Master thesis

Biogas production from ensiled maize with and without hydrothermal pretreatment

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ABSTRACT

Energy crops are suitable substrates for anaerobic digestion. In the future their use will probably increase, and among other things optimum storage and pretreatment technologies should be developed. In this study eight maize silage varieties grown in Piikkiö, Southern Finland were used as substrates in anaerobic digestion. Also hydrothermal pretreatment was used to improve the biogas yield. Composition of the maize silage and the pretreated maize silage were analyzed and inhibitors were analyzed from the pretreated maize silage. The maize silages produce methane as much as fresh maizes in previous studies, when losses during the ensiling are taking into account and even 25-50 % more when losses were ignored. KXA7251 produced most methane per volatile solids analyzed before ensiling ($VS_{original}$) (408 ± 7 l CH_4/kgVS) and per cultivation area, whereas Amara produced most methane per volatile solids analyzed after ensiling (VS_{added}) (470 ± 4 l CH_4/kgVS). However, no variety jumps out when comparing the results of this study and the results of previous studies for fresh maizes. The pretreated silages produce less methane than the untreated silages, probably because of inhibition. Probable reasons for the inhibition were furans and carboxylic acids. More cellulose, insoluble lignin and ash were analyzed from the solid fraction of the pretreated silage than from the untreated silage, probably caused by hemicellulose degradation during the pretreatment.

TIIVISTELMÄ

Energiakasvit soveltuvat hyvin biokaasuntuotannon raaka-aineeksi. Tulevaisuudessa niiden
käyttöä tullaan luultavasti lisäämään ja mm. sopivia varastointi- ja esikäsittelymenetelmiä
tullaan tarvitsemaan. Tässä työssä tutkittiin kahdeksan Piikkiössä, Etelä-Suomessa kasvate-
tun ja märkävarastointimenetelmällä säilötyn maissilajikkeen metaanintuottopotentiaaleja.
Lisäksi hydrotermistä esikäsittelyä käytettiin metaanintuoton parantamiseksi ja esikäsitel-
lyn ja käsittelemättömän maissisäilörehun rakennetta analysoitiin. Esikäsitellyn säilörehun
nestemäisestä osiosta analysoitiin myös metaanintuottoon vaikuttavia inhiboivia yhdisteitä.
Maissisäilörehu tuotti metaania yhtä hyvin kuin tuore maissi aiemmissa tutkimuksissa, kun
varastoinnin aikaiset häviöt otettiin huomioon ja jopa 25–50 % enemmän, kun häviöitä ei
huomioitu. Paras metaanintuotto ennen säilöntää määritettyä orgaanista kuiva-ainetta kohti
($VS_{alkuperäinen}$) oli lajikkeella KXA7251 (408±7 l CH_4/kgVS) ja säilönnän jälkeen määritet-
tyä orgaanista kuiva-ainetta kohti ($VS_{lisätty}$) lajikkeella Amara (470±4 l CH_4/kgVS). Mi-
kään lajikkeista ei kuitenkaan erottunut selvästi muista, kun otetaan huomioon myös ai-
emmat tutkimukset. Esikäsitellyt säilörehut tuottivat metaania käsittelemättömiä vähem-
män, mikä luultavasti johtuu käsittelyn aiheuttamasta inhibitiosta. Furaanit ja karboksyyli-
hapot olivat luultavasti merkittävimpiä inhibition aiheuttajia. Esikäsitellyssä maississa oli
enemmän selluloosaa, liukenematonta ligniiniä ja tuhkaa, mutta vähemmän hemiselluloo-
saa kuin käsittelemättömästä maississa, mikä johtuu luultavasti hemiselluloosan hajoami-
sesta esikäsittelyn aikana.

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1 INTRODUCTION

1.1 Biogas production from energy crops

Biogas is produced by anaerobic digestion, where organic matter is broken down by microbiological activity in the absence of air (Balat 2008). Among the various feedstocks, energy crops are highly suitable substrates for anaerobic digestion and in the future biogas production from energy crops will increase (Amon et al. 2007, Balat 2008). Among other things optimum conservation and pretreatment technologies must be developed as well as specific genotypes are required when crops are used as renewable energy sources (Amon et al. 2007).

Fresh maize (*Zea mays L.*) and maize silage are good substrates for anaerobic digestion (Bruni et al. 2010a). Maize cultivation has been shown to be possible even in southern Finland (Seppälä et al. submitted).

1.2 The composition of lignocellulosic crops

Lignocellulosic material consists of mainly three different types of polymers: cellulose, hemicelluloses and lignin (Hendriks & Zeeman 2008). Carbohydrates (cellulose and hemicelluloses) can be structural or non-structural (Sluiter et al. 2008). Structural carbohydrates are bound in the matrix of the biomass, while non-structural carbohydrates can be removed using extraction or washing steps (Sluiter et al. 2008).

Cellulose is a linear polysaccharide polymer of glucose and the cellulose chains are packed by hydrogen bonds (Taherzadeh & Karimi 2008). In plants cellulose consists of parts with a crystalline (well-organized) structure and parts with an amorphous (not well-organized) structure (Hendriks & Zeeman 2008).

