermented by the anaerobic bacteria into a mixture of short-chain organic acids (volatile fatty acids), carbon dioxide, alcohols and hydrogen. /5/, /6/

2.4 Acetogenesis

The volatile fatty acids from acidogenesis along with ethanol are transformed into acetic acid (ethanoic acid), carbon dioxide and hydrogen by hydrogen-producing acetogenic bacteria. Below is an example of how propionic acid (propanoic acid) is converted in acetogenesis:

$$CH_3(CH_2)COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$$

Hydrogen partial pressure has to remain low in order for the acetogenesis to function properly, meaning the concentration of H_2 has to remain also low. The hydrogen concentration is kept low with the efficient removal by the hydrogenotrophic methanogens and/or homoacetogens. /5/, /6/

2.5 Methanogenesis

In methanogenesis, or methanogenic phase, methane is formed in strictly anaerobic conditions. Acetotrophic or aceticlastic methanogens create methane from acetate which is formed in acetogenesis. Hydrogenotrophic methanogens generate the rest of the methane from hydrogen and carbon dioxide. As an example here are two different reactions, first for acetotrophic and second for hydrogenotrophic methanogenesis:

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3$$

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$

There are three suitable temperatures for the methanogenesis to work in: psychrophilic, mesophilic and thermophilic. Mesophiles, methanogens that operate in mesophilic temperature, has their highest efficiency at 35-40 °C. Most of the methanogens are mesophilic and in this experiment the temperature of 35°C, 308.15K, was chosen to be used. /5/, /6/

2.6 Formulas

2.6.1 The Ideal Gas Law

The basis for all the calculations in the thesis is the ideal gas law:

$$\text{Ideal Gas Law: } n = \frac{p \times V}{R \times T}$$

n = number of moles of gases formed (mol)

p = gas pressure in Pascal (N/m^2)

V = gas volume (m^3)

R = gas constant (8,314 J/(mol*K))

T = incubation temperature (K)

Since the pressure in this experiment is in hPa, volume in ml and temperature 35 °C (308,15K) the formula can be simplified into: /7/

$$n = p \times V \times 3{,}903 \times 10^{-8}$$

n = number of moles of gas formed (mol)

p = pressure (hPa)

V = volume (ml)

2.6.2 Carbon Content of the Gaseous Phase

Carbon (carbon dioxide and methane) content of the **gaseous phase** can be calculated with the following formula: /7/

$$n = \frac{\Delta p \times Vg}{R \times T} \times 10^{-4}$$

n = number of moles of gas formed, CO_2 and CH_4 (mol)

Δp = the difference of the gas pressure in the sample bottle at the end of the experiment (plateau) minus the pressure in the beginning of the experiment minus the difference of the blank values (hPa)

Vg = gas volume of the headspace (ml)

10^{-4} = conversion factor Pa in hPa and m^3 into ml

The formula for the calculation of the moles in the gaseous phase of the sample bottle can, if the pressure is in hPa, volume in ml and temperature 35 °C, be simplified into: /7/

$$n = \Delta p \times Vg \times 3{,}903 \times 10^{-8}$$

n = number of moles of gas formed, CO_2 and CH_4 (mol)

Δp = the difference of the gas pressure in the sample bottle at the end of the experiment (plateau) minus the pressure in the beginning of the experiment minus the difference of the blank values (hPa)

Vg = gas volume of the headspace (ml)

2.6.3 Differentiation Between CO_2 and CH_4

Differentiation between carbon dioxide and methane: /7/

$$n = \left(\frac{p3 \times (Vg - V(KOH)) - p \times Vg}{R \times T}\right) \times 10^{-4}$$

n = number of moles of carbon dioxide (mol)

$\mathbf{p_3}$ = absolute gas pressure after the injection of 30% v/v KOH-solution (hPa)

p = absolute gas pressure before the addition of KOH (hPa)

$\mathbf{V_g}$ = gas volume (ml)

$\mathbf{V_{KOH}}$ = volume of KOH-solution added

R = gas constant (8,314 J/(mol*K))

T = incubation temperature (K)

$\mathbf{10^{-4}}$ = conversion factor Pa in hPa and m^3 into ml

The formula can be simplified into: /7/

$$n = [p3 \times (Vg - V(KOH)) - p \times Vg] \times 3{,}903 \times 10^{-8}$$

n = number of moles of carbon dioxide (mol)

$\mathbf{p_3}$ = absolute gas pressure after the injection of 30% v/v KOH-solution (hPa)

p = absolute gas pressure before the addition of KOH (hPa)

$\mathbf{V_g}$ = gas volume (ml)

$\mathbf{V_{KOH}}$ = volume of KOH-solution added

3. Laboratory Tests

3.1 Principles of Oxitop® Method

The Oxitop® method is based on pressure changes in the graduated flasks (Figure 1) when anaerobic degradation takes place. The sample substances are placed into the flasks with an appropriate amount of distilled water and additional substances, sugar or digested sludge in this experiment, and a magnetic stirrer into each bottle. The bottles are then placed into an incubation chamber at a constant temperature of 35°C in the dark, onto an inductive stirring system, Stirrer IS

12, which keeps the samples moving constantly, thus increasing the rate of biogas production.

Figure 1. The Oxitop® Sample Bottle with a measuring head

As a result the anaerobic degradation forms methane (CH_4) and carbon dioxide (CO_2) which leads to the increase of pressure inside the head space of the gas proof sample flasks. This pressure change is recorded at 160 minute or 56 minute intervals automatically, depending on the time of the whole experiment, but can also be measured manually whenever. The results are stored into the Oxitop®-C measuring head, as seen in figure 2, and from there the results are read and transferred to a computer for analyzing with the controller OC 110 that can be seen in figure 2.

The changes in the pressures allow us to calculate the amount of total carbon produced and then to differentiate between carbon dioxide and methane according to the formulas in chapter 2.6. The differentiation of methane and carbon dioxide in the head space of the sample bottles is possible after the carbon dioxide has been absorbed by a CO_2 absorber, in this case potassium hydroxide (KOH 30 % v/v), injected trough a septum nozzle into a rubber stopper in the flask, seen in figure 4, with an injection syringe. After the carbon dioxide has been absorbed, in 18-24 hours, the remaining

over pressure gives us the needed results to calculate the amount of methane produced. For this reason it is preferable for the pressure level of the sample to reach a plateau stage before the injection of potassium hydroxide in order to see the pressure differences accurately.

Figure 2. The Oxitop®-C Measuring Head

Figure 3. The Oxitop® OC 110 Controller

Figure 4. The Septum Nozzle and the Rubber Stopper

Before the sample substances are placed into the graduated flasks the volume of the flasks must be known. This can be done in two different ways that also serve as a confirmation for each other. First method is to weigh the dry weight of the bottle, including all the rubber stoppers and the magnetic stirrer, then fill the bottle with bubble free water and weigh it again. The resulting weight difference in grams is parallel to the volume in milliliters. The second way of measuring the volume is to fill the graduated flask with bubble free water and then simply poor the water into a measuring cylinder.

3.2 Trial Run

The trial run was done in order to find out the appropriate amounts of horse manure and biowaste in order to have readable results of pressure changes in the sample bottles. Eleven samples with different water/sample mixtures were prepared, sugar was added into some of the samples to see if the degradation process would develop faster. The samples were divided so that first samples were prepared with only horse manure, then samples with both horse manure and biowaste, and finally samples with only biowaste. The consistency of the biowaste from TAMK University of Applied Sciences cafeteria was mostly potato, leftover salad and carrot, with a mixture of other food

supplies in lesser amounts. In Table 1 the trial run setup, with all the variables, is shown. There were only 11 samples in the trial run, for one of the graduated flasks had been broken by an unknown assailant.

Table 1. Trial Run Setup 08.04.2009.

Sample Number	Test Substance	Water (Distilled H_2O)	Sugar ($C_{12}H_{22}O_{11}$)
1.	20ml Horse Manure	150ml	-
2.	40ml Horse Manure	150ml	-
3.	20ml Horse Manure	150ml	4g
4.	40ml Horse Manure	150ml	4g
5.	10ml Horse Manure, 20ml Biowaste	150ml	-
6.	20ml Horse Manure, 10ml Biowaste	150ml	-
7.	10ml Horse Manure, 20ml Biowaste	150ml	2g
8.	40ml Biowaste	150ml	
9.	20ml Biowaste	150ml	
10.	40ml Biowaste	150ml	4g
11.	20ml Biowaste	150ml	4g

3.3 Trial Run Test Results

The results of pressure changes, and thus biogas production, in the trial run in April 2009 can be seen in figures 5-16 where the pressure in hectopascals (hPa) is plotted against time in minutes.

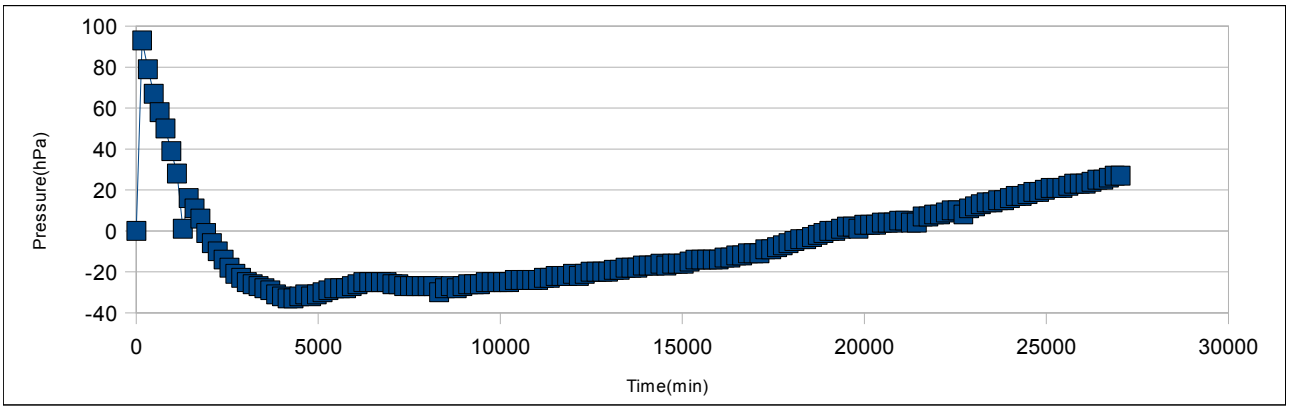

Figure 5. The change in pressure (hPa) over time (minutes) in trial run of sample 1 (20 ml of horse manure)

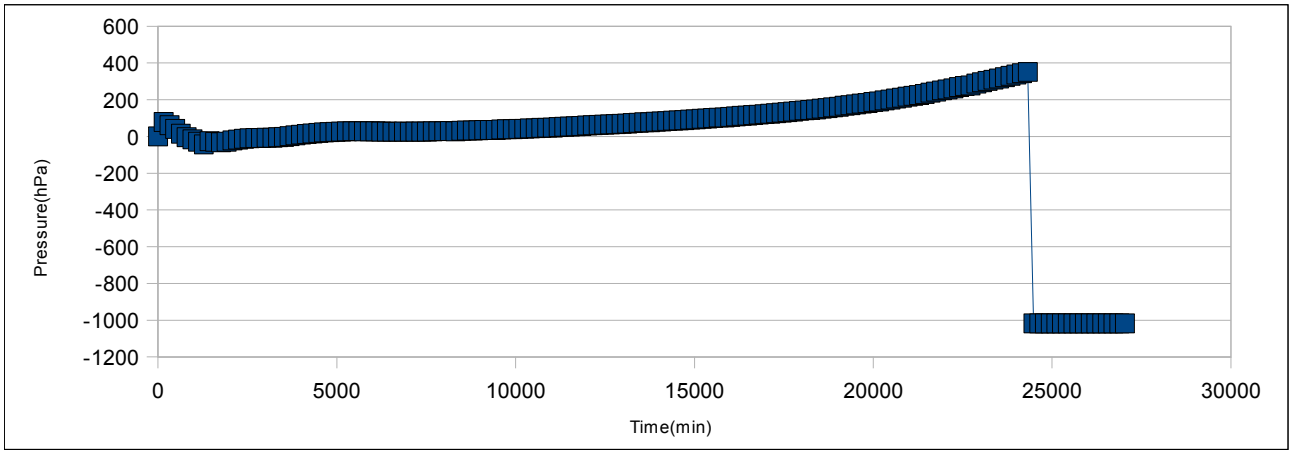

Figure 6. The change in pressure (hPa) over time (minutes) in trial run of sample 2 (40 ml of horse manure)

