Biogas production from municipal waste mixed with different portions of orange peel

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Abstract

Orange cultivation is a huge industry which increasing for each year. By the year 2010 the orange production of the world is expected to reach 66.4 million tons per year. Most of the oranges are used for orange juice production. Consequently, a large amount of organic wastes, including seeds, segment membranes and peel, counting up to half of the weight of used oranges, are generated. As alternatives to land filling and incineration, source separation and composting together with biogas production are being considered as suitable methods for treating this fraction of wastes, because it holds a high amount of organic materials in form of valuable carbohydrate polymers. However, the presence of peel oil, limonene, known to have antimicrobial effects, has showed to be a strong inhibitor for the biogas producing microorganisms. Therefore the orange peel waste (OP) was mixed with the organic fraction of municipal solid waste (MSW) in this study to keep the concentration of this inhibitory compound at low level.

Based on the results from previous batch experiments, this study was performed in order to confirm and develop the possible use of orange peel waste in biogas production. Since the batch experiments showed that the methane production was not affected, when a mixture of 70% MSW and 30% OP (calculated on the basis of volatile solids (VS) content added) was used as substrate, a continuous anaerobic digestion experiment was performed using the same mixture of MSW and OP as a substrate in this study. Furthermore, a reactor utilizing only MSW was used as a control. Both reactors were operated during 35 days at thermophilic conditions (55°C), with an organic loading rate of 3gVS/L/day and a hydraulic retention time of 21 days. The methane production was around 0.5 Nm³/kgVS/day in both reactors during the first period of operation. However, the production of methane started to decrease after 20 days followed by a sharp decrease during the last 5 days in the orange peel-containing reactor. Furthermore, a steadily increase (from 4.85g/L to 6.51g/L) in the total content of volatile fatty acids (VFA) could be observed here, while the total content of VFA in the control reactor remained at low levels (0.84g/L). A second experimental set up using a decreased amount of OP (20% OP and 80% MSW) in the substrate mixture and operating at the same conditions as the previous experiment was also performed and showed similar trends in the results. The failure of the process can be explained by inhibition in the system, which led to the accumulation of VFA’s resulting in a decreased and finally no methane production. This inhibition might be caused by the accumulation of the inhibitory compound, limonene, presented in the orange peel waste. Therefore some treatment of the OP is necessary prior to digestion to avoid this inhibition. Batch digestion of treated vs untreated OP showed that the methane production of treated OP could be increased to 0.628 Nm³/kgVS compared to that of 0.408 Nm³/kgVS from the untreated sample. Further investigations utilizing this treated OP fraction in continuous biogas process are necessary in the future.
Executive Summary

Biogas typically refers to the gas that has been produced during the breakdown of organic materials without presence of oxygen, which consist of mainly methane and carbon dioxide. This process is known as anaerobic digestion and is performed by microorganisms present in the anaerobic digester. This phenomenon occurs also naturally in anaerobic environments, like in ponds and marshes.

The history of biogas production from organic waste goes back to 3000 years ago [7]. There are a lot of evidences that have been found, which show that biogas has been used both in Assyria in 10th century BC and Persia in 16th century AD. Nevertheless, it was not until 1808 that the organic waste was recognized as a source of energy by Davy, who documented that methane, was produced during the breakdown of cattle manure. However, it was not until the end of 19th century that methanogenesis was associated to microbial activity [9].

Biogas is produced in three main steps; hydrolysis, acidification and methane production. The active microorganisms consist of a large mixture of differently acting species that live under symbiotic relationship. The first step of biogas production called hydrolysis is known as the polymer breakdown stage. The second step is the process of acidification where the acid-producing bacteria convert the monomers produced in the first step to different fermentation products, mainly acids. In the second stage of this process, called acetogenesis, the different fermentation products will be converted to acetic acid, which serve as one of the substrate for the methane production. In third and the last step methane-producing bacteria are utilizing either acetate, or carbon dioxide and hydrogen to form methane and carbon dioxide. There are many factors that play a significant role in this process, like pH value, temperature, organic loading rate, retention time, C/N ratio, the amount of available nutrients and toxicity.

Gathering chopping and grounding the biomass or waste in order to reduce the size starts the process of anaerobic digestion. Occasionally, some pre-treatment is needed to improve the biodegradation process and than the feed can directly be added or it can be added as slurry if a continuously stirred tank reactor is used. A secondary anaerobic digester can also be used after the separation of the solid part in order to be recycled which can be done directly or after post-treatment. The settled solid and the supernatant are furthermore processed and used as a fertilizer or animal feed. The final gas product is a mixture of 40-70% methane, 30-60% CO\textsubscript{2} and 1-5% of other gases depending of the composition of substrate used for biogas production. The biogas can be used directly or after upgrading and elimination of hydrogen sulphide traces for a variety of applications.

One of the most important benefits of biogas is energy production, including electricity, light and heat. It also can be used as an alternative fuel for vehicles. Elimination and conversion of organic waste into useful and valuable products is another major benefit which leads to another advantage like improving hygienic conditions as a result of reduced pathogens, and protection of air, water and vegetation in the environment. The technology used for the biogas production also contributes to economical and social development. Despite of all benefits, biogas and biogas technology has not yet been accepted in many countries. One of the reasons
for this is the high investment capital cost and the other reason is the unrealistic expectation of the users. The fact is that this technology cannot solve all problems.

Organic waste consists generally of household waste, agricultural waste, and human and animal waste. The produced amount of organic waste is increasing dramatically for each year. Today, source separation, composting together with biogas production is more and more being considered as a replacement to land filling and incineration strategies.

A large quantity of solid waste is also generated from fruit processing industries. The orange production is predicted to reach 66, 4 million tones by 2010 [12]. Around half of this amount after extraction of the juice consists of orange peels, segment membranes and seed. The peel holds a range of different carbohydrate polymers. Because the organic material content is high in orange waste, it makes it idyllic as a source of renewable energy through anaerobic digestion. However, investigations for the utilization of orange waste in thermophilic digestion can be considered as a new research area. The main problem is the presence of peel oil, limonene, which through its antimicrobial properties causes a sever inhibition in the digestion process.

In order to confirm and develop the usage of orange peel in biogas production batch digestion experiments were performed in our laboratory using different mixtures of organic fraction of municipal solid waste (MSW) and orange peel waste (OP) as substrates.

Based on the results of these previous batch experiments, continuous biogas experiments were carried out in this thesis work. Different portions of orange peel was mixed with municipal solid waste, and utilized in continues stirred tank reactors. A mixed substrate, there 70% of the added volatile solids (VS) was coming from MSW and 30% from OP, respectively, resulted in a methane production up to 0,6 m³CH₄/kgVS. The same result was obtained when a substrate containing 80% MSW and 20%OP (on VS basis) was used. However, the methane production started to decrease after 20 days of operation in continues stirred tank reactors in both cases leading to a failure of the process, while previous results showed that up to 50% (on VS basis) untreated OP mixed with MSW could be utilized in a batch method. The failure of the performance is caused by steadily increased total content of volatile fatty acids (VFA). The accumulation of VFA is an indicator for problem in the process and has an inhibition effect, which stops the production of methane. Nevertheless, batch experiments performed on pre-treated vs untreated OP showed promising results for the use of industrial orange peel waste as a suitable source for biogas production after pre-treatment of the OP prior to the digestion
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1. Introduction

Wherever there is human habitation, organic waste is produced, which consist of mainly household food waste, agricultural waste, human and animal waste. For each year the amount of produced organic waste is increasing dramatically, source-separation, composting and anaerobic digestion with related biogas production is increasingly being considered as a substitute for waste management strategies as land-filling and incineration of municipal solid waste (MSW) [5].