Hemicellulose serves a connection between the lignin and the cellulose fibers. It is more soluble than cellulose (Cloete & Malherbe 2002, Hendriks & Zeeman 2008). Hemicellulose consists of different polymers like pentoses (C-5) and hexoses (C-6). The dominant component of hemicelluloses from agricultural plants is xylose. Xylose and arabinose belong to pentoses, and mannose, glucose and galactose to hexoses (Hendriks & Zeeman

2008). Hemicellulose is a physical barrier which surrounds the cellulose fibers and can protect the cellulose from enzymatic attack (Taherzadeh & Karimi 2008).

Lignin is a complex molecule constructed of phenylpropane units (Taherzadeh & Karimi 2008). It is a highly irregular and insoluble polymer whose main purpose is to give the plant structural support, impermeability and resistance against microbial attack and oxidative stress (Cloete & Malherbe 2002, Hendriks & Zeeman 2008).

1.3 Ensiling as a preservation method

Silage is the material produced by the controlled fermentation of a crop of high moisture content. The name of the process is ensiling (McDonald et al. 1990). Silage has been defined as an acidic, fermented, stored product from an agricultural crop (Buxton et al. 2003).

Two main objectives in preserving crops by natural fermentation are to achieve anaerobic conditions and to discourage the activities of undesirable microorganism (McDonald et al. 1990). Lactic acid bacteria are the microorganisms responsible for successful ensiling (Egg et al. 1993). The fermentation of sugars within the plant material produces organic acids, principally lactic acid (Buxton et al. 2003). That causes acidification of the crop, which inhibit growth of undesirable microorganisms (McDonald et al. 1990, Buxton et al. 2003). The inhibiting effect depends not only on pH, but also the moisture content and the temperature. The wetter the material, the lower will be the critical pH value at which the inhibiting effect occurs (McDonald et al. 1990).

Many of storage methods used in agricultural industry can be adapted to the storage of energy crops, particularly when crops are used for methane production. Silage systems offer many advantages for anaerobic digestion feedstocks. Losses during ensiling can be controlled by good management and after ensiling little or no additional processes is needed prior to digestion (Egg et al. 1993).

Maize is the most popular cereal crop conserved as silage. Maize has relatively high dry matter content and low buffering capacity, and it contains adequate levels of water soluble carbohydrates for satisfactory fermentation to lactic acid. Thus it can be regarded as an ideal crop for ensiling (McDonald et al. 1990).

1.4 Pretreatments to improve biogas yield

Lignocellulosic materials are resistant to anaerobic digestion because of their composition and structure (Bruni et al. 2010b). Pretreatments of lignocellulosic material could accelerate the hydrolysis process and improve the final biogas production by increasing the accessible surface area and pore size, decreasing the cellulose crystallinity and decreasing the degree of polymerization (Taherzadeh & Karimi 2008, Fernandes et al. 2009).

Pretreatments of can be classified into mechanical, thermal, chemical and biological pretreatments. Mechanical pretreatments are e.g. milling (cutting the biomass into smaller pieces) and irradiation (using e.g. gamma rays, electron beam or microwaves) (Taherzadeh & Karimi 2008, Hendriks & Zeeman 2008). Steam or liquid hot water is used in thermal (hydrothermal) pretreatments. There are two kinds of steam pretreatments: steam pretreatment and steam explosion (Hendriks & Zeeman 2008). Chemical pretreatments can be divided into acid (e.g. sulfuric acid and nitric acid) and alkaline (e.g. lime, aqueous ammonia and sodium hydroxide) pretreatments (Kim et al. 2003, Kim & Holtzapple 2005, Hendriks & Zeeman 2008, Zheng et al. 2009). Several fungi have been used in biological pretreatments as well as enzymes (Taherzadeh & Karimi 2008). Combination pretreatments (e.g. thermal pretreatment with alkaline) have also been used to improve the effect of the pretreatments (Hendriks & Zeeman 2008).

During the hydrothermal pretreatment the lignocellulosic material is heated. When temperature increases above 150-180 ℃, first hemicelluloses and shortly after that lignin starts to dissolve (Hendriks & Zeeman 2008). Kaparaju & Felby (2010) used corn stover and wheat straw as substrates and treatments conditions time 6 min, temperature 195 ℃ and pressure 8 bar. They found out that pretreatment improved the cellulose and lignin content with substantial removal of hemicellulose.

1.5 Inhibitors from pretreatments and their effect on anaerobic digestion

Degradation products from pretreatment of lignocellulosic materials can be divided into the following classes: primary intermediates (soluble hemicelluloses and lignin), secondary intermediates (phenols and furans), tertiary intermediates (carboxylic acids) and end prod-

ucts (carbon dioxide and water) (Klinke et al. 2002, Thomsen et al. 2009). Some of those byproducts are potential inhibitors of anaerobic digestion (Chen et al. 2008).