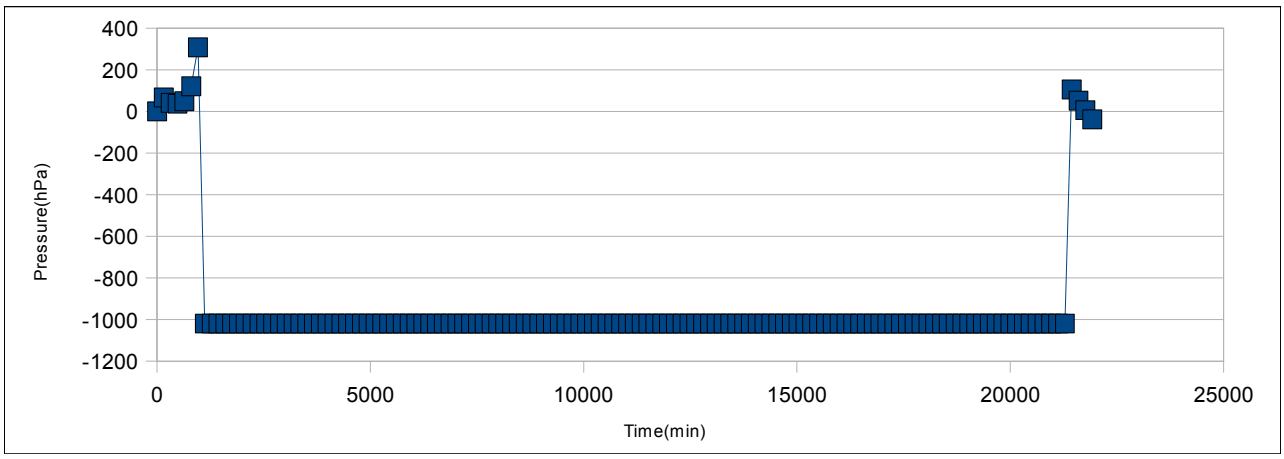

Figure 7. The change in pressure (hPa) over time (minutes) in trial run of sample 3 (20 ml of horse manure and 4 g of sugar)

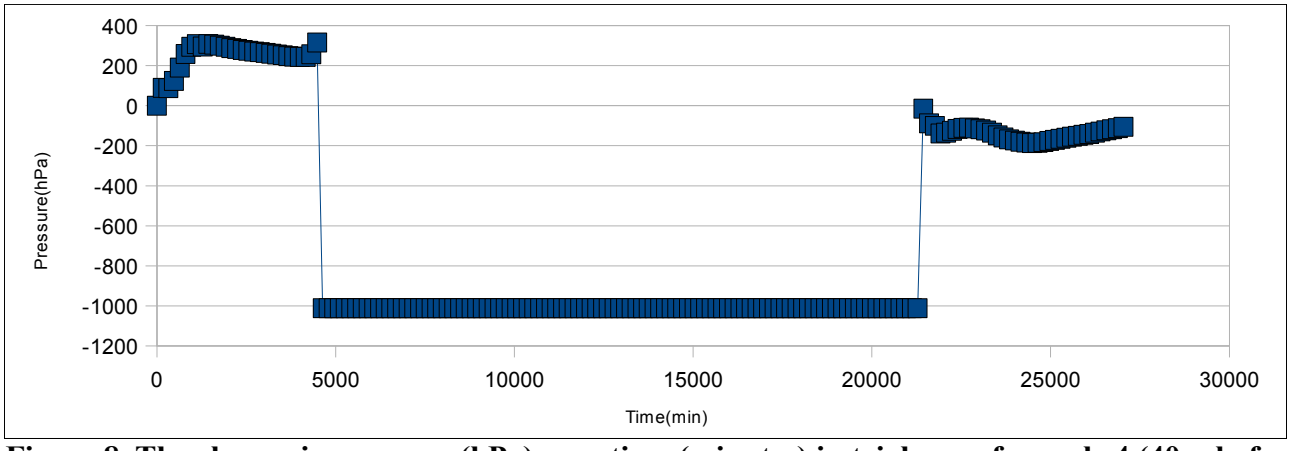

Figure 8. The change in pressure (hPa) over time (minutes) in trial run of sample 4 (40 ml of horse manure and 4 g of sugar)

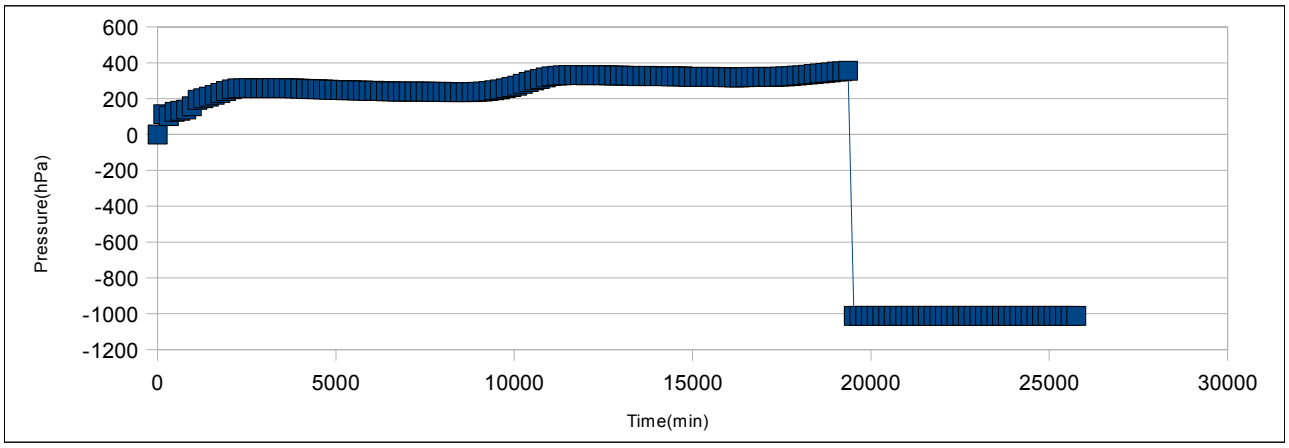

Figure 9. The change in pressure (hPa) over time (minutes) in trial run of sample 5 (10 ml of horse manure and 20 ml of biowaste)

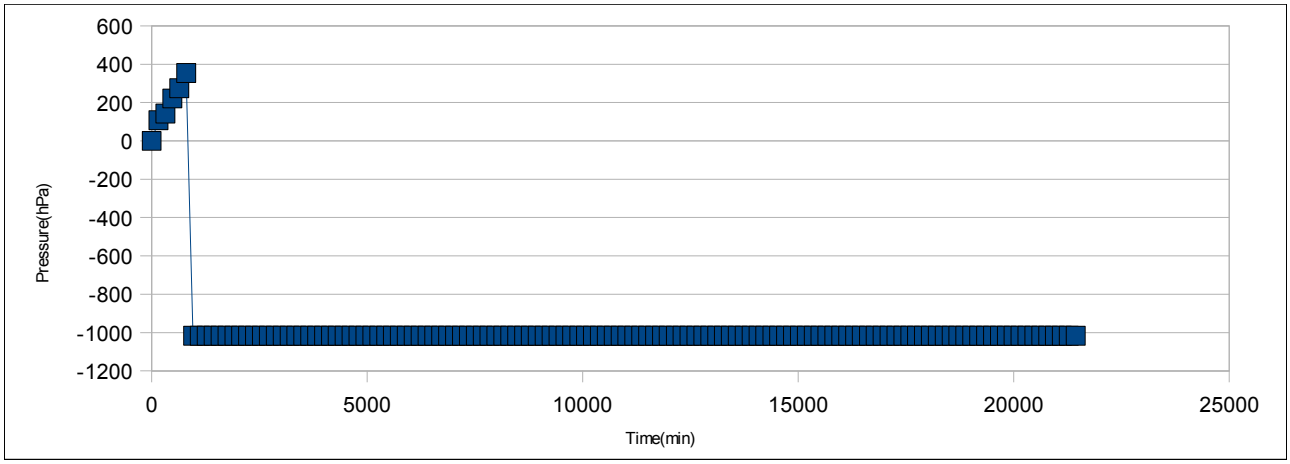

Figure 10. The change in pressure (hPa) over time (minutes) in trial run of sample 6 (20 ml of horse manure and 10 ml of biowaste)

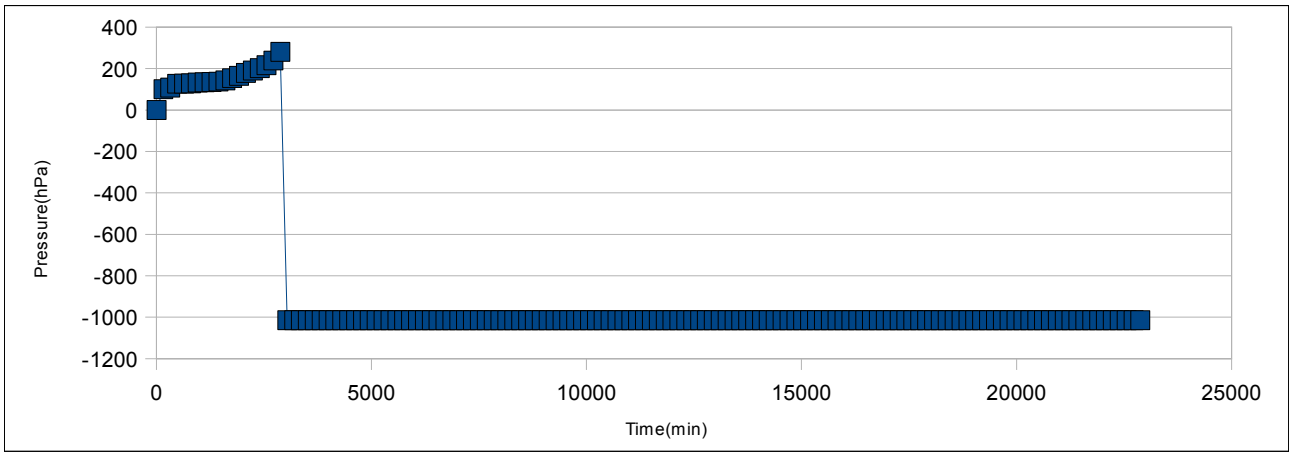

Figure 11. The change in pressure (hPa) over time (minutes) in trial run of sample 7 (10 ml of horse manure, 20 ml of biowaste and 2 g of sugar)

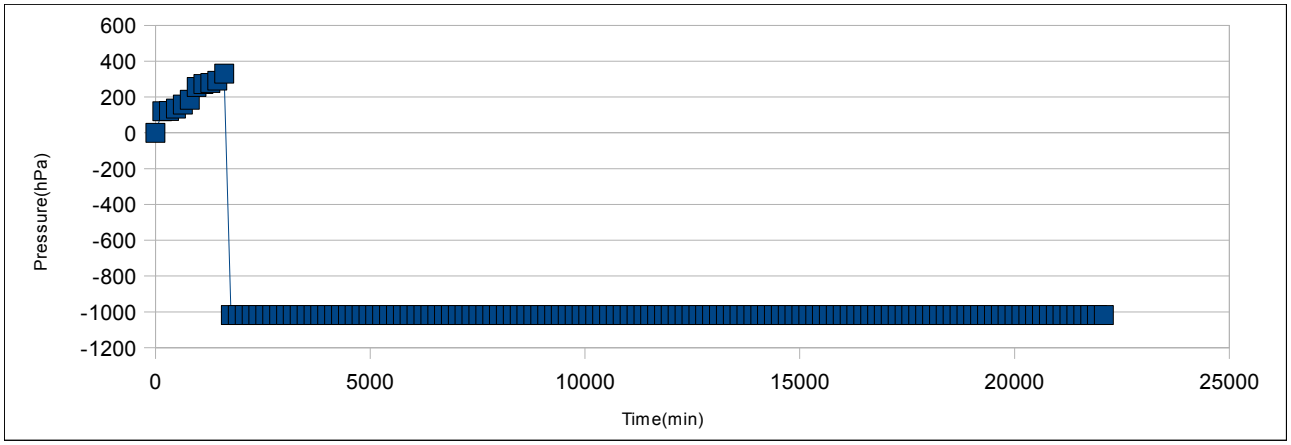

Figure 12. The change in pressure (hPa) over time (minutes) in trial run of sample 8 (40 ml of biowaste)