Breakdown of organic materials in the absence of oxygen produces methane. The process is known as anaerobic digestion and performed through the biological activity of microorganisms. This phenomenon naturally occurs at the bottom of ponds and marshes, which results in production of methane [4]. The production of natural biogas is the main part of biochemical carbon cycle [6]. Temperature and the composition of the feedstock are two important factors that should be taken into consideration due to the sensitivity of the process [4]. The involved bacteria are active within limited range of temperature, especially methanogens that are the methane-producing bacteria. These bacteria are the final link in a chain of a micro-organism that degrade organic materials and return the decomposed products into the environment. A source of renewable energy is produced when biogas is generated during this process [6].

One of the benefits of using waste in digestion processes is that the produced methane can be used as a fuel. The rest product, the digested slurry, contains also a high amount of nutrients and can be used as a fertiliser. Some of the most common applications for biogas include lighting, electricity, cooking, and utilization as an alternative vehicle fuel [4].

The objective of this study was to confirm and develop the usage of orange peel in biogas production during thermophilic conditions. Batch digestion experiments were performed previously in our laboratory using different mixtures of organic fraction of municipal solid waste (MSW) and orange peel waste (OP) as substrates [14]. Based on the results of these previous batch experiments, continuous biogas experiments were carried out in this work. Different portions of orange peel was mixed with municipal solid waste, and utilized in continues stirred tank reactors. Parallel with this project different pretreatment of the orange peel was also performed. In the end of this work batch experiments were carried out to compare the biogas production from the treated and orange peel.

2. Anaerobic digestion

Humans have used anaerobic digestion for centuries. Biogas was produced as early as 3,000 years ago from animal dung, human sewage and organic waste [7]. Evidences point out that biogas was used for water bath heating in Assyria in the 10th century BC. Persia, on the other hand, started to use biogas in the 16th century AD [9].

However, it was not until 1630, when Van Helmont recorded the arising of an inflammable gas from breakdown of organic matter. Shirley further described this gas in 1667, but Volta was the one who placed methane digestion on a scientific map. The chemical composition of methane was determined by Dalton in 1804. Organic waste was recognized as source of
energy for the first time, when Davy in 1808 recognized that methane was produced from decomposition of cattle manure [9].

Methanogenesis was found to be connected to microbial activity in the end of the 19th century. In 1884, Pasteur studied biogas production from animal residues (horse litter). The produced biogas was proposed to be used for street lighting [6]. In 1906 the first anaerobic waste water treatment plant was established in Germany but it was not until 1920 when the first sewage plant in Germany collected biogas and connected it into the public gas supply system [6].

Baker’s biochemical studies contributed considerably to the identification and knowledge of the methane bacteria, which is still relevant and utilized in many established biogas plants all over the world. However, the present technology of anaerobic digestion has up till now not attained its full potential for energy production [9].

2.1 Microbiology

Biogas production is based on three main steps and the active microbes consist of a large variety group of complex and differently acting species. Figure 1 illustrates the three steps of biogas production process that consist of hydrolysis, acidification, and methane formation [6].

![Figure 1. The anaerobic fermentation of biomass [15].](image)

2.1.1 Hydrolysis

In hydrolysis, which is the first step of biogas production, the extracellular enzymes, such as celluloses, amylases, proteases and lipases released by the bacteria, hydrolyse the organic material. The hydrolysis stage is also known as the polymer breakdown stage. The complex chain of carbohydrates, proteins and lipids are decomposed into shorter parts. Polysaccharides are at this stage transformed into monosaccharides and proteins are converted into peptides
and amino acids. Large amount of organic acids are also produced by acid forming bacteria in this initial period of fermentation [6].

2.1.2 Acidification
Acidification is the second step in the process where acid-producing bacteria transforms the monomers and fermentation products produced in stage I, into acetic acid (CH₃COOH), hydrogen (H₂) and carbon dioxide (CO₂). Since these bacteria are facultative anaerobic they are able to grow in acidic environment. For production of acetic acid, these bacteria need oxygen and carbon. The solved oxygen in the solution or the bounded oxygen is used for this propose. An anaerobic environment is hereby obtained by acid producing bacteria, which is vital for the methane-producing microorganisms. Furthermore, the acid producing bacteria also reduce the compounds with a low molecular weight into organic acids, alcohols, amino acids, carbon dioxide, traces of methane and hydrogen sulphide. The main acids produced in this stage are acetic acid, propionic acid, and butyric acid; furthermore ethanol is also produced [6].

2.1.3 Methane formation
The methane producing bacteria decompose further the compounds with a low molecular weight. For example, in order to form methane and carbon dioxide, the methane producing bacteria utilise hydrogen, carbon dioxide and acetic acid. The methane producing bacteria exist under natural conditions under water, in ruminant stomachs and in marshes, where anaerobic conditions are present. Theese microorganisms are very sensitive to environmental variations since they are obligatory anaerobic. The methanogenic bacteria are included in the archeabacteria genus in contrast to acidogenic and acetogenic bacteria. As shown in Figure 2, there are three types of methanogenic bacteria involved in the methane producing process;

- Methanosarcina genus (spherically shaped)
- Methanothrix bacteria (long and tubular)
- Bacteria that catabolize furfural and sulfates (short and curved rods) [6].
The equations below illustrate that various products, by-products and intermediates products that are formed in the digestion process of an anaerobic state can be converted to the final product, which is methane.

The acids produced in Stage II are processed by methanogenic bacteria to generate methane, which is described in the following equations [6]:

\[
\begin{align*}
\text{CH}_3\text{COOH} & \rightarrow \text{CH}_4 + \text{CO}_2 \\
\text{Acetic acid} & \quad \text{methane} & \quad \text{carbon dioxide} \\
2\text{CH}_3\text{CH}_2\text{OH} + \text{CO}_2 & \rightarrow \text{CH}_4 + 2\text{CH}_3\text{COOH} \\
\text{Ethanol} & \quad \text{Carbon dioxide} & \quad \text{Methane} & \quad \text{Acetic acid}
\end{align*}
\]

\[
\begin{align*}
\text{CO}_2 + 4\text{H}_2 & \rightarrow \text{CH}_4 + 2\text{H}_2\text{O} \\
\text{Carbon dioxide} & \quad \text{Hydrogen} & \quad \text{Methane} & \quad \text{Water}
\end{align*}
\]

### 2.2 Symbiosis of bacteria

Usually methane producing bacteria and acid producing bacteria live in a symbiotic relationship. While acid producing bacteria produce an ideal environment like anaerobic conditions and low molecular weight compounds for methane producing bacteria, the methane producing bacteria utilize the intermediates that are produced from acid producing bacteria during their activity. Without this symbiotic activity the concentration of the intermediates would increase and leading to a toxic condition for acid producing bacteria.
2.3 Facilitating and inhibiting factors

Apparently, there are many factors that play important role in this process; these are discussed below.

2.3.1 PH value
In order to obtain the best-optimized condition for biogas production, where the methane-producing bacteria exist, the pH value of input mixture in the digester should be between 6 and 7. After stabilization of the fermentation process under anaerobic condition, the pH value end up between 7.2 and 8.2 due to the buffer effect of increased ammonium concentration [6]. When large amounts of organic acids are produced in the beginning of the fermentation, the pH inside the digester might decrease below 5. Since the digester has a high concentration of volatile acids the methane fermentation process in the digester will be inhibited and even stopped. The medium with low pH (below 6, 5) will have a toxic effect on the methanogenic bacteria [6].

2.3.2 Temperature
A wide range of temperature is possible for anaerobic fermentation, usually between 3°C and 70°C. In general, three temperature ranges is common, the psychrophilic (below 20°C), the mesophilic (between 20°C and 40°C) and the thermophilic (above 40°C) ranges [6].