Inhibitory substances are often found to be leading cause of anaerobic reactor failure (Chen et al. 2008). Palmquist & Hahn-Hägerdal (2000) divided inhibitors in three major groups: weak acids, furan derivatives and phenols. When hemicellulose is degraded, xylose, mannose, acetic acid and glucose are liberated. Cellulose is degraded to glucose. At high temperature and pressure xylose is further degraded to furfural and 5-hydroxymethyl furfural (5-HMF) is formed from hexose degradation. Furfural and 5-HMF can further degrade to levulinic acid, formic acid and 2-furoic acid (Palmquist & Hahn-Hägerdal 2000, Klinke et al. 2002). Furfural, 5-HMF, formic acid and levulinic acid are potential inhibitors of anaerobic digestion (Chen et al. 2008).

Phenols, e.g. vanillic acid, vanillin, syringaldehyde, syringic acid and 4-hydrobenzoic acid, are generated from partial breakdown of lignin (Palmquist & Hahn-Hägerdal 2000, Klinke et al. 2002). Phenols are inhibitory to microorganism and are reported to be toxic to anaerobic processes (Palmquist & Hahn-Hägerdal 2000, Chen et al. 2005).

1.6 Objectives

The objective of this study was to evaluate the methane potential of eight maize silage varieties. In addition, the effect of hydrothermal pretreatment on methane yield and composition of two maize silage varieties was studied.

2 MATERIALS AND METHODS

2.1 Materials

Eight maize varieties (Ronaldino, Cannavaro, Amara, KXA 7211, Amadeo, KXA 7254, KXA 7251 and Valdez) used in this study were grown in Piikkiö, southern Finland by MTT Agrifood Research Finland. The varieties were harvested at 12.9.2009, harvesting yields were determined and the varieties were chopped to a particle size less than 15 cm. Total solids (TS) content of the varieties was analyzed. The varieties were ensiled in barrel silos, weighted and stored at 4 ℃.

The maize varieties were stored for 9-10 weeks. After that the silos were opened and weighted. Appearance of the silages was evaluated and parts with mold were removed. After opening, the silages were stored at -20 ℃.

Prior to pretreatments, batch assays and analysis, the maize silages were chopped to a particle size less than 2 cm using Wolf Garden SD 180E garden chopper and scissors. Inoculum used in first batch assays was obtained from a mesophilic pilot biogas reactor treating energy crops. In other batch assays, inoculum from a mesophilic biogas reactor treating cow manure and confectionary by-products were used. Both reactors were at a farm at Laukaa, Finland.

2.2 Pretreatments

Hydrothermal pretreatments were done using two maize silage varieties, Amadeo and KXA 7251, as substrates. High temperature and pressure reactor (Berghof with Berghof DTR 841 heating system) was used with substrate loading of 30 g and water loading of 187.5 g. The content of the reactor was flushed with N_2 (100 % N_2, Aga). The pretreatment conditions were temperature 185 ℃ for 10 min plus temperature rising time 110 min. After the pretreatment, substrate was left to cool down till next day.

Solid fraction and liquid fraction (hydrolysate) of the hydrothermally pretreated substrate were separated using Harrier 18/80 centrifuge (rate 4000 rpm for 10 min) (Figure 1). The fractions were stored at 4 ℃.
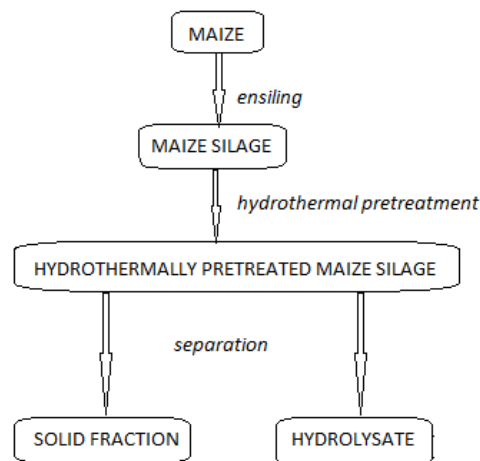

Figure 1. Materials used in this study: methane potentials of maize silage, hydrothermally pretreated maize silage, solid fraction and hydrolysate were determined.

2.3 Batch assays

Methane potentials of maize silages and hydrothermally pretreated maize silages were determined using batch assays. The batch assays using the maize silage varieties as substrates were performed in triplicates, using 1 l glass bottles. Volatile solids of substrate ($VS_{substrate}$) to volatile solids of inoculum ($VS_{inoculums}$) ratio of 1 were used.

A liquid volume of 0.75 l was used including 300 g of inoculum, substrate (according to inoculum to substrate VS:VS-ratio) and tap water. $NaHCO_3$ (3 g/l) was used as a buffer. The content of the bottles were flushed with N_2 (100 % N_2, Aga) for 5 min to create anaerobic conditions and finally the bottles were sealed with silicone stoppers with Master Flex Tygon tubes. Aluminum gas bags were used to collect the gas produced. The assays were incubated at 35 ℃ for 97 days.

Assays with inoculums and tap water were used as a control. The methane produced from controls was subtracted from the assays with maize silage.

In second batch assays the pretreated maize silage, solid fraction and hydrolysate of the pretreated maize silage were used as substrates. The batch assays were performed in triplicates using 120 ml serum bottles. $VS_{inoculum}$ to $VS_{substrate}$ ratio of 1 was used in case of the solid fraction and hydrolysate. In the assays with the pretreated maize silage (containing both the solid fraction and hydrolysate), $VS_{inoculum}$ to $VS_{substrate}$ (added to pretreatment reactor) ratio of 1 was used.

