



# **BIOETHANOL POTENTIAL OF PRESERVED BIOWASTE**

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Engineering

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## **ABSTRACT**

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The development of alternatives to fossil fuels like oil and natural gas is becoming increasingly urgent with the depletion of resources of fossil fuels and the steadily worsening state of our atmosphere and natural environment. The usage of biofuels is one possibility to decrease greenhouse gas emissions in the nearer future, while other environmentally friendly vehicle technologies are still under development. Bioethanol can be used in fuels for vehicles without any modifications of the engines in concentrations up to 5 per cent, and even 10 per cent in newer engines. Different possible raw materials for the production of bioethanol have been studied during the last few decades.

The handling of waste produced by human society is becoming more and more difficult due to a growing world population and an increase in living standards world-wide. The aim of this study is to show the bioethanol production potential of preserved food waste in an institution like Tampere University of Applied Sciences. It investigates if the biowaste from the TAMK kitchen, after being stored over longer time periods, is suitable for bioethanol production.

The change in bioethanol yield was studied over a time period of three months, during which the food residues were preserved and stored in anaerobic conditions. The bioethanol yield, as well as other factors such as chloride content, pH, conductivity, and dry matter content, and their fluctuation over time were analyzed over the whole three month period.

The study showed that even though factors like chloride content, pH and conductivity were kept at desirable levels, the bioethanol yield itself fluctuated a lot during the 3 month period. The method of adding the biowaste to the vessel - in terms of amounts and adding rhythm - seems to have an effect on the ethanol yield. An assumption of early fermentation taking place was not confirmed. The dry matter content could not be analyzed accurately enough with the used method and needs to be studied further in the future. For future projects, it would also be necessary to find out the glucose content of the raw material to make the results more comparable to already existing studies.

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Key words: Bioethanol yield, biowaste, hydrolysis, fermentation

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## 1 GLOSSARY

Cl	Chloride
CO <sub>2</sub>	Carbon dioxide
C <sub>5</sub>	Xylose-sugars
C <sub>6</sub>	Glucose-sugars
EU	European Union
EtOH	Ethanol
EPA	US Environmental Protection Agency
MSW	Municipal Solid Waste
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NTNU	Norwegian University
RDF	Refuse Derived Fuel
RFA	Renewable Fuels Association
RFA	Renewable Fuels Association
rpm	revolutions per minute
TAMK	Tampere University of Applied Sciences

## 2 INTRODUCTION

While the world is running short on fossil fuels in the near future, the production of solid waste and biowaste is growing steadily at the same time due to a growing world population and a rising standard of living in developing countries as well as a growing consumerism in developed countries. At the same time the challenge of reducing greenhouse gas emissions asks for alternatives to fossil fuels. Global energy policies respond to the urgent situation by setting up targets, like the European Union which is demanding a share of renewable fuels of at least 10 per cent of the fuel consumption in the EU by 2020. To answer the demand for new sources of energy and manage the growing amounts of waste, there has been done research on the utilization of waste for energy production in the past and will become more and more important in the future.

Ethanol, an alcohol, can be made from basically any kind of biomass which contains glucose. Bioethanol can be used in fuels for vehicles without any modifications of the engines in concentrations up to 5 per cent and even 10 per cent in newer engines, and is therefore a good option in the fuel industry for the nearer future when other technologies are still to be developed.

The basic process of winning ethanol from biomass is described as follows,

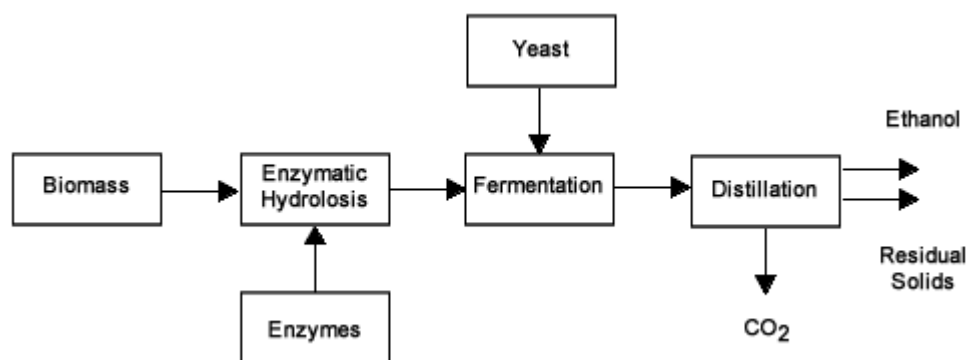
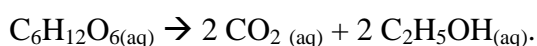


FIGURE 1. Ethanol production process (RFA 2007)

where the most important chemical reaction, from glucose to ethanol, is



The aim of this study is to show the bioethanol potential of preserved food waste in a larger institution like Tampere University of Applied Science, where its composition should be comparable to biowaste produced in other similar institutions. The study is part of a larger project investigating the possibilities of the Jäte-Aate vessel for re-use of the kitchen waste of the TAMK kitchen and cafeteria which is serving approximately 6000 students. As can be seen in the sketch by the project manager Pirkko Pihlajamaa presented in figure 2, the possible future application of the vessel is the production of the raw material for the production of bioethanol, biodiesel, biogas or biocellulose, which would be produced by larger companies, who buy the raw material for their production and sell the end product further on to the end user. The vessel would be installed in the institutions providing the feedstock for the vessel. For the application of the vessel all places are suitable where large amounts of food are handled, like schools, universities, hospitals, grocery stores, food producers and similar institutions. The vessel would be installed on-site and the left-over food fed to the vessel directly and stored there, and the vessel emptied after certain periods of time.

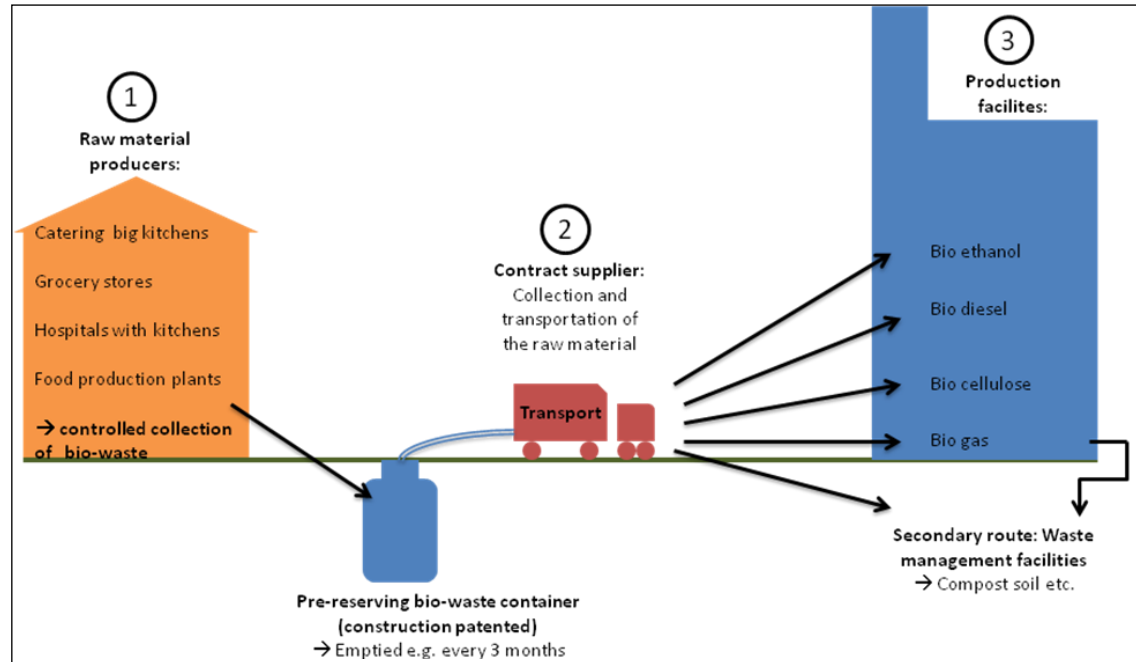


FIGURE 2. Usage of biowaste as a raw material (Draft by Pirkko Pihlajamaa, 2011).

In this experiment, the potential production of raw material for the bioethanol production is analysed by studying the change in bioethanol yield over a time period of three months, during which the food residues are preserved and stored in anaerobic

conditions. The bioethanol yield as well as other factors such as chloride content, pH and conductivity, and their fluctuation over the time are analyzed over the whole period. In an ideal case the bioethanol yield stays constant also over longer time periods due to the preservation in order to guarantee a constant quality and reliable characteristics of the raw material for the ethanol production. Influences on fluctuation in the ethanol yield will be analyzed and possible improvements in the process pointed out.

This study is a continuation of earlier studies on the project. In the previous study implemented by Luis Gonzáles Martos (2011), the influence of two different preservation agents (LactoFast and formic acid) were compared when being applied for storage periods of ten days each. It was decided to use formic acid for this study, based on the results of the earlier study presented in the thesis of Martos.

### 3 BIOETHANOL

Ethanol, also called ethyl alcohol, is an alcohol derived from sugars by fermentation and distillation. Therefore basically any feedstock containing a sufficient amount of sugar or materials which can be converted into sugar is suitable for ethanol production. Referred to as bioethanol is all ethanol obtained from biomass. (Schnepf 2006, 4-5.) According to Demirbas (2006), bioethanol as an alternative fuel can be used either as a gasoline additive or substitute and can be produced from wood, straw, crops and household waste by the alcoholic fermentation of the sugars which are produced by hydrolysis of the biomass. (Demirbas 2006, 1) Dependent on the feedstock for the production of bioethanol, it can be referred to as a first generation or second generation biofuel. First generation biofuels are produced from food crops, while second generation (or advanced) biofuels are derived from non-food feedstocks, as can be seen in table 1. (Demirbas, Balat & Balat 2011, 1817.)

TABLE 1. Classification of biofuels (Demirbas et al. 2011, 1817, modified)

<b>Generation</b>	<b>Feedstock</b>	<b>Example</b>
First generation biofuels	Sugar, starch, vegetable oils, or animal fats	Bioalcohols, vegetable oil, biodiesel, biosyngas, biogas
Second generation biofuels	Non food crops, wheat straw, corn, wood, solid waste, energy crop	Bioalcohols, biooil, bio-dmf, biohydrogen, bio-fischer-tropsch diesel, wood diesel
Third generation biofuels	Algae	Vegetable oil, biodiesel
Fourth generation biofuels	Vegetable oil, biodiesel	Biogasoline

Demirbas defines any biofuel as a "non-polluting, locally available, accessible, sustainable and reliable fuel obtained from renewable sources" (Demirbas 2008, 2106), which makes them and especially bioethanol interesting in the future for the industry as is explained more detailed in the following.



### 3.1. Bioethanol as an alternative fuel in the past, nowadays and in the future

Bio-ethanol, along with other biofuels, became increasingly interesting for research and commercial production in the 1970's after the first oil crisis which showed the need for alternatives in cases of shortening in the oil supply. Fanchi and Fanchi present the development of the the crude oil prize over the last 4 decades, where the first peak in prize occurred in 1974. (Fanchi & Fanchi 2011, 87.)

Approximately at the same time the world reached the first peak oil point in 1978 and first serious doubts about the limitless abundance of fossil fuels were raised. In figure 3 the world production rate of oil is presented along with a forecast of the future production. The peak in the late 70's as well as the prediction according to the Gausssian curve can be seen.