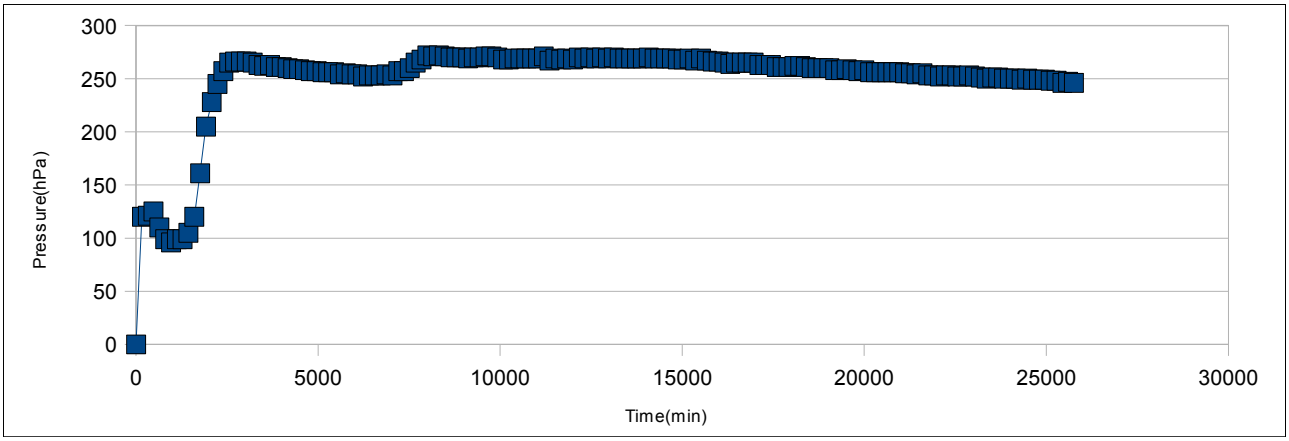

Figure 13. The change in pressure (hPa) over time (minutes) in trial run of sample 9 (20 ml of biowaste)

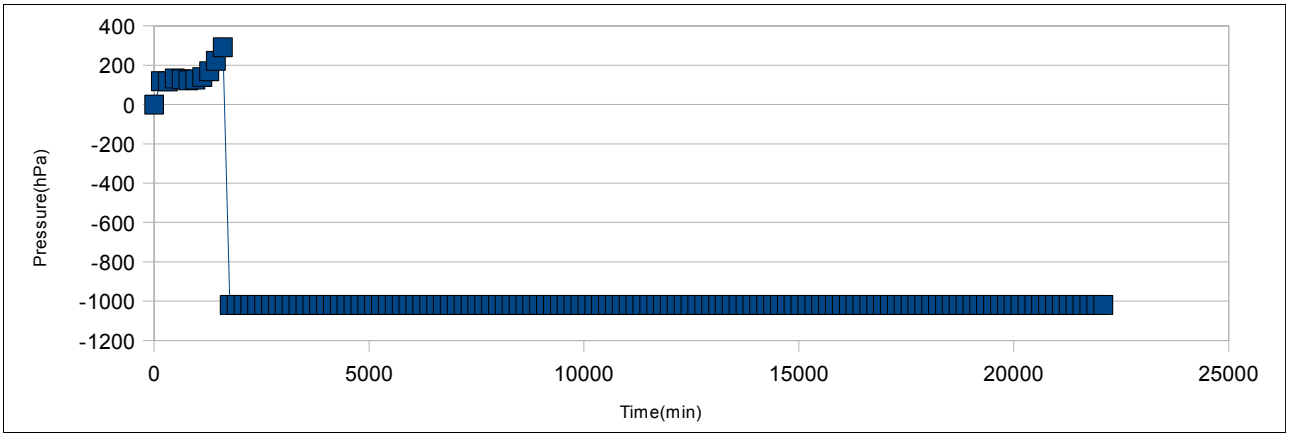

Figure 14. The change in pressure (hPa) over time (minutes) in trial run of sample 10 (40 ml of biowaste and 4 g of sugar)

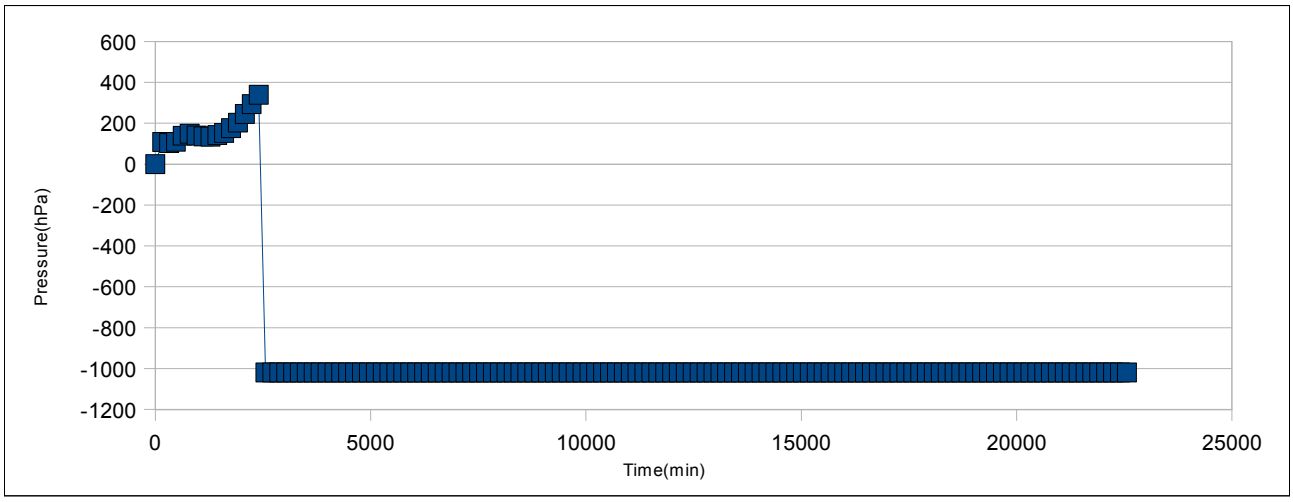

Figure 15. The change in pressure (hPa) over time (minutes) in trial run of sample 11 (20 ml of biowaste and 4 g of sugar)

3.4 Analyzing the Trial Run

3.4.1 Sample 1. 20 ml of horse manure

The first sample of the trial run with 20ml of horse manure started out with a significant lag phase of over seven days, which can be seen in figure 5. After the lag phase the biological degradation started to produce gases in to the graduated flask and the pressure slowly increased until the end of the experiment. The rate of degradation of this sample proved to be too slow for it to be useful in the actual experiment and was disregarded when the sample substances and amounts were decided.

3.4.2 Sample 2. 40 ml of horse manure

The second sample had a lag phase of only a bit over a day and from there on biological degradation took place increasing the pressure of the graduated flask at a fairly constant rate that was easy to follow, as seen in figure 6. After 17 days the pressure increased to a level that exceeded the threshold of the Oxitop®-C measuring head's measuring capacity of about 400hPa. The

degradation of this sample was so constant that the amount of 40ml of horse manure was chosen to be one the sample amounts in the actual experiment of biogas production.

3.4.3 Sample 3. 20 ml of horse manure and 4 g of sugar

The third sample was the same as the first one with an additional four grams of sugar placed into the flask in order to make the process of degradation a little faster. As seen in figure 7 the process did indeed start faster and already after less than a day the pressure in the flask increased too high and passed the measuring threshold. It became clear that the addition of sugar should be ablated from the final experiment.

3.4.4 Sample 4. 40 ml of horse manure and 4 g of sugar

Sample four also started with a rapid increase in pressure as sample three, but experienced a lag phase at the same time when sample three exceeded the pressure threshold at 18,5 hours into the experiment. The lag phase was most likely due to fact that in sample four the amount of horse manure was double of that in sample three, and so the mixing of the sample was slower and the process took more time reach its potential. Nevertheless the additional sugar made the process still too fast and after three days the pressure threshold was exceeded, as can be seen in figure 8, and so reassurance of omitting sugar from the final experiment was presented.

3.4.5 Sample 5. 10 ml horse manure and 20 ml of biowaste

In sample five a significant lag phase was not experienced, but the pressure started to increase from the beginning until it seemed to plateau after a day and a half at 258 hPa. From there on the pressure started a slight decline which seemed to end after six days when the pressure started to increase again. The second increase appeared to plateau at approximately 330 hPa after a little over eight days into the trial run. Eventually the pressure started to increase again and exceeded the pressure threshold after 13,5 days. These three stages of degradation can clearly be seen in figure 9. The

results of sample five were excellent considering the final experiment for the reason that the pressure levels of the sample plateaued and thus the amounts of 10 ml horse manure mixed with 20 ml of biowaste were later used.

3.4.6 Sample 6. 20 ml of horse manure and 10 ml of biowaste

Sample six proved to have a rapid rate of biological degradation and the pressure levels grew immeasurable in just over 13 hours, as seen in figure 10, and thus the just as sample three, the substance amounts of sample six were discarded for the final experiment.

3.4.7 Sample 7. 10 ml of horse manure, 20 ml of biowaste and 2 g of sugar

Figure 11 shows that sample seven had reached pressure levels that exceed the measuring capacity fairly quickly. This gave even more evidence for omitting sugar from upcoming experiments, especially since the substance amounts used in sample seven, excluding sugar, were the same as in sample five, which proved to be an excellent candidate for the future experiment.

3.4.8 Sample 8. 40 ml of biowaste

The curve of sample eight in figure 12 resembles the curve of sample six, even though the substances used were different. Sample eight consisted only of 40 ml of biowaste and water and it reached the pressure threshold after a bit more than a day, and this sample consistency was thus excluded from the final experiment.

3.4.9 Sample 9. 20 ml of biowaste

Sample nine, in figure 13, started a rapid increase in pressure after a lag phase of approximately 21 hours. The increase seemed to eventually stabilize at 270 hPa, after six days of degradation. The plateau looked excellent when considering the final experiment, and this led to the conclusion that samples with 20 ml of biowaste mixed with water were going to be used in the in the later phase of the thesis work.

3.4.10 Sample 10. 40 ml of biowaste and 4 g of sugar

Sample number ten, in figure 14, resembles all the other samples where sugar was placed into the graduated flasks. And as the others also sample ten reached a level of immeasurable pressure very early on in the trial run and was overlooked when the samples for the final experiment were decided.

3.4.11 Sample 11. 20 ml of biowaste and 4 g of sugar

Sample 11 appears to have the same qualities than sample ten, as seen in figure 15, and sample 11 was then also overlooked when deciding the samples for the final experiment.

3.5 Second Experiment

More information on running a biogas experiment with the Oxitop® method were received after the trial run had been completed. The first, and an important fact, was that the carbon dioxide absorber is to be injected into the graduated flask, when a plateau stage is reached, as mentioned in chapter 3.1, instead of placed into the bottle in the form of granules as was thought earlier. Another fact was that if the pressure levels grow too high a simple aeration through the septum nozzle has to be performed. This releases the over pressure, but the experiment can continue until a plateau is reached. Also digested sludge from the communal waste water treatment plant was used in part of

the samples, which was neglected from the trial run, to start the degradation process as it would when producing biogas in large scale.

The sample substances and amounts that were to be used in the final experiment were derived from the results of the trial run. The three samples chosen, all three times with additional variables, were sample one, sample five and sample nine. In addition to these also two blank samples with only distilled water, in order to have blank values for the calculations for total carbon in the gas phase, and a sample with only digested sludge were included in the experiment. With these facts in mind the final experiment, with a set 14 day experiment time, was started on the 22nd of September in 2009. Below, in table 2, all the variables, substances and volumes for the final experiment are presented.

Table 2. Experiment setup 22.09.2009.