In the assays a liquid volume of 50 ml was used. Amount of the inoculum was 20 ml and in addition to that substrate, distilled water and buffer ($NaHCO_3$ 3 g/l) was added to the bottles. The bottles were flushed with N_2 and sealed with butyl rubber stoppers and aluminum crimps. The assays were incubated at 35 ℃ for 77-79 days.

2.4 Analysis and calculations

TS and VS were analyzed according to standard SFS 3008 (Finnish Standard Association 1990). All pH measurements were done using Seven Easy Mettler Toledo pH-meter except ammonium nitrogen (NH_4-N) titrations were done using Metrohm 774 pH-meter.

Extractions for the maize silage varieties were done according to modified Finnish standard SFS-EN 12457-4 (Finnish Standard Association 2002). After the extractions, samples were filtered with VWR filter papers (glass microfibers filter 691, particle retention 1.6 µm). Total chemical oxygen demand (TCOD) and soluble chemical oxygen demand (SCOD) (using filtered samples) were analyzed according to Finnish standard SFS 5504 (Finnish Standard Association 1988). Total nitrogen (N_{tot}) and NH_4-N were analyzed according to the Tecator application note (Perstorp analytical/ Tecator AB 2005) with a Kjeltec system 1002 distilling unit (Tecator AB). The protein content was calculated as follows (Bruni et a. 2010b):

$$protein = \left(N_{tot} - \left(NH_4 - N\right)\right) \cdot 6.25 \qquad (1)$$

Gas samples from batch assays were taken with a pressure lock syringe. Methane content of the gas samples were analyzed with Perkin Elmer Clarus 500 gas chromatograph

equipped with a flame-ionization detector (Perkin Elmer Alumina column length 30 m*0,53 mm, injector and detector temperature 225 °C, oven temperature 100 °C, carrier gas argon). Water displacement method was used to measure gas volume in the assays with gas bags.

The methane potentials of the untreated maize silage varieties were calculated per VS_{added} (amount of VS added to the bottle), per $VS_{original}$ (taking into account losses during the storage) and per cultivation area (using harvest yields). Two of replicates, which produce most methane, were taking into account in calculations. The VS before ensiling was calculated according to Pyykkönen 2009.

The methane potentials of the hydrothermal pretreated maize silages were calculated per $VS_{original}$ (VS added to the pretreatment reactor). The methane potentials of the hydrolysate and the solid fraction of the pretreated maize silages were calculated per VS_{added} (VS added to the assays).

The methane yields of all batch assays were calculated corresponding to normal liters (conditions T= 273 K, p= 1,013 bar).

2.5 Analysis of carbohydrates and lignin

Structual carbohydrates (sugars) and lignin content of maize silage (KXA 7251), untreated and hydrothermally pretreated, was determined by two-step strong acid hydrolysis with sulfuric acid according to National Renewable Energy Laboratory (Sluiter et al. 2008). Each sample was analyzed in duplicates. Prior to analysis, the samples (the untreated maize silages and the solid fraction of the hydrothermally pretreated maize silages) were dried at 35-40 °C for 2-3 days. After drying the samples were milled with coffee bean miller (Krups F203) and TS of the samples was analyzed.

In first stage, about 0.5 g of sample and 6 ml of 72 % H_2SO_4 was added to tube and the tubes were placed in a water bath at temperature 30 °C. The samples were stirred during the treatment. After the hydrolysis, the acid was diluted to a 4 % concentration by adding 168 ml Millipore-grade water. In second stage the sample tubes were autoclaved (autoclave Melag 23) for 1 h in pressure 1 bar (at 121°C). After autoclaving the samples were

separated using sinter glasses and vacuum flask system. The samples were flushed with hot Millipore-grade water and finally the solutions were diluted in a 1000 ml volume.

Acid-insoluble lignin (Klason lignin) was determined from the precipitate. The sinter glasses with the precipitate were dried at 105 °C for 12 h and weighted. Acid-soluble lignin was determined from the solution using UV-Visible spectrometer (Becman Du-600). Sulfuric acid (4 %) was run as a background. The results were calculated as follows:

$$c = \frac{A}{a \cdot b} \tag{2}$$

where c is concentration of the sample (g/l), A is absorbance, a is absorptivity (110 $\mathrm{lg^{-1}cm^{-1}}$) and b is length of light path (cm). Total amount of lignin was calculated adding the acid-insoluble lignin to the acid-soluble lignin.

During the hydrolysis the polymeric carbohydrates are hydrolyzed into the monomeric forms (Sluiter et al. 2008). Monomeric carbohydrates were analyzed from the solution by high-performance liquid chromatography (HPLC). Fucose was used as a calibration verification standard and arabinose, galactose, glucose, xylose and mannose were used in sets of calibration standards. The samples were filtered (0.2 µm filter) and analyzed using Dionex HCPL system with CarboPac PA1 and Quard PA1 columns (column temperature 30 °C, mobile phase water and 100 mM NaOH, flow rate 0.30 ml/min).

