Pre-treatment of straw and forest residue for biogas production;

Recycling and Reuse of NMMO

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Abstract

N-methylmorpholine-N-oxide has shown a positive effect for the pretreatment of lignocelluloses. Pretreatment by NMMO was developed to enhance the digestibility of lignocellulosic biomass.

Barely straw and forest residue were pretreated by N-methylmorpholine-N-oxide (NMMO) prior to anaerobic digestion. The effectiveness of NMMO-treatment on straw and forest residue was examined as well as the recycling and reuse of NMMO for the next pretreatment process. During the first experimental series pretreatments were performed at 90 °C for 3h and 30h, followed by digestion of the pretreated material for 41 days. Low methane yield was found in these experiments due to high organic loading rate. In the second series the recycling and reuse of NMMO was investigated on straw. The pretreatments were carried out at 90 °C for 30 hr and the recycling and reuse were performed in three times. After treatments with fresh, as well as 1, 2, 3 times recycled NMMO methane yield of 0.45, 0.42, 0.38 and 0.4 Nm$^3$/kg VS were obtained.

Keywords: NMMO pretreatment, biogas production, anaerobic digestion, barley straw, forest residues, NMMO recycling and reuse.
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1. Introduction

Renewable energy resources are a major pillar for the protection of climate and for sustainable development; they are key elements for energy production.

Currently, the energy demand in the world is growing; around 88% of the energy used is employed by fossil fuels, which causes an increase in the concentration of greenhouse gases. In order to reduce this impact on climate the emission of the greenhouse gases must be decreased to the half of the global emission today (Weiland 2010). Figure 1 shows how the oil production varied and expected to vary in the future in the World. It is still a disagreement on how the production and use of fossil fuels will continue. One says that “peak oil production” has already been obtained while according to others it is assumed to occur in the near future.

Therefore, one of the most current challenges is to reduce the dependency on fossil resources and simultaneously the emissions of greenhouse gases. The adoption of clean energies like wind and solar energies, hydroelectric power and biogas would assist the supply of the diversification, diminishing the release of greenhouse gases as well as developing job opportunities in both urban and rural areas (Martins das Neves, Converti et al. 2009).

Figure 1: scenario of World oil production and “peak oil” adapted from Al Seadi, Rutz et al. (2008).
At present around 12.7% of the biomass produced globally is used for renewable energy production (Herrmann 2012). Biogas is an attractive renewable energy source to replace the fossil fuels as vehicle fuel. It is produced by the activity of different microorganisms, by means of an anaerobic process, also assigned as anaerobic digestion. Compared to other biomass generated fuels such as biodiesel or bioethanol, biogas is characterized by both better resource use and higher energy efficiency. Germany is considered as a largest biogas producer in the world (Herrmann 2012).

The advantages of biogas production can be viewed through the biogas cycle presented on Figure 2, which shows how biogas can be produced and utilized. Biomass produced through carbon fixation with the help of light source can be utilized in agriculture, as animal feed or in food production. The waste generated after these kinds of utilizations as well as cultivated energy crops directly can then be used as raw materials for the biogas process (Wilkie 2011). Furthermore, the nutrient rich digested residue generated can be utilized as bio fertilizer and recycled back to agricultural land.

Other residues from diverse social activities, such as: agricultural residues, industrial residues, forestry waste, municipal sewage or domestic refuse can also be employed as raw materials to produce biogas by anaerobic digestion (Pankhurst 1983).

The large scale of wastes or biomass obtained from the so named “energy crops”, such as barely, wheat, maize, or sugar beet, indicates a good substitute as raw materials for biogas production (Pankhurst 1983). Moreover, the handling of several kinds of waste to biogas production express a good benefit for society by creating jobs, strengthening local economies and protecting the environment (Wilkie 2006).
Simultaneously, changeable, fixed factors can affect the potential of biofuels; such as energy policy, government policy, land area, population growth, climate, fresh water area, geography or agriculture policies leading to complete change in the generation of biogas (Pankhurst 1983).

1.1 Biogas

Biogas consists mostly of methane and carbon dioxide. Depending on the type of processes or waste characteristics up to 75% of the total content of biogas can be methane (CH₄) and up to 50% can be carbon dioxide (CO₂) as it is shown in Table 1. Moreover, biogas also contains traces of other components, such as: hydrogen, nitrogen, hydrogen sulphide, and oxygen. Other general features of biogas summarized in Table 2.

The amount of methane produced can be theoretically assumed by applying the empirical formula below (Lübken, Gehring et al. 2010).

\[
C₉H₆O₃N₄ + \left( \frac{a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{3e}{3}}{2} \right) H₂O \rightarrow \left( \frac{a - \frac{b}{2} - \frac{c}{4} + \frac{3d}{3} + \frac{3e}{3}}{2} \right) CO₂ + \left( \frac{a - \frac{b}{2} - \frac{c}{4} + \frac{3d}{3} + \frac{3e}{3}}{2} \right) CH₄ + dNH₃
\]

However, the final methane content in biogas depends also upon other parameters, like the organic matter content of the substrate, in addition to other factors like pretreatment or process conditions.

If compared to other alternatives of renewable energy, when needed biogas can be produced and can be stored easily and it has several applications.

1. Transportation fuel
2. Distributed into the natural gas grid (Panwar, Kaushik et al. 2011).
3. Electricity generation
4. Production of chemicals
### Composition of Biogas

<table>
<thead>
<tr>
<th>Component</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methane ($\text{CH}_4$)</td>
<td>50-75%</td>
</tr>
<tr>
<td>Carbon dioxide ($\text{CO}_2$)</td>
<td>25-50%</td>
</tr>
<tr>
<td>Water ($\text{H}_2\text{O}$)</td>
<td>0-10%</td>
</tr>
<tr>
<td>Hydrogen sulphide ($\text{H}_2\text{S}$)</td>
<td>0-3%</td>
</tr>
<tr>
<td>Ammonia ($\text{NH}_3$)</td>
<td>0-1%</td>
</tr>
<tr>
<td>Nitrogen ($\text{N}_2$)</td>
<td>0-10%</td>
</tr>
<tr>
<td>Oxygen ($\text{O}_2$)</td>
<td>0-1%</td>
</tr>
<tr>
<td>Hydrogen ($\text{H}$)</td>
<td>0-1%</td>
</tr>
</tbody>
</table>

Table 1: Composition of biogas, adapted from Martinka (2012)

### General Features of Biogas

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Content</td>
<td>6-6.5k Wh/m³</td>
</tr>
<tr>
<td>Fuel Equivalent</td>
<td>0.6-0.651 oil/m³ biogas</td>
</tr>
<tr>
<td>Explosion Limits</td>
<td>6-12% biogas in air</td>
</tr>
<tr>
<td>Ignition Temperature</td>
<td>650-750°C</td>
</tr>
<tr>
<td>Critical Pressure</td>
<td>75-89 bar</td>
</tr>
<tr>
<td>Critical Temperature</td>
<td>-82.5°C</td>
</tr>
<tr>
<td>Normal Density</td>
<td>1.2 kg/m³</td>
</tr>
<tr>
<td>Smell</td>
<td>Bad eggs</td>
</tr>
</tbody>
</table>

Table 2: General features of Biogas adapted from Vij (2011).
1.2 Biogas production in the world

Today, as the alternative energy sources are gaining importance as the energy demand increasing and as the world’s economy has been growing; biogas production is spread in many countries around the world (Martins das Neves, Converti et al. 2009).