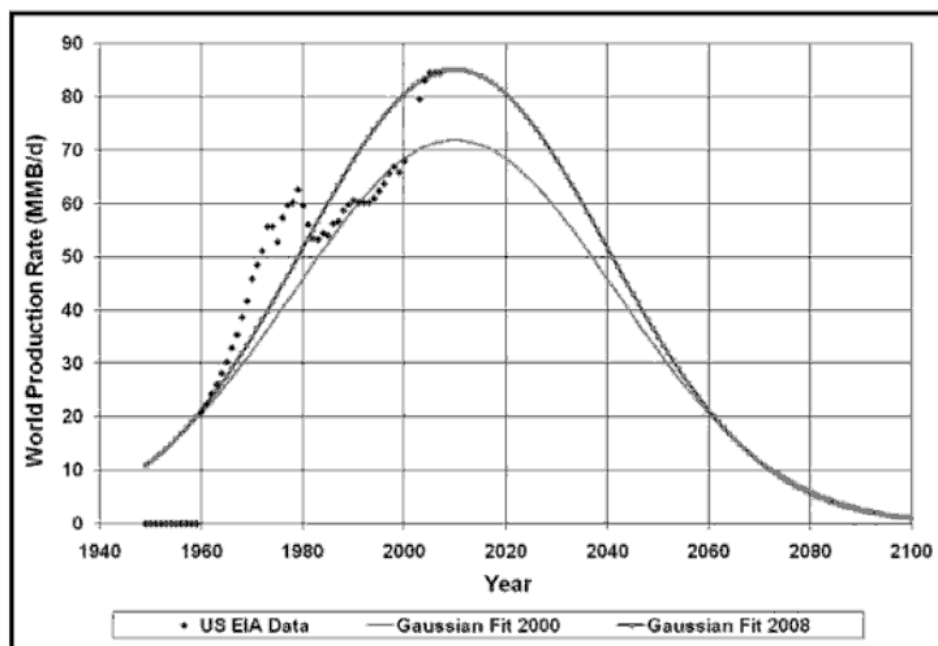


FIGURE 3. World Oil Production Rate Forecast Using Gaussian Curve (Fanchi & Fanchi 2011, 87)

Those two factors, the dependency on international trading and political relations as well as the possible future shortage in oil and gas resources were therefore in the 1970's the main driving forces towards the development of biofuel production. Environmental concerns about greenhouse gas emissions related to the use of fossil fuels were existent already by that time, but became more important only later when the world policies started to address environmental issues and especially the climate change as a result of

traffic- and industry-born air pollution. As stated by Türe, Uzun and Türe (1997), the world-wide energy consumption grew 17-fold during the 20<sup>th</sup> century and, resulting mainly from the combustion of fossil fuels, CO<sub>2</sub>, SO<sub>2</sub> and NO<sub>x</sub> became the main causes of atmospheric pollution (Türe et al. 1997). The Kyoto protocol, signed in 1997 and put in force in 2005, as the first big international agreement on fighting global warming, along with the oil peak being predicted for the time around the year 2000, caused an increase in global biofuel production after 2000. In 2009, the EU published a directive on the promotion of the use of energy from renewable sources in the European Union, which contains a binding target of a share of 20 per cent of renewable energy by 2020 in the final energy consumption in the European Union. It also includes a binding target for each member state of a minimum 10 per cent share of renewable energy sources in transport. (Koponen, Soimakallio & Sipilä 2009, 3.) This directive is most likely going to increase the pace of development of biofuel technologies even further. Figure 4 shows the world-wide production of fuel ethanol from 1975 to 2003.

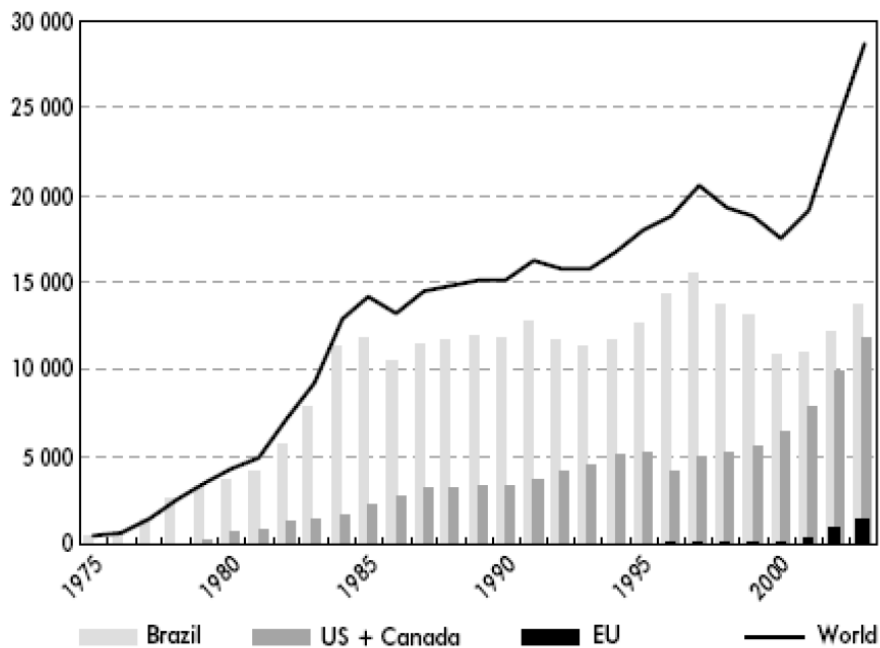


FIGURE 4. World and regional fuel ethanol production, 1975-2003, million liters per year (Vessia 2005, 14)

Recently, bioethanol starts to become economically profitable and competitive with fossil fuels and is according to Demirbas (2009) the world-wide most used biofuel. The global production of bio-fuels was 68 billion l in year 2007, where the main feedstocks for the bio-ethanol production are sugar cane, produced in Brazil with a 60 per cent

share of overall bio-fuel production, and other crops. (Demirbas, 2009, 2239.) Nevertheless, even with the increasing oil prices, biofuels are still more expensive than fossil fuels, but the biofuel industry is expected to be shaped in the coming century in the same way the fossil fuel industry was shaped in the last century. Predictions for the availability of modern transportation fuels are presented in table 2, where the availability of bioethanol in the future is estimated to be excellent. Governments can support this process with methods like for example the reduction of taxes on biofuels and obligatory usage of biofuels. (Demirbas 2008, 2113.)

TABLE 2. Availability of modern transportation fuels (Demirbas 2009, 2240)

Fuel type	Availability	
	Current	Future
Gasoline	Excellent	Moderate-poor
Bioethanol	Moderate	Excellent
Biodiesel	Moderate	Excellent
Compressed natural gas	Excellent	Moderate
Hydrogen for fuel cells	Poor	Excellent

### 3.2. Application, restrictions and advantages of bioethanol fuel

Bioethanol as a fuel can be used according to the EU standard EN 228 as a 5 per cent blend with petrol without any required modifications of the engine and in higher blends of up to 85 per cent with engine modifications. In modern engines, E10 containing 10 per cent ethanol can be used. It is therefore a gasoline additive or substitute. The environmental properties of bioethanol result in a net release of no carbon dioxide and very little sulphur, due to a higher octane number, higher flame speed and evaporation heat, and broader limits for flammability. These lead to a higher compression ratio and a shorter burning time as well as leaner burn engine, which result in better efficiency in internal combustion engines compared to petrol. Only anhydrous ethanol is suitable for this use, while hydrated ethanol, containing more than 2 per cent of water, is only to some extent miscible with gasoline and requires therefore further treatment. Bioethanol which is produced biologically contains around 5 per cent of water and therefore falls under this category. The energy density of ethanol is lower than that of gasoline. Ethanol is more corrosive, has a lower vapour pressure which makes it more difficult to start the engine in low temperatures, is miscible with water, and increases the emissions

of acetaldehyde and evaporating emissions when blending with gasoline. (Demirbas et al. 2006, 2008, 2011.)

### **3.3. Sources of bioethanol**

As it was said already earlier, ethanol can be won from any feedstock which can be converted into sugars. Bioethanol is produced from renewable feedstocks. The value of the biomass for the ethanol production is defined by how easily the conversion to sugars takes place. This makes feedstocks with a high content of starch and sugars easily convertible, while cellulosic materials require more pre-treatment. (Demirbas et al. 2011, 1818). Until now, mainly food crops are used for the bioethanol production, but there is frequently active research done on the investigation of non-food crops as raw materials due to different socio-economic effects such as increasing food prices, shortages in food for cattle, and growing competition for land (Stichnothe & Azapagic 2009, 624)

#### **3.3.1 Food crops**

Food crops are suitable for the bio-ethanol production due to their high contents on fats, proteins and carbohydrates. The production of bio-ethanol from food crops is criticized due to the fact that its production reduces the resources for the food production and therefore increases food prices. (Kessler 2008, 274-275) They are therefore referred to as first generation bio-fuels, since they are sustainable only to a certain extent, as was presented in table 1. Any food crop can be used for the ethanol production, but the currently most used food crops are corn and sugar cane, where Brazil is the leading ethanol producer using sugar cane, followed by the US deriving ethanol from corn. (United States Department of Energy 2006, 39)

#### **3.3.2 Common crops and lignocellulosic materials**

Lignocellulosic materials are materials containing cellulose and lignin which are formed during photosynthesis. They occur in wood as well as other woody tissue like for example agricultural residues, grasses, and water plants. They are referred to as

biomass, but since biomass generally includes all kind of living substances, lignocellulosic materials are just one specific form of biomass. (Rowell 1992, 12.)

Hu (2008) defines lignocellulosic materials as a “natural, abundant and renewable resource”. Due to recent need for biofuels, lignocellulosic materials became increasingly interesting as a raw-material for the production of such and especially in the sector of bioethanol production. He also says that there are no effective and economical ethanol production methods yet due to a lack of knowledge about the structures of lignocellulosic materials, and that improved methods for their characterization still need to be developed. (Hu 2008)

There are different lignocellulosic materials used for the bioethanol production. One example is woodchips, the residues of the forest and timber industry in form of scraps of tree stems, shredded twigs and similar. Another lignocellulosic material used is agricultural waste material, which is the leftovers of agricultural production of crops and represents the remaining part of the plants which are of no use for the food industry or others (Najafi et al., 2008). Research is lately done on the usage of different grasses, like for example switchgrass, a grass growing in North-America and Canada having high contents of cellulose and growing very high, making it a suitable feedstock for ethanol production (Rinehart 2006, 1). Another grass used is *Miscanthus*, which is also a high yielding energy crop and only recently being researched for the use for bioethanol production. (Sørensen et al. 2007, 6602)

### **3.3.3 Municipal waste**

According to Stichnothe and Azapagic (2009), municipal waste and especially organic waste becomes due to its qualities increasingly interesting for the energy production industry, since the environmental and economical benefits of bioethanol derived from cultivated crops are questionable. Waste materials used as feedstock for the bioethanol production decrease the stress on landfills, increase the re-use of materials and reduce the greenhouse gas emissions from landfill sites. By this they help to fulfil requirements of legislations such as the European Waste Framework Directive. (Stichnothe & Azapagic 2009, 624)

The production of bioethanol from biowaste has been researched only little until now and therefore needs further investigation. In the study from Stichnothe and Azapagic (2009), the greenhouse gas emissions of the production process of bioethanol from both household waste Refuse Derived Fuel and Biodegradable Municipal Waste was analyzed with the result, that even though the production of bioethanol from RDF reduces emissions compared to current waste management practice in the UK, it nevertheless does not save any emissions when comparing the RDF derived ethanol fuel with petrol. On the other hand, there is a reduction in greenhouse gas emissions of 92,5 per cent from the fuel combustion process comparing the ethanol produced from BMW with petrol. Bioethanol derived from Brazilian sugar cane reached only savings of up to 70 per cent compared to petrol. (Stichnothe & Azapagic 2009, 624.) This makes the biodegradable waste, which is analyzed in this study at TAMK, especially interesting as a future raw material for the fuel ethanol production.

### **3.4. Production of bioethanol**

According to Demirbas et al. (2006), the process of deriving ethanol from biomass consists of two main steps: the hydrolysis of carbohydrates to simple sugars glucose and xylose, and the fermentation of the sugars to alcohol. Carbohydrates can be the cellulose and hemicellulose in plant matter for example. Cellulose is an organic polymer which occurs in long molecular chains, consisting of units of anhydro glucose. During hydrolysis it is split up into glucose, where the conversion efficiency is dependent mostly on the chemical and mechanical pre-treatment of the cellulose. Hemicelluloses occur in much shorter chain molecules than cellulose and act as bindings between the cellulose molecules. They are soluble in alkali, which enhances the hydrolysis. The hemicelluloses occurring in woody tissues break down much easier during thermal treatment. (Demirbas et al. 2006, 9.)

The enzymatic hydrolysis process is presented in figure 5, where after the hydrolysis of the carbohydrates with the help of acid and cellulase enzymes both the C<sub>5</sub> and C<sub>6</sub>-sugars are fermented and the resulting ethanol is distilled to obtain higher concentrations.