The concentrations of the polymeric carbohydrates ($c_{polymer}$) were calculated from the concentrations of the monomeric carbohydrates ($c_{monomer}$) as follows:

$$c_{polymer} = x \cdot c_{monomer} \tag{3}$$

where x is correction factor, 0.88 for pentoses (xylose and arabinose) and 0.90 for hexoses (glucose, galactose and mannose) (Sluiter et al. 2008).

Weak acid hydrolysis was used to determine the contents of non-structural carbohydrates from the hydrolysate of the hydrothermally pretreated maize silage. Sulfuric acid (72 % 1 ml) and Millipore-grade water (25 ml) was added to the liquid sample (5 ml). The sample were autoclaved in pressure 1 bar for 5 min plus warming and cooling time 11 min. The

sample was diluted (three different dilutions, 1:50, 1:100 and 1:200) and analyzed as the structural carbohydrates.

2.6 Analysis of inhibitors

Furfurals and 5-HMF were analyzed from the hydrolysates of the hydrothermal pretreated maize silages (Amadeo and KXA 7251) by HCPL using Waters 996 photodiode Array detector, Waters 717 plus autosampler, Waters 501 pumps, Phenomenex Gemini C18 kolumn (wave length 280 nm, mobile phase Millipore-grade water and acetonitrile, flow rate 1.0 ml/min).

Phenolic compounds were analyzed also from the hydrolysates. pH of the samples was adjusted to 2 with 1 M sulfuric acid and phenolic compounds were extracted with dichloromethane using liquid-liquid-extraction. After that dichloromethane was evaporated using rotating evaporator (Heidolph WB/VV 2000). The dried samples were dissolved to pyridine and silylated with NO,O-bis(trimethylsilyl)trifluoroacetamine (BSTFA) and trimethylchlorosilane (TMCS). BSTFA and TMCS were added to the samples and the samples were shaked with Stuart SFI flash shaker (660 osc/min). The samples were analyzed with Shimadzu GC-2010 gas chromatograph with DP-1701 column (length 60 m, initial diameter 0.32 mm, film 0.25 µm) to determine the amounts of the phenolic compounds. Helium was used as carrier gas, column temperature used was 290 °C and flow rate used was 2.65 ml/min. Mass spectrometry (Agilent 5973 mass selective detector with HP-5 column: length 30 m, initial diameter 0,25 mm and film 0,25 µm) was used to identify the compounds. Same analyzing conditions as in gas chromatography were used.

3 RESULTS

3.1 Characteristics and methane potentials of maize silage varieties

Methane potentials of eight maize silage varieties were determined. KXA 7251 had the highest methane yield per $VS_{original}$ (408±7 l CH_4/kgVS) and per cultivation area (7780±130 m^3 CH_4/ha). Ronaldino had the highest yield per $WW_{original}$ (92±2 l CH_4/kgWW) (Table 1).

The lowest methane yields per $VS_{original}$ were obtained from KXA 7254 (255±6 l CH_4/kgVS) and Amadeo (287±60 l CH_4/kgVS), which had also the lowest yields per cultivation area (4260±100 and 4350±910 m^3 CH_4/ha). KXA 7254 had the lowest yield per $WW_{original}$ (57±2 l CH_4/kgWW) (Table 1).

Five maize silage varieties (Ronaldino, Cannavaro, Amara, KXA 7211 and KXA 441) had methane yield per VS_{added} over 400 l CH4/kgVS. The highest methane yield per WW_{added} had Ronaldino and KXA 7251 (Figure 2, Table 1).

Table 1. Methane yields per original and added VS and WW as well as cultivation area (±standard error).

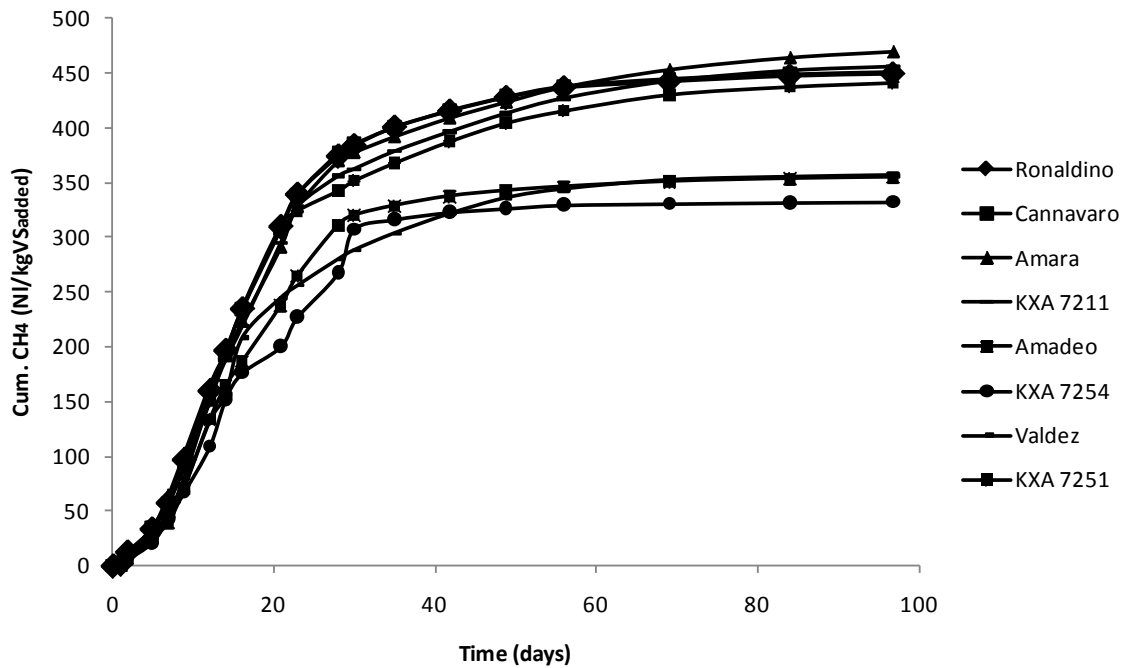| Variety	CH_4 per $VS_{original}$ (l CH_4/ kgVS)	CH_4 per VS_{added} (l CH_4/ kgVS)	CH_4 per $WW_{original}$ (l CH_4/ kgWW)	CH_4 per WW_{added} (l CH_4/ kgWW)	CH_4 per cult. area (m^3 CH_4/ ha)
Ronaldino	356±6	449±7	92±2	95±2	5200±80
Cannavaro	398±37	452±42	78±8	81±8	6680±610
Amara	336±3	470±4	77±1	79±1	6300±60
KXA 7211	345±18	456±24	84±5	87±5	5740±300
Amadeo	287±60	355±74	69±15	70±15	4350±910
KXA 7254	255±6	333±8	57±2	59±2	4260±100
KXA 7251	408±7	441±8	88±2	92±2	7780±130
Valdez	349±7	357±8	75±2	80±2	6930±140