The rate of methane production increases with increased temperature. On the other hand, the increased temperature in turn will also increase the concentration of free ammonia. As a consequence, the process will be inhibited and the production will be reduced.

The methane formation process is extremely sensitive to temperature alternation. In general three changes in temperature ranges are accepted as still un-inhibitory effects concerning the process. The limits for fluctuation should not exceed the given ranges; there are ± 2°C/h for the psychrophiles, ± 1°C/h for the mesophiles, and ± 0, 5°C/h for the thermopiles.

2.3.3 Loading rate
The amount raw materials fed per unit volume of digester capacity per day is known as loading rate. It is important to optimize the loading rate due to avoid overfeeding which leads to inhibited methane production. However, underfeeding the plant would lead to low gas production and economically ineffective process as well.

2.3.4 Retention time
The retention time can be correctly distinct only in batch type facilities. By dividing the digester volume by the daily influent rate, the mean retention time can be determined in continuous systems. Based on the vessel geometry, the mixing rate and the actual substrate component, the effective retention time differs significantly. Therefore, the appropriate retention time is determined due to considering two factors;

- Process temperature
- Substrate type
2.3.5 Toxicity
Some of the toxic materials that might inhibit the normal growth of pathogens in the digester include mineral ions, heavy metals and detergents. However, low concentrations of the mineral ions, such as sodium, potassium, calcium, magnesium, ammonium and sulphur, are needed for stimulation of bacterial growth. At the same time, if the concentration of these ions were too high, it would lead to toxification. Addition of substances including soap, antibiotics, organic solvents, etc should be avoided, since this would lead to inhibition of the activity of methane producing bacteria [6].

2.3.6 Available nutrients
Apart from providing a source of carbon and energy through organic substances for the bacteria to be able to grow, they require other mineral nutrients as well. Except from carbon, oxygen and nitrogen for the production of biomass a sufficient amount of nitrogen, sulphur, phosphorous, potassium, calcium, magnesium and a little amount of trace elements such as manganese, molybdenum, cobalt, zinc, selenium and nickel etc are also needed. Generally agricultural residue and municipal sewage as substrate have enough amounts of these elements.

However each of these substances at high concentrations typically would have an inhibitory effect. So it is important to investigate the nutrient characteristics in order to decide the amount of the nutrients needed [6].

2.3.7 C/N Ratio
Both nitrogen and carbon is essential for microorganisms in order to assimilate these into their cell structure. Based on studies, the metabolic activity of methanogenic bacteria is possible to be optimized at a C/N ratio around 8-20. However, depending on the characteristics of the substrate, the optimum point can vary [6].

3. Biogas process technology
An anaerobic digestion process is in general based on methane fermentation and is started with gathering the biomass or waste for chopping and grounding in order to reduce the size. In some cases different kind of pre-treatment is also needed in order to improve the biodegradation. If a continuously stirred tank reactor is used, the feed can both be added directly or as slurry. The loading rate is depended on whether the process is mesophilic or thermophilic. An anaerobic secondary digester might be used after settling the solids in the effluent and being recycled. This can be done directly or after post-treatment. The solids can furthermore be processed as fertilizer or animal feed. It is also possible to be subjected to thermal conversion. The supernatant might be recycled as well or it can also be used as fertilizer and animal feed supplement. Furthermore, it can be processed into appropriate form for suitable disposal. The final gas-product that comes out can either be used directly or it can be treated to eliminate carbon dioxide and hydrogen sulphide traces in order to obtain pure methane for utilization as fuel. Figure 3 illustrates a generalized anaerobic digestion process that can be found in a wastewater treatment plant for the treatment of sludge. However, a variety of variations of this scheme are common [10]. Figure 4 describes a typical biogas system configuration. The source of substrate for producing biogas might come from organic industry waste, agro-waste and animal waste [6].
Figure 3. A typical anaerobic digestion process scheme [10]
Figure 4. A general biogas system configuration [6].

3.1 Process evaluation

Methane yield, methane production rate, organic matter reduction, stability of the culture, thermal efficiency and process economics are known factors that can be used in order to evaluate performance of anaerobic digestion of different feedstock’s or new designs of the digester reactors. The amount of methane produced in volumes of methane per active reactor volume per day is referred as methane production rate. Organic reduction efficiency directly affects the methane yield and is inversely associated to the quantity of process residues that needs further processing or disposal. A process material balance that is explained in terms of organic matter reduction determines this important performance parameter. Failure or reduction of digester performance can be depend on certain mixture of feedstock’s and operating conditions that result in disturbing the balance of microbial population which leads to reduced gas production, decreased pH and increased volatile acids concentrations [10].

3.2 Biogas technology limitations

Biogas systems all over the world are presently performing under different climatic conditions. The need and demand of poor rural populations and urban communities correspond to the biogas systems. However, the commercialization of biogas technology has not yet been accepted in many countries. The high investment capital cost is one of the reasons for this low acceptant. Another reason is also the unrealistic and high expectation of the users. Since biogas technology is not yet able to solve all kind of problems on a village, farm or animal production, the limitations should be clear to avoid disappointments [6].
There are many competitors to biogas technology, since energy can also be produced from fuel wood plantations with positive side effects. Solar systems, micro-hydro power and a variety of other different renewable technologies are considered to be competitors to biogas technology [6].

3.3 Composition and application of biogas

Biogas consists of a mixture of methane (40-70 vol %), carbon dioxide (30-60 vol %) and other gases (1-5 vol %) like hydrogen (0-1 vol %) and hydrogen sulphide (0-3 vol %). The pressure and temperature and the composition of raw materials as well determine the characteristic properties of biogas. The moisture content affects the properties as well. In this case, the most interesting factors are change in volume, change in water vapour content and change in calorific value as a function of temperature and pressure. Calorific value also depends on a function of water vapour content [6].

A wide range of application is common for biogas, which can be used like any other fuel gas for household energy and industrial use. Some common applications include gas cookers, refrigerators, engines, incubators, radiant heaters and biogas lamps [6].

4. Upgrading of biogas

In a number of countries biogas is used as transportation fuel. However the major breakthrough has reached only in Sweden and Switzerland. The biogas plants that are in the planning or construction phase in Sweden is capable of delivering upgraded biogas with a high quality. This upgraded biogas is used directly as vehicle fuel or for injection into the natural gas grid.

In order to use the biogas in normal vehicles that is designed to use natural gas, it has to be upgraded to natural gas quality. Water Scrubber Technology and Pressure Swing Adsorption (PSA) technology are the most widespread technologies for upgrading of biogas. Normally there are two main steps performed for gas upgrading. The first step in the process is to remove CO₂ from the gas. Other contaminants like sulphur compounds are usually removed before removing the CO₂. Water removal can be done before or after the removing the CO₂, this part is highly depending on the process [17].

4.1 Water vapour

The biogas produced from anaerobic digestion is usually saturated with water. Drying of the gas is most often necessary since some upgrading processes need relatively dry gas. Water vapour can be problematic. For example when the water vapour passes from high to lower pressure it might condense into ice and be corrosive. The other problem is that it clogs pressure regulator in the distribution system. However, different utilisation systems have different standards for water vapour tolerance. For example the water vapour is not a problem in boilers and in Combined Heat and Power (CHP) plants, while it is very problematic in fuel applications and grid injections [18].

There are a variety of methods that can be used to reduce water vapour in the biogas that include:
**Refrigeration:** In this method heat exchangers are used to cool the biogas to suitable dew point to condense water vapour. For further dryness, the biogas is pressurized and the condensate is removed and disposed of. The wastewater is then recycled back to the digester.