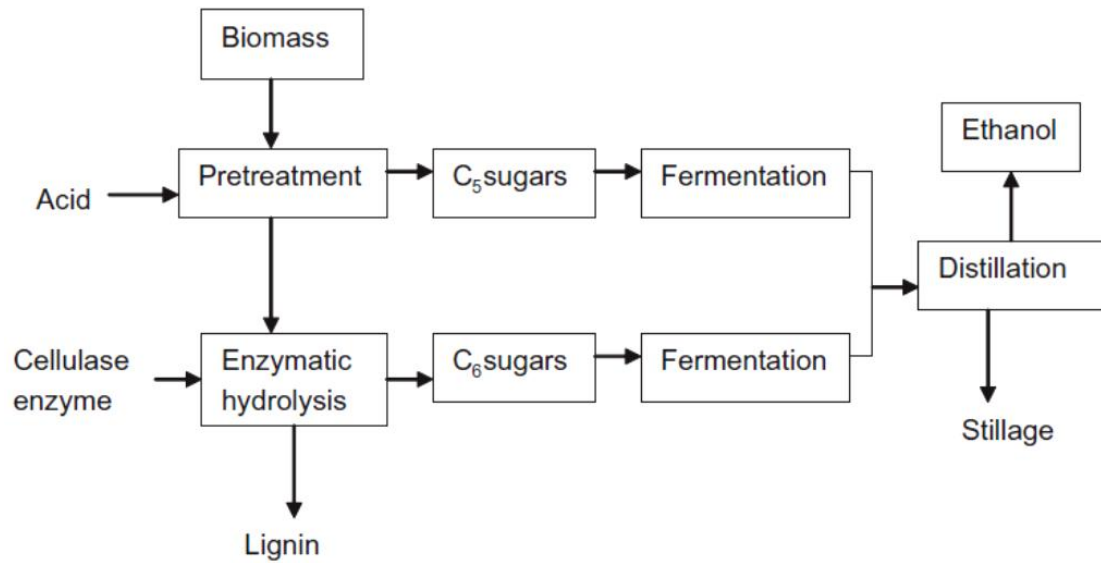
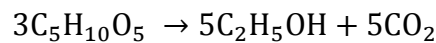
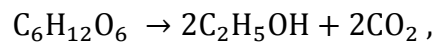


FIGURE 5. Enzymatic hydrolysis process (Demirbas et al. 2011, 1819)

The reactions taking place during the fermentation are according to Vessia (2005)



and



where the reaction from xylose to ethanol is more complicated and has been researched successfully only since the 1980's. Both reactions are needed in order to increase the ethanol yield. These two reactions result in a theoretical maximum ethanol yield of 0,51 kilogram per kilogram of xylose and glucose. According to Vessia (2005), a biological process has certain advantages compared to a chemical catalytical process, like a higher specificity, higher yields, less catalyst poisoning, and lower energy inputs. Even though the process is slower than the chemical reaction, it is an irreversible process which allows complete conversion, hence there are no thermodynamic equilibrium relations. (Vessia 2005, 29-30.)

## **4 EXPERIMENTAL STUDIES**

### **4.1. Implementation of the experiment**

The experimental set-up was the same as in the two previous studies on this project implemented by Esther Posadas Olmos (2011) and Luis Gonzáles Martos (2011). In the facilities of the TAMK laboratories a vessel, which can be seen in figure 6, with a volume of 0,8 cubic meters provided and patented by Aate Virtanen was installed and tested within the Jäte-Aate project at TAMK since 2010 over periods of two weeks for the anaerobic storage of preserved biowaste. Kitchen waste is fed to the vessel via a grinder (model imc 726) which can be seen in figure 7 with addition of water in order to ensure that the waste does not block the pipe. Samples of the vessel content can be taken from two valves at different heights on the vessel. Pressure as well as temperature is measured constantly, and a valve on the top of the vessel allows the measurement of the gas composition inside the vessel.





FIGURE 6. Biowaste preservation installation in the TAMK greenhouse (Photo: Luis González Martos 2011)



FIGURE 7. Structure of the feeding grinder (Photo: Luis González Martos 2011)

Waste was collected from the TAMK cafeteria, where it was stored in an air-conditioned room outside the kitchen, and brought in closed buckets to the laboratories. This was done twice a week in the time from 1.11.2011 until 31.1.2012 aiming at collecting a waste mass of 40 kilograms a week dependent on the quality of the available kitchen waste. Large amounts of paper waste were avoided since they could have caused possible blockings of the feeding grinder. There were slight fluctuations in volumes of waste fed to the vessel over the time due to an occasional lack of useable waste. The waste was composed of food products, where salad, potato products and grain products were dominating components. An accurate list of all materials added can be found from the appendix 2 of this thesis.

In total a minimum of 400 kilograms of waste had to be collected during the period of three months. While in the previous studies the testing periods lasted only for a few weeks, this time the changes in the bioethanol yield over a longer time period were studied.

The collected waste was weighed and preserved using liquid formic acid AIV 2 plus in a ratio of 5 millilitres per kilogram of waste. The material safety data sheet of the product is included in the appendix 1 of this thesis. The formic acid was handled under the hood using a volumetric pipette. After the addition of the formic acid the waste was mixed thoroughly and screened in order to avoid feeding accidentally disposed non-biodegradable or too large pieces into the grinder. The water flow was kept below 0,5 litre per kilogram of waste in order not to dilute the raw material too much. Nevertheless it was sometimes needed to exceed this limit when the material was too dry, other times, when having rather moist waste samples, much less water was used. The overall addition of water stayed therefore within the given range.

The pH of the vessel content was measured three times a week with a Mettler Toledo pH meter when sampling the preserved biowaste. pH measurements were done according to the international standard ISO10390. Samples of the vessel content could be measured straight with the instrument, whereas the biowaste samples had to be diluted with distilled water (dilution factor 1:5) and stirred for at least 15 minutes before measuring the pH.

The conductivity of the preserved biowaste was measured three times a week using a Mettler Toledo conductivity meter.

The gas composition inside the vessel was measured three times a week with the help of the Gas Analyzer Geotech GA 2000PLUS. The instrument was measuring CH<sub>4</sub>, O<sub>2</sub>, and CO<sub>2</sub> content.

## **4.2. Analytical methods**

After the implementation of the testing period of three months, the samples taken during that time were analyzed regarding their bioethanol yield and their chloride content. Not all samples taken during the testing period could be analyzed due to a tight schedule. It was decided to use for the analysis two samples of the first month of the experiment, and four samples of each the second and third month, since it was more interesting to see the development of the ethanol potential during later stages of the experiment. The bioethanol yield was analyzed using the testing procedure described below, including also the measurement of pH and dry matter content of the samples before and after the fermentation process. The chloride content of the samples was measured using potentiometric titration as is described in chapter 4.2.2.

### **4.2.1 Bioethanol potential**

The basic principle of the bioethanol potential test is the hydrolysis of carbohydrates to sugars and the fermentation of the glucose in the raw material, and the calculation of the ethanol produced by the determination of the loss in weight of the raw material during the fermentation. In order to make the glucose in the raw material available for fermentation, enzymes were used which degrade the long-chained starch in the sample. Acid Alpha Amylase GC 626 and Glucoamylase Diazyme® SSF2 were used for this purpose. The influence of the  $\alpha$ -Amylase in combination with a suitable pre-treatment temperature on the ethanol yield can be seen from figure (Genencor 2010).

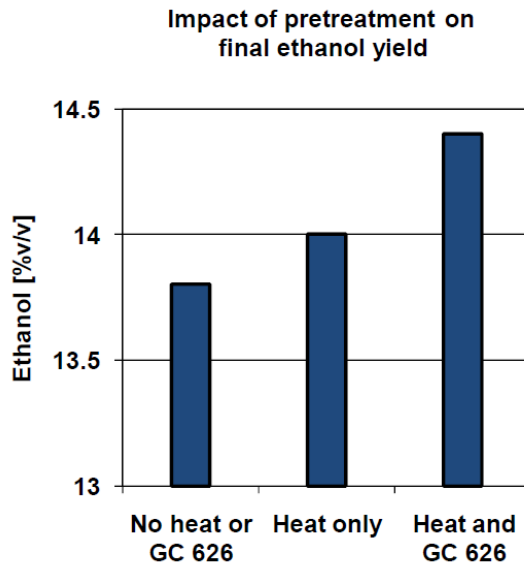


FIGURE 8. Impact of pre-treatment on final ethanol yield (Genencor 2010)

The procedure used for analyzing the bioethanol yield was based on a study comparing different treatment methods (Lemuz et al. 2009, 356), and adjusted according to the instruction on the dosages of enzymes and yeast recommended by the producers of the products. The resulting procedure was applied equally to all samples.

First, the pH of the samples was adjusted to 4.25 at room temperature using 0.5M NaOH. 3-4 replicates of each sample, according to the initial volume of sample available, with a volume of 80-100 millilitres were placed in 250 or 300 millilitre Erlenmeyer flasks and 5.24 micro litres of  $\alpha$ -Amylase per 10 millilitres of sample added. The samples were then heated and kept at 65°C in a water bath (see figure 9) for one hour while swirling them regularly. After that, 14.6 micro litres of glucoamylase per 10 millilitres of sample were added and the flasks swirled again to mix the sample. The samples were then left to cool down, and at a temperature below 32°C 0.05 grams of fresh yeast per 10 millilitres of sample were added. The samples were again mixed well and the flasks closed with water locks. pH and dry matter content of the samples were determined, as well as the initial weight of each Erlenmeyer flask and content.

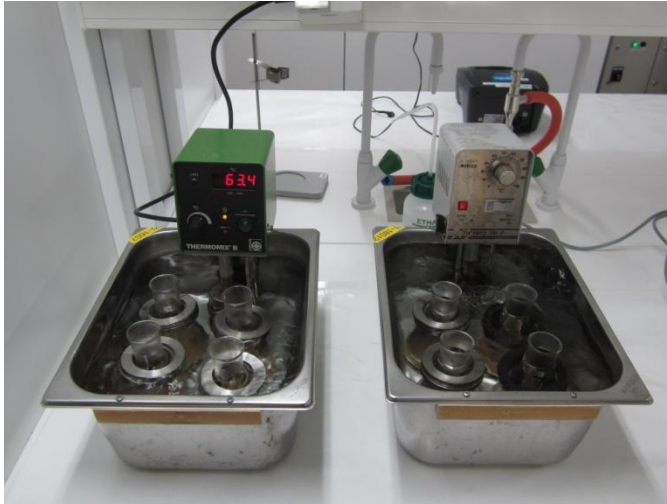


FIGURE 9. Water bath with ethanol samples (Photo: Magdalena Gerlach 2012)

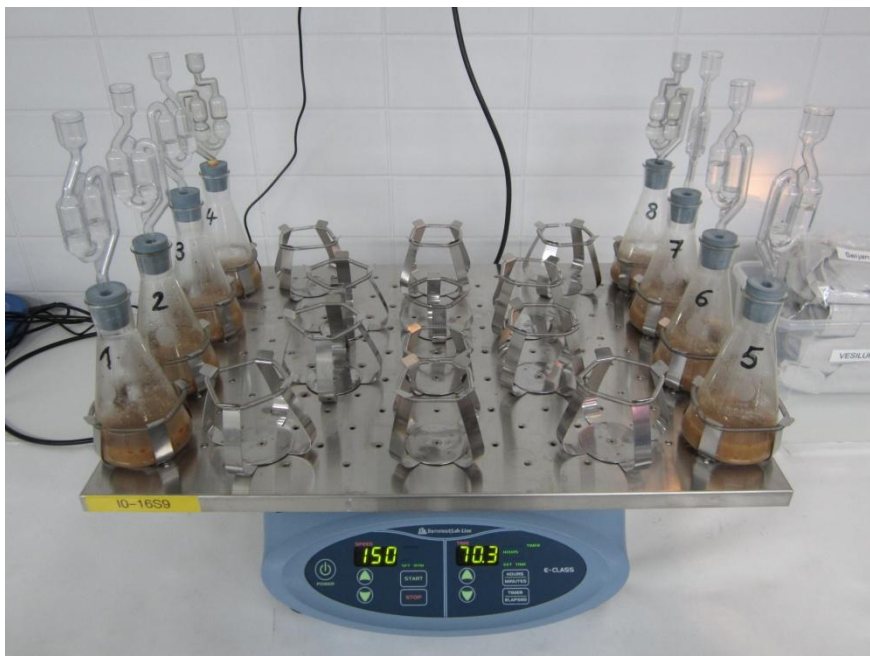


FIGURE 10. Barnstead|Lab-Line MaxQ 2000 Shaker (Photo: Magdalena Gerlach 2012)

The samples were then left for 72 hours for fermentation on a Barnstead|Lab-Line MaxQ 2000 Shaker, as presented in figure 10, at 150rpm. Every four hours, if possible, the samples were weighed again and the reduction in mass monitored. After 72 hours the final mass was determined and again pH and dry matter content analyzed. The total reduction in mass defines the reaction of glucose to ethanol, so that the amount of ethanol produced can be calculated as can be seen in the results part of this thesis.