Figure 2. Cumulative CH$_4$ production of maize silage varieties.

Losses during the ensiling were estimated. Valdez had the highest mass losses, but the lowest TS losses. Cannavaro, KXA 7254 and KXA 7251 had also high mass losses (4-5 %). TS losses were highest with Amara, and KXA 7211 and KXA 7254 had also high TS losses (Table 2).

Table 2. Harvesting yields, and mass and TS losses during ensiling

Variety	Harvest yield (tTS)	Mass losses during ensiling (%)	TS losses during ensiling (%)
Ronaldino	15.8	3	21
Cannavaro	18.1	4	15
Amara	20.1	3	29
KXA 7211	17.9	3	26
Amadeo	16.2	2	19
KXA 7254	18.2	4	26
KXA 7251	20.5	4	10
Valdez	21.5	5	5

Condition of the maize silages was evaluated. pH's of the maize silage varieties were between 4.2-4.4 (Table 3). There was some mold growth in top of all maize silage varieties.

In three of the varieties (KXA 7254, KXA 7251 and Valdez) there were moldy parts also inside the silo. The moldy parts were removed.

TS and VS content of the silages were analyzed. Ronaldino had the highest TS content as fresh maize (28 %) and also as silage (23 %). Cannavaro had the lowest TS content as fresh maize (21 %), when Amara had the lowest TS content when comparing the silage varieties (18 %). VS of the silages vary between 17 and 22 % and VS of the fresh maize varieties were not determined (Table 3).

Nitrogen content and SCOD was analyzed. NH_4-N and N_{tot} were analyzed from two varieties, Amadeo and KXA 7251. Amadeo had higher NH_4-N, N_{tot} and protein content than KXA 7251. SCOD was analyzed from all varieties of silages and it varied between 207 (Cannavaro) and 370 mg/gTS (KXA 7254) (Table 3).

Table 3. pH, TS, VS, COD, NH_4-N, N_{tot} and protein content of maize silage varieties and TS of fresh maizes.

Variety	pH	TS (%) (fresh maize)	TS (%) (silage)	VS (%) (silage)	SCOD (mg/gTS) (silage)	NH_4-N (mg/ gTS)	N_{tot} (mg/ gTS)	Protein (mg/ gTS)
Ronaldino	4.3	28	23	21	213	n.d.	n.d.	n.d.
Cannavaro	4.3	21	19	18	207	n.d.	n.d.	n.d.
Amara	4.3	25	18	17	331	n.d.	n.d.	n.d.
KXA 7211	4.4	26	20	19	247	n.d.	n.d.	n.d.
Amadeo	4.3	26	21	20	208	0.51	16.7	102
KXA 7254	4.2	24	19	18	370	n.d.	n.d.	n.d.
KXA 7251	4.2	23	22	21	275	0.46	16.1	98
Valdez	4.2	23	23	22	239	n.d.	n.d.	n.d.

3.2 Properties of hydrothermally pretreated maize silages

Methane potentials of two hydrothermally pretreated maize silage varieties were determined. In the beginning of the assays, the controls started to produce methane faster than the treated silages. Also the solid fractions started faster than assays with both solid fraction and hydrolysate (Figure 3).

Hydrothermally pretreated maize silages produce methane 291-349 l CH_4/kgVS. Controls (untreated maize silages) produced methane 8 % (Amadeo) and 23 % (KXA 7251) more

than the hydrothermally pretreated maize silages. Solid fraction of the hydrothermally pre-treated silages produced 39 % less (Amadeo) and as much (KX 7251) methane as the whole hydrothermally treated silages. Hydrolysates produced no methane under current experimental conditions (Figure 3, Table 4).