Sample Number	Test Substance	Water (Distilled H_2O)	Digested Sludge	Total Volume
1.	40ml Horse Manure	150ml	10ml	178ml
2.	40ml Horse Manure	150ml	5ml	174ml
3.	40ml Horse Manure	150ml	-	170ml
4.	10ml Horse Manure, 20ml Biowaste	150ml	10ml	184ml
5.	10ml Horse Manure, 20ml Biowaste	150ml	5ml	180ml
6.	10ml Horse Manure, 20ml Biowaste	150ml	-	178ml
7.	20ml Biowaste	150ml	10ml	184ml
8.	20ml Biowaste	150ml	5ml	176ml
9.	20ml Biowaste	150ml	-	174ml
10.	-	-	150ml	150ml
11. Blank	-	150ml	-	150ml
12. Blank	-	150ml	-	150ml

3.6 Second Experiment Results

The results of pressure changes in the graduated sample flasks are presented in figures 16-18. All the different operations such as aerations and the addition of the carbon dioxide absorber, KOH, are clearly visible from the curves. Again the pressure in hectopascals is plotted against time in minutes.

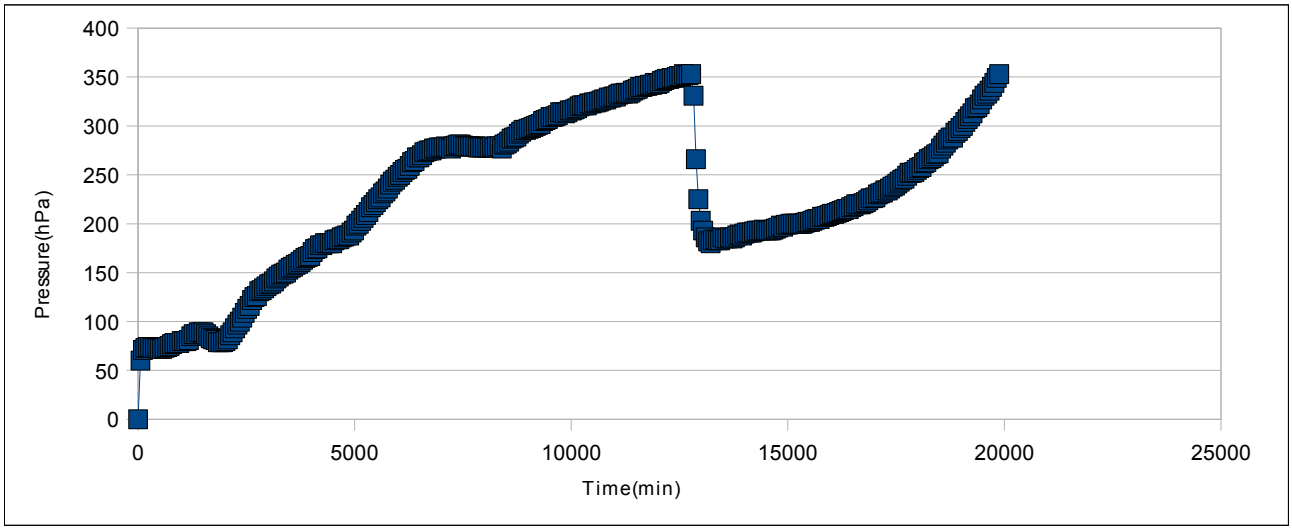

Figure 16. The change in pressure (hPa) over time (minutes) of sample 1 in the second experiment (40 ml of horse manure and 5 ml digested sludge)

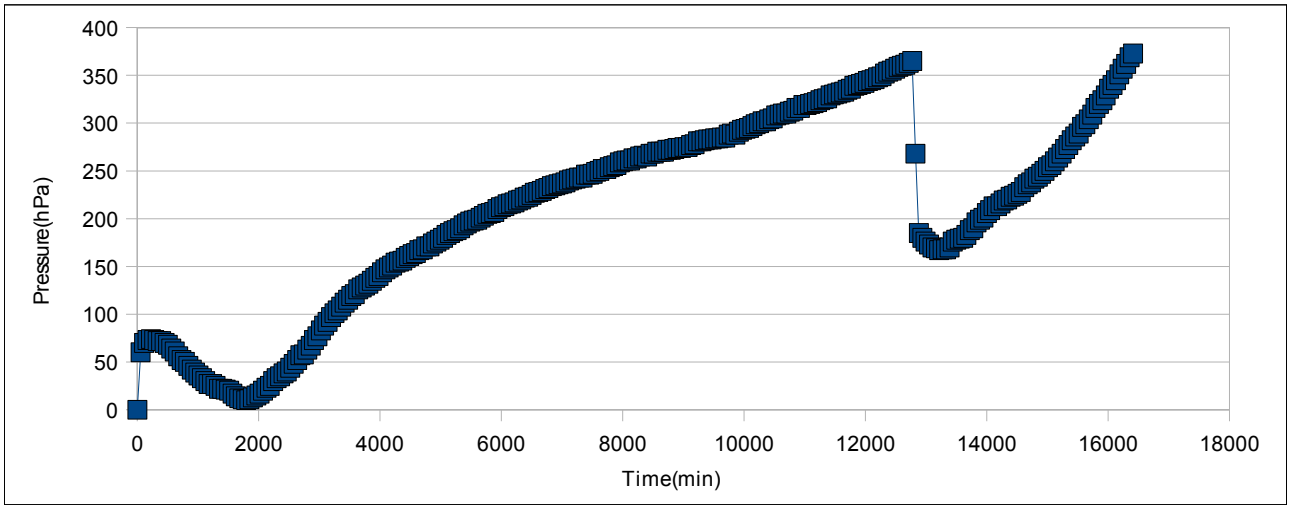

Figure 17. The change in pressure (hPa) over time (minutes) of sample 2 in the second experiment (40 ml of horse manure and 10 ml digested sludge)

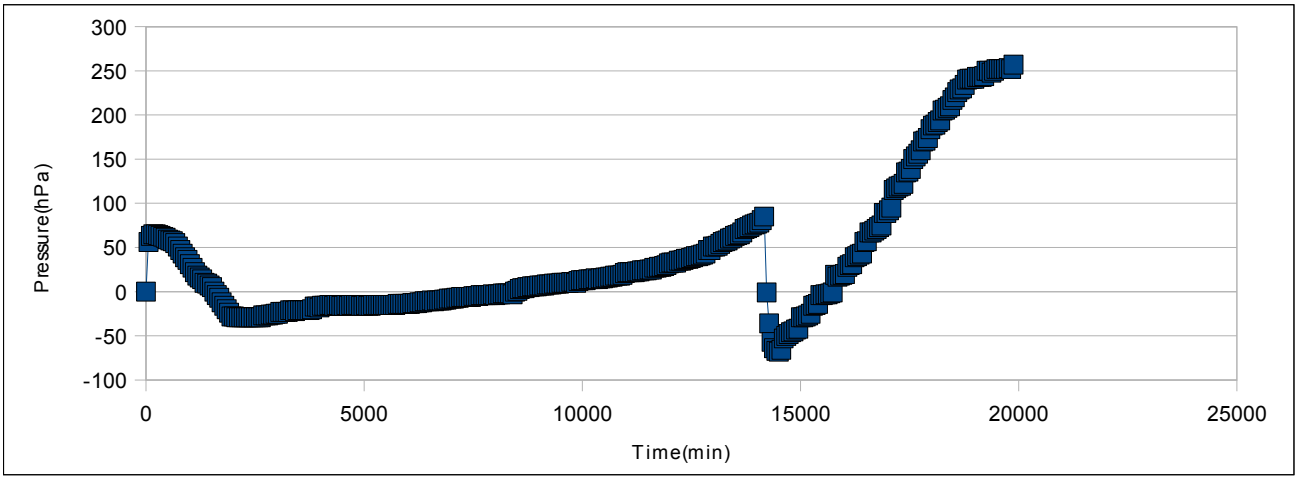

Figure 18. The change in pressure (hPa) over time (minutes) of sample 3 in the second experiment (40 ml of horse manure)

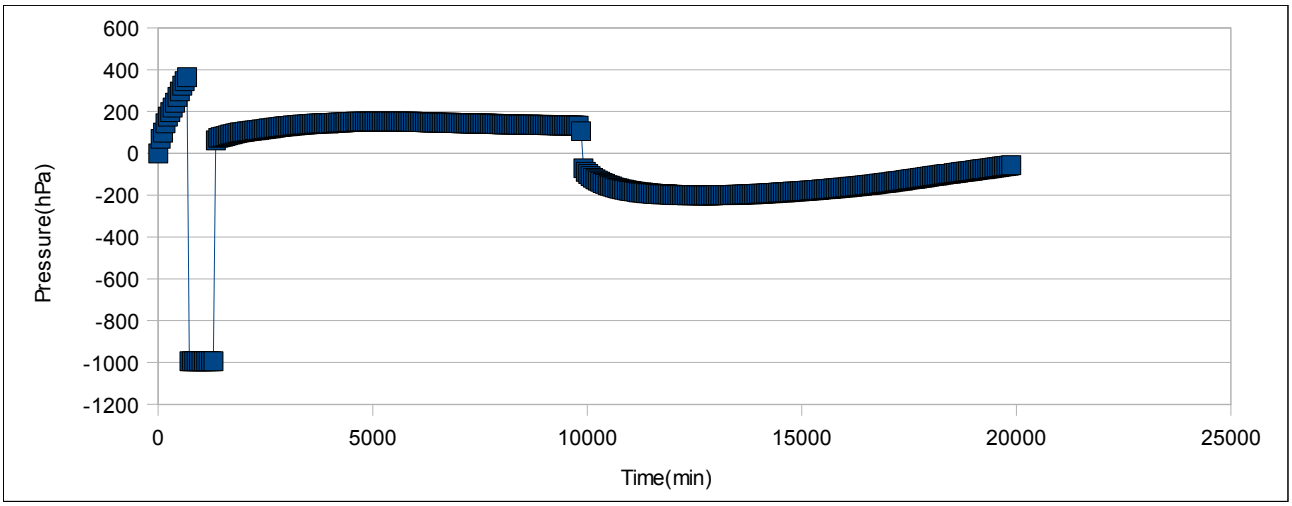

Figure 19. The change in pressure (hPa) over time (minutes) of sample 4 in the second experiment (10 ml of horse manure, 20 ml of biowaste and 10 ml digested sludge)

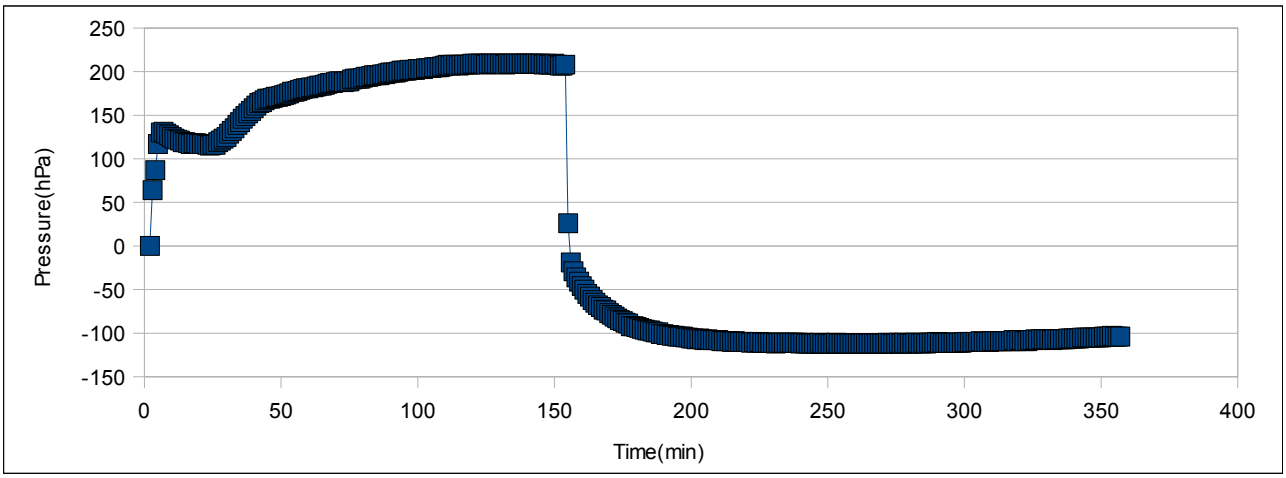

Figure 20. The change in pressure (hPa) over time (minutes) of sample 5 in the second experiment (10 ml of horse manure, 20 ml of biowaste and 5 ml digested sludge)