**Absorption:** Commonly there are two methods of water absorption processes that are used for gas upgrading from anaerobic digestion. These processes are called single pass absorption and regenerative absorption. The most important differences in these two processes are that the water in the single pass processes used just on time. In the case of regenerative absorption the water can be recycled and a stripper column must be integrated in the process [17]. Salts like glycol or hygroscopic salts is used to absorb water and the medium is dried at high temperature after the medium is regenerated.

**Adsorption:** to reduce the moister adsorption drying agents can also be used. Drying agents like silica gel or aluminium oxides can reduce moister levels that is low enough for use in for example in vehicle fuel. Here packed media in two vessels are use there one vessel is regenerated while the other one is used for drying which is done at high pressure that usually preferable. If not so air is need to be injected for regeneration [18].

4.2 Water scrubbing

The most common solvent is water and thereby the name water scrubbing. From bottom of the column the compressed biogas is fed into the column where it meets a counter flow of water. To create a large surface area between the two phases (gas and liquid), the column is filled with packing.

The solubility of carbon dioxide and hydrogen sulphide in water is higher than methane. The biogas that comes out from the top of the column is contain mostly methane and is saturated with water, which needs to be dried in order to reduce water. The water that is enriched with carbon dioxide is fed to a flash tank. In the flash tank the pressure is decreased and the most of carbon dioxide is released. To enhance the process occasionally air stripping or vacuum is used. In air stripping the oxygen is brought to the system, which causes problem if the gas is to be used as fuel or when it is going to be fed into the grid. When the gas that is released in the flash tank it goes into the inlet if a compressor.

The released hydrogen sulphide goes to the air and causes emission problems and some of the sulphur that is accumulated in the water creates some problems after sometime with fouling and plugging of pipe work. To prevent these types of problems the hydrogen sulphide is need to be separated before hand and to prevent the plugging installation of automatic washing equipment is also recommended for the column [19].

4.3 Carbon dioxide removal

Prior to use biogas as a vehicle fuel the carbon dioxide level must be reduced, even though it is possible to use the biogas in vehicles without removing the carbon dioxide if the engine is particularly adjusted to it. However, there is number of reasons for carbon dioxide removal. Removal of carbon dioxide would elevate the heating value, which would also elevate the driving distance for gas storage volume. Furthermore it would lead to a reliable gas quality between different plants and comparable quality to natural gas. In order to reach the necessary wobbe index of the gas it is common to remove the carbon dioxide prior to addition of the biogas to natural gas grid. During the removal process of carbon dioxide from the gas stream,
little amount of methane is removed as well, which should be kept minimal for economical as well as environmental reasons. The most common commercial methods for carbon dioxide reduction are absorption or adsorption processes. Membrane separation and cryogenic separation are other techniques that can be mentioned [19].

4.4 Membrane separation

Several processes are used with membrane separation. One process is a separation on both sides of the membrane with a gas phase, which is called dry membranes. In the other process a liquid (usually an amine) absorbs the carbon dioxide that diffuses through the membrane, which is called gas-liquid absorption. This method has a high selectivity in contrast to solid membrane systems. The separation is done around atmospheric pressure.

On the other hand, the dry membrane process can both work at high pressure > 20 bar and at low pressures 8-10 bar. The separation is done by different size of molecules with different permeability characteristics diffuses through the membrane because of the temperature and the pressure differences between the both sides of the membrane. In this process carbon dioxide and hydrogen sulphide are able to pass through the membrane, while methane is kept on the inlet side. However, some amounts of methane do pass to the other side of the membrane. In the upgraded gas, high levels of methane can be reached by using larger size membranes or using several membranes connected in series to each other. As a consequence, this last method would lead to increased methane loss to the permeate stream. However, the methane loss during this process can be used if the permeate stream is used in a joint heat and power plant simultaneously with raw gas or in a flox burner which would decrease the investment cost and at the same time reduce the energy consumption for the upgrading process.

Before the biogas goes through the membrane where the carbon dioxide and some amounts of hydrogen sulphide are separated, it is compressed and dried. Additional hydrogen sulphide separation is necessary before the biogas is utilized for vehicles or before it fed to the gas grid [19].

4.5 Pressure Swing Adsorption

To separate CO$_2$ from the biogas, materials like activated carbon or molecular sieves are used to adsorb carbon dioxide. Different mesh sizes are used to get selectivity of adsorption. This technology is thereby called Pressure Swing Adsorption-, (PSA), since the adsorption is done at high pressure and material regeneration is done through pressure reduction. Dry gas is obligated for this process. Typically the water vapour is condensed in a cooler. Furthermore, hydrogen sulphide has to be separated prior to feed the gas to the bottom the adsorption vessel. This can be done by an extra tank using activated carbon. Generally, the material replacement takes place when its surface is saturated with hydrogen sulphide. To increase the lifetime of the sulphide removal unit, air is added to the biogas stream, even though there is a risk of oxygen traces in the upgraded biogas.

In the following step carbon dioxide is adsorbed in the vessels that are pressurized. The gas is enriched with methane when it leaves at the top of the vessel. The biogas is than switched and directed to a new vessel, when the material in the first vessel becomes saturated. There are a number of vessels, usually four vessels, which are connected together to ensure a continuous
operation and to decrease the energy required for the gas compression. First, in order to connect the vessel with a previously regenerated vessel the regeneration pressure is reduced and than the pressure is decreased to approximately atmospheric pressure. In this step the released gas holds a considerable amount of methane and therefore it is recycled to the gas inlet.

The vessel is at last evacuated totally with a vacuum pump. The gas leaving this step is primarily consisting of only carbon dioxide. In one system the gas is released to the atmosphere, while in the other it is burned. However, when the gas is released to the atmosphere more upgraded biogas is produced, whereas in the second system requires less absorber surface and at the same time there is no methane emission to the atmosphere [19].

4.6 Removal of hydrogen sulphide

During the digestion of proteins, hydrogen sulphide and other sulphur containing materials are formed. Hydrogen sulphide is corrosive and thereby it should be separated rather early in the upgrading process. It can be separated in the digestion chamber, in the gas stream or it can be removed in the upgrading process. The most usual methods are the internal separation. As an example iron chloride or air/oxygen addition to the digester can be mentioned [19].

4.6.1 Biological desulphurisation

Microorganisms can also be used to decrease the hydrogen sulphide level in the biogas by converting it to elementary sulphur and some sulphate. The microorganisms that oxidize sulphur belong to the family of thiobacillus. These organisms do not have to be inoculated since they are usually present in the digestion material. These microorganisms are autotrophic and use carbon dioxide as carbon source from the biogas.

In biological desulphurization the oxygen that has to be added to the biogas should be in stoichiometric amount and the needed amount is depending on the hydrogen sulphide concentration.

The easiest method is to add oxygen or air directly to the digestion chamber, which reduces hydrogen sulphide level up to 95%. However there are factors that affect the reduction rate like place and amount of air that is need to be added, temperature and reaction time.

Separate bio filter filled with plastic bodies with attached desulphurizing micro-organisms can also be used in biological desulphurization. In this method the up flow gas meets the counter flow liquid. The liquid consists of gas condensate and liquid from separated effluent slurry or mineral solution. 5-10% air is added to the unit before the biogas is entered into it. The level of hydrogen oxide can be decreased from 3000-5000 ppm to 50-100 ppm. If ammonia is present in the gas it can be separated at the same time [19]

4.6.2 Iron chloride dosing to digestor slurry

In the digestion chamber iron chloride (FeCl₂) can be added which can reduce the level of hydrogen sulphide in the biogas. The reaction between iron (Fe²⁺) and sulphide ions (S²⁻) forms iron sulphide (FeS). The hydrogen sulphide level decreases to 100-150ppm at most which depends on the iron chloride amount added to the digestion chamber [19].
4.6.3 Impregnated activated carbon
Using activated carbon can do the catalytic conversion of hydrogen sulphide to elementary sulphur and water. To increase the rate of reaction the carbon is impregnated with potassium iodide (KI) or with sulphuric acid. When the sulphur containing carbon is saturated it can be replaced or regenerated. Impregnated activated carbon method is usually used before the upgrading system with PSA and it is a common method for separating hydrogen sulphide [19].