Table 4. Methane yields of hydrothermally pretreated (HT) maize silages.

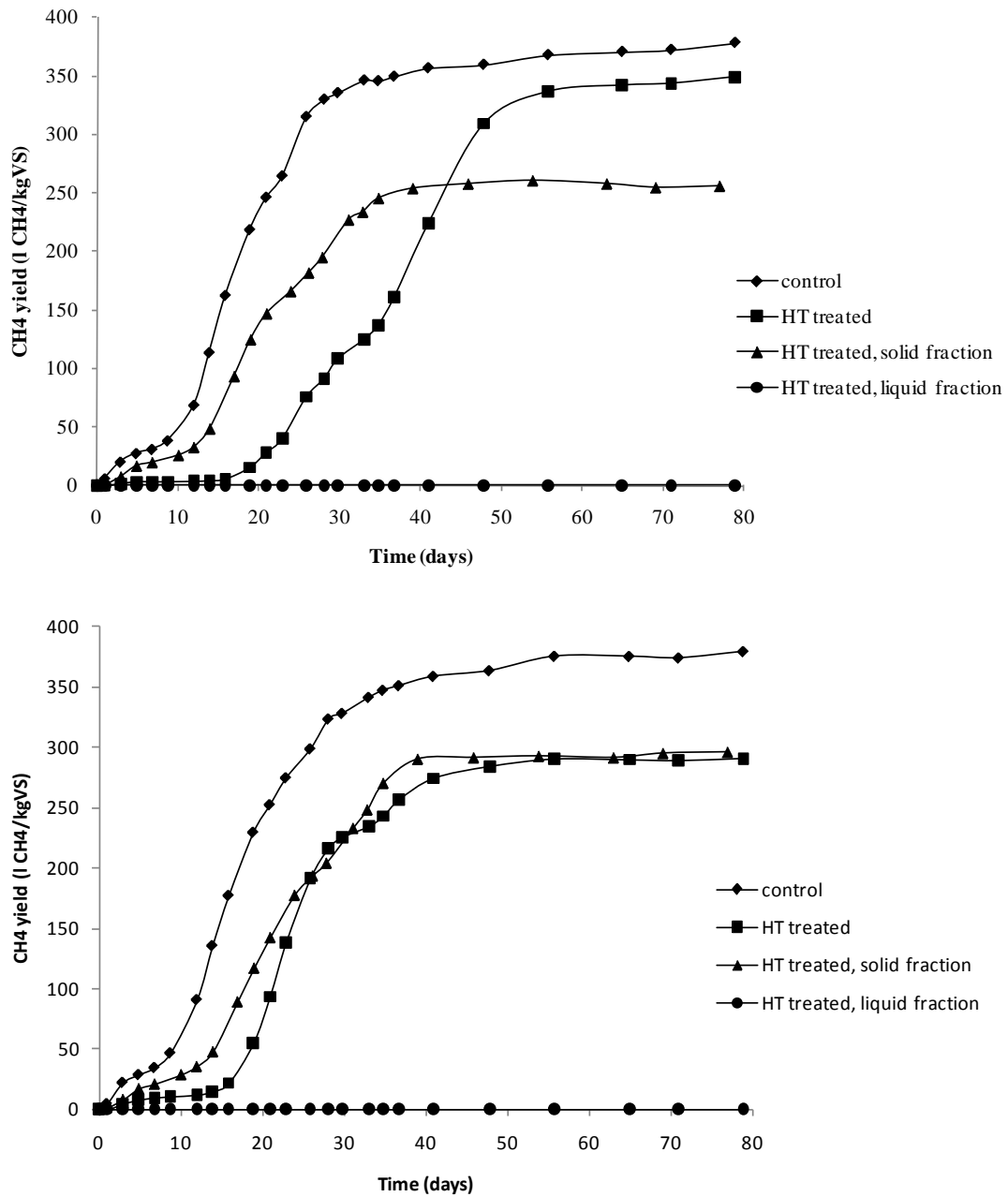| Variety	CH_4 control (untreated) (l CH_4/kgVS)	CH_4 hydrothermal treated (l CH_4/kgVS)	CH_4 HT solid fraction (l CH_4/kgVS)	CH_4 HT liquid fraction (l CH_4/kgVS)
Amadeo	378±10	349±23	251±12	0
KXA 7251	379±8	291±20	297±19	0

Figure 3. Methane yield of hydrothermal treated (HT), untreated (control), solid fraction and liquid fraction of HT Amadeo (up) and KXA 7251 (down).

pHs of hydrothermally pretreated maize silages was analyzed. pHs were between 3.90 and 3.98, when pHs of untreated maize silages were 4.2-4.4.

3.3 Composition of hydrothermally treated and untreated maize silage

Composition of untreated and hydrothermally pretreated maize silage (variety KXA 7251) was determined. Comparing the original maize silage and solid fraction of the hydrother- mally pretreated maize silage, there was more glucose (cellulose), Klason lignin (insoluble lignin) and ash, and less hemicellulose (arabinose, galactose, mannose and xylose) in the pretreated silage (Table 5). In the original maize silage, cellulose content (of TS) was 36.4 % and hemicellulose content was 17.9 %, whereas there was 58.9 % cellulose and 7.2 % hemicellulose in the pretreated maize silage.