#### 4.2.2 Chloride content

The chloride content defines the quality of the raw material for bioethanol production significantly. Due to industrial process related reasons, the material is required to have a chloride content of below 1%.

The chloride content was analysed according to the International standard SFS-EN ISO 5943 by potentiometric titration of the preserved food waste. In order to obtain the total chloride stored also in the solid parts of the raw materials which are not dissolved, a standard for the analysis of milk products was applied.

Four samples from different stages of the testing period were analysed by using the automated titrator Mettler Toledo DL50 as can be seen in figure 11. Three replicates of each sample were taken. A dilution of the raw material with distilled water in a ratio 1/5 due to the thickness of the raw material was necessary in order to get analysis results.



FIGURE 11. Mettler Toledo DL50 (available at [http://www.globalspec.com/NpaPics/42/92833\\_110420036371\\_ExhibitPic.jpg](http://www.globalspec.com/NpaPics/42/92833_110420036371_ExhibitPic.jpg), accessed 24.4.2012)

## 5 RESULTS AND CONCLUSIONS

### 5.1. Presentation of the measurements

In the following there are the measurements and analyses which were conducted during and after the testing period presented, as well as possible reasons for the results analyzed. An overview of the measurements taken during the testing period can be found from appendix 3.

#### 5.1.1 pH of the preserved biowaste

The pH was measured over the whole testing period starting from day 17. It was kept around 3,5 over the whole period by the addition of the formic acid. The reason for this procedure was the prevention of the formation of microorganisms in the vessel which would support the fermentation of the food waste when it is not desired yet. The pH was successfully kept low and did not vary significantly as can be seen from figure 12. The small raise of the pH in the end of the testing period could be a result of the last addition of food waste, which was with 40 kilograms rather big compared to earlier additions of usually around 20 kilograms at a time.

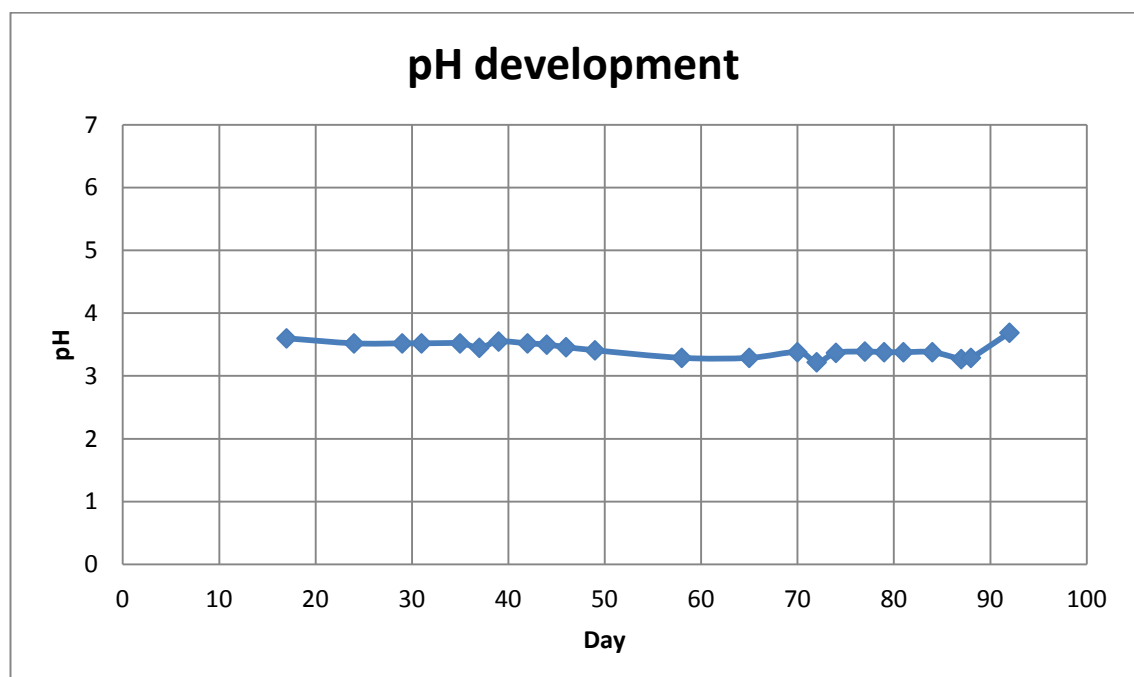


FIGURE 12. pH development over the testing period

### 5.1.2 Conductivity of the preserved biowaste

According to the United States Environmental Protection Agency, the conductivity is “a measure of the ability of water to pass an electrical current”, which results from inorganic or organic compounds dissolved in the water, where the inorganic compounds, like also the chloride, conduct easily electric charges and result therefore in higher conductivity, and organic compounds lower it. (EPA 2012).

It stayed, as also the pH, rather stable around 12 milli Siemens per centimetre as can be seen from figure 13. It was not influenced from outside and is a result of the composition of the vessel content.

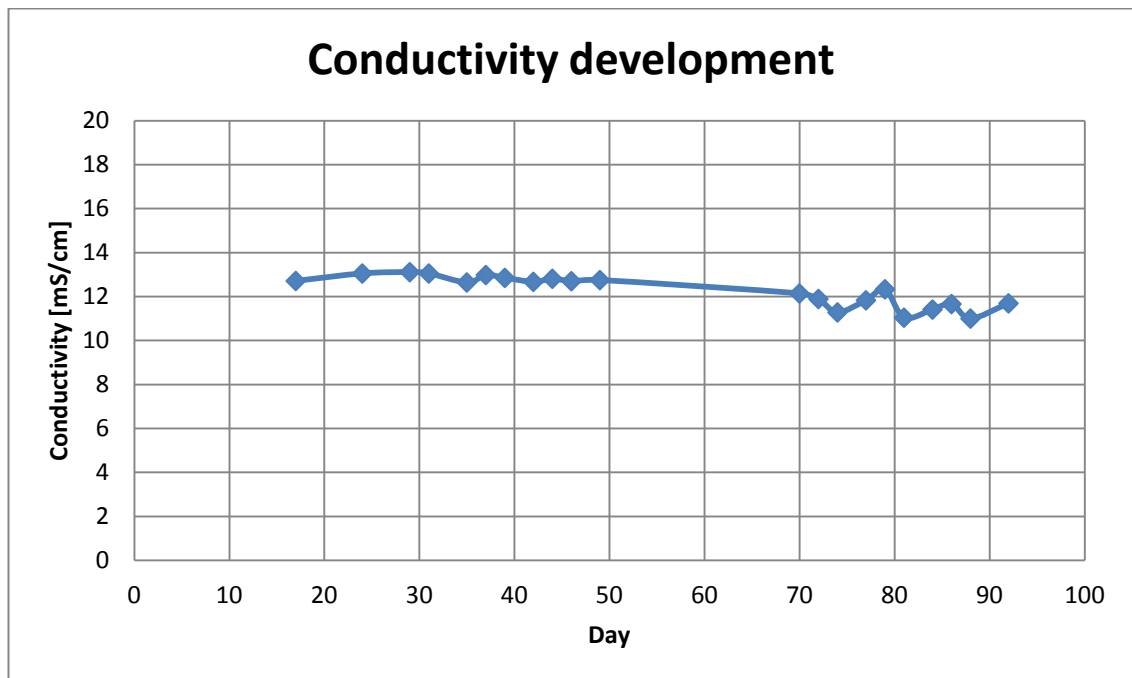


FIGURE 13. Conductivity development over the testing period

### 5.1.3 Gas composition inside the vessel

The gas composition inside the vessel gives information about the reactions happening in the preserved biowaste. The most interesting gas to observe is the carbon dioxide, since it is formed as a result of the fermentation in the vessel. As can be seen in figure 14, the carbon dioxide content was peaking three times during the testing period. It was raising nearly linearly during the first 40 days of the experiment to drop then very



rapidly. Simultaneously, the oxygen content was raising. This can be explained only by the fact that someone must have opened the vessel cover. Otherwise oxygen could have neither entered the vessel nor is there any reaction which possibly could have resulted in oxygen being formed. The oxygen content went back to close to zero per cent within only 15 days again. At the same time the carbon dioxide was rising steeply again up to nearly 60% to slowly go down then again, and was rising in the end of the testing period again up to nearly 70%. In the following it has to be examined what was causing the rise in carbon dioxide, and if the feeding procedure of the biowaste to the vessel could possibly have an effect on the early fermentation. The methane content stayed close to 0% over the whole testing period as was desired, which indicates that no anaerobic digestion took place.

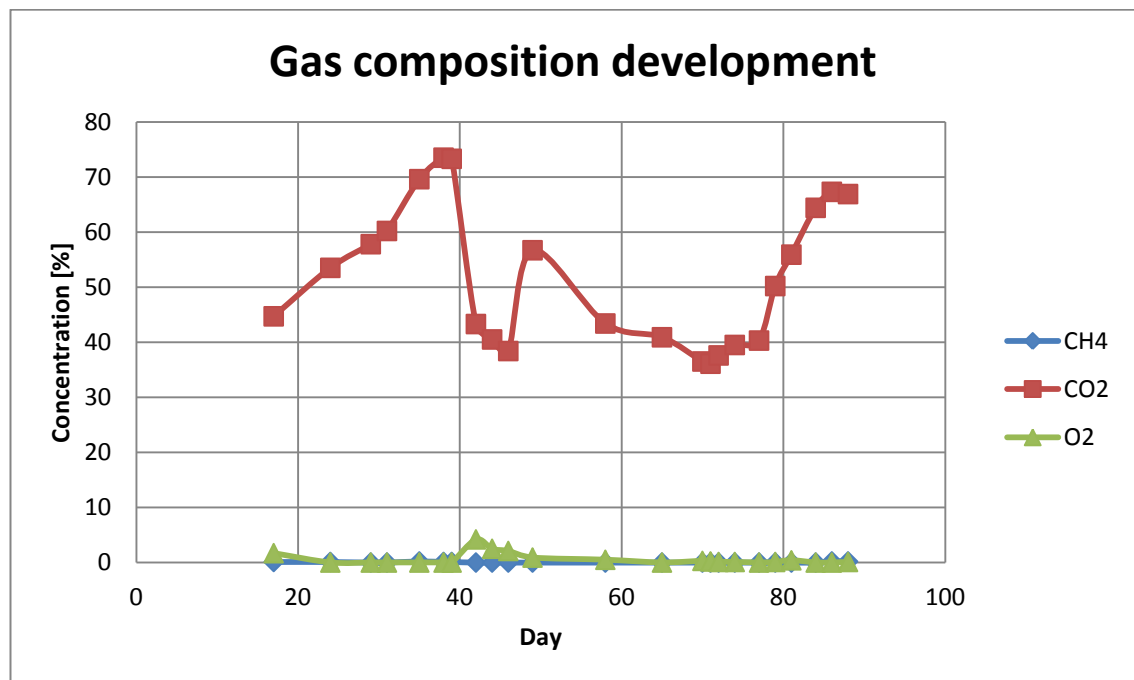


FIGURE 14. Gas composition inside the vessel over the testing period

#### 5.1.4 Chloride content of the preserved biowaste

The chloride content was given by the Mettler Toledo DL 50 in grams per litre of sodium chloride as can be found from the appendix 4-7, which needed to be converted into the concentration of chloride only. The sodium chloride compound has a molar mass of 58,44 grams per mole, out of which the Sodium is responsible for 22,99 grams per mole, and the chloride for 35,45 grams per mole. The chloride has therefore a

percentage of the overall mass of 60,6. The concentration was then converted as follows:

$$c \left[ \frac{g}{l} Cl \right] = c \left[ \frac{g}{l} NaCl \right] \cdot 0,606$$

Dilution factors were taken into account where they had been used, and the average of the three replicates was calculated. The results are presented in table 3. As it can be seen, the chloride content did vary only between 0,179 and 0,207 per cent over the whole testing period. Given a requirement of a chloride content of the raw material below 1 per cent, these results are more than favourable in this sense.