4.6.4 Iron hydroxide or oxide
Here the reaction of iron hydroxide or oxide with hydrogen sulphide forms iron sulphide (FeS). After saturation the material can be regenerated or replaced. The iron oxide or hydroxide and elemental sulphur are recovered, when the iron sulphide is oxidised with air during the regeneration process [19].

4.6.5 Sodium hydroxide scrubbing
In this method a water solution of sodium hydroxide (NaOH) is used to separate hydrogen sulphide. Sodium hydroxide and hydrogen sulphide react with each other and form sodium sulphide or sodium hydrogen sulphide, which both are insoluble salts [19].

5. The benefits of biogas
A major benefit of a well-functioned biogas system is that it eliminates and converts organic waste into useful and valuable products. One of the main benefits with biogas is the production of energy, including heat, light and electricity. Beside of energy production other valuable products, such as high quality fertilizer for replacement of expensive mineral fertilizer, are also obtained. Some advantages are connected to waste removal through the biogas system, like improving hygienic conditions due to reduced pathogens and worm eggs. Protection of soil, water, air and woody vegetation can be mentioned as environmental advantages. If the actual conditions are satisfactorily, the biogas technology can contribute to conservation and development [6].

The fact that a biogas digester can be build and operated locally creates opportunities to decrease the waste solid collection volume and land disposal costs. Furthermore, there are countries, like Sweden; there the land disposal of organic materials is forbidden since 2002 [16]. The technology has also a potential to create job opportunities locally for several thousands of people. Finally, the standard of living can be enhanced which directly contributes to social and economical development of a country [11].

6. Orange waste
A large amount of solid waste is generated from fruit processing industries [3]. For example the production of orange is estimated to reach 66.4 million tons by the year of 2010, which represent an increase of 14% within 12 years. The waste of orange after extraction of the juice consists of peels, segments, membranes and seed.

Today some fraction of this waste is dried out and pelletized to be used as low value cattle feed. However, dehydration process is not economical since the peels contain a high moister
content. Consequently a big portion of this unwanted waste is discarded into the environment, which causes both economical and environmental problems [12]. Additionally, the accumulation of this industrial orange waste cause also problems for fruit processing industries, such as odour and soil pollutions [3].

The peel holds a variety of carbohydrate polymers, which makes it an attractive choice for utilization in biological processes [12]. Therefore, the orange waste can represent a probable energy source if it can be accurately treated in order to transform it biologically into methane. This makes the waste a source of renewable energy, and the output of carbon dioxide to the atmosphere is zero [13].

In recent times, anaerobic digestion of ligno-cellulosic wastes as a treatment of this renewable energy has emerged. Additionally, anaerobic digestion seems to be one of the best sustainable technologies existing for treatment of fruit and vegetable wastes and has been acknowledged to be the most suitable option to incineration and composting.

The high organic matter content in the orange waste makes it idyllic for anaerobic digestion. However, previous investigations reported that orange waste might affect the thermophilic digestion process negatively, due to the presence of peel oil, limonene, which has antimicrobial effects [3].

6.1 The objective of this study

The aim of the current project was to investigate whether orange peel waste can be utilized for biogas production by mixing it with municipal waste and on this way keeping the limonene concentration low during the digestion process. Two different methods, -batch and continuous experiments were performed. The possible use of a suitable treatment of orange peel prior to the digestion was also evaluated in this study.
7. Experimental methods and material

7.1 Origin of substrate and inoculum

Source sorted organic fraction of municipal solid waste from a full-scale biogas plant (Sobacken, Borås; Sweden) was used as one of the substrates. In order to achieve a purified digester feed that was homogenized and enriched in biodegradable organics without large pieces of plastic and glass, the substrate was sorted in multiple steps at the biogas plant. Additionally, the substrate was further treated in the lab by shredding it into smaller pieces and homogenized it in a mixer in order to prevent clogging the reactors. The homogenization is also significant to ensure representative sampling. For storage of the finished feed, plastic cups were used. Each cup containing approximately 200g was stored at -20ºC [1].

Orange peel waste needed, was obtained from Brämhults Juice in Borås; Sweden. The orange peel that already was shredded at Brämhults Juice were further mixed and mashed in the lab in order to make it easier for digestion. The prepared orange peel were added into municipal waste in different portions and stored at -20ºC for usage as feed.

The inoculum, used in all of the experiment, was also obtained from the large-scale biogas plant, Sobacken in Borås. The plant operates under thermophilic condition and treats the organic fraction of household waste, industrial residues and sorted waste from recycling stations. The inoculum was transported in 10-liters vessels and during the delivery some temperature drops was considered. Therefore, the inoculum was stored at 55ºC incubator for three days before using in the experiments [2].

7.2 Determination of characteristics of the substrate and inoculum

7.2.1 Total solid (TS) and volatile solid (VS) measurements

An appropriate number of crucibles were placed in oven at 105ºC over night and then cooled down in an executor before balancing the empty crucibles and measuring the weight of the sample. The crucibles with the sample were also dried at 105ºC for minimum 8h but preferably over night. The dried samples were cooled down in an executor and then balanced before placing them in muffle furnaces at 550ºC +- 25ºC for an hour. After one hour the crucibles was removed from the furnace directly into an executor and cooled down approximately for one hour. After cooling down the weight of crucibles was measured and recorded. Table 1 illustrates the result from the TS and VS measurements from the inoculum and different substrates. Equation used for calculating TS and VS values is described below:

TS: \[\frac{(\text{Dried crucible} + \text{dried sample} - \text{dried crucible})}{\text{sample weight}} \times 100\]

LoI (Loss on ignition): \[\frac{(\text{Dried crucible} + \text{sample}) - (\text{Dried sample} + \text{dried crucible})}{(\text{Dried crucible} + \text{sample}) - (\text{Dried crucible})}\]

VS: LoI * TS
### Table 1- The characteristic of substrate and inoculum

<table>
<thead>
<tr>
<th>Substrate</th>
<th>TS %</th>
<th>VS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Municipal solid waste (MSW)</td>
<td>26.96</td>
<td>21.03</td>
</tr>
<tr>
<td>Orange peel</td>
<td>20.13</td>
<td>15.07</td>
</tr>
<tr>
<td>Inoculum</td>
<td>1.69</td>
<td>1.09</td>
</tr>
<tr>
<td>Feed: 100% MSW</td>
<td>12.58</td>
<td>7.77</td>
</tr>
<tr>
<td>Feed: 30% OP 70% MSW</td>
<td>12.07</td>
<td>7.47</td>
</tr>
</tbody>
</table>

#### 7.3 Batch experiment

The batch experiments were carried out according to the method described by Hansen et al [2]. As reactor, two liter glass bottles with a rubber septum were used. Each reactor contained 400 ml inoculum from a thermophilic biogas plant and 200 ml of a mixture of substrate and tap water corresponding to VS content of 0.75%. Gas mixture of 80% N\textsubscript{2} and 20% CO\textsubscript{2} were used by flushing the headspace for three minutes in order to obtain anaerobic conditions in the reactors and also to prevent pH-change in the solution. The experiments were carried out in triplicates while the flasks were incubated at 55ºC in an incubator as shown in figure 5. Throughout the experiment period, the bottles were shaken and moved around in the incubator once a day.