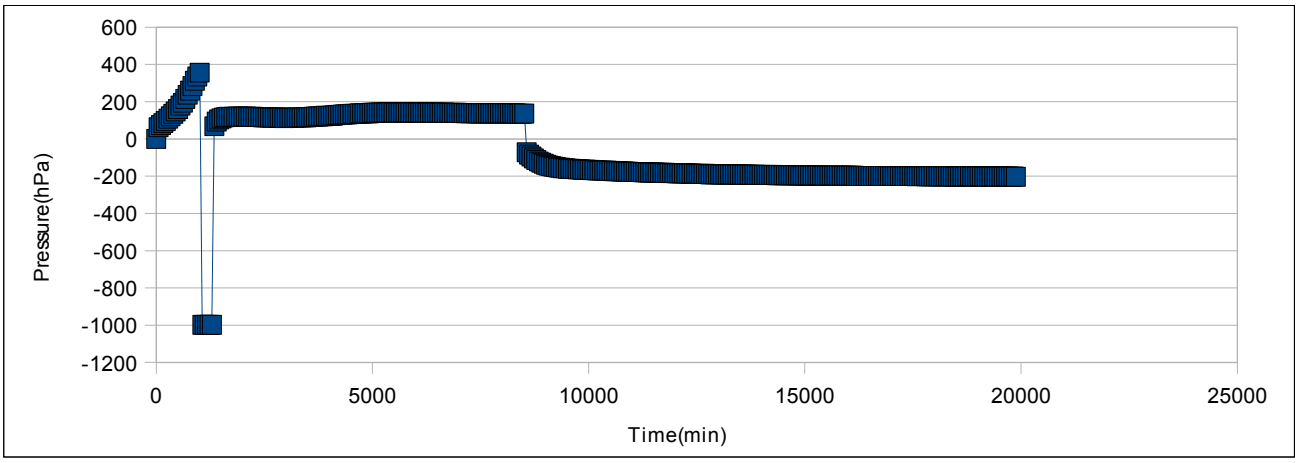

Figure 21. The change in pressure (hPa) over time (minutes) of sample 6 in the second experiment (10 ml of horse manure and 20 ml of biowaste)

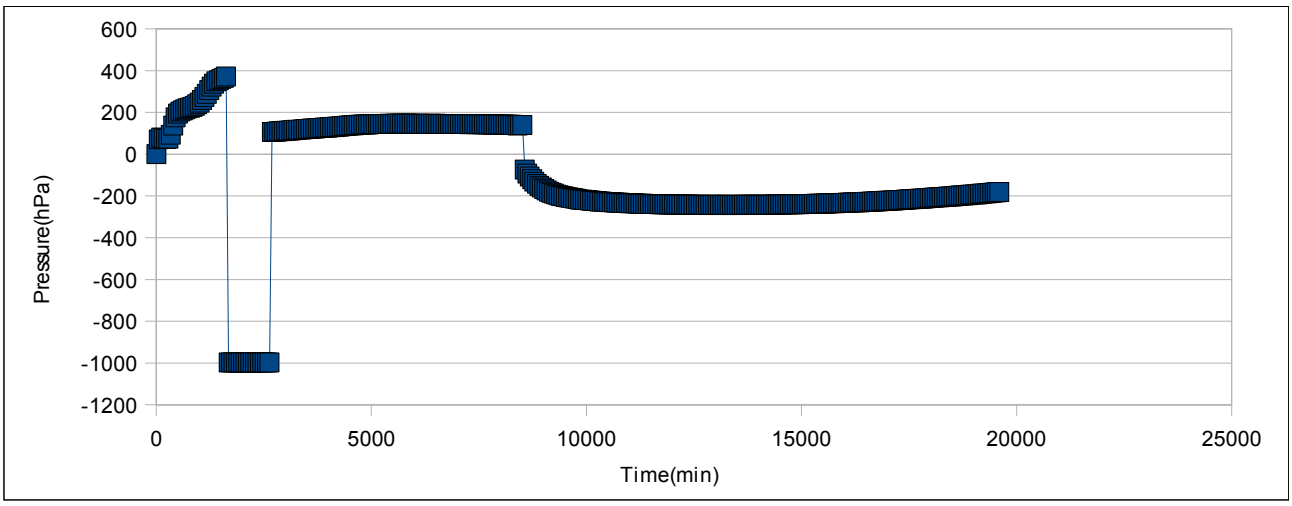

Figure 22. The change in pressure (hPa) over time (minutes) of sample 7 in the second experiment (20 ml of biowaste and 10 ml digested sludge)

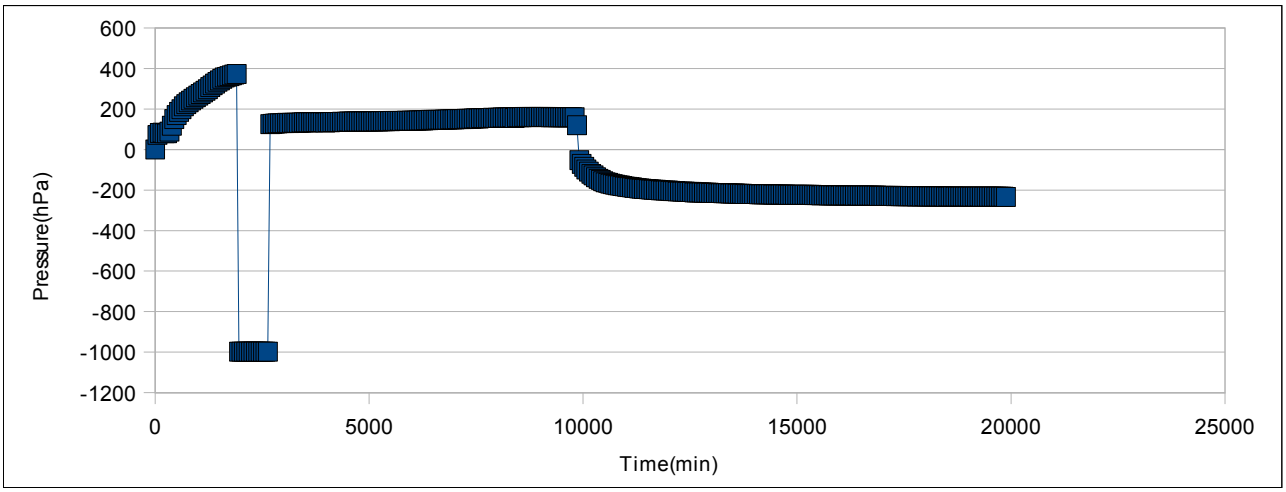

Figure 23. The change in pressure (hPa) over time (minutes) of sample 8 in the second experiment (20 ml of biowaste and 5 ml digested sludge)

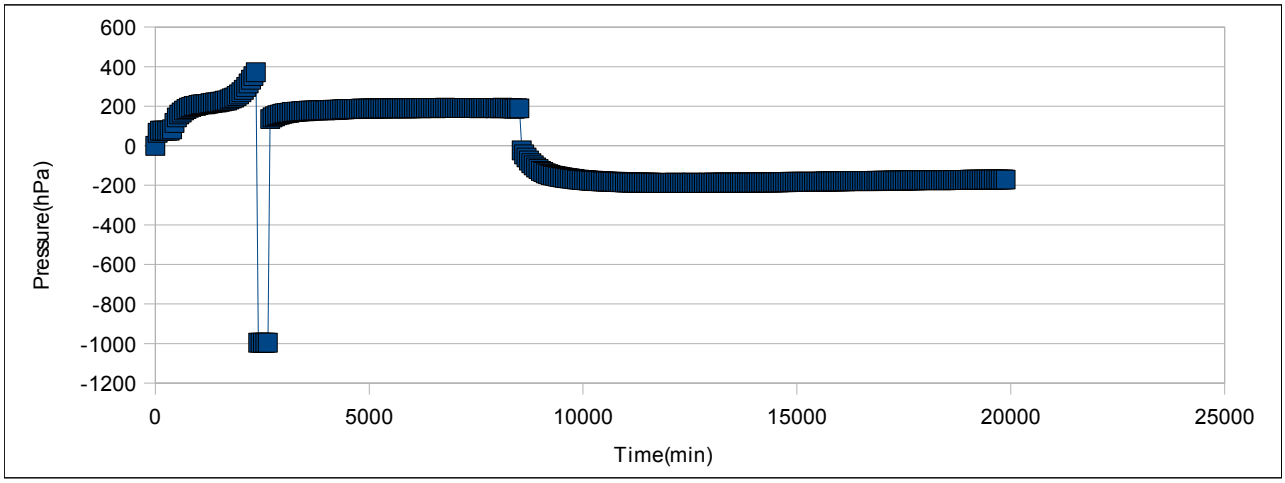

Figure 24. The change in pressure (hPa) over time (minutes) of sample 9 in the second experiment (20 ml of biowaste)

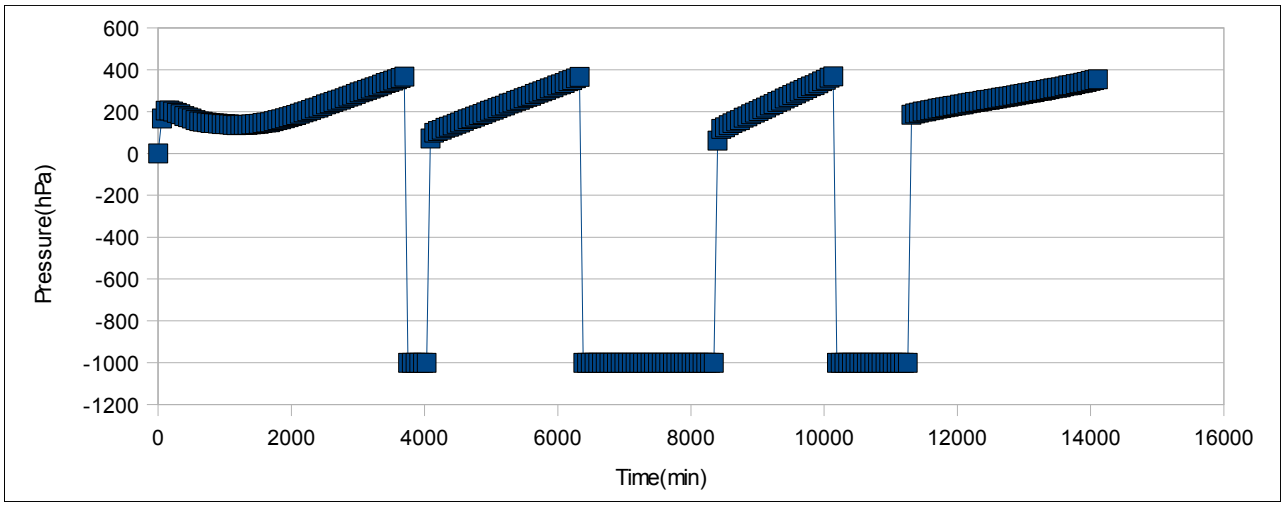

Figure 25. The change in pressure (hPa) over time (minutes) of sample 10 in the second experiment (150 ml digested sludge)

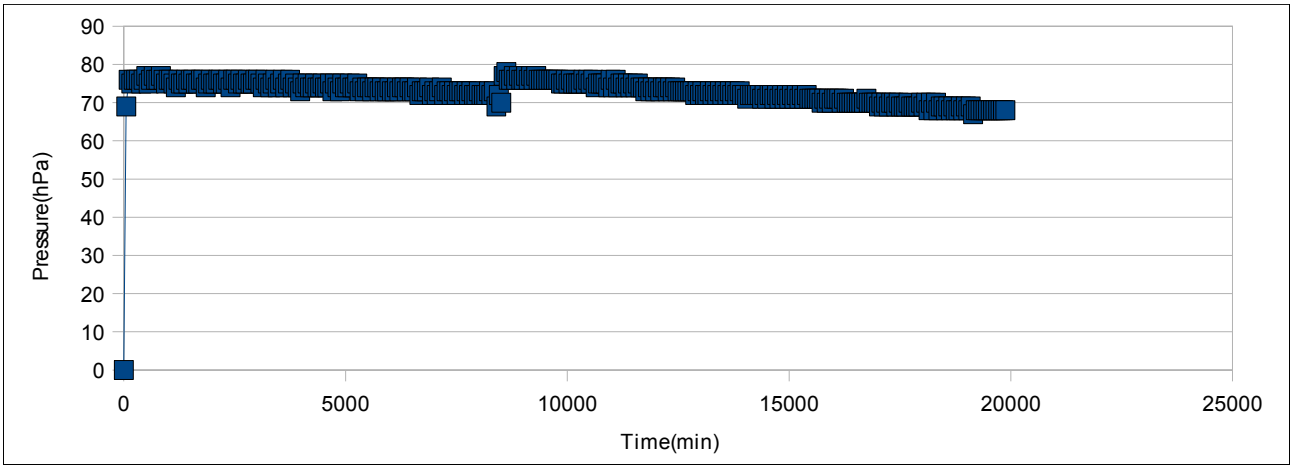

Figure 26. The change in pressure (hPa) over time (minutes) of sample 11 in the second experiment (blank sample, 150 ml distilled water)