TABLE 3. Chloride content of the preserved biowaste

<b>Day of the experiment</b>	<b>Date</b>	<b>Chloride conc. [g/l]</b>	<b>Chloride conc. [%]</b>
24	24.11.11	2,068	0,207
58	28.12.11	1,791	0,179
79	18.1.12	1,886	0,188
92	31.1.12	1,793	0,179

### 5.1.5 Dry matter content of the preserved biowaste

Unfortunately the results for the dry matter content are questionable as the measurements were fluctuating crucially, up to over 9 per cent over a time span of only two weeks, and can therefore be used for interpretation only to a certain extent. Rough conclusions have to be drawn from the results available.

The fluctuation of the results might have several reasons. One possible explanation is that the fluctuating values are a result of the analysis method, which works with very small sample sizes around 1 gram. The small sample size means that the sample is not fully representative for the original sample, since the solid content of the raw material, although grinded when added, is not dissolved in the water. The dry matter content of the small sample taken can therefore vary tremendously. Due to limited time resources it was not possible to analyse more replicates. It would have been more favourable to conduct the analysis with a standard gravimetric method for determination of dry matter

content which uses bigger sample sizes. The standard method SFS-EN 12145 could be used for example.

The sampling from the vessel had an influence on the dry matter content of the sample as well. Inside the vessel, the solid is suspected to separate from the liquid and settle at the bottom of the vessel. This hypothesis is supported by the fact that the samples taken during the first weeks were rather liquid and started to contain solids only after a few weeks, when the level of the vessel content was rising. Once the solid level had reached the outlet valve, it was noticed that from time to time the valve got blocked and the texture of the sample became more liquid again. This does not affect the dry matter content analysis as such, but makes it more difficult to draw conclusions on the relation between time, dry matter content and bioethanol potential.

The dry matter content as it was measured before and after the fermentation of the samples is presented in figure 15. It is assumed that the first peak in the dry matter content before the fermentation is an outlier and does not represent the real situation.

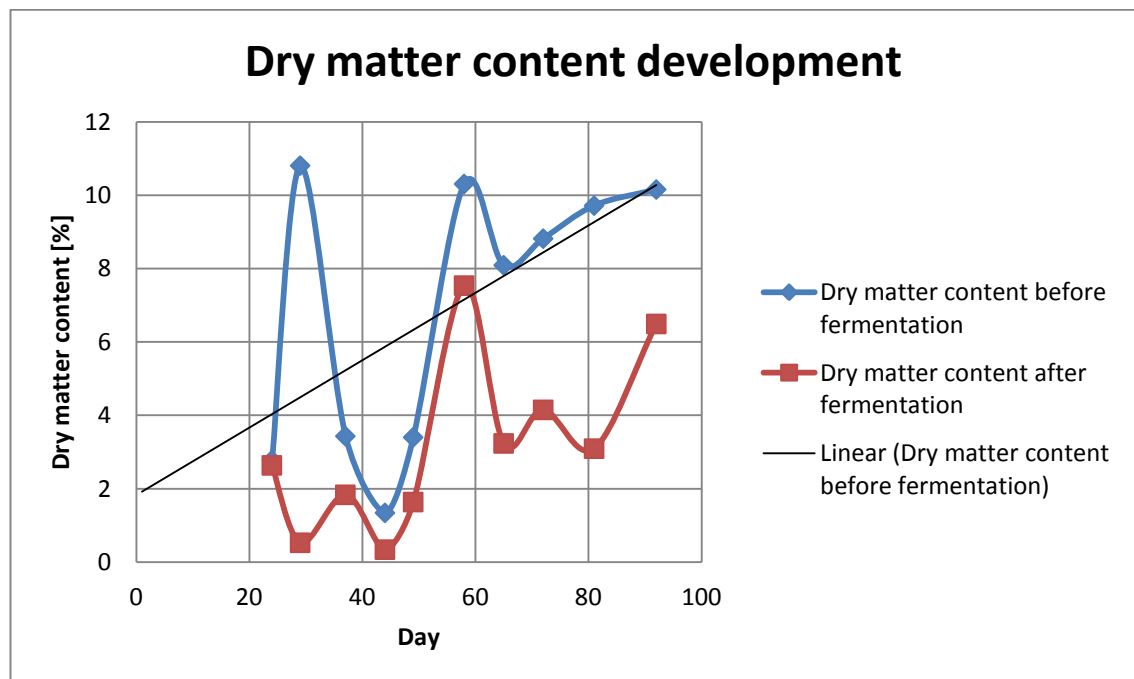


FIGURE 15. Dry matter content over the testing period

In order to make in the following parts of this work assumptions based on the dry matter content, trendlines have been drawn for the dry matter content after fermentation which allow to calculate rough values of the dry matter content over the whole testing period.

The equation for the dry matter content based on the trendline before fermentations is:

$$y = 0,0919x + 1,82 ,$$

where x is the day of sampling. This trend is also only approximate since it is based on the measurement results. Furthermore it could be assumed that the rising of the dry matter content follows, in contrast to the proposed linear trend, in reality rather an exponential trend with progressing time, since the settling of the dry matter follows the rule of gravity and it therefore can be assumed that from bottom to the top the speed of settling as well as the density of the raw material decreases. Nevertheless the drawn trend lines seem to be reasonable compared to the measurement results and will therefore be used.

### **5.1.6 Ethanol yield**

In order to analyze the ethanol yield, the mass loss of the samples during the 72 hours of the fermentation process was studied as described in chapter 4.2.1. The results of the change in mass over the time are presented in figure 16. In the measuring procedure, two samples could be analyzed at a time, and the results show that there is no visible correlation between the mass loss behaviour and the analysis session. Several samples had infrequently a little raise in the mass where it was expected to decline constantly. One possible reason for this behaviour could be the scale itself in case it was used by others in between the measurements and somehow moved or in some other way influenced. This theory is supported by the fact that the changes could be seen in many cases similarly at the same time in all replicates analyzed at a time, as for example can be seen in the samples from day 24 and 29, which both gained in mass after around 48h of fermentation. Another possible reason is the dropping of water from the water locks on top of the Erlenmeyer beakers used. For the first analysis session there were water locks used which apparently did not always prevent the water from dropping into the sample. The amounts of water added to the sample were small, but nevertheless crucial for the total mass loss. Affected samples were excluded from the calculation of the average.

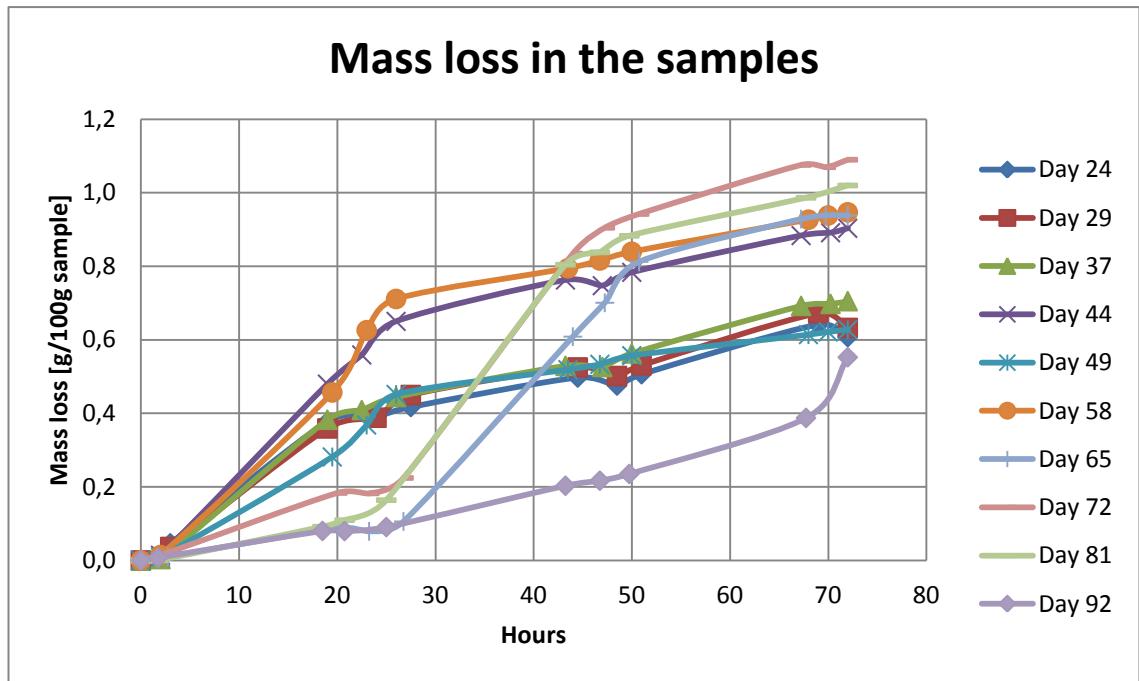


FIGURE 16. Comparison of the mass loss in grams per 100 grams of raw material in the ethanol samples

The ethanol which was produced during fermentation was calculated from the loss in mass. It is known that the reaction from glucose to ethanol and carbon dioxide results in a quantitative mass of 0,51 grams of ethanol and 0,49 grams of carbon dioxide per gram of glucose.

The reaction from glucose to ethanol is described as:



$$180 \frac{\text{g}}{\text{mol}} = 2 \cdot 46 \frac{\text{g}}{\text{mol}} + 2 \cdot 44 \frac{\text{g}}{\text{mol}}$$

$$\text{yield} = \frac{92\text{g}}{180\text{g}} = 0,51 \frac{\text{g ethanol}}{\text{g glucose}}$$

(Dien 2010, 218)

Therefore the loss in mass of the sample represents the amount of carbon dioxide being formed during fermentation, and could be transferred into the conversion rate of

biomass to ethanol. In table 4, the measurements of dry matter content before and after fermentation as well as the ethanol yields in grams per 100 grams of wet sample are presented. It becomes clear that there was in nearly all cases a reduction in the dry matter content, which indicates along with the mass loss that solids were decomposed and fermentation took place.

TABLE 4. Analyses done after the testing period (dry matter content of the sample before and after fermentation, ethanol produced)

<b>Day</b>	<b>DM before fermentation</b> [%]	<b>DM before fermentation (calculated)</b> [%]	<b>DM after fermentation</b> [%]	<b>Ethanol yield</b> [g/100g of wet sample]
<b>24</b>	2,78	3,98	2,64	0,028
<b>29</b>	10,81	4,43	0,53	0,006
<b>37</b>	3,44	5,15	1,84	0,019
<b>44</b>	1,35	5,78	0,34	0,004
<b>49</b>	3,41	6,23	1,64	0,017
<b>58</b>	10,31	7,04	7,54	0,079
<b>65</b>	8,10	7,67	3,24	0,034
<b>72</b>	8,82	8,3	4,15	0,043
<b>81</b>	9,72	9,11	3,10	0,032
<b>92</b>	10,16	10,1	6,50	0,068

The results for the ethanol yield of the wet samples in grams of ethanol per 100 grams of sample are presented in figure 17. As can be seen from the graph, the ethanol yield was reaching its peak on the 58th day of the experiment with 0,079 grams of ethanol per 100 grams of sample, after being close to zero only two weeks before that. After the peak the ethanol yield declines again to 0,043 grams of ethanol per 100 grams of sample, but raises at the end of the experiment again.