The main reason behind the implementation of biogas sites is the increasing demand for waste treatment technologies from municipalities, farmers, or industrial activities as well as, the increasing opportunities provided for natural gas by means of transportation and cogeneration applications. However, one of the main drawbacks of raw biogas is the presence of carbon dioxide, moisture and corrosive gases, such as hydrogen sulphide, which can easily reduce the life time of the equipment there biogas is utilized. The main corrosive agents are chlorine salts or potassium can be reduced by removing them entirely, however this will increase the total energy costs within the production (Martins das Neves, Converti et al. 2009).

The biogas market is growing, since a number of countries are accessing up biogas targets as a main approach for treating a variety of waste streams. Moreover, biogas production and its utilization can decrease the environmental and socioeconomic impact of decomposing organic compounds and beside a numerous valuable co-products can be produced (Lawrence 2012).

The power generation from biogas is constantly increasing, counting up to around 20% of renewable energies for energy consumption in the European Union, as it is shown in Figure 3. About 52% of the biogas plants produces biogas from agriculture waste, and about 36% are utilizing sewage sludge and the remaining 12% are landfills treatment plants. Germany is by far the major biogas producer in the world (BisypIn 2012).
Figure 3: Primary energy production from biogas per capita in the European Union presented in MWh per 1000 inhabitants adapted from Bisypln (2012).
2. Biogas production process

Biogas is produced through a common biochemical process called anaerobic digestion (AD), where the complex organic matter will be decomposed into methane by different types of anaerobic microorganisms. The schematic process of anaerobic digestion is shown on Figure 4. The four main stages within the decomposition are: hydrolysis, acidogenesis, acetogenesis, and lastly methanogenesis.

![Anaerobic Digestion Process](image)

Figure 4: Anaerobic Digestion Process adapted from Al Seadi, Rutz et al. (2008)

2.1 Hydrolysis

Hydrolysis is the first step of anaerobic digestion. During hydrolysis complex substances, such as carbohydrates, proteins and lipids will be broken down into sugars, amino acids and volatile fatty acids (VFA), since they cannot be used directly as food source by the anaerobic microbes due to their large polymer size (Neves 2009).

Hydrolysis is operated by hydrolytic microorganisms which excrete exoenzymes, such as protease, lipase, cellulase, cellobiase, amylase and xylanase as it is shown in Table 3 and these enzymes convert the complex polymers into simpler compounds.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Breakdown Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipase</td>
<td>Lipids</td>
<td>Fatty acids and glycerol</td>
</tr>
<tr>
<td>Protease</td>
<td>Proteins</td>
<td>Amino acids</td>
</tr>
<tr>
<td>Cellulase, cellobiase, xylanase and amylase</td>
<td>Polysaccharide</td>
<td>Monosaccharide</td>
</tr>
</tbody>
</table>

Table 3: Hydrolytic enzymes (Al Seadi, Rutz et al. 2008).

2.2 Acidogenesis and Acetogenesis

Products from hydrolysis, enters into acidogenesis. During acidogenesis phase the substrate will be degraded into carbon dioxide, hydrogen gas, alcohols, organic acids, a few organic-nitrogen compounds and organic-sulphur compounds. These products can be used directly or indirectly as a substrate by the methane-forming bacteria.

During acetogenesis, the simple molecules created through the acidogenesis phase are further digested by acetogenic fermentative bacteria to produce acetic acid, carbon dioxide and hydrogen. (Neves 2009).

2.3 Methanogenesis

Lastly methanogenesis takes the acetate, hydrogen and carbon dioxide, formed during the earlier steps, and produces both methane and carbon dioxide.

Methanogenesis is a biochemical process achieved by Methanogenic archaea. Formation of methane occurs either through the acetoclastic pathway or produced by the hydrogenotrophic methanogens. The primary ones take acetate as substrate and the secondary ones convert hydrogen and carbon dioxide to methane.

The hydrogenotrophic methanogens utilize the hydrogen, produced by the acetogenic bacteria during the previous step. The process of managing a low pressure of hydrogen is a key role for the hydrogen utilizing methanogens, preventing the inhibition of acetogenic activity caused by the accumulation of hydrogen (Beam 2011).
Methanogenesis is extremely accessed by operation conditions such as temperature, composition of feedstock, pH, carbon/nitrogen (C/N) and carbon/ phosphorus (C/P) ratio, which are if not at optimal level will result in a decrease or failure in methane production (Esposito, Frunzo et al. 2012).

3. **Lignocelluloses as possible feedstock for biogas production**

Lignocellulose is the major part of plant bodies and plays an important role in the energy production. It is also a natural, abundant and renewable resource with potential for conversion to transportation fuels to replace petroleum. Lately, a significant progress has been made to convert lignocellulosic material to biogas and bioethanol as well as utilizing them in multi-production facilities, such as bio-refineries (FitzPatrick, Champagne et al. 2010). Lignocellulosic materials such as wood, agricultural, forest, crop residues, other plant substances, animal manures and municipal solid waste exist in a large fraction. Lignocelluloses are composed of three major parts, such as cellulose, hemicelluloses and lignin, together with additional minor inorganic materials (Carrier, Loppinet-Serani et al. 2011).

3.1 **Cellulose**

Cellulose is made of D-glucose subunits that are connected together by β-1, 4 glycosidic bonds. Cellulose can be found in the plant in two forms, like in crystalline (organized) and/or in amorphous structure. The cellulose chains are bound together by hydrogen bonds and packed into micro fibrils; these fibrils are attached to each other by hemicelluloses, which are amorphous polymers of different sugars, as well as other polymers such as pectin. The structure of cellulose fibrils is rather crystalline than amorphous, thus this phenomena make cellulose more resistant to enzymatic hydrolysis through the limited accessibility of the cellulose chains (Fan, Lee et al. 1980).
3.2 Hemicelluloses

Hemicellulose has a complex carbohydrate structure with lower molecular weight than cellulose. Hemicellulose is composed of different polymers of sugars, as pentoses (xylose and arabinose) and hexoses (mannose, glucose and galactose), as well as sugar acids. Dominant of these component in hemicelluloses is mannose in softwoods and xylose in hardwoods and in agricultural residues (Schädel, Richter et al. 2010).