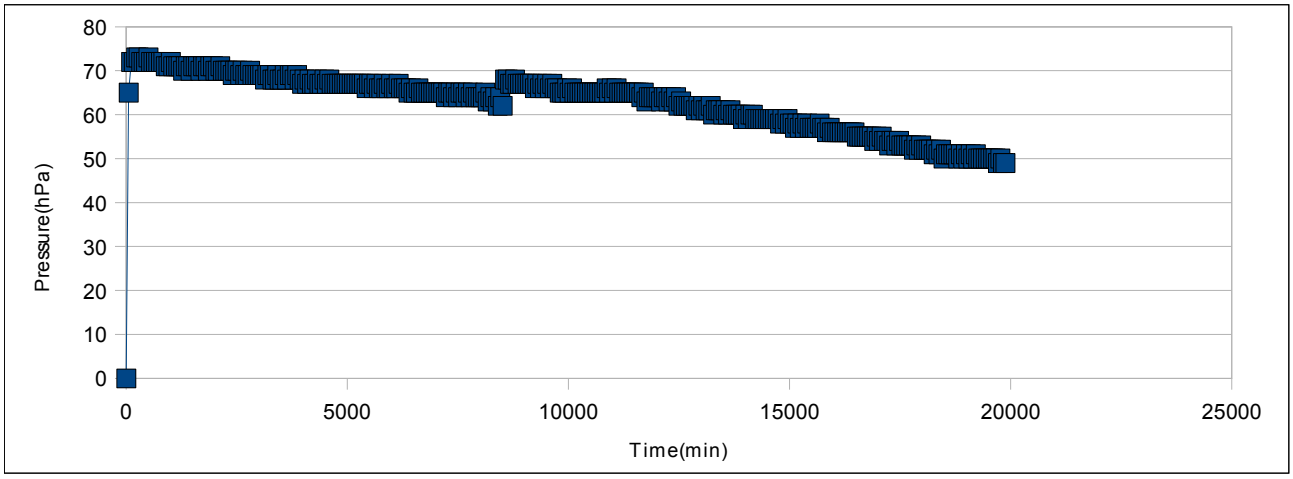

Figure 27. The change in pressure (hPa) over time (minutes) of sample 12 in the second experiment (blank sample, 150 ml digested sludge)

3.6 Analyzing the Final Experiment

The results will be presented in the order in which the processes were completed. The calculations were done according to the formulas given in chapter 2.6. The volume of the graduated flask, 320 ml, was measured according to the instructions in chapter 3.1, and when the total volume of the flask and the sample volume were known, the volume of the head space i.e. gas volume was easily calculated.

On the 28th of September 2009, approximately 8500 minutes after the start of the experiment, samples 5, 6, 7, 9, 11 and 12 had reached a plateau state and were ready for potassium hydroxide (KOH 30% v/v) addition to absorb the carbon dioxide. The KOH was prepared by dissolving 30 grams of solid KOH into distilled water and then filled up to 100 ml in a volumetric flask. On the 29th of September 2009, 9800 minutes after the start, samples 4 and 8 had plateaued. On the 1st of October 2009, 12768 minutes from the beginning, KOH was added to samples 1 and 2 even though they had not reached a plateau state, but because they were on the border of excess pressure and aeration was not an option at this phase. A day later, 14168 minutes from the beginning, on the 2nd of October sample 3 was ready for KOH addition, this sample had not plateaued either nor was it close to exceeding the pressure limit, but because of the time frame KOH was added. Sample 10 had automatically finished itself and so any further measurements or calculations were impossible on this sample.

3.6.1 Sample 5. 10 ml of horse manure, 20 ml of biowaste and 5 ml of digested sludge

Sample five, 10 ml horse manure, 20 ml of biowaste and 5 ml activated sludge, showed an increase of pressure in the graduated flask once the lag phase of about 22 hours was completed. Sample five did not need aeration at any time during the experiment, and so the total carbon in the flask is all the carbon that was produced. Here an example of the calculations is introduced, later only the results are shown. The calculations for total carbon, in moles, were done according to the formula in chapter 2.6.2.

$$n = \Delta p \times Vg \times 3{,}903 \times 10^{-8}$$

The pressure difference:

$$\Delta p = 207\,hPa - 64\,hPa - (71\,hPa - 63\,hPa)$$

$$\Delta p = 135\,hPa$$

The gas volume:

$$Vg = 320\,ml - 180\,ml$$

$$Vg = 140\,ml$$

The amount of total carbon (C) in gaseous phase:

$$n = 135\,hPa \times 140\,ml \times 3.903 \times 10^{-8}$$

$$n = 0{,}0007373667\,mol$$

$$n \approx 0{,}738\,mmol$$

Table 3. Results of Measurements and Calculations of sample 5 (10 ml of horse manure, 20 ml of biowaste and 5 ml of digested sludge)

	Beginning	**End**
Time (min)		8512 min (6 days)
Pressure (hPa)	64 hPa	207 hPa
Gas volume (ml)	140 ml	140 ml
Total carbon, CO_2+CH_4 (mmol)		**0,738 mmol**
KOH volume (ml)	1 ml	1 ml
KOH reaction (min)	8512 min	9968 min (7 days)

Gas volume before and after KOH (ml)	140 ml	139 ml
Pressure before and after KOH (hPa)	207 hPa	-162 hPa
CO_2 absorbed (mmol)		-

As can be seen from table 3 as well as from figure 20 the pressure in the graduated flask sample five drops below zero after the addition of 1ml of KOH. This can lead us to the conclusion that the amount of KOH was excessive for the gas volume. Unfortunately since many of the samples plateaued roughly the same time, the same amount of CO_2 absorbent KOH was added into the samples. This lead to the decrease of pressure below zero in many of the samples and there for the calculations for CO_2 and CH_4 differentiation could not be, only the total carbon in the gas could be calculated.

3.6.2 Sample 6. 10 ml of horse manure and 20 ml of biowaste

Early on in the experiment the pressure in sample six, 10 ml of horse manure and 20 ml of biowaste, grew too high, already after 17 hours into the experiment the pressure was immeasurable and after 22 hours it was aerated., which can be seen from figure 21. No lag phase was visible in sample six as there was in sample five, which came as a surprise since there was no added activated sludge in sample six and in sample five there was. The

Table 4. Results of the Measurements and Calculations of sample 6 (10 ml of horse manure and 20 ml of biowaste)

	Beginning	**End**
Time (min)		8512 min (6 days)
Pressure (hPa)	63 hPa	136 hPa
Gas volume (ml)	142 ml	142 ml
Total carbon, CO_2+CH_4 (mmol)		**0,360mmol**
KOH volume (ml)	1 ml	1 ml
KOH reaction (min)	8512 min	9968 min (7 days)
Gas volume before and after KOH (ml)	142 ml	141 ml
Pressure after KOH (hPa)	136 hPa	-166 hPa
CO_2 absorbed (mmol)		-

3.6.3 Sample 7. 20 ml of biowaste and 10 ml of digested sludge

The curve of sample seven, 20 ml of biowaste and 10 ml of digested sludge, in figure 22 is very similar, although the process was a little slower, to the curve of sample six. No distinct lag phase was apparent, the pressure grew too high in 28 hours and aeration was done after 44 hours. From there on the pressure stabilized almost immediately, and no further biogas production was seen.

Table 5. Results of the Measurements and Calculations of sample 7 (20 ml of biowaste and 10 ml of digested sludge)

	Beginning	**End**
Time (min)		8512 min (6 days)
Pressure (hPa)	56 hPa	140 hPa
Gas volume (ml)	136 ml	136 ml
Total carbon, CO_2+CH_4 (mmol)		**0,324 mmol**
KOH volume (ml)	1 ml	1 ml
KOH reaction (min)	8512 min	9968 min (7 days)
Gas volume before and after KOH (ml)	136 ml	135 ml
Pressure after KOH (hPa)	140 hPa	-220hPa
CO_2 absorbed (mmol)		-

3.6.4 Sample 9. 20 ml of biowaste

The curve of sample nine, 20 ml of biowaste, resembles the previous two curves, but a clearer short lag phase can be seen in the beginning of the experiment in figure 24. Again the pressure grew too high and aeration was done during the same time as sample seven, from where the pressure seemed to stabilize almost immediately.

Table 6. Results of the Measurements and Calculations of sample 9 (20 ml of biowaste)

	Beginning	**End**
Time (min)		8512 min (6 days)
Pressure (hPa)	65 hPa	189 hPa
Gas volume (ml)	146 ml	146 ml
Total carbon, CO_2+CH_4 (mmol)		**0,661 mmol**

KOH volume (ml)	1 ml	1 ml
KOH reaction (min)	8512 min	9968 min (7 days)
Gas volume before and after KOH (ml)	146 ml	145 ml
Pressure after KOH (hPa)	189 hPa	-173 hPa
CO_2 absorbed (mmol)		-

3.6.5 Samples 11 and 12. blank samples of 150 ml of distilled water

Samples 11 and 12 were blank samples with only distilled water inside the graduated flasks, and when looking at figures 26 and 27 this becomes clear. The pressure inside the flasks stays around 75 hPa for sample 11 and varies from 60 to 70 hPa for sample 12, throughout the experiment. The reducing pressure in sample 12 could be explained a leaking nozzle that would decrease the pressure at a slow rate. When the KOH was added into the flasks the pressure, instead of decreasing, increased, which can be explained by the decreasing gas volume when KOH was injected.

Table 7. Results of the Measurements and Calculations of samples 11 and 12 (blank samples, 150 of distilled water)

	Beginning	**End**
Time (min)		8512 min (6 days)
Pressure (hPa)	69/65 hPa	70/62 hPa
Gas volume (ml)	170 ml	170 ml
Total carbon, CO_2+CH_4 (mmol)		-
KOH volume (ml)	1 ml	1 ml
KOH reaction (min)	8512 min	9968 min (7 days)
Gas volume before and after KOH (ml)	170 ml	169 ml
Pressure before and after KOH (hPa)	70/62 hPa	76/66 hPa
CO_2 absorbed (mmol)		-

3.6.6 Sample 4. 10 ml of horse manure, 20 ml of biowaste and 10 ml of digested sludge

Sample four, 10 ml of horse manure, 20 ml of biowaste and 10 ml of activated sludge, was aerated during the initial phases of the experiment, same time as sample six, as can be seen from figure 19.

No lag phases could be seen, evidently because of the amount of activated sludge in the sample, although sample six seemed to posses similar traits without activated sludge reaching excessive pressure only 5,5 hours later than sample four. Towards the end of the experiment the pressure of sample four was increasing, which could mean that the degradation process had not been complete and that the organic material was still degrading but had experienced a lag phase where the process had been halted.

Table 8. Results of the Measurements and Calculations of sample 4 (10 ml of horse manure, 20 ml of biowaste and 10 ml of digested sludge)

	Beginning	**End**
Time (min)		9800 min (7 days)
Pressure (hPa)	69 hPa	132 hPa
Gas volume (ml)	136 ml	136 ml
Total carbon, CO_2+CH_4 (mmol)		**0,292 mmol**
KOH volume (ml)	1 ml	1 ml
KOH reaction (min)	9800 min	11256 min (8 days)
Gas volume before and after KOH (ml)	136 ml	135 ml
Pressure before and after KOH (hPa)	132 hPa	-187 hPa
CO_2 absorbed (mmol)		-

3.6.7 Sample 8. 20 ml of biowaste and 5 ml of digested sludge

Sample eight, 10 ml horse manure, 20 ml of biowaste and 5 ml of activated sludge, resembles sample seven a great deal, even the needed aeration was done at the same time, as can be seen in figure 23. The 5 ml less activated sludge in sample eight only made the process of degradation slightly slower, and eventually the pressure grew a little higher in sample eight.