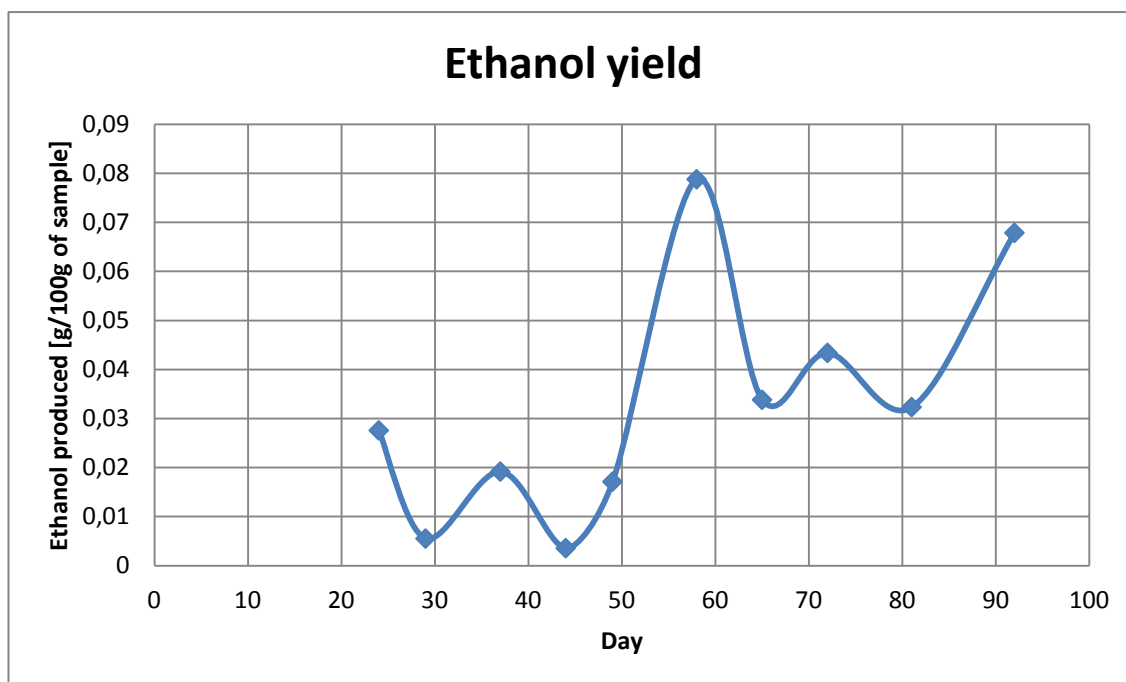


FIGURE 17. Ethanol produced in grams per 100 grams of the samples

Unfortunately it was not possible to determine the glucose content of the raw material. It would have been interesting to define the conversion rate of glucose to ethanol.

## 5.2. Conclusions on the ethanol yield results

There can be many reasons for the behaviour of the ethanol yield, out of which only a few can be analysed in this study. The correlation between the ethanol yield and the carbon dioxide being emitted during the experiment is observed, as well as the correlation between ethanol yield and the dry matter content of the raw material. In addition, the amounts and times of the adding of raw material to the vessel will be analysed in order to find a possible influence on the ethanol potential.

The carbon dioxide content of the gas composition inside the vessel, as already said, was fluctuating irregularly. This is supposed to be an indicator for early fermentation. When looking at figure 18, which presents both the carbon dioxide content and the change in ethanol yield in grams per 100 grams of sample, there nevertheless does not seem to exist a clear correlation between those two measurements.

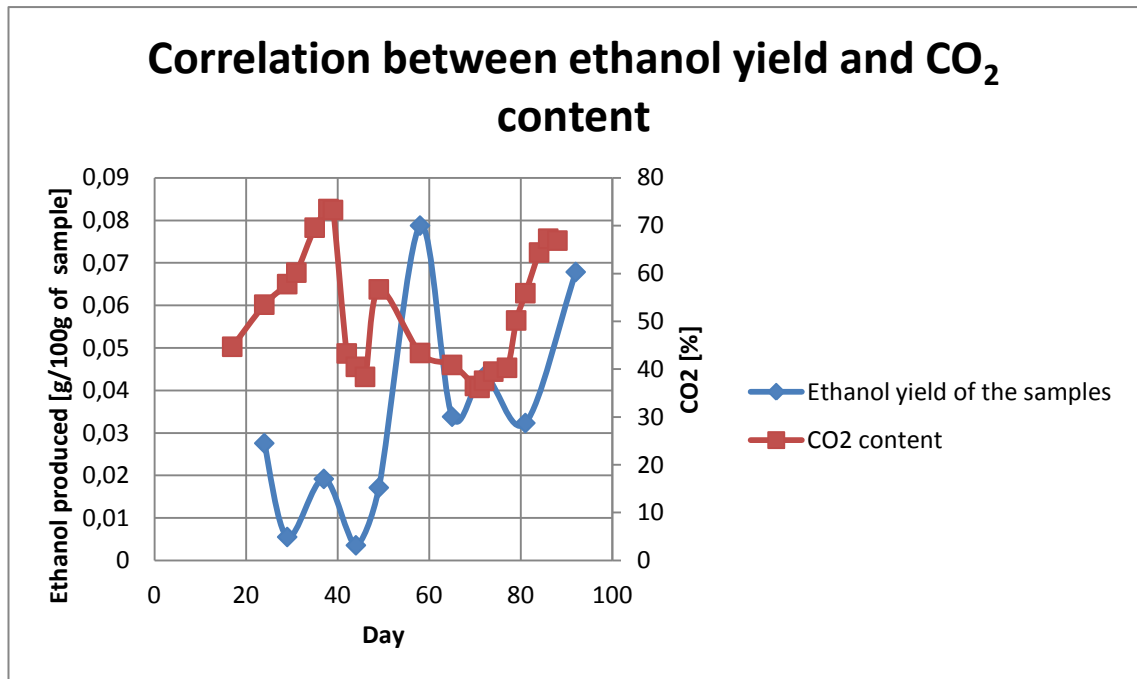


FIGURE 18. Correlation between ethanol yield of the samples and CO<sub>2</sub> emitted

The dry matter content of the raw material is much likely to have an influence on the bioethanol potential. A study conducted by Byung-Hwan and Hanley (2008) on the ethanol yield and conversion of lignocellulosic biomass by conventional fermentation showed that the best conversion was achieved with a dry matter content of 10 per cent as can be seen in table 5. In the study there was used *Zyomonas mobilis*, and the fermentation took place for 48h. Tested were solid concentrations of 10, 15 and 20 per cent. (Byung-Hwan & Hanley 2008, 1257-1265.) These results are comparable only to some extent to this study case, since the used biomass was different, but nevertheless it could be expected that this tendency is applicable for all raw materials.



TABLE 5. Ethanol yield and conversion in per cent by *Z. mobilis* after 48 h (Byung-Hwan & Hanley 2008, 1264)

<b>Substrate concentration</b>	<b>10%</b>	<b>15%</b>	<b>20%</b>
Initial glucose after enzyme reaction [g/l]	42.6	55.5	58.4
Final ethanol concentration after 48h [g/l]	18.2	19.7	6.3
Conversion of consumed glucose into ethanol [%]	83.6	73.4	21.8
Theoretical ethanol yield [%]	80.5	68.6	19.1
Total fermentation time based on portion method [h]	106	110	114

Comparing the ethanol yield obtained in this study to the dry matter content as can be seen in figure 19, it can be said that there is some correlation between them. The dry matter values obtained by calculation according to the trend line were used, which were presented earlier. It is assumed that the tremendous fluctuations in the ethanol yield are a result of the analysis method. The analysis method includes a big number of influencing factors like the yeast, enzymes, temperature of the water bath and others, which make the results very vulnerable. Nevertheless there can still be seen a slight raise in the ethanol yield over the time between those fluctuations, which seems nearly linear with the calculated dry matter content. Unfortunately the dry matter content did not rise above 10 per cent. It is therefore not known if the conversion would have grown further on with higher dry matter content or declined again as the study of Byung-Hwan and Hanley (2008) showed.

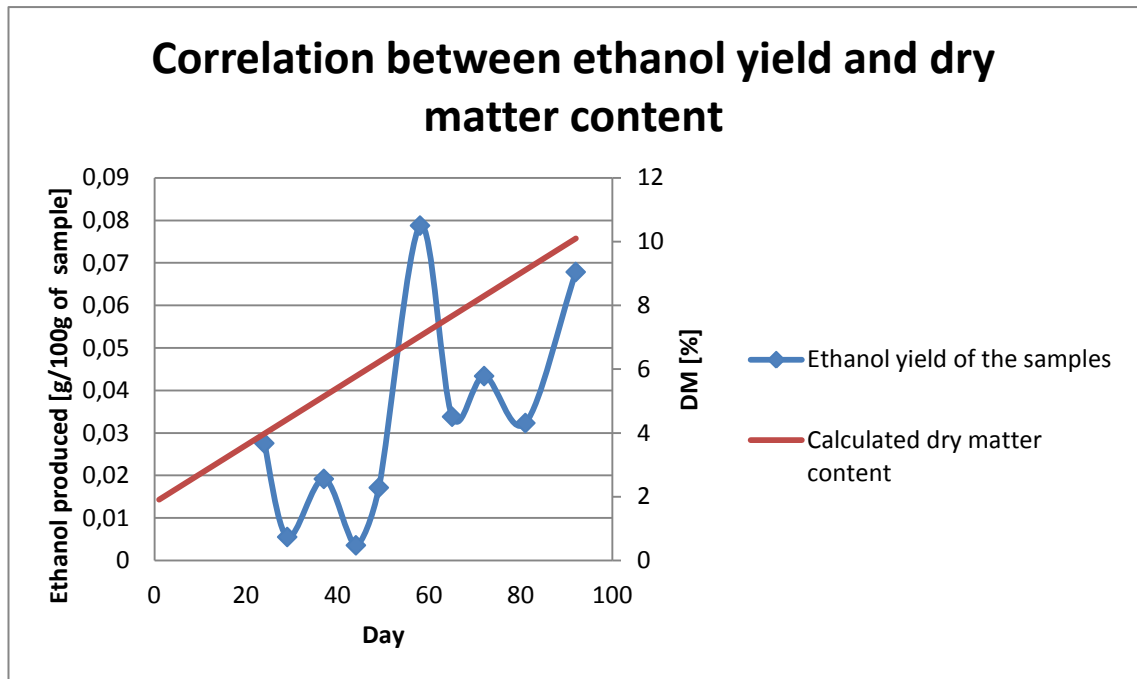


FIGURE 19. Correlation between the ethanol yield of the samples and the dry matter content

Looking at the addition of food waste to the vessel presented in figure 20, it can be seen that there were two short breaks in the feeding rhythm; one around the 40th day of the experiment due to a lack of available biowaste, and another longer break around the 60th day of the testing period resulting from the Christmas holiday during which the TAMK kitchen was out of service. It can be seen that additions of biowaste exceeding 20-25 kilograms resulted in peaks in the ethanol potential, while breaks in the adding lead to a decrease in ethanol yield. This is an interesting observation since the addition of the acid and the dilution with water in combination with the grinding should result in a rather homogenous mixture of the waste inside the vessel. The peaks did not show immediately after the additions but only some days later, which also precludes the assumption of the food waste not having settled down yet which could influence the sample.