In contrast to cellulose (crystalline and strong), hemicelluloses have a random, amorphous and branched structure with low resistant to hydrolysis, hence they can be easily converted to their monomers by acid hydrolysis.

Hemicelluloses create a connection between the cellulose fibers and lignin; furthermore give more rigidity to the lignocellulose’ structure. The solubility of the lignocellulose depends on several factors, such as temperature, moisture content, pH and the compounds present in hemicelluloses. For instance the solubility of different sugars from higher to lower is: mannose, xylose, glucose, arabinose, and galactose, respectively (Hendriks and Zeeman 2009).

3.3 Lignin

After cellulose and hemicellulose, lignin is the most abundant polymer in nature and covers the hemicelluloses and cellulose in the cell wall. Together they form lignin holocellulose which is less accessible to microorganisms. Holocellulose is less biodegradable or even is completely resistant to digestion. Among the plants most of the agricultural residues, as well as softwood contain more lignin and fewer biodegradables.

Lignin is an amorphous heteropolymer that is constructed of three different phenylpropanes (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different kinds of linkages making lignin difficult to degrade (Rencoret, Gutiérrez et al. 2011). It is non-water soluble and optically inactive.

Just like hemicellulose lignin is soluble in acid, neutral or alkaline environment depending on the components (p-coumaryl, coniferyl and sinapyl alcohol) it is made of, (Hendriks and Zeeman 2009).
4. Pretreatment methods

Bioconversion of lignocellulosic materials to biogas is significantly prevented by the structural and chemical complexity of lignocelluloses which make them refractory to enzymatic attack; hence this is a main challenge for cellulosic biogas production. To overcome these problems several pretreatment methods have been suggested aiming to enhance the digestibility of lignocellulosic materials (Agbor, Cicek et al. 2011).

The best pretreatment method depend on the type of lignocelluloses, since several parameters such as crystallinity, accessible surface area, protection by lignin and hemicellulose, degree of cellulose polymerization, and degree of acetylation of hemicelluloses, will affect the biological degradation of lignocelluloses (Taherzadeh and Karimi 2008). Around 2/3 of the total cellulose is in crystalline form, and the crystallinity of cellulose is the main factor that prevent cellulose from enzymatic hydrolysis. Previous research showed that enzymes can only readily degrade the cellulose in its more accessible amorphous form. If the cellulose is more crystalline it is therefore more resistant to hydrolysis (Fan, Lee et al. 1980).

Pretreatment is therefore needed to open up the compact structure, convert the crystalline form to a more accessible amorphous one to make lignocellulosic materials suitable for the utilization in renewable energy production processes. The pretreatment methods can be classified into physical, chemical, physico-chemical, and biological pretreatments; however each of the pretreatments has different effects on the lignocelluloses.

4.1 Physical pretreatment

The goal of the physical pretreatments is to breakdown the biomass (lignocellulosic material) into smaller pieces.

4.1.1 Milling

Milling is a simple pretreatment method that has been applied prior to enzymatic hydrolysis or other pretreatment methods. Milling reduces the particle size and the crystallinity. The reduction of particle size is associated with the increase of available specific surface area and decreased degree of polymerization (DP) (Mshandete, Björnsson et al. 2006). The increase of specific surface area and the reduction of DP can increase in most cases the total hydrolyses yield of lignocelluloses by 5–25% (Hendriks and Zeeman 2009). Milling can increase both
methane and ethanol yield and produces no microbial inhibitors (like furfural and HMF hydroxyl methyl furfural), indeed these pretreatments methods have high energy demands (Brodeur, Yau et al. 2011).

4.1.2 Irradiation

Three sources, such as gamma rays, electron beam and microwaves, can be used to improve enzymatic hydrolysis of lignocelluloses. Irradiation of cellulose by $\gamma$-rays, leads to the cleavage of $\beta$-1,4-glycosidic bonds resulting in a larger surface area and a lower crystallinity (Kumar, Barrett et al. 2009). Irradiation can be combined with other methods such as acid pretreatment. However, the irradiation methods will be very expensive and are associated with huge amount of environmental and safety concerns (Agbor, Cicek et al. 2011).

4.2 Physico-chemical pretreatment

Physico-chemical pretreatment is a combination of both physical and chemical pretreatments.

4.2.1 Steam explosion

It is a hydrothermal pretreatment in which the biomass is subjected to high pressure and temperatures for a short duration time after which the system will be rapidly depressurized. This makes the material undergo an explosive decompression. In steam explosion the temperature and pressure are adjusted to between 160-260°C and 0.69-4.83 MPa, respectively, for a few minutes (Kumar, Barrett et al. 2009).

In these circumstances hemicelluloses will be the predominant fraction of the carbohydrates solubilized into the liquid phase and lignin is transformed to degradation compounds. As the results the cellulose in the solid fraction becomes more accessible to the enzymatic hydrolysis.

Steam explosion can be performed with addition of $\text{H}_2\text{SO}_4$, $\text{CO}_2$ or $\text{SO}_2$ as a catalyst. Addition of acid in steam explosion process can effectively improve the hydrolysis of hemicelluloses during the pretreatment enhancing cellulose digestibility (Agbor, Cicek et al. 2011). The major drawback of steam explosion pretreatment is that lignin will not be completely transformed. Hence there is a risk of condensation and precipitation of soluble lignin components which in turn makes the biomass less digestible and leads to the production of inhibitory compounds (Hendriks and Zeeman 2009).
4.2.2 Liquid Hot Water-Pretreatment

Liquid hot water (LHW) pretreatment is another hydrothermal pretreatment method that is similar to steam explosion but uses liquid hot water at elevated temperature instead of steam. In this process hot water under high pressure can penetrate into the biomass which causes an effective hydrolysis of hemicelluloses together with removal of lignin. The water and biomass are kept in contact for 15 min at temperatures of 200–230 °C. Acids are released during biomass hydrolysis by breaking the hemiacetal linkages, this phenomena could help or hinder LHW pretreatment (Agbor, Cicek et al. 2011). Weak acids released in the process help to further hydrolyze the biomass and the removal of oligosaccharides can further hydrolyze hemicelluloses to monomeric sugars. On the other hand, they can be degraded to aldehydes (i.e. furfural and 5-hydroxymethyl furfural (HMF)) that are major inhibitors in the following microbial processes (Palmqvist and Hahn-Hägerdal 2000).