Only inoculum and water was present in the reactors that were used as controls in order to determine the methane production obtained from the inoculum itself. The aim was to determine methane production from fresh substrate and therefore the methane produced from the controls was subtracted from the methane produced in the reactors containing samples. Each experiment was completed after approximately 50 days when no considerable increase in the gas production could be observed and full degradation of the degradable organic matter could be ensured. After the experiment period the pH was measured in the reactors [1], [2].
7.3.1 Monitoring
During the experiment period, the methane content of the reactors was measured on a regular basis. Totally around 10 measurements were made throughout the incubation time. However, in the beginning of the experiment, the measurements were done in every third day and at the end of the incubation time it was adequate to measure only once a week.

A pressure-tight gas syringe (VICI, Precision Sampling Inc., USA) was used to take samples (0.25ml) from the headspace of the reactors. The samples were then directly injected into the gas chromatograph (Auto System, Perkin Elmer, USA) equipped with a packed column (Perkin Elmer, 6’ x 1.8” OD, 80/100, Mesh, USA) and a thermal conductivity detector (Perkin Elmer, USA) with inject temperature of 150°C. The carrier gas was nitrogen operated with a flow rate of 20 ml/min at 60°C. After releasing the overpressure in the reactor by a hospital needle, another sample were taken and measured at the same way by the GC. The release of overpressure gas prevents also the build up of too high pressure in the system and the leakage of the gas. By using the measurements done before and after the release, the amount of the gas produced during the period of between two measurements could be calculated [2].

7.3.2 Data treatment and presentation
The produced amount of methane gas could be determined based on the gas volume of each reactor and the methane content in the sample of 0.25ml, which were measured on the GC. The results are shown as the accumulated methane production in the function of the
incubation time. The final result represents just the methane production of the substrate since the methane production from the inoculum was subtracted.

Furthermore, the determined gas content was recalculated for standard conditions (STP: 22 ºC, 1 atmosphere) based on the ideal gas law and the produced methane is given as Nm³/kgVS [2].

Following equation was used for the calculations:

\[ X_{STP} = X_m \times \frac{(T_{std} \times P_m)}{(T_m \times P_{std})} \]

Where

\[ T_m = \text{actual temperature} \]
\[ P_m = \text{atmospheric pressure} \]
\[ X_m = \text{measured methane content} \]
\[ X_{STP} = \text{methane content at standard temperature and pressure conditions} \]

7.4 Semi-continuous experiment

Two 5-liters continuously stirred reactors were operated parallel in a water bath at 55ºC as shown in figure 6. Reactor 1 (R1) was considered as control-reactor and was only fed with 100% MSW. The second reactor (R2) was used for investigating the effect of mixing different proportions of orange waste with MSW. Throughout the experiment period R2 was fed with two different mixtures of OP and MSW. In the first experiment 70% of the feed VS were coming from MSW and the rest, 30% of VS from industrial orange peel waste. In the second experiment the orange peel part of the total VS was decreased to 20%. Both experiments were carried out during approximately 35 days in order to confirm the actual time for preferable biogas production conditions in the reactors.

The procedure started with introducing 5 l inoculum at day one and the feeding started at day 2. The inlet at the top of the reactors was used to take out approximately 240mL of the waste matter in the reactors. The reactors were fed from the same inlet with an equal volume of substrate, in order to achieve a retention time of ca 21 days. Furthermore, during a start-up period of 10 days the organic loading was increased gradually from 1.5gVS/L/day to a final loading rate of 3gVS/L/day.

The experiment ended when the pH of the effluent from R2 reached around 5, and the production of biogas was inhibited due to the accumulation of volatile fatty acids (VFA).
7.4.1 Monitoring
A gas meter was connected to each reactor, which measured the total biogas production during the whole day. Samples of gas were taken every day by a 250μl syringe for analyses in GC with TCD detection. The pH of the effluent, which was taken out before a new feeding, was also measured daily, after the temperature of the sample dropped to room temperature.

Samples from the effluent were also used for TS and VS measurements once a week. Furthermore, total alkalinity (TA) was determined two times a week by filtrate the effluent and diluting it with milli Q water in volume ratio of 1:10. The sample were then titrated with a 0,05M HCl until the pH reached 5,75 in the first step, which corresponds to the bicarbonate alkalinity. In the second step, the total alkalinity was determined by continuing the titration until the pH of 4,0 was reached.

At the same time samples from the effluent were frozen down for further VFA analysis. Before VFA measurement the samples were centrifuged and filtrated in order to analyze them by high performance liquid chromatography (HPLC) once a month. HPLC (Waters 2695, Millipore, Milford, USA) equipped with a refractive index (RI) detector (Waters 2414) was used for the analyses. The separation was performed on an ion-exchange column (Aminex
HPX-87H column, Bio-Rad, USA), at 60°C using 5 mM H$_2$SO$_4$ as eluent with a flow rate of 0.6 ml/min.

7.4.2 Data treatment and presentation
Based on the final counter nr from the gas meters, the total biogas production per day could be determined. The methane content in the biogas on the other hand was calculated based on the GC measurements. The result is shown as produced volume of CH$_4$/gVS/d and produced volume of biogas/gVS/d as a function of time.

The alkalinity results were calculated in mg/l and analyzed based on the quota of VFA/TA. The results from the HPLC analysis were shown as the concentrations (g/l) of the different fatty acids presented in the reactors.

8. Results
The results from the anaerobic digestion of source separated organic household waste mixed with untreated or treated industrial orange peel waste in both semi-continuous and batch experiments are presented below.

8.1 Semi-continuous experiment
To study the biogas potential of industrial orange peel waste using thermophilic digestion, different mixtures of industrial orange waste and municipal solid waste was used as a substrate in two parallelly operating continuously stirred tank reactors. Methane production started after a short initial lag period from day one when the feeding started. Both reactors were fed daily.

8.1.1 Feeding with 70% MSW and 30% OP
The feeding started with an organic loading rate (OLR) of 1.5gVS/L/d on day 2 and increased gradually to 3gVS/L/d until day 10 of the operation, keeping it at this level until the end of the experiment. In order to obtain the same inoculum characteristics in both reactors, the content of R1 and R2 were mixed and divided within the reactors again on day 9. Thus, the R1 was considered as control reactor and was there-after fed only with 100% MSW. As shown in Figure 7, the biogas production increased when the OLR increased. The process was stable for approximately 20 days in both reactors; however the production of biogas started to decrease after 20 days in R2.

The production of methane during the same period is illustrated in Figure 8. During the stable period of the process, the specific production of methane was kept between 0, 5 Nm$^3$/kgVS/d and 0, 6 Nm$^3$/kgVS/d for R2 while R1 was less fluctuating around 0, 6 Nm$^3$/kgVS/d. After 20 days of feeding at constant rate, decline in methane production and pH was obtained. The pH started at around 8 for both reactors, and held constant for R1 while the pH for R2 started to decrease significantly at day 20 and dropped to 5.5 until day 33 as shown in Figure 9. At this point the process was considered to be failed.
Figure 7. Biogas production from semi-continuous digestion of industrial orange waste mixed with MSW.

Figure 8. Methane production from a mixture of MSW (70% of total VS) and OP (30% of total VS) in the feed.
Figure 9. pH measurements of continuously stirred tank reactors during the feeding period of 70% MSW and 30% OP of total VS.

As shown in Table 2 the specific methane yield decreases in relation to the increased TS content in R2. The decrease of methane production is evident in R2 while it is almost stable in R1.