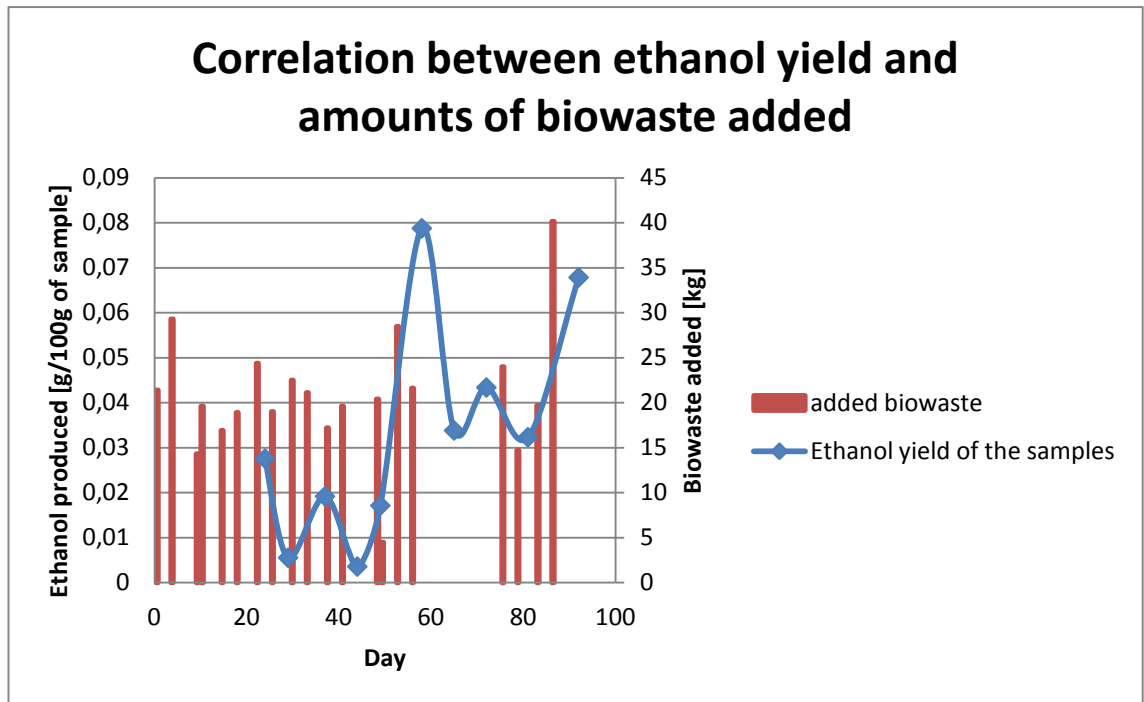


FIGURE 20. Correlation between the ethanol yield of the samples and the amounts of biowaste added

## 6 DISCUSSION

The study showed that it is possible in general to store the preserved biowaste over 3 months without losing the properties needed for bioethanol production. Further on it should be studied whether even longer periods of storage for this purpose would be possible. The qualities of the pre-served biowaste concerning pH, conductivity and chloride content were as desired, meaning stable values for the pH and the conductivity without fluctuations, and a chloride concentration of below 1 per cent over the whole testing period, which makes it easier to focus on other possible reasons for the fluctuations in the bioethanol yield. In this study it could not yet be fully investigated how efficient exactly the hydrolysis and fermentation procedures were, as well as the factors influencing the processes, and thus how valuable the preserved biowaste is as raw material.

The dry matter content seems to have an influence on the bioethanol yield, as was also confirmed by the study of Byung-Hwan and Hanley (2008, 1264), but unfortunately only solid contents of up to 10 per cent could be achieved with the procedure applied in this study. It would have been interesting to know how the ethanol yield changes for higher solid contents. The addition of water during the feeding process of the biowaste had maintenance-related reasons, and it needs to be determined if it is possible even to reduce the volumes of water added without causing problems to the grinder. However, the dry matter content itself was not the problem in this study but inaccuracies in the measurement procedure. For the future, a method should be applied using bigger sample volumes in order to obtain more reliable results.

There were fluctuations in the carbon dioxide content inside the vessel, which might stand in some correlation to the bioethanol yield. This correlation needs to be further investigated and also possible reasons for the changes in the carbon dioxide content examined. There is a possibility that the addition of the biowaste has an influence on the carbon dioxide content, and maybe even the bioethanol yield directly, when the feeding rhythm was irregular or the masses of biowaste added were varying. The impacts of the adding behaviour should be analyzed further on.

To make the results more comparable to already existing data, it would be necessary to analyze also the glucose content of the preserved biowaste. Nevertheless the conversion rate of the raw material to ethanol is of main interest for ethanol producers.

Comparing the obtained results on the ethanol yield with already existing studies, it becomes clear that the ethanol yield obtained with our procedure is relatively small. Kim et al. (2008) analyzed in their study the optimization of enzymatic saccharification and ethanol fermentation of food waste with the help of a statistical model and experimental verification. Food waste of a university cafeteria was used in the study of Kim et al. (2008), and its composition can be assumed to be similar as the composition of the raw material used in the Jäte-Aate project. In the study of Kim et al. (2008), the food waste was diluted with water in a ratio 1:1, resulting in a dry matter content of 12,9 per cent. The resulting optimum conditions for the hydrolysis were according to Kim et al. (2008) a pH of 5,20 and an enzyme reaction temperature of 46,3°C. For the fermentation the optimum conditions were found to be a pH of 6,85 and a temperature of 35,3°C. The enzyme which was used was glucoamylase, with an optimum concentration of 0,16 per cent. Ethanol fermentation was conducted in anaerobic conditions, handling the samples in a vacuum anaerobic chamber. (Kim et al. 2008, 1308.) Comparing these conditions to the procedure applied in the study conducted at TAMK, it can be seen that even though there are some similarities, the methods nevertheless differ. The enzyme concentration used in this study was with 0,146 per cent very similar to the optimum concentration found by Kim et al. (2008). The reaction temperature for the glucoamylase should have been similar, since the glucoamylase was added after taking the samples out of the waterbath in the Jäte-Aate study, hence when cooling down from 65°C to room temperature. Fermentation took place at room temperature, which was around 21°C and therefore below the optimum 35,3°C found by Kim et al. (2008). The maximum ethanol yield obtained with the optimized method by Kim et al. (2008) was 57,6 grams of ethanol per litre of raw material. Comparing this to the maximum yield obtained in the Jäte-Aate study of around 0,79 grams of ethanol per litre of diluted waste (assuming a density of around 1 kilogram per litre), the deficit in the used method becomes clear.

It can be said that the rather low ethanol yield results were achieved due to a lack of insufficient knowledge in this field. The ethanol production method by hydrolysis and

fermentation is a biological process, which is influenced by a huge variety of factors. Their influences have to be studied further on. The Jäte-Aate vessel in the TAMK laboratories provides a suitable frame to study the behaviour of the bioethanol yield and different influencing factors on the process, so that the system could be improved further on. In general the Jäte-Aate vessel seems suitable for the production of raw material, which can be converted into bioethanol. Therefore the Jäte-Aate vessel should be further improved for on-site applications.

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## APPENDICES

## Appendix 1. MATERIAL SAFETY DATA SHEET Formic Acid



## MATERIAL SAFETY DATA SHEET

2522 Plantation Center Drive  
 Matthews, NC 28105  
 (704) 845 9440

www.ciscochem.com

1. PRODUCT NAME: Formic Acid

2. CHEMICAL NAME: Formic Acid

3. SYNONYMS:

4. CAS NUMBER: 64-18-6

5. COMPOSITION: Formic Acid 90-100%

6. PROPERTIES: ODOR & APPEARANCE: clear, colorless, fuming liquid with intense, pungent, pungent, penetrating odor  
 ODOR THRESHOLD: 13ppm – 340ppm – wide range, suggesting unreliable data VAPOUR PRESSURE: 33.5mmHg/4.5kPa (20 c)  
 EVAPORATION RATE (butyl Acetate=1): 2.1  
 VAPOR DENSITY (air=1): 1.6  
 BOILING RANGE: 100.8 c (100%), 105 c (90%)  
 FREEZING POINT: 8.4 c (100%), approx -6 c (90%)  
 SPECIFIC GRAVITY: 1.22 (100%), 1.20 (90%) (20/20 c)  
 WATER SOLUBILITY: complete  
 IN OTHER SOLVENTS: soluble in acetone, methanol, ethanol, diethyl ether, glycerol, ethyl acetate  
 VISCOSITY: 1.8 centipoise (20 c)  
 PH: 2.4 (0.1M sol'n)

7. HAZARDS: MATERIAL USE: silage additive, leather tanning

8. FIRE FIGHTING INFORMATION: FLASH POINT: 46 c (100%), 58 c (90%) - (closed cup)  
 AUTOIGNITION TEMPERATURE: 480 c (100%), 434 c (90%)  
 FLAMMABLE LIMITS: 12% - 30% (100%), 18% - 57% (90%)  
 COMBUSTION PRODUCTS: carbon monoxide and water below 150 c; above 150 c, hydrogen gas also forms  
 FIREFIGHTING PRECAUTIONS: foam, dry chemical, water fog, water spray only to cool & dilute, product floats on water – water jet spreads flames; firefighters must wear SCBA  
 STATIC DISCHARGE: will not accumulate a static charge  
 MECHANICAL IMPACT: not sensitive  
 CHEMICAL STABILITY: stable; will not polymerize  
 REACTIVE WITH: strong oxidizing agents; alkalies, reacts vigorously with powdered metals producing hydrogen gas, reacts vigorously with aluminium, corrodes most metals; nickel or palladium/carbon catalyses rapid decomposition to hydrogen gas  
 DECOMPOSITION PRODUCTS: slowly decomposes to carbon monoxide – decomposes more rapidly when warm

9. PERSONAL PROTECTION MEASURES: HANDS: butyl rubber or neoprene rubber gloves – other materials also suitable; check with supplier  
 EYES: safety glasses with side shields or chemical goggles  
 RESPIRATOR: not required if ventilation is adequate, or use organic vapor cartridge  
 CLOTHING: impermeable (hands, above) boots, apron, and long sleeves if splashing is anticipated

10. FIRST AID PROCEDURES: SKIN: Wash with soap and plenty of water. Remove contaminated clothing and do not reuse until thoroughly cleaned or laundered.  
 EYES: Wash eyes with plenty of water, holding eyelids open. Seek medical assistance promptly if there is irritation.

IN CASE OF  
 TRANSPORT EMERGENCY  
 CONTACT CHEMTREC  
 USA: 1-800-424-9300  
 INTERNATIONAL: 1-703-527-3887

INHALATION: Remove from contaminated area promptly. CAUTION: Rescuer must not endanger himself! If breathing stops, administer artificial respiration and seek medical aid promptly.

INGESTION: Give plenty of water to dilute product. Do not induce vomiting (NOTE below). Keep victim quiet. If vomiting occurs, lower victim's head below hips to prevent inhalation of vomited material. Seek medical help promptly.

NOTE: Inadvertent inhalation of vomited material may seriously damage the lungs. The risk and danger of this is greater than the risk of poisoning through absorption of this relatively low-toxicity product. The stomach should only be emptied under medical supervision, after the installation of an airway to protect the lungs.

11. EXPOSURE TWAEV ppm: 5  
LIMITS: LD50 ORAL: 700  
SKIN (mg/kg): not known  
LC50ppm INHALATION: 825
12. TOXICOLOGICAL EFFECTS ACUTE EXPOSURE  
INFORMATION: SKIN CONTACT: dangerous corrosive to skin  
SKIN ABSORPTION: probably not; - skin damage too severe to allow absorption  
EYE CONTACT: severely irritating, corrosive, likely to damage eyes  
INHALATION: severely irritating; may cause choking, coughing, difficult breathing  
INGESTION: severely irritating & corrosive to digestive tract; burning sensation in mouth, vomiting of dark blood may occur; may cause asphyxia from oedema of larynx  
EFFECTS OF CHRONIC EXPOSURE  
GENERAL: may cause blood or albumin in urine  
SENSITISING: not a sensitizer  
REPRODUCTIVE EFFECT: probably not a mutagen, no known effect on humans  
SYNERGISTIC WITH: not known  
ESTIMATED LD50: 700mg/kg (oral, mouse), 1100mg/kg (oral, rat), 4000mg/kg (oral, dog)  
ESTIMATED LC50: 825ppm (inhalation, mouse), 2000ppm (rat), 3300PPM (MOUSE)
13. ECOLOGICAL INFORMATION: This product cannot accumulate in living tissue; this product is readily and rapidly biodegradable in the presence of oxygen.
14. DISPOSAL CONSIDERATIONS: DO NOT FLUSH TO SEWER; may be incinerated in approved facility.
15. CARCINOGENIC PROPERTIES & NOTIFICATIONS: Not a tumorigen, not a carcinogen.
16. TRANSPORT INFORMATION: USA 49 CFR  
Product identification number: UN - 1779  
Shipping name: formic acid  
Classification: Class 8 (9.2); Packing Group II  
WHMIS Class: B3, E
17. HANDLING & STORAGE: Store and use a cool dry environment, away from sources of ignition, heat and oxidizing agents. Pure (100%) formic acid tends to decompose into carbon monoxide. The pressure of gas accumulating within a drum can rupture the drum. Formic acid diluted to 95% or 90% is more stable. When diluting, always add the acid to the water. Use with adequate mechanical exhaust ventilation. All people working in the vicinity of this product should carry escape respirators with acid gas filter cartridge. Do not cut, drill, weld

or grind on or near this container. Avoid contact with skin and wash work clothes frequently. An eye bath and safety shower should be available near the workplace.

18. ACCIDENTAL LEAK PRECAUTION: dyke to control spillage and prevent environmental contamination.  
RELEASE HANDLING SPILL: ventilate contaminated area; recover free liquid with explosion-proof pumps; absorb residue on an inert sorbent, pick up using non-sparking plastic shovel, & store in closed containers for disposal.