4.2.3 CO₂ Explosion

Supercritical carbon dioxide has been considered as an extraction solvent for non-extractive purpose. This pretreatment method is used due to its several advantages such as availability at relatively low cost, non-toxicity, non-flammability, easy recovery after extraction, and environmental acceptability (Taherzadeh and Karimi 2008). Low temperature supercritical carbon dioxide pretreatment does not cause decomposition of monosaccharaides compared to the steam explosion pretreatment were decomposition occurs due to the high temperature involved (Kumar, Barrett et al. 2009).

The moisture content of lignocelluloses will affect the efficiency of this pretreatment. It was shown that in the presence of water, supercritical carbon dioxide have more capability for enhancing the enzymatic digestibility of aspen (hardwood) and southern yellow pine (softwood) (Kim and Hong 2001). The requirement of high pressure makes a disadvantage point in this method.

4.2.4 Ammonia Fiber Explosion (AFEX)

Ammonia Fiber Explosion (AFEX) is one of the alkaline physico-chemical pretreatment methods. In this process liquid ammonia is used for improving the susceptibility of lignocelluloses biomass to enzymatic attack (Bals, Wedding et al. 2011). During this process the biomass is subjected to liquid anhydrous ammonia at moderate temperature (60°C to 100°C) and high pressure for a period of e.g. 30 min (Brodeur, Yau et al. 2011). Then by immediate reduction of pressure the ammonia is vaporized allowing it to be recycled. In AFEX process hemicelluloses and cellulose may remain intact while the lignin fraction can be
either modified or removed, therefore this process has significant effects on biomass that contains less lignin (Taherzadeh and Karimi 2008).

4.3 Chemical Pretreatments

4.3.1 Alkaline pretreatment

The basis of alkaline pretreatment method refers to the use of alkaline solutions such as sodium, potassium, calcium, and ammonium hydroxide, for the degradation of lignocelluloses. In alkaline pretreatment the biomass is soaked by alkaline solution at relatively low temperature for a certain amount of time. The main effect of alkaline pretreatment on lignocelluloses is delignification by breaking the ester bonds cross-linking between lignin, hemicellulose and cellulose, thus increasing the accessibility of cellulose to enzymatic attack (Taherzadeh and Karimi 2008). Sodium hydroxide pretreatment is an effective pretreatment method (Silverstein, Chen et al. 2007), which causes cellulose swelling, increasing the internal surface of cellulose and decreasing the degree of polymerization and crystallinity.

4.3.2 Acid pretreatment

Acid pretreatment is a simple and direct way to hydrolyze lignocelluloses. During this treatment concentrated or diluted acid (usually between 0.2% to 2.5% w/w) is added to the biomass at temperatures between 130°C to 210°C. H₂SO₄ is the most effective acid which has been commercially used to pretreat a wide variety of biomass types. Sun and Cheng (2005) reported pretreatment of rye straw and bermudagrass with sulfuric acid at various concentrations (0.6, 0.9, 1.2and 1.5%, w/w) and residence times (30, 60, and 90 min). By increasing the severity of the pretreatment condition, more than 50% of the hemicellulose was hydrolyzed into monomeric sugars, while the glucose concentration of ray straw remained constant. However, the use of high acid concentration in the process is associated with extremely corrosive nature and toxicity extensive washing and/or detoxification step is therefore needed before the following enzymatic hydrolyses (Brodeur, Yau et al. 2011). Moreover, acid pretreatment or other pretreatments performed at low pH may involve the production of fermentation inhibitors, like furfural and HMF (hydroxymethyl furfural). These byproducts usually inhibit the microbial growth, thus result in less yield and productivity of biogas or ethanol (Taherzadeh and Karimi 2008).
4.4 Biological pretreatment

Many microorganisms exist in nature are able to degrade lignocelluloses. These microorganisms degrade lignin as a sole carbon and energy source, thus making easy access to cellulose. Several organisms, such as brown-, white- and soft-rot fungi, have been suggested for these kinds of pretreatments. White-rot fungi was found to be the most effective microorganism for biological pretreatment of lignocelluloses (Sánchez 2009). The biological pretreatment has been extensively studied due to its several advantages, such as non-toxicity, environmental friendly, no chemical requirement, mild environmental conditions and low energy requirements. However, long pretreatment time is needed, which is the main disadvantage of biological pretreatment methods (Taherzadeh and Karimi 2008).

4.5 Pretreatment by NMMO

Many pretreatment methods have been reported to enhance the digestibility of lignocelluloses by disrupting the cellulose crystallinity. Generally most of them involve high energy requirements and/or generates toxic by-products. Recently, pretreatment with an organic solvent, N-methylmorpholine- N-oxide (NMMO) has been suggested to improve digestibility of lignocellulosic materials, Meister and Wechsler (1998), Kuo and Lee (2009), Fink, Weigel et al. (2001), Shafiei, Karimi et al. (2011).

Today, NMMO is commercially used as a cellulose solvent, with an industrial application in the lyocell process for making fiber in the textile industry. NMMO can physically dissolve cellulose, furthermore with the use of this solvent cellulose is not derivatized but mainly dissolves to give a homogeneous polymer solution. The high polarity N–O bond in the chemical structure of NMMO (Figure 5) will cause the breakdown of the hydrogen bond network of the cellulose, which instead will make a strong hydrogen bond interaction with the solute (Fink, Weigel et al. 2001).

![Figure 5: NMMO is formed by the oxidation reaction of the ternary N-methylmorpholine (Fink, Weigel et al. 2001).](image-url)
4.5.1 Interaction between Cellulose-NMMO-water

Normally, the melting point of NMMO is 170°C and it can be decreased by adding water. Obviously by adding one molecule water the melting point can be decreased to less than 76 °C. Since N-O bond in NMMO is very stable, the oxygen in the N-O bond can therefore form a N-O---H-O-H hydrogen bond, and these kind of bonds are also formed with cellulose (Zhao, Kwak et al. 2007). There is a competition reaction between cellulose and water to take oxygen from NMMO. Depending on NMMO concentration swelling and dissolution of cellulose in NMMO were observed in a study by Cuissinat and Navard (2008). They have also found that cellulose fibres will be fully dissolved at the highest concentration of NMMO used.

4.5.2 Various modes of swelling and dissolution of cellulose pretreated with NMMO

NMMO-water mixture with three different water content were used for the investigations (Cuissinat and Navard 2006). Five modes of structural changes of wood and cotton cellulose fibres in NMMO were found (Cuissinat and Navard 2006).