Table 9. Results of the Measurements and Calculations of sample 8 (20 ml of biowaste and 5 ml of digested sludge)

	Beginning	**End**
Time (min)		9800 min (7 days)
Pressure (hPa)	72 hPa	159 hPa

Gas volume (ml)	144 ml	144 ml
Total carbon, CO_2+CH_4 (mmol)		**0,444 mmol**
KOH volume (ml)	1 ml	1 ml
KOH reaction (min)	9800 min	11256 min (8 days)
Gas volume before and after KOH (ml)	144 ml	143 ml
Pressure after KOH (hPa)	159 hPa	-190 hPa
CO_2 absorbed (mmol)		-

3.6.8 Sample 1. 40 ml of horse manure and 10 ml of digested sludge

As can be seen from figure 16 sample one was much slower in its biogas production than the previous samples. There is a 33 hour lag phase in the beginning of the curve before the pressure started to increase. Most likely this is due to the fact that 40 ml of horse manure has a tendency to settle on the bottom of the graduated flask, thus making it hard for the magnetic stirrer to keep the sample moving at a constant rate. Another reason might be that horse manure is full of hey and hey contains long-chain carbohydrates, which take longer to go through hydrolysis. Since sample one did not reach a plateau phase during the experiment for its slow start up, the KOH was added later than for the previous samples, and in the middle of biogas production after about nine days in to the experiment. It became clear from the previous samples that the amount of KOH should be revised, as mentioned in chapter 3.6.1. For sample one, two and three the amount of KOH was decreased to 0,5 ml, which proved to be an important adjustment, for it allowed for the absorbed carbon dioxide to be calculated, and thus the amount of methane could be revealed. Below are the calculations for sample one according to the formulas in chapter 2.6.3, the calculations, for total carbon, were done as in the case of sample five.

$$n = [p3 \times (Vg - V(KOH)) - p \times Vg] \times 3{,}903 \times 10^{-8}$$

$$n = [180\,hPa \times (142\,ml - 0{,}5\,ml) - 354\,hPa \times 142\,ml] \times 3{,}903 \times 10^{-8}$$

$$n = -0{,}0009679\,mol$$

$$n \approx 0{,}968\ mmol$$

Table 10. Results of the Measurements and Calculations of sample 1 (40 ml of horse manure and 10 ml of digested sludge)

	Beginning	**End**
Time (min)		12768 min (9 days)
Pressure (hPa)	60 hPa	354 hPa
Gas volume (ml)	142 ml	142 ml
Total carbon, CO_2+CH_4 (mmol)		**1,585 mmol**
KOH volume (ml)	0,5 ml	0,5 ml
KOH reaction (min)	12768 min	13216 min (7,5 hrs later)
Gas volume before and after KOH (ml)	142 ml	141,5 ml
Pressure after KOH (hPa)	354 hPa	180 hPa
CO_2 absorbed (mmol)		**0,968mmol**

The amount of carbon dioxide in the graduated flask was 0,968 mmol out of 1,585 mmol of total carbon, from which we can derive that 0,617 mmol of that carbon was methane.

3.6.9 Sample 2. 40 ml of horse manure and 5 ml of digested sludge

The curve of sample two, in figure 17 resembled the one of sample one in every way excluding the more intensive lag phase, probably due to the 5ml less of digested sludge in the 40 ml of horse manure. The reason for the KOH reaction time to be so short is because the biogas production did not plateau, but was still increasing so the lowest pressure point after the addition was used for the calculations, in both sample one and two.

Table 11. Results of the Measurements and Calculations of sample 2 (40 ml of horse manure and 5 ml of digested sludge)

	Beginning	**End**
Time (min)		12768 min (9 days)
Pressure (hPa)	60 hPa	365 hPa
Gas volume (ml)	146 ml	146 ml
Total carbon, CO_2+CH_4 (mmol)		**1,692 mmol**
KOH volume (ml)	0,5 ml	0,5 ml
KOH reaction (min)	12768 min	13216 min (7,5 hrs later)
Gas volume before and after KOH (ml)	146 ml	145,5 ml
Pressure after KOH (hPa)	365 hPa	167 hPa

CO_2 absorbed (mmol)		**1,132 mmol**

The amount of carbon dioxide absorbed by KOH was 1,132 mmol from 1,692 mmol of total carbon, and so the remaining 0,56mmol was presumably methane.

3.6.10 Sample 3. 40 ml of horse manure

Lack of digested sludge and constant movement in sample three caused it to have a long lag phase where after the production of biogas was very slow, which becomes clear when looking at figure 18. No plateau phase was reached in the case of sample three for its slow production rate. The peak production was reached only after the addition KOH, after almost ten days from the beginning, when finishing the experiment.

Table 12. Results of the Measurements and Calculations of sample 3 (40 ml of horse manure)

	Beginning	**End**
Time (min)		14168 min (10 days)
Pressure (hPa)	56 hPa	85 hPa
Gas volume (ml)	150 ml	150 ml
Total carbon, CO_2+CH_4 (mmol)		**0,139 mmol**
KOH volume (ml)	0,5 ml	0,5 ml
KOH reaction (min)	14168 min	14504 min (5,6 hrs later)
Gas volume before and after KOH (ml)	150 ml	149,5 ml
Pressure after KOH (hPa)	85 hPa	-68 hPa
CO_2 absorbed (mmol)		-

4. Conclusions

It can be said that all the samples in the final experiment, excluding the two blank samples , produced carbon. Unfortunately in most of the samples after the addition of KOH the pressure dropped below zero and the amount of methane remained a mystery. Also many of the samples had to be aerated in order to decrease the pressure that had grown out of proportion. Obviously the aeration results in the release of the biogas produced so far, thus eventually not giving precise information on how much carbon dioxide or methane could the studied sample produce altogether. Sample 10, with the composition of only activated sludge, had to be aerated several times and

eventually no results were studied since the Oxitop® control automatically finished the measurements of this sample.

Even though the production had not reached a plateau phase in samples one, two and three, the KOH was added and momentary values of total carbon and methane could be calculated for one and two, the pressure of the third sample dropped below zero and the methane calculation was impossible. Since the pressure was still increasing while the KOH was added into these samples, one and two, the results are not absolutely precise, but give a fairly accurate estimate of the momentary biogas composition in the head space of the graduated flask. Neither of these samples were aerated so the values of carbon are what the anaerobic degradation of the samples had produced so far, unlike in many of the samples were aeration had to be preformed because of overpressure and the results reflect the values of production after the aeration. The reason for samples one, two and three develop slower than the others was discussed in chapter 3.6.8.

As mentioned in chapter 2.1 the composition of biogas is generally 71% methane and 29% carbon dioxide. In this experiment the two samples from which the composition could actually be calculated had the ratios of 61% CO_2 and 39% CH_4 for sample one, and 67% CO_2 and 33% CH_4 for sample two.

There are many factors that contribute to the composition of biogas. For example the addition of materials rich in fat increase the percentage of methane in the gas, and thus the quality of the gas. In this case the most important factor that affected the biogas composition seems to be fact that the degradation of biological material tends to improve over time, this means that the production of methane increases especially after the carbon dioxide producing phase of hydrolysis finishes. It might be so that in the experiment here the hydrolysis phase was so overwhelming in the samples which were ready sooner than samples one, two and three, that the graduated flasks were filled with CO_2 and needed aeration before any CH_4 was produced. In samples five, seven and nine the curves seem to be slightly increasing towards the end of the experiment which could have eventually lead to an increased amount of methane in the graduated flasks. /5/

5. Recommendations

A few recommendations for future biogas studies conducted with Oxitop® control:

- Smaller sample sizes. The smaller the sample size is the larger the gas volume is and so aeration becomes less necessary and more gas can be produced.

- For samples excluding one, two and three in the second experiment the added KOH was in excess, the volume of KOH should be kept as low as 0,5 ml or less.

- The samples, especially horse manure, should be carefully disintegrated before starting the process, because the lignin structure in horse manure takes longer to degrade if not.

- Longer experiment time for samples that start the degradation process slower, in order to reach the plateau phase.

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Appendices

Appendix 1

For future experiments the amounts of water, test substance, KOH, and digested sludge could be changed from the amounts in this experiment. According to the recommendations given the experiment setup could resemble the amounts in the table below.

Table 13. Possible Future Experiment Setup

Sample Number	Test Substance	Water (Distilled H_2O)	Digested Sludge	KOH (30% V/V)
1.	20ml Horse Manure	120ml	5ml	0,5ml
2.	20ml Horse Manure	120ml	5ml	0,5ml
3.	20ml Horse Manure	120ml	-	0,5ml
4.	10ml Horse Manure, 10ml Biowaste	120ml	5ml	0,5ml
5.	10ml Horse Manure, 10ml Biowaste	120ml	5ml	0,5ml
6.	10ml Horse Manure, 10ml Biowaste	120ml	-	0,5ml
7.	20ml Biowaste	120ml	5ml	0,5ml
8.	20ml Biowaste	120ml	5ml	0,5ml
9.	20ml Biowaste	120ml	-	0,5ml
10.	-	120ml	5ml	0,5ml
11. Blank	-	120ml	-	0,5ml
12. Blank	-	120ml	-	0,5ml

Appendix 2

Instructions on starting an experiment with the Oxitop® system. As an example, sample 4 from appendix one is used as the basis for the instructions.

1) Measure the dead weight of the graduated flask including the magnetic stirrer and other parts, measuring head is not needed, in order to find out the volume of the bottle.

2) Fill the graduated flask with distilled water (bubble free) and measure the weight again. The weight difference corresponds the volume in ml, which is needed for calculations later.

3) Take 10 ml of horse manure and try to disintegrate it as much as possible by physical force. Do the same with 10 ml of biowaste, and the combine the substances.

4) Add 120 ml of distilled water and 5 ml of digested sludge as an inoculum and mix thoroughly.

5) Measure the total volume of the mixture and place the mixture into the graduated flask.

6) Place the graduated flask, including the magnetic stirrer, into the incubation chamber where the temperature is set at 35 ºC, on the magnetic stirrer platform. (The sample must be started with Controller OC-110 before placing into the incubation chamber, see Controller instructions.)

7) Check the pressure with Oxitop® OC-110 controller daily, or whenever possible. If the pressure grows too high, and cannot be measured, aeration must be done by opening the septum nozzle and releasing the pressure.

8) When the sample reaches a plateau phase, i.e. the pressure does not change any more, which can be seen from Achat OC 2.03 program and also from OC-110 controller (see controller instructions),

place 0,5 ml of KOH (30% V/V) into the rubber stopper through the septum nozzle with an injection syringe and allow the pressure to settle again (18-24 hours). From the plateau pressure the amount of total carbon in the gaseous phase can be calculated using the following formula:

$$n = \Delta p \times Vg \times 3{,}903 \times 10^{-8}$$

n = number of moles of gas formed, CO_2 and CH_4 (mol)

Δp = the difference of the gas pressure in the sample bottle at the end of the experiment (plateau) minus the pressure in the beginning of the experiment minus the difference of the blank values (hPa)

Vg = gas volume of the headspace (ml)

9) When the pressure has settled the remaining overpressure in the graduated flask can be used to distinguish the amount of methane (CH_4) from carbon dioxide (CO_2) using the following formula:

$$n = [p3 \times (Vg - V(KOH)) - p \times Vg] \times 3{,}903 \times 10^{-8}$$

n = number of moles of carbon dioxide (mol)

$\mathbf{p_3}$ = absolute gas pressure after the injection of 30% v/v KOH-solution (hPa)

p = absolute gas pressure before the addition of KOH (hPa)

$\mathbf{V_g}$ = gas volume (ml)

$\mathbf{V_{KOH}}$ = volume of KOH-solution added

Appendix 3

Before starting the experiment with the OC-110 Controller all settings must be checked once for the whole batch. In order to do this:

1) Press

2)Press

Select Settings:

→ **Operation mode** → select **pressure p**

→ **Limit pressure** → 100 hPa

→ **Measuring time** → 25 days → back to GLP

3) Select **maintenance → erase sample → all**

Now the OC-110 Controller is ready for a new batch of biogas samples.