Table 2- Specific methane yield and TS content in the R1 and R2 operated at 55°C

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>TS content R1 % Controll reactor</th>
<th>Specific methane yield R1(Nm³/kgVS)</th>
<th>TS content R2 % Test reactor (OP)</th>
<th>Specific methane yield R2(Nm³/kgVS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>2,2</td>
<td>0,66</td>
<td>2,25</td>
<td>0,69</td>
</tr>
<tr>
<td>17</td>
<td>2,31</td>
<td>0,58</td>
<td>2,26</td>
<td>0,63</td>
</tr>
<tr>
<td>24</td>
<td>2,37</td>
<td>0,59</td>
<td>2,39</td>
<td>0,54</td>
</tr>
<tr>
<td>31</td>
<td>2,22</td>
<td>0,53</td>
<td>2,71</td>
<td>0,41</td>
</tr>
</tbody>
</table>

Table 3 compares the concentration of the total volatile fatty acids in both reactors. The developments in the concentration of different kind of VFAs during the experiment period are also shown in the table. Total VFA in R1 decreased from 3, 83 g/L to 0, 84 g/L during 31 days of operation, while the concentration in R2 increased from 4, 85 g/L to 6, 51 g/L during the same period. Especially the concentration of propionic acid increased from 0, 96 g/L to 2, 03 g/L during this time, parallely there was a considerable increase in the concentration of other longer chain fatty acids as capriorate.
Table 3- The concentration (g/l) of Volatile Fatty Acids (VFA) in R1 and R2

<table>
<thead>
<tr>
<th>Time</th>
<th>2d</th>
<th>21d</th>
<th>33d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R1</td>
<td>R2</td>
<td>R1</td>
</tr>
<tr>
<td>Total VFA (g/l)</td>
<td>3,83</td>
<td>4,85</td>
<td>1,59</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>1,24</td>
<td>2,42</td>
<td>0,6</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>1,68</td>
<td>0,96</td>
<td>0,55</td>
</tr>
<tr>
<td>Iso-butyrate</td>
<td>0,21</td>
<td>0,16</td>
<td>0,01</td>
</tr>
<tr>
<td>Butyrate</td>
<td>0,07</td>
<td>0,09</td>
<td></td>
</tr>
<tr>
<td>Iso-valerate</td>
<td>0,52</td>
<td>0,32</td>
<td>0,15</td>
</tr>
<tr>
<td>Valerate</td>
<td>0,12</td>
<td>0,21</td>
<td>0,1</td>
</tr>
<tr>
<td>Capriorate</td>
<td>0,78</td>
<td>0,19</td>
<td>0,44</td>
</tr>
</tbody>
</table>

8.1.2 Feeding with 80% MSW and 20% OP

The start up of feeding R2 with 80% MSW and 20% OP of the total VS, began with adding fresh inoculum at day 1 and then mix the content of R1 (control reactor) with the new inoculum in R2 in order to obtain similar type of inoculum in both reactors before the experiment started. The feeding started with an OLR of 1.5 gVS/L/d and gradually increased to 3gVS/L/d during the first week of operation in both reactors. Similar trend as in the previous experiment could be observed even here. According to both Figure 10 and Figure 11 the production of biogas and methane was stable for approximately 20 days in both reactors. However, the production of methane was found to drop in R2 while in R1 the production was kept constant. Moreover, accumulation of H₂ was observed at day 28 in R2, which confirmed the failure of the process, since H₂ is one of the degradation products from the degradations steps prior to the final step, the methanogenesis in an anaerobic digestion process (Figure 1). Obviously, the activity of methane producing bacteria was inhibited. Furthermore, in R2 the pH was also noticed to start to decrease after 15 days and a sharp decline was observed after 20 days of operation as it is shown in Figure 12.
Figure 10. Biogas production from semi-continuous digestion of industrial orange waste mixed with MSW.

Figure 11. Methane production from a mixture of MSW (80% of the total VS) and OP (20% of the total VS) in the feed.
Table 4 illustrates how the specific methane yield decreases in relation to the increased TS content in the reactors. The decrease of methane production is evident in R2 while the production of methane remains at a stable level in R1.

**Table 4- Specific methane yield and TS content in the R1 and R2 operated at 55°C**

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>TS content R1 %</th>
<th>Specific methane yield R1(Nm³/kgVS)</th>
<th>TS content R2 %</th>
<th>Specific methane yield R2(Nm³/kgVS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>2,34</td>
<td>0,57</td>
<td>2,27</td>
<td>0,67</td>
</tr>
<tr>
<td>24</td>
<td>2,48</td>
<td>0,53</td>
<td>2,46</td>
<td>0,40</td>
</tr>
<tr>
<td>29</td>
<td>2,55</td>
<td>0,49</td>
<td>2,82</td>
<td>0,05</td>
</tr>
</tbody>
</table>

According to Table 5 the concentration of VFA in R1 did not show any significant change, the concentration kept constant around 4 g/L while the concentration in R2 increased noticeably from 5,18 g/L to 10,5 g/L during an operating period of 24 days. Also the developments of the concentration of different kind of VFA’s are shown during the experiment period in the table. The increased amount of long chained fatty acids in R2 also indicates some imbalance in the system leading to the inhibition in the production of methane.

**Figure 12.** pH measurements of continuously stirred tank reactors during the feeding period of 80% from MSW and 20% from OP of the total VS.
Table 5- The concentration (g/l) of Volatile Fatty Acids (VFA) in R1 and R2

<table>
<thead>
<tr>
<th>Time</th>
<th>Constituents</th>
<th>R1</th>
<th>R2</th>
<th>R1</th>
<th>R2</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total VFA (g/l)</td>
<td>4.3</td>
<td>5.18</td>
<td>4.57</td>
<td>5.74</td>
<td>4.78</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>Acetic acid</td>
<td>1.92</td>
<td>2.82</td>
<td>1.79</td>
<td>2.46</td>
<td>2.03</td>
<td>6.09</td>
</tr>
<tr>
<td></td>
<td>Propionic acid</td>
<td>1.51</td>
<td>1.55</td>
<td>1.7</td>
<td>2.5</td>
<td>1.59</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>Iso-butyrate</td>
<td>0.19</td>
<td>0.2</td>
<td>0.34</td>
<td>0.4</td>
<td>0.35</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Butyrate</td>
<td>0.05</td>
<td>0.02</td>
<td>0.02</td>
<td>0.07</td>
<td>0.04</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>Iso-valerate</td>
<td>0.55</td>
<td>0.51</td>
<td>0.68</td>
<td>0.67</td>
<td>0.75</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>Valerate</td>
<td>0.08</td>
<td>0.07</td>
<td>0.03</td>
<td>0.06</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Capriorate</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The bicarbonate alkalinity (BA) and total alkalinity (TA) test indicates at early stage (within 10-15 days) an expected decreased production of biogas and thereby the failure of the process. As seen in Figure 13 both the BA and TA drop slightly already after 10 days of operation, which signifies that the reactor will fail in a near future. This observation gives an opportunity to recover the process by adding buffers in order to increase the pH and prevent the failure of the experiment. However, the recovering part was not performed in this study, since this treatment is expected to result in a stable condition only for a short period of time if the reason for the decreased alkalinity is not diminished from the process.

Figure 13. Bicarbonate alkalinity (BA) and total alkalinity (TA) in R1 and R2.
8.2 Batch experiment

Parallel to the continuous experiments different pre-treatment of orange peel samples were carried out in our laboratory within another project (data are not shown). As a final investigation batch experiments on treated vs untreated OP samples were performed in this study to verify the effect of the treatments, and also the addition of nutrients into the system.