Mode 1: Fast dissolution by disintegration into rod-like fragments
Mode 2: Large swelling by ballooning, and then dissolution of the whole fibre
Mode 3: Large swelling by ballooning, and partial dissolution of the fibre, still keeping its fibre shape
Mode 4: Homogeneous swelling, and no dissolution of any part of the fibre
Mode 5: No swelling and no dissolution (case of a non-solvent)

It was reported that mixture with low percentage of water (around 16% of water) causes complete dissolution of the cellulose fibres, such as ramie and cotton (Figure 6). On the other hand by increasing the percentage of water (to around. 19% for ramie in the mixture), the cellulose fibres exhibited a homogeneous irreversible swelling (Figure 7). In this case the crystalline structure of cellulose was affected by the solvent and cellulose II, the amorphous form of cellulose, was produced after the removal of the swelling agent consequently. Finally in the presence of more than 20% of water swelling was observed but not everywhere along the fibre (Figure 8).
Figure 6: Borregaard fibre swollen in NMMO-Water at 17% water content, optical microscopy in transmitted light (Cuissinat and Navard 2006).

Figure 7: Cotton fibre swollen in NMMO–water at 35% water content, optical microscopy in transmitted light (Cuissinat and Navard 2006).
NMMO is an environmentally friendly solvent which can be recovered by more than 98%. Refer to previous studies by Akhand and Méndez Blancas (2012), the NMMO pretreatment method has shown a positive effects on straw for biogas production. The purpose of this present study was therefore to investigate the effects of the pretreatment after the recycling and reuse NMMO on straw samples. For the economical point of view of the implementation of NMMO pretreatment in future large scale processes it is important to determine whether, the employment of recycled NMMO will result in as much positive effects as the fresh one during the following anaerobic digestion of the pretreated lignocellulosic materials.

5. Materials and Methods

5.1 Raw Materials

The two cellulosic materials investigated were barely straw and forest residues including branches, small trees, or tops. The straw was achieved from farmland (Figure 9) and other was obtained from a forest area (Figure 10) in Sweden in 2012 September.
In order to achieve appropriate particle size for the pretreatments prior to anaerobic digestion, both straw and forest residues were milled and sieved to 5mm and 2 mm size particles, respectively.

5.2 NMMO Pretreatment

Pretreatment of these different lignocellulosic materials at different duration times were carried out in order to analyze which material and which pretreatment conditions could achieve better results during the following anaerobic digestion assays. A commercial grade NMMO with a concentration of 50% w/w in aqueous solution (BASF, Ludwig-Shafen, Germany) was used for the pretreatments. It was first concentrated to 85% w/w using a
Rotary evaporator (Heidolph, Germany). In order to prevent degradation of NMMO during the pretreatment, 0.625 g/kg of proplygallate was added (Bang, Lee et al. 1999).

For the pretreatment 6gr of dry weight straw with 5 mm particle size or forest residue with 2 mm particle size were mixed with 94gr of 85% w/w NMMO solution in 500ml bottles. These samples were then kept at 90°C using an oil bath for treatment times of 3 hours or 30 hours. During pretreatment to enhance proper mixing or to skip the formation of clusters, the samples were stirred continuously.

To stop the reaction, boiling water was added immediately in the end of the duration time in every pretreatment. Then, the pretreated straw and forest residue were collected in textile bags which were used for filtration. To remove any remaining rest of NMMO, the pretreated samples were again washed with boiling water. The filtration and washing steps were repeated at least 3 times to make sure that all the NMMO was removed.

The pretreated straw and forest residue samples were carefully marked individually and stored in the fridge at 4 °C until they were used in anaerobic digestion assays. In supplement, parts of the samples were freeze dried for further structural and compositional analyses.

5.3 The recycling and reuse of NMMO for pretreatment of straw

The experiments were performed with 3 times recycling of NMMO. Fresh NMMO was concentrated to 85% by vacuum evaporator, operating at a total pressure of 100 mbar and a maximum temperature of 130°C. Each gram of straw (dry weight) was then pretreated with 15.6 gram of 85% NMMO solution (Lennartsson, Niklasson et al. 2011), in an oil bath at 90°C and atmospheric pressure for 30 h. The sample was mixed during the time of the pretreatment. After the pretreatment the NMMO-solution was separated from the straw by filtration. The collected NMMO solution had a concentration of about 50% and was recycled by concentrating it to 85% again using the vacuum evaporator, and it was then reused for the next treatment.

After each treatment step the concentration of NMMO was determined by a simple titration method. In this titration method, HCL (0.1M) was used as a reagent. A standard curve has created using known NMMO concentrations (1w/w%, 0.5w/w%, 0.25w/w%) as it is shown
on Figure 11. The concentration of the NMMO is estimated when a small change in HCL volume near the equivalence point results in a large pH change (Figure 12).

Figure 11: Standard curve for NMMO concentration

Figure 12: Equivalence point between Concentration of NMMO and Acid HCL (0.1M)
5.4 Anaerobic digestion

After the NMMO pretreatment, anaerobic batch digestion assays were carried out according to the method described by Hansen, Schmidt et al. (2004). Both untreated and pretreated straw and forest residue were investigated in triplicate samples at thermophilic conditions (55°C). The inoculum used was obtained from a large scale biogas plant Sobacken in Borås, treating the organic fraction of municipal solid waste.

The samples were placed in sealed bioreactors (small glass bottles of about 118 ml). Each bioreactor consisted of 30 ml inoculum and untreated or pretreated straw or forest residue as substrate. The amount of substrate for each sample was determined according to its VS content, keeping the VS ratio as 2:1 between the inoculum and substrate (Table 4).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Amount of material</th>
<th>Amount of Inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated Straw</td>
<td>0.30 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>Straw treated 3h</td>
<td>1.04 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>Straw treated 30h</td>
<td>0.91 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>Untreated Forest Residue</td>
<td>0.28 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>Forest residue treated 3h</td>
<td>0.69 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>Forest residue treated 30h</td>
<td>0.75 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>Cellulose (Control substrate)</td>
<td>0.25 g</td>
<td>30 ml</td>
</tr>
<tr>
<td>Inoculum (blank)</td>
<td>0.00 g</td>
<td>30 ml</td>
</tr>
</tbody>
</table>

Table 4: Bioreactor setup for Straw and Forest residue as materials.

In order to test the quality of inoculum, there was a control bioreactor which consisted of cellulose as substrate, and furthermore a blank was also prepared containing only inoculum and water. This was used to determine the methane produced by the inoculum itself. All experimental setups were running in triplicates.
Lastly, the headspace of all reactors was flushed for 2 minutes, by injecting a gas mixture of 20% CO₂ and 80% N₂. The sealed bioreactors were then placed in the incubator at 55°C and were frequently shaken and moved around the incubator during the experimental period. The entire incubation time was 41 days, to ensure a complete degradation process of the organic substances.