Appendix 4

Instructions for Oxitop® Controller OC-110 in the pressure mode for biogas determination and analyzing the results with Achat OC 2.03 program.

Starting the sample:

1) Press

2)Press

→ select **start sample**

→ choose **ID number** (the controller will do it automatically)

→ select **start** (while pointing to the measuring head of the graduated flask)

Checking pressure:

1) Press

2) Press

→select the desired sample

→select **momentary value** → you can save up to 10 values, otherwise select stop

Calling up all data:

1) Press

2) Press

→ select **call up all data** (samples that have exceeded the set limit pressure are shown and all results are transmitted to the controller memory for analyzing)

Checking pressure curves on the OC-110:

1) Press

2) Press

→ select the desired sample (do not press enter at this point)

3) Press

- Move cursor to see different pressures on the curve

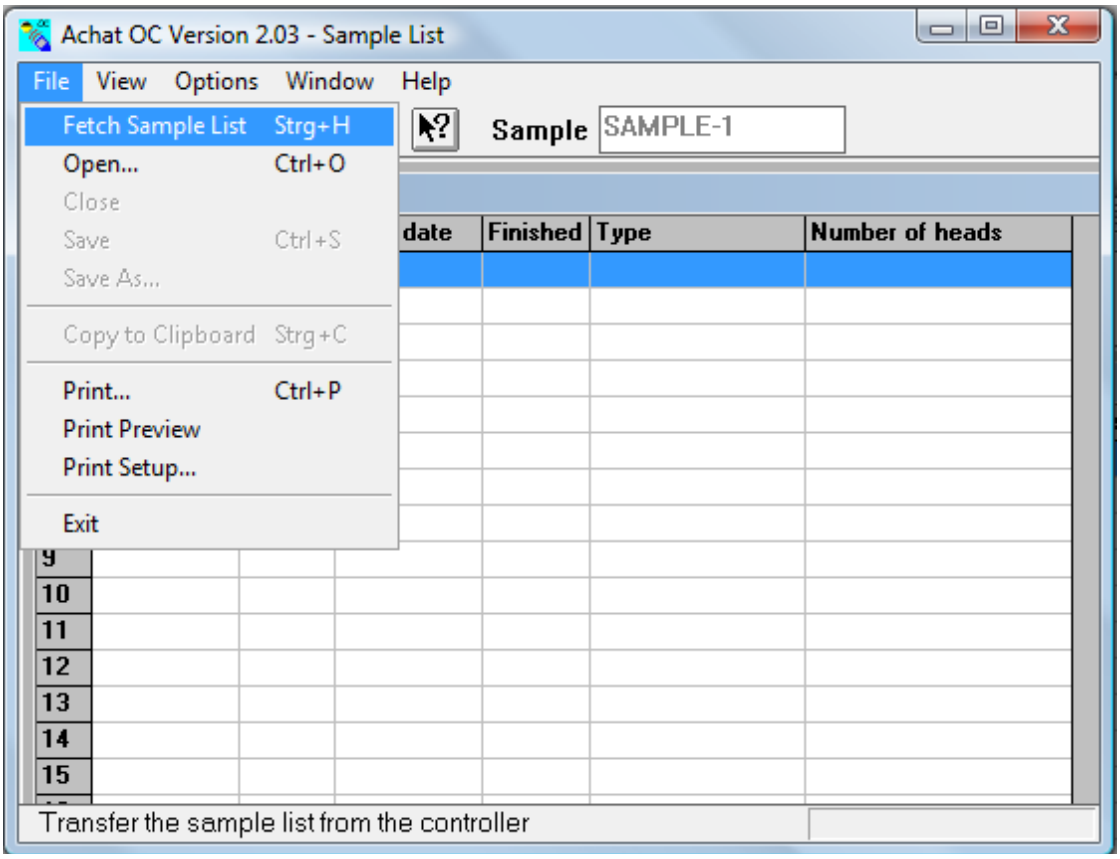
Transferring and checking data with Achat OC 2.03:

1) Attach OC-110 to the computer with the cable.

2) Turn on the program Achat OC 2.03 (Icon on the desktop).

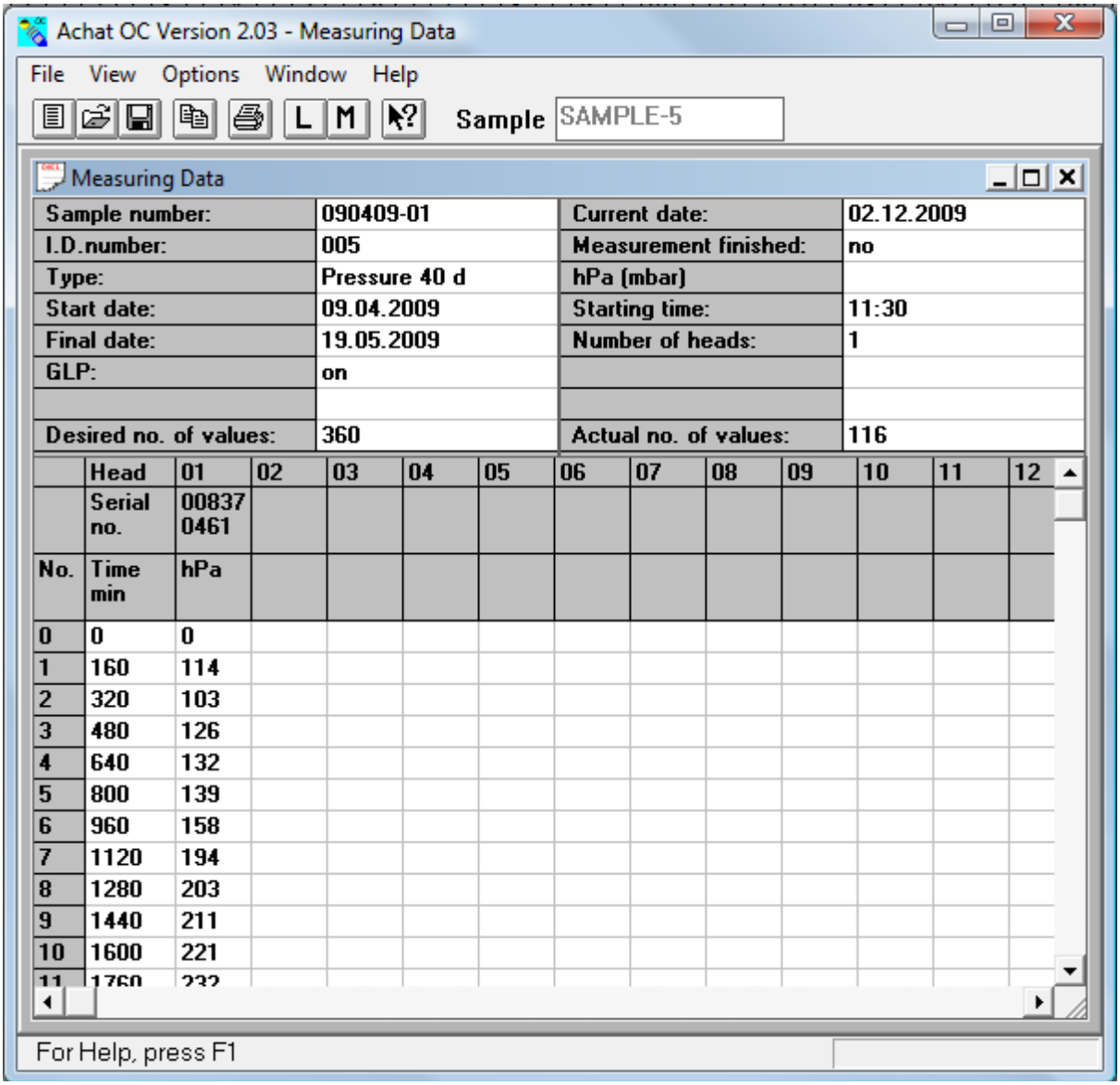
3) Turn on the OC-110 controller.

4) Go to File → Fetch Sample List

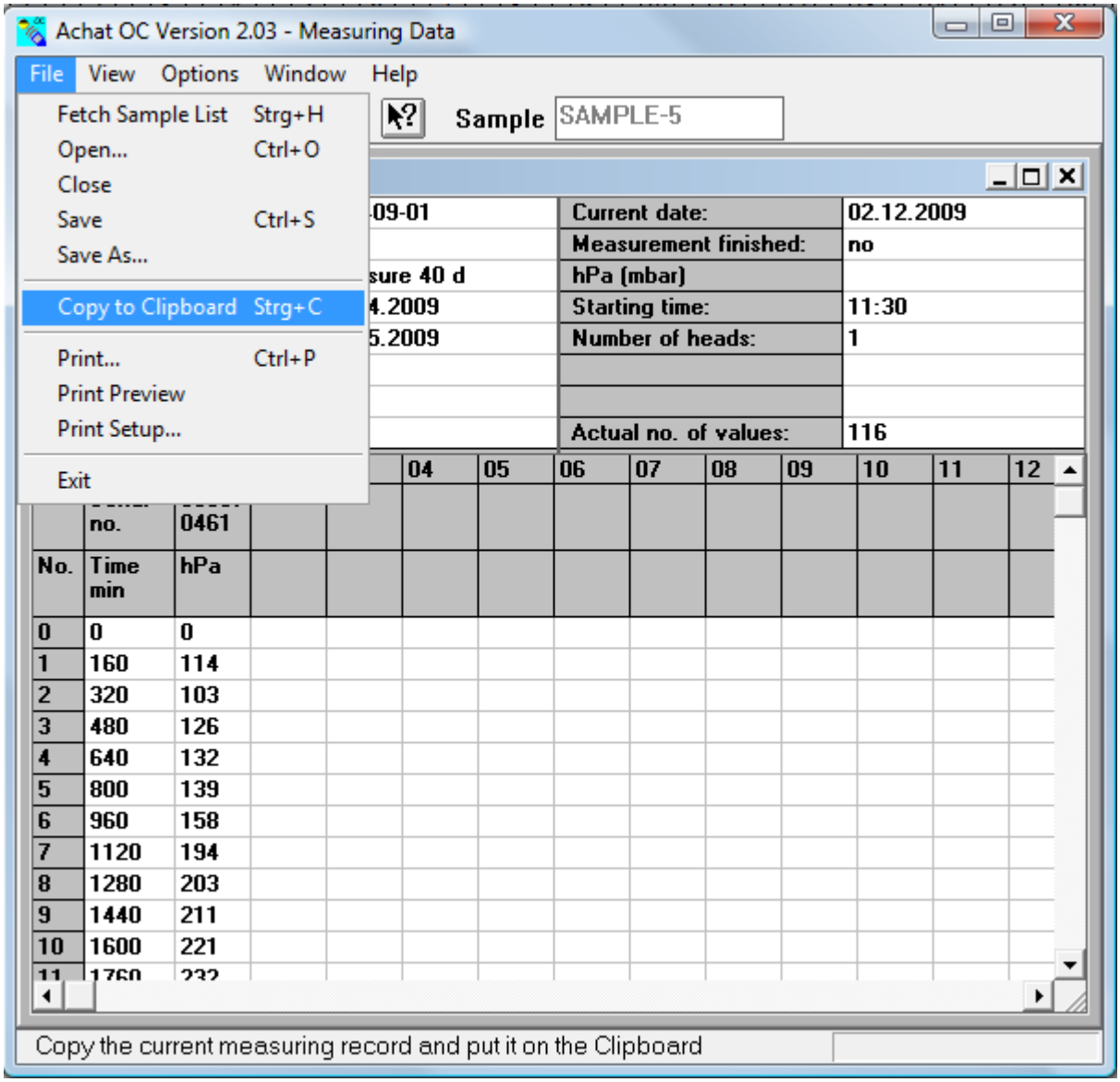


5) The program shows the list of samples saved on the controller. Choose the wanted sample and double click.

6) The measuring data of the chosen sample stored on the OC-110 will then appear.



7) Go to File → Copy to Clipboard



8) Go to Microsoft Excel or Open Office Calc and paste the results for further processing.