Figure 14 shows the average of triplicates for the accumulated methane production measured with untreated orange peel as well as after the different kind of treatments. The average values represent the accumulated methane production from the different samples in the function of time. The final result presents here the accumulated methane production after correction based on the methane produced by the inoculum. However, it was also observed a significant methane production from the inoculum itself during this experiment. Therefore, as it is shown in Fig 14, negative values were also obtained as a consequence of removal of the methane produced from the inoculums itself. The experiment stopped after 50 days, when no increasing methane production could be observed. The methane production was on average 0,408 Nm³ CH₄/kgVS for the untreated orange peel samples and 0,628 Nm³ CH₄/kgVS for the orange peel treated with method one (treated op 1), while the second treatment (treated op 2) was less effective and resulted in only 0,489 Nm³ CH₄/kgVS (Table 6). Furthermore, not only the accumulated methane production but also the kinetics of the methane production process could be improved by using pre-treatments. In general, the present of nutrients resulted in less production of methane, indicating that some imbalance in the system appeared caused by this addition of extra nutrients.
**Figure 14.** Methane production curves for different pre-treatments of orange peel

**Table 6- Methane yields and initial methane production rates of the different samples.**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Methane Yield (Nm³/kgVS)*</th>
<th>Initial methane production rate (Nm³/kgVS/day)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% untreated OP</td>
<td>0.408</td>
<td>0.013</td>
</tr>
<tr>
<td>100% untreated OP + nutrients</td>
<td>0.371</td>
<td>-0.003</td>
</tr>
<tr>
<td>Treated OP 1</td>
<td>0.628</td>
<td>0.046</td>
</tr>
<tr>
<td>Treated OP 1 + nutrients</td>
<td>0.603</td>
<td>0.021</td>
</tr>
<tr>
<td>Treated OP 2</td>
<td>0.489</td>
<td>0.020</td>
</tr>
<tr>
<td>Treated OP 2 + nutrients</td>
<td>-0.146</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*After 50 days of incubation

**Average initial production per day after 7 days of incubation.
9. Discussion

Investigations on thermophilic digestion of industrial orange waste are a relatively new research area. Further efforts are needed for the development, evaluation and improvement of this technology.

Based on earlier batch experiments there the biogas potential of MSW mixed with different portions of orange peel was investigated [14]; in this study a continuous experimental set up was designed. Results from the batch experiments showed that the biogas production was not effected for samples there 70% of the total VS in the substrate was coming from MSW and 30% from OP, respectively. The methane production, around 0.5 Nm$^3$ CH$_4$/kgVS was comparable with the control sample where only MSW was used as a substrate [14]. However, further, increase in the portion of OP led to inhibition of CH$_4$ production [14].

Therefore in our continuous experimental set up we started with a mixture of 70% from MSW and 30% from OP on a total VS basis. After a short period the methane production started up due to degradation of the organic compounds. The reactors were operated during a period of approximately 35 days until the procedure failed due to a steadily increased total content of VFA (from 4.85 to 6.51 g/L) in R2. During the same period the total content of VFA in the control reactor (R1) that was fed only with 100% MSW, remained under 4 g/L. The accumulation of VFA is considered to be an indicator in the system, which shows the possible inhibition of the process, leading to a stop in the production of biogas.

On the other hand, the control reactor, there only MSW was used as substrate for the biogas production worked properly as it is also indicated from the concentration of accumulated VFAs in R1 which was gradually decreased from 3.83 to 0.84 g/L and remained at this low level.

Since the feeding of reactors on a basis of total VS of 70% from MSW and 30% from OP failed, a new setup was started up with a decreased OP amount in the feed. The total VS of the substrate contained now 80%, of MSW, and 20%, of OP, respectively. However, once again the same trend as in the previous experiment was observed.

The reactors were operated during 33 days before the procedure failed. The total content of VFAs increased from 5.18 to 10.5 g/L within 24 days in R2. During the same period the total content of VFAs in the control reactor R1 remained on a stable level i.e. under 5 g/L.

In the digestion of orange waste, the acid condition and low pH of the orange waste is considered to cause some problems, since the anaerobic digestion needs a pH of 7-8 for successful performance. If the pH in the reactors drops below 6, the methanogenesis will be inhibited and as a consequence the process will fail. Furthermore, factors such as limonene present in orange peel and other aromatic acids present in the peel oil might have negative affect on the digestion process at thermophilic conditions [3]. There is a possibility to maintain a stable pH within accurate range by addition of chemicals like NaHCO$_3$. However, it would help only for a while because there seemed to be an accumulated inhibition effect, coming from the fact that the inhibitory compound, limonene, obviously could not be degraded during the continuous operation, leading to an accumulated high concentration which caused the failure of the process. Previously, it was reported that feeding limonene, at higher concentrations then 67 μL/L /d into an anaerobic digestion process at mesophilic conditions caused failure of the process [20]. According to our analysis of the limonene content of orange peel waste, the calculated feed of limonene in our case was only 30 μL/L /d.
The possible reason for the failure could be explained by the fact that our process was performed under thermophilic conditions and it is known that the thermophilic process is much more sensitive than the mesophilic one [6].

After the failure of feeding the reactors with untreated substrate, another set up of batch experiments was performed in order to investigate the opportunity of using treated OP instead in a future continuous experiment. Three different treatments were carried out and treatment 1 showed the highest production of methane as illustrated in Table 6 and Fig 14. The methane production increased from $0.408 \text{ m}^3 \text{ CH}_4/\text{kgVS}$ for the untreated orange peel samples to $0.628 \text{ m}^3 \text{ CH}_4/\text{kgVS}$ for the orange peel treated with method one (treated op 1). The second treatment (treated op 2) was less effective and resulted in only $0.489 \text{ m}^3 \text{ CH}_4/\text{kgVS}$. Furthermore, as it can be seen in Table 6, the initial methane production is faster when we use treated orange peel in the system ($0.046 \text{ m}^3 \text{ CH}_4/\text{kgVS/day}$ and $0.020 \text{ m}^3 \text{ CH}_4/\text{kgVS/day}$ compared with only $0.013 \text{ m}^3 \text{ CH}_4/\text{kgVS/day}$ for untreated orange peel). Due to this aspect the organic loading can therefore be increased when using treated orange peel. These promising positive results could be the basis of a future continuous experiment; there this pre-treated orange peel would be utilized in a continuous anaerobic digestion.

10. Conclusion

This study illustrates that thermophilic digestion of industrial orange waste mixed with municipal solid waste in a continuous stirred tank reactors can produce a methane yield up to $6 \text{ m}^3 \text{ CH}_4/\text{kgVS}$ for both mixtures of 30-70 % and 20-80 % OP and MSW, respectively. The portion of mixing was calculated on the basis of total VS added. However, a process would fail after around 20 days of operation, which was indicated by the decreased pH, decreased alkalinity and increased VFA concentrations determined during the operation period. The methane production stops due to the accumulation of peel oil, limonene, which has a known antimicrobial effect, affecting the most sensitive group of bacteria, the methanogens in the system.

However, the batch experimental studies using untreated vs treated orange peel waste samples as substrate for the anaerobic digestion demonstrate that industrial orange peel waste can be a suitable source for biogas production when an appropriate pre-treatment is performed prior to the digestion process.

11. Future work

The experiments including untreated OP in the semi-continuous anaerobic digestion method failed after 20 days of operation. Therefore, we can conclude that pre-treatment of OP is necessary in order to avoid accumulation of inhibitory components. Several pre-treatment methods were tested within a parallel project and two of the pre-treated samples were evaluated in the end of this study using batch anaerobic digestion experiments. The promising results reported previously in chapter 8.2 will lead to a naturally continuation of this work, by testing the performance of these pre-treated OP samples even in a continuous biogas process.
References