5.5 Analytical Methods

According to Sluiter, Hames et al. (2008) the percentage of Total solids (TS) and Volatile Solids (VS) in the samples were determined by drying them first at 105°C in an oven until constant weight was achieved and then further continue the ignition of the dried sample in a furnace at 575°C.

The compositional analyses of barely straw determining the cellulose, hemicellulose, lignin and extractives was carried out by standard procedures of National Renewable Energy Laboratory (NREL) and explained in chapter 5.5.2.

Using a gas chromatography (GC), the amount of methane in the biogas was analyzed by taking the samples regularly from the sealed head space of the bioreactors as described by Teghammar, Yngvesson et al. (2010) and explained in details in chapter 5.5.1.

5.5.1 Biogas Production

The amount of methane and carbon dioxide produced in a sample was analyzed twice a week during the first two weeks and then it was measured only once a week during the rest of the experimental period.

Using a pressure tight gas syringe (VICI, Precision Sampling Inc., USA) shown in Figure 13, gas samples were obtain from the head space of the bioreactor, then the individual samples were directly loaded into the gas chromatograph (Auto System Perkin Elmer, USA), equipped with a packed column (Perkin Elmer, 6’×1, 8”OD, 80/100, Mesh, USA), a thermal conductivity detector (Perkin Elmer, USA) and with the inject temperature of about 150°C. The carrier gas was nitrogen with a flow rate of 20 ml/min at 60°C.
To skip over pressure inside the bioreactors, the excess biogas was released systematically, using a needle, keeping the overpressure less than 2 bars in the headspace of the glass flasks, after the release a new sample was taken and the methane content was measured again (Teghammar, Yngvesson et al. 2010).

The amount of methane and carbon dioxide (CO₂) which produced was calculated by using ideal gas law using the volume of the headspace of the bioreactor and the volume of the sample that was taken by the syringe and injected into the gas chromatograph (GC).

The produced amount of methane by the sealed bioreactor containing only inoculum was then deducted from the total methane produced in the individual samples and the values were plotted to get the accumulated methane production curve. All of these values for determining the methane production were calculated at normal conditions.
The composition of straw, determining the carbohydrate and lignin content was determined according to Sluiter, Hames et al. (2008). In addition, the extractives were determined and removed in order to prevent interference with further analytical stages (Sluiter, Ruiz et al. 2005).

The non-structural components were removed by achieving water extraction for 24 hours using a Soxhlet apparatus. Using rotary evaporator the water which used as solvent was removed and then the remaining materials were dried in an oven at 35°C for 24 hours to evaluate the non-structural components in the sample. After that, the samples were cooled to room temperature, and the amount of non-structural components was calculated.

After removing of the non-structural component the straw samples were hydrolyzed, by using 72% H₂SO₄ at 30°C for about 1 hour in a water bath. By adding 84 ml deionized H₂O, the acid concentration was then diluted to 4%.

The straw samples were autoclaved, and after cooling down, the assays for determining acid insoluble lignin were carried out. The autoclaved samples were first filtered by using vacuum filtration crucibles.

The filtration crucibles which contain the acid insoluble lignin were dried at 105°C for 4 hours and the samples were weighted and then finally kept at a muffle furnace at 575°C for about 24 hours.

The achieved aliquot was stored in the refrigerator and further allowed to evaluate the acid soluble lignin by using a UV spectrophotometer (Libra biochrom, Nordic Bio labs).

Furthermore, the aliquot was also analyzed, for determining the different sugars by using high performance liquid chromatography. The HPLC (Waters 2695, Millipore, Milford, USA) was equipped with a refractive index (RI) detector, and using an ion exchange column (Aminex HPX-87P, Bio Rad USA). Glucose and xylose were measured using an eluent of pure water with a flow rate of 0.6 ml/min at 85°C.

5.5.3 Crystallinity determination by FTIR

Fourier Transform Infrared Spectroscopy (FTIR) was used to measure the cellulosic crystallinity of untreated straw and pretreated straw.

The straw samples were made into powder to attain proper homogeneity, and the spectra were performed by using the FTIR apparatus (Impact, 410, Nicolet instrument Corp. Madison, Wisconsin).
W1). The spectra of the samples were obtained with an average of 64 scans from 600 to 4000 cm\(^{-1}\) and a resolution of 4 cm\(^{-1}\).

The crystallinity was measured by using the absorption bands at 1420 and 898 cm\(^{-1}\), and these bands were assigned to the respective crystalline cellulose I and cellulose II. Their absorption ratio (A1422/A898) gives the Crystallinity Index or Lateral Order Index (LOI) (Carrillo, Colom et al. 2004).

## 6. Results and Discussion

The aim of this study was to determine the methane production of untreated and pretreated materials through anaerobic batch digestion assays. NMNO was then recycled after the pretreatment and the straw was treated with recycled NMNO. This procedure was then repeated for three times to evaluate the effect of recycled NMNO. The raw straw and forest residue sample were milled into 5 mm and 2 mm size pieces, respectively, to promote the pretreatment and the following digestion process.

Anaerobic digestion was attained for 41 days with untreated and pretreated (with 85% NMNO at 90ºC) straw and forest residue samples to study the effect of different pretreatment conditions on the degradability of lignocellulosic materials.

The work started with the determination of Total Solids (TS %) and Volatile Solids (VS %) content of untreated and pretreated straw and forest residue samples, and the results are summarized in Table 5 below.
Sample | TS% | VS%
--- | --- | ---
Untreated Straw | 90.21 | 81.83
Treated Straw (3Hrs) | 26.04 | 23.91
Treated Straw (30Hrs) | 30.14 | 27.53
Untreated Forest residue | 89.78 | 88.68
Treated FR (3Hrs) | 36.34 | 36.19
Treated FR (30Hrs) | 33.48 | 33.43

Table 5: TS% and VS% values

Before the anaerobic digestion assays, NMMO needs to be removed from the materials because it might affect the anaerobic process during methane production (Kabir, del Pilar Castillo et al. 2013).
6.1 Methane Production/ Biogas Production

The biogas production was recorded and calculated in array to estimate the accumulative methane production. The results are presented in Table 6 and Figure 14.

The highest biogas production obtained was 0.27 Nm³ Methane/kg VS from the pretreated straw samples with NMMO for 3 hours, and in the case of forest residue, the highest biogas production of 0.09 Nm³ Methane/kg VS was obtained after treatment at the same condition, i.e. after 3 hours.