19. REGULATORY  
INFORMATION:

## Appendix 2. Biowaste materials added to the Jäte-Aate vessel over the testing period

<b>Carbohydrate-rich materials</b>	<b>Vegetables and fruits</b>	<b>Protein-rich materials</b>	<b>Other</b>
Potatoe, rice, pasta, bread, beans, corn, pizza crust	Salad, cabbage, carrots, tomatoes, cucumber, beetroot, onion, apple, orange peel, pineapple, paprika, egg plant, water melon	Ham, minced meat, egg, feta cheese, cottage cheese, sausage	Coffee ground, paper

Appendix 3. Measurements taken during the testing period (pH, conductivity, CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>)

Day	Date	pH	conductivity [mS/cm]	CH <sub>4</sub> [%]	CO <sub>2</sub> [%]	O <sub>2</sub> [%]
17	17.11.2011	3,6	12,71	0,1	44,7	1,7
24	24.11.2011	3,52	13,05	0,1	53,5	0
29	29.11.2011	3,52	13,11	0	57,8	0
31	1.12.2011	3,52	13,05	0	60,2	0
35	5.12.2011	3,52	12,64	0,2	69,6	0
37	7.12.2011	3,45	12,98	-	-	-
38	8.12.2011	-	-	0,1	73,5	0
39	9.12.2011	3,55	12,85	0,1	73,3	0
42	12.12.2011	3,52	12,67	0	43,3	4,2
44	14.12.2011	3,5	12,81	0	40,5	2,5
46	16.12.2011	3,46	12,7	0	38,4	2,1
49	19.12.2011	3,41	12,75	0	56,7	0,9
58	28.12.2011	3,29	-	0	43,4	0,5
65	4.1.2012	3,29	-	0	40,9	0
70	9.1.2012	3,38	12,14	0	36,5	0,3
71	10.1.2012	-	-	0	36,1	0,2
72	11.1.2012	3,22	11,89	0	37,6	0,1
74	13.1.2012	3,37	11,28	0	39,5	0,1
77	16.1.2012	3,39	11,83	0	40,3	0
79	18.1.2012	3,38	12,33	0	50,2	0,1
81	20.1.2012	3,38	11,04	0	55,9	0,4
84	23.1.2012	3,38	11,41	0	64,4	0
86	25.1.2012	-	11,66	0,2	67,3	0
88	27.1.2012	3,29	11	0,2	66,9	0,1
92	31.1.2012	3,69	11,69	-	-	-

Appendix 4. Chloride content analysis results of the sample from 24.11.2011

24.11.11

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 10:24  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 10.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 6.6169 mL  
 Q1 = 0.59060 mmol  
 EPOT1 = 91.1 mV  
 Excess VEX = 0.5831 mL  
 QEX = 0.05204 mmol

CALCULATION

Result R1 = 6.6169 mL Content

CALCULATION

Result R2 = 3.451 g/L Content

CALCULATION

Result R3 = 0.345 %

*NaCl*  
 $3.451 \times 0.606 = 2.0913 \text{ g/kg}$   
 $= 2091 \text{ mg}$

ALL RESULTS

No.	ID	Sample size and results			
1	300	10.0	mL		
		R1 = 6.6169	mL		Content
		R2 = 3.451	g/L		Content
		R3 = 0.345	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-51.3			0:03
	0.2000	0.2000	-50.4	0.9	4.5	0:06
	0.4000	0.2000	-49.2	1.2	5.8	0:09



24.11./2

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 10:37  
 User admin

## RAW RESULTS

## SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 10.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

## EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 6.4778 mL  
 Q1 = 0.57818 mmol  
 EPOT1 = 93.9 mV  
 Excess VEX = 0.5222 mL  
 QEX = 0.04661 mmol

## CALCULATION

Result R1 = 6.4778 mL Content

## CALCULATION

Result R2 = 3.379 g/L Content

## CALCULATION

Result R3 = 0.338 %

## ALL RESULTS

No.	ID	Sample size and results			
1	300	10.0	mL		
		R1 = 6.4778	mL	Content	
		R2 = 3.379	g/L	Content	
		R3 = 0.338	%		

## MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-48.3			0:03
	0.2000	0.2000	-48.3	0.0	0.0	0:06
	0.4000	0.2000	-46.7	1.6	8.1	0:09

24.11.13

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 10:48  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 10.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 6.5382 mL  
 Q1 = 0.58357 mmol  
 EPOT1 = 94.1 mV  
 Excess VEX = 0.4618 mL  
 QEX = 0.04122 mmol

CALCULATION

Result R1 = 6.5382 mL Content

CALCULATION

Result R2 = 3.410 g/L Content

CALCULATION

Result R3 = 0.341 %

ALL RESULTS

No.	ID	Sample size and results			
1	300	10.0	mL		
		R1 = 6.5382	mL	Content	
		R2 = 3.410	g/L	Content	
		R3 = 0.341	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-52.2			0:03
	0.2000	0.2000	-50.8	1.4	7.1	0:06
	0.4000	0.2000	-49.9	0.9	4.5	0:09



## Appendix 5. Chloride content analysis results of the sample from 28.12.2012

28.12./A

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 13:29  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.5154 mL  
 Q1 = 0.49228 mmol  
 EPOT1 = 100.7 mV  
 VEX = 0.4846 mL  
 QEX = 0.04325 mmol

Excess

CALCULATION

Result R1 = 5.5154 mL Content

CALCULATION

Result R2 = 0.575 g/L Content

CALCULATION

Result R3 = 0.058 %

ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.5154	mL		Content
		R2 = 0.575	g/L		Content
		R3 = 0.058	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-50.1			0:03
	0.2000	0.2000	-49.1	1.0	4.8	0:06
	0.4000	0.2000	-48.0	1.1	5.5	0:09

Page

28.10./2

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 13:36  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.5178 mL  
 Q1 = 0.49250 mmol  
 EPOT1 = 99.8 mV  
 Excess VEX = 0.4822 mL  
 QEX = 0.04304 mmol

CALCULATION

Result R1 = 5.5178 mL Content

CALCULATION

Result R2 = 0.576 g/L Content

CALCULATION

Result R3 = 0.058 %

ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.5178	mL	Content	
		R2 = 0.576	g/L	Content	
		R3 = 0.058	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-50.0			0:03
	0.2000	0.2000	-49.2	0.8	3.9	0:06
	0.4000	0.2000	-48.0	1.2	5.8	0:09

28.12./3

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 13:42  
 User admin

## RAW RESULTS

## SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

## EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.6053 mL  
 Q1 = 0.50031 mmol  
 EPOT1 = 57.4 mV  
 Excess VEX = 0.3947 mL  
 QEX = 0.03523 mmol

## CALCULATION

Result R1 = 5.6053 mL Content

## CALCULATION

Result R2 = 0.585 g/L Content

## CALCULATION

Result R3 = 0.058 %

## ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.6053	mL	Content	
		R2 = 0.585	g/L	Content	
		R3 = 0.058	%		

## MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-53.2			0:03
	0.2000	0.2000	-52.7	0.5	2.6	0:06
	0.4000	0.2000	-51.8	0.9	4.5	0:09



## Appendix 6. Chloride content analysis results of the sample from 18.1.2012

18.1./1

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 13:03  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.9595 mL  
 Q1 = 0.53192 mmol  
 EPOT1 = 101.8 mV  
 VEX = 0.4405 mL  
 QEX = 0.03932 mmol

Excess

CALCULATION

Result R1 = 5.9595 mL Content

CALCULATION

Result R2 = 0.622 g/L Content

CALCULATION

Result R3 = 0.062 %

ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.9595	mL		Content
		R2 = 0.622	g/L		Content
		R3 = 0.062	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-56.7			0:03
	0.2000	0.2000	-55.3	1.4	6.8	0:06
	0.4000	0.2000	-54.0	1.4	6.8	0:09

Pag

18.1/2

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 13:10  
 User admin

## RAW RESULTS

## SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

## EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.9655 mL  
 Q1 = 0.53246 mmol  
 EPOT1 = 101.2 mV  
 Excess VEX = 0.4345 mL  
 QEX = 0.03878 mmol

## CALCULATION

Result R1 = 5.9655 mL Content

## CALCULATION

Result R2 = 0.622 g/L Content

## CALCULATION

Result R3 = 0.062 %

## ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.9655	mL	Content	
		R2 = 0.622	g/L	Content	
		R3 = 0.062	%		

## MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-54.9			0:03
	0.2000	0.2000	-53.9	1.0	5.2	0:06
	0.4000	0.2000	-52.9	1.0	5.2	0:09

~~28.12.13~~  
18.1.13

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 13:16  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.9763 mL  
 Q1 = 0.53342 mmol  
 EPOT1 = 101.2 mV  
 Excess VEX = 0.4237 mL  
 QEX = 0.03782 mmol

CALCULATION

Result R1 = 5.9763 mL Content

CALCULATION

Result R2 = 0.623 g/L Content

CALCULATION

Result R3 = 0.062 %

ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.9763	mL	Content	
		R2 = 0.623	g/L	Content	
		R3 = 0.062	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-53.2			0:03
	0.2000	0.2000	-52.3	0.9	4.5	0:06
	0.4000	0.2000	-51.3	1.0	4.8	0:09



## Appendix 7. Chloride content analysis results of the sample from 31.1.2012

31.1./1

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 11:21  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.6921 mL  
 Q1 = 0.50805 mmol  
 EPOT1 = 84.8 mV  
 Excess VEX = 0.5079 mL  
 QEX = 0.04534 mmol

CALCULATION

Result R1 = 5.6921 mL Content

CALCULATION

Result R2 = 0.594 g/L Content

CALCULATION

Result R3 = 0.059 %

ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.6921	mL		Content
		R2 = 0.594	g/L		Content
		R3 = 0.059	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-51.9			0:03
	0.2000	0.2000	-51.2	0.6	3.2	0:06
	0.4000	0.2000	-50.5	0.8	3.9	0:09

Page

31.1./2

METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 11:31  
 User admin

RAW RESULTS

SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.6683 mL  
 Q1 = 0.50593 mmol  
 EPOT1 = 86.6 mV  
 Excess VEX = 0.5317 mL  
 QEX = 0.04745 mmol

CALCULATION

Result R1 = 5.6683 mL Content

CALCULATION

Result R2 = 0.591 g/L Content

CALCULATION

Result R3 = 0.059 %

ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.6683	mL	Content	
		R2 = 0.591	g/L	Content	
		R3 = 0.059	%		

MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-52.0			0:03
	0.2000	0.2000	-51.2	0.8	4.2	0:06
	0.4000	0.2000	-50.2	1.0	4.8	0:09



31.1/3

## METTLER TOLEDO DL50 Titrator V2.4

Method 300 Equivalence point titr'n 09-Jan-2008 12:08  
 Measured 04-Apr-2012 11:37  
 User admin

## RAW RESULTS

## SAMPLE

No. 1  
 Identification 300  
 Titration stand Stand 1  
 Volume 50.0 mL  
 Correction factor f 1.0  
 Mol.mass M 58.44 g/mol  
 Equivalent number z 1  
 Temperature sensor Manual

## EQP TITRATION

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Consumption EQP 1 VEQ1 = 5.6589 mL  
 Q1 = 0.50509 mmol  
 EPOT1 = 87.7 mV  
 Excess VEX = 0.5411 mL  
 QEX = 0.04829 mmol

## CALCULATION

Result R1 = 5.6589 mL Content

## CALCULATION

Result R2 = 0.590 g/L Content

## CALCULATION

Result R3 = 0.059 %

## ALL RESULTS

No.	ID	Sample size and results			
1	300	50.0	mL		
		R1 = 5.6589	mL	Content	
		R2 = 0.590	g/L	Content	
		R3 = 0.059	%		

## MEASURED VALUES EQP TITRATION[1]

Titrant AgNO3 0.1 mol/L t = 0.89256  
 Drive No. 2 10 mL  
 Sensor DM141  
 Termination condition  
 n EQPs 1

	Volume mL	Increment mL	Signal mV	Change mV	1st deriv. mV/mL	Time min:s
ET1	0.0000		-50.9			0:03
	0.2000	0.2000	-49.9	1.0	4.8	0:06
	0.4000	0.2000	-49.0	0.8	4.2	0:09