<table>
<thead>
<tr>
<th>day</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>13</th>
<th>17</th>
<th>22</th>
<th>27</th>
<th>34</th>
<th>41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated straw</td>
<td>0.00</td>
<td>0.06</td>
<td>0.1</td>
<td>0.13</td>
<td>0.14</td>
<td>0.17</td>
<td>0.21</td>
<td>0.23</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>Treated straw 3H</td>
<td>0.00</td>
<td>0.07</td>
<td>0.12</td>
<td>0.16</td>
<td>0.19</td>
<td>0.20</td>
<td>0.22</td>
<td>0.24</td>
<td>0.25</td>
<td>0.27</td>
</tr>
<tr>
<td>Treated Straw 30H</td>
<td>0.00</td>
<td>0.08</td>
<td>0.13</td>
<td>0.17</td>
<td>0.17</td>
<td>0.16</td>
<td>0.18</td>
<td>0.20</td>
<td>0.20</td>
<td>0.23</td>
</tr>
<tr>
<td>Cellulose</td>
<td>0.00</td>
<td>0.008</td>
<td>0.05</td>
<td>0.11</td>
<td>0.17</td>
<td>0.19</td>
<td>0.19</td>
<td>0.20</td>
<td>0.21</td>
<td>0.20</td>
</tr>
<tr>
<td>Untreated Forest residue</td>
<td>0.00</td>
<td>0.02</td>
<td>0.005</td>
<td>0.006</td>
<td>0.002</td>
<td>-7.23</td>
<td>0.015</td>
<td>0.01</td>
<td>0.013</td>
<td>0.016</td>
</tr>
<tr>
<td>Treated Forest</td>
<td>0.00</td>
<td>0.01</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
<td>0.05</td>
<td>0.07</td>
<td>0.08</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>Treated Forest</td>
<td>0.00</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.05</td>
<td>0.05</td>
<td>0.04</td>
<td>0.4</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 6: Methane production of the different samples of batch anaerobic process
According to previous reports, specific methane production values of untreated straw is in between 240 L kg\(^{-1}\) VS to 370 L kg\(^{-1}\) VS (Møller, Sommer et al. 2004) which can be expressed as 0.240 Nm\(^3\) Methane/kg VS and 0.370 Nm\(^3\)/kg VS and these are comparable to our results of 0.26 Nm\(^3\)/kg VS.

High biogas production was achieved from straw compared to forest residue. Due to the highly compact structure and high lignin content of forest residue, it is difficult to get higher methane yields from this substrate.

However, there was a more than 5-fold increase in the methane production from forest residues as well compared to that from untreated samples after NMMO treatment, while the treatment did not increase the methane yield from straw remarkably, only 5% increase could be observed (Figure 15).
6.2 Composition analysis

The contents of straw such as carbohydrates, lignin and ash were determined by following the NREL methods’ protocol.

Table 7 shows the composition of untreated and pretreated straw. The data indicates that the material was not severely affected by the NMMO pretreatment. However the cellulose content was increasing while the lignin content was decreasing as the result of the treatment. Hemicellulose was also decreasing as treatment time was increasing. Surprisingly, increased treatment time did not resulted in increased methane production, probably due to the presence of minerals such as silica in straw, which was affected by the treatment (Antongiovanni and Sargenti 1991). The mineral content can vary a great extent depending on soil contaminants and diverse soil conditions.
### Table 7: Straw compositions

<table>
<thead>
<tr>
<th>Sample</th>
<th>Untreated (% of TS)</th>
<th>3H pretreatment (% of TS)</th>
<th>30H pretreatment (% of TS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose</td>
<td>29.39</td>
<td>34.75</td>
<td>32.42</td>
</tr>
<tr>
<td>Hemicellulose</td>
<td>14.03</td>
<td>14.23</td>
<td>13.85</td>
</tr>
<tr>
<td>ASL</td>
<td>4.43</td>
<td>4.35</td>
<td>4.22</td>
</tr>
<tr>
<td>AISL</td>
<td>22.98</td>
<td>21.91</td>
<td>19.51</td>
</tr>
<tr>
<td>Ash</td>
<td>6.65</td>
<td>7.14</td>
<td>6.83</td>
</tr>
</tbody>
</table>

#### 6.3 Structural Analysis

In array to determine the structural changes in the sample as a result of the NMMO treatment, a method called **Fourier transform infrared spectroscopy** (FTIR) was applied.

The spectra of untreated straw and treated straw are shown on Figure 16 and Figure 17, respectively.
The Crystallinity index (CI) was determined with the help of two bands values at 1420 and 898 cm\(^{-1}\), which are correspond to the crystalline cellulose I and cellulose II, respectively.

Figure 16: FTIR spectra approached from untreated straw showing in the range from 600 to 4000 cm\(^{-1}\).

Figure 17: FTIR spectra approached from pretreated straw for 3 Hours showing in the range from 600 to 4000 cm\(^{-1}\).
The Crystallinity index (CI) shown in the Table 8 was determined by calculating the ratio $A_{1420}/A_{898}$.

This shows that the CI for the samples treated under 3 hours was obtained to be the lowest, which is in good correspondence with the methane yield, which was the highest after 3h NMMO treatment. After the 30 hours-treatment as well as for the untreated sample, the same CIs were obtained, corresponding to the methane yield which could not be increased after 30h treatment.
7. **Pretreatment after recycling of NMMO**

The setups of for the pretreatments with fresh VS recycled and reused NMMO are shown in Table 9. The pretreated straw was then washed with boiling water until no NMMO remained and finally the straw was freeze-dried and stored at 4 °C until use.

<table>
<thead>
<tr>
<th>pretreatment</th>
<th>Straw (g)</th>
<th>NMMO (g)</th>
<th>Pretreatment time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh NMMO</td>
<td>177.3</td>
<td>2500</td>
<td>30</td>
</tr>
<tr>
<td>First reuse NMMO</td>
<td>127.66</td>
<td>1800</td>
<td>30</td>
</tr>
<tr>
<td>Second reuse NMMO</td>
<td>92.19</td>
<td>1300</td>
<td>30</td>
</tr>
<tr>
<td>Third reuse NMMO</td>
<td>58.12</td>
<td>900</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 9: pretreatment setups for treatment with fresh and recycled and reused NMMO
7.1 Biogas production after recycling and reuse of NMMO

Anaerobic batch digestion assays were performed to determine the biogas production from untreated and pretreated samples as described earlier. The experiments were operated at thermophilic condition (55°C), and the organic loading rate (OLR) was 6grVS/l. The amount of substrate for each bottle was determined according to their VS% content (Table 10).

<table>
<thead>
<tr>
<th>Applied pretreatment</th>
<th>TS%</th>
<th>VS%</th>
<th>Substrate (gr)</th>
<th>Inoculum(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-treated straw</td>
<td>90.2</td>
<td>81.8</td>
<td>0.1832</td>
<td>15</td>
</tr>
<tr>
<td>Straw treated with-fresh NMMO</td>
<td>19.39</td>
<td>18.1</td>
<td>0.8317</td>
<td>15</td>
</tr>
<tr>
<td>Straw treated with-first reuse NMMO</td>
<td>23.1</td>
<td>21.4</td>
<td>0.7013</td>
<td>15</td>
</tr>
<tr>
<td>Straw treated with-second reuse NMMO</td>
<td>21.4</td>
<td>20.1</td>
<td>0.7495</td>
<td>15</td>
</tr>
<tr>
<td>Straw treated with-third reuse NMMO</td>
<td>30.6</td>
<td>28.3</td>
<td>0.5299</td>
<td>15</td>
</tr>
<tr>
<td>Cellulose</td>
<td>100</td>
<td>100</td>
<td>0.15</td>
<td>15</td>
</tr>
<tr>
<td>Inoculum</td>
<td>3.71</td>
<td>1.94</td>
<td>_</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 10: Experimental setups for the anaerobic digestion assays

Triplicate samples for each pretreatment setup were prepared including three bottles of blank (with only inoculum and water) and another three for untreated samples. Sample containing cellulose was used to test the quality of the inoculum, furthermore in each bioreactor 10 ml water was added to achieve a final reaction volume of 25 ml. The anaerobic digestion assays were monitored during 41 days; at the first two weeks the methane production was measured in every third day, and afterward samples were taken once a week.
The cumulative methane production of each sample was calculated in terms of the specific methane yield and is shown in Table 11. The anaerobic digestion was completed in 41 days since there was no further increase in methane production. The amount of specific methane yield depend on the total mass of VS that was initially added (Le Roux, Wakerley et al. 1979).

<table>
<thead>
<tr>
<th>day</th>
<th>0</th>
<th>3</th>
<th>6</th>
<th>10</th>
<th>13</th>
<th>19</th>
<th>21</th>
<th>27</th>
<th>36</th>
<th>41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Straw treated with-fresh NMMO</td>
<td>0</td>
<td>0.16</td>
<td>0.24</td>
<td>0.31</td>
<td>0.34</td>
<td>0.4</td>
<td>0.42</td>
<td>0.44</td>
<td>0.45</td>
<td>0.45</td>
</tr>
<tr>
<td>Straw treated with-first reuse NMMO</td>
<td>0</td>
<td>0.15</td>
<td>0.23</td>
<td>0.29</td>
<td>0.31</td>
<td>0.37</td>
<td>0.4</td>
<td>0.42</td>
<td>0.41</td>
<td>0.42</td>
</tr>
<tr>
<td>Straw treated with-second reuse NMMO</td>
<td>0</td>
<td>0.18</td>
<td>0.21</td>
<td>0.26</td>
<td>0.26</td>
<td>0.32</td>
<td>0.34</td>
<td>0.36</td>
<td>0.38</td>
<td>0.38</td>
</tr>
<tr>
<td>Straw treated with-third reuse NMMO</td>
<td>0</td>
<td>0.14</td>
<td>0.22</td>
<td>0.3</td>
<td>0.29</td>
<td>0.35</td>
<td>0.39</td>
<td>0.38</td>
<td>0.41</td>
<td>0.4</td>
</tr>
<tr>
<td>UN.TR.SRAW</td>
<td>0</td>
<td>0.1</td>
<td>0.14</td>
<td>0.17</td>
<td>0.16</td>
<td>0.21</td>
<td>0.24</td>
<td>0.25</td>
<td>0.26</td>
<td>0.27</td>
</tr>
<tr>
<td>pure Cellulose</td>
<td>0</td>
<td>0.02</td>
<td>0.03</td>
<td>0.17</td>
<td>0.21</td>
<td>0.35</td>
<td>0.37</td>
<td>0.38</td>
<td>0.38</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Table 11: Accumulated methane production (Nm$^3$/kg VS) obtained in the end of 41 days long incubation period

In Figure 18, it can be seen that pretreated straw gave more methane yield than untreated straw. The methane production from treated straw by fresh NMMO was obtained to approximately 0.45 Nm$^3$/kg VS, comparing this yield with that of 0.24 Nm$^3$/kg obtained during the previous experiment we can conclude that the difference can be explained by the higher organic load used during the first assay. The organic loading rate for experiment 1 and experiment 2 were 8.3 grVS/l and 6grVS/l respectively. Menardo, Gioelli et al. (2011) showed that an increase in organic loading rate can cause a decrease in biogas production rates by overloading the system.
Figure 18: Average cumulative methane production of triplicates sample measured during 41 days of incubation for straw.

Figure 18 illustrated accumulative methane production from different straw samples. Methane production after treatment with fresh NMMO was found to increase to a level of 0.45Nm³/kgVS. This is an almost twofold increase compared to that of untreated straw (0.27 Nm³/kgVS). Treatment with recycled NMMO after the first, second and third recycling and reuse resulted in less significant differences in methane production compared to that obtained after treatment with fresh NMMO (Figure 19)
Figure 19: Methane production after NMMO pretreatments compared to the methane production of untreated straw.
8. Conclusions

NMMO pretreatment of the straw and forest residue prior to anaerobic digestion can significantly increase the methane yield depending on the substrate and treatment conditions. As a result of NMMO-treatment the crystalline structure of the cellulose will be disrupted thus higher accessibility of the bacteria to the cellulose and hemicellulose will be achieved. Milling can further increase the affectivity of the treatment and the methane production during the following anaerobic digestion due to increased accessible surface area resulting in a better digestibility of the cellulose. Moreover, NMMO is an environmentally friendly solvent, the treatment can be performed with low energy consumption and the solvent can be recycled and reused after the treatment (Shafiee, Karimi et al. 2010). These are the main advantages of this pretreatment method compare to other pretreatment methods used for lignocelluloses.

This study shows the possibility for an economically-feasible lignocellulosic biogas production using NMMO pretreatment.

NMMO pretreatment at 90 °C increased the methane yield of both straw and forest residues. Performing the pretreatment at 90 °C results in low energy consumption, therefore this process can be applied in industrial scale processes in the future having commercial benefits. The yield of methane was higher for straw than that of forest residues due to the structural differences between these two substrate.

Mixing the samples continuously during the pretreatment time have shown positive effects on the treatment of the substrates. Treatment of the straw with recycled and reused NMMO resulted in approximately the same methane yields as treatment with fresh NMMO

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