Table 5. Structual carbohydrates, Klason lignin, soluble lignin and ash, analyzed from solid fraction of hydrothermally pretreated (HT) maize silage and untreated maize silage.

	Untreated (% TS)	HT (% TS)
Arabinose	2.1	0.2
Galactose	0.9	0.2
Glucose	36.4	58.9
Mannose	14.7	6.6
Xylose	0.2	0.2
Klason lignin	15.1	24.3
Soluble lignin	3.4	3.0
Ash	4.4	5.2
Rest	22.8	1.4
Total	100	100

Amount of non-structural carbohydrates, analyzes from liquid fraction of the hydrothermal pretreated maize silage, was 4.74 g/l. Hemicellulose content was 2.44 g/l whereas cellulose content was 2.30 g/l (Table 6).

Table 6. Soluble sugars (non-structural carbohydrates) analyzed from liquid fraction of hydrothermal pretreated maize silage.

Arabinose	Galactose	Glucose	Mannose	Xylose
		g/l		
0.13	0.12	2.30	2.14	0.05

3.4 Inhibitors

Inhibitory compounds, furans (2-furfural and 5-HMF) and phenols, were analyzed from liquids fractions of two hydrothermally pretreated maize silage varieties. There were more furans in Amadeo than in KXA 7251 (Table 7).

Table 7. Furans analyzed from liquid part of hydrothermal pretreated maize silages.

	2-Furfural	5-HMF	Total furans
	g/100g crop		
Amadeo	0.27	0.10	0.37
KXA 7251	0.17	0.04	0.21

Total amounts of phenols were 0.13 g/100 g crop and 0.12 g/100 g crop (Table 8). The amounts of unknown peaks were calculated using the biggest unknown peaks. Retention times of the unknown peaks were 12.945, 14.180, 19.182 and 13.684.

Table 8. Phenols analyzed from liquid fraction of hydrothermal pretreated maize silages.

Phenolic compound	Amadeo (g/100g crop)	Amadeo (%)	KXA 7251 (g/100g crop)	KXA 7251 (%)
2-Furoic acid	0.0033	2.4	0,0039	2,8
Guaicol	0.0007	0.5	0.0014	1,0
4-Hydroxybenzaldehyde	0.0029	2.1	0.0050	3,6
Vanillin	0.0064	4.7	0.0084	6,2
Syringaldehyde	0.0015	1.1	0.0021	1,5
Vanillic acid	0.0009	0.7	0.0011	0,8
Coumaric acid	0.0022	1.6	0.0030	2,2
Ferulic acid	0.0024	1.7	0.0032	2,3
2,4-dihydroxybenzoic acid	0.0106	7.8	0.0157	11,5
Unknown peaks	0.0624	45.5	0.0569	41,6
Total phenols	0.1254	100	0.1197	100

4 DISCUSSION

4.1 Maize silage varieties

Ensiling appears to make maize more appropriate substrate for anaerobic digestion. The maize silages produced methane as much as 25-50 % more than fresh maizes (also from Piikkiö) in previous studies (Table 9) (Pyykkönen 2009). The methane yields (per VS_{added}) were near to result from previous study, 410 ± 10 l CH_4/kgVS (Bruni et al. 2010).

Ensiling does not seem to affect methane potential of maize negatively, even if losses during the ensiling are taking into account. The methane yields of maize silage varieties per $VS_{original}$ are in agreement with previous studies for fresh maize varieties (Table 9) (Pyykkönen 2009). Hence the ensiling has not changed the methane potential of the varieties significantly, except in case of some varieties. Amara produced almost 30 % more methane as silage than as fresh maize in previous studies (Pyykkönen 2009) when Amadeo produces almost 30 % less methane as silage than as fresh maize in previous studies (Pyykkönen 2009). However, Amadeo has bigger standard error (±60 l CH_4/kgVS) than other maize silages (±3-±37 l CH_4/kgVS), so comparing is not trouble-free. It must also take into account that the fresh maize varieties from previous studies (Pyykkönen 2009) are from cultivation of year 2008 and their batch assays lasted longer, 126-127 days.   In this study maize silages were also frozen before the batch assays, which can affect the results.

KXA 7251 and Amara produced most methane among the maize silage varieties. However, no variety jump out when comparing the results of this study and the results of previous studies (Table 9) (Pyykkönen 2009).

Table 9. Methane yields of maize silage varieties and fresh maize varieties (Pyykkönen 2009) (±standard error).

Variety	CH$_4$ per VS$_{original}$ (silage) (l CH$_4$/kgVS)	CH$_4$ per VS$_{added}$ (silage) (l CH$_4$/kgVS)	CH$_4$ (fresh maize, 2008 [*]) (l CH$_4$/kgVS)
Ronaldino	356±6	449±7	336±7
Cannavaro	398±37	452±42	-
Amara	336±3	470±4	236±18
KXA 7211	345±18	456±24	-
Amadeo	287±60	355±74	370±22
KXA 7254	255±6	333±8	295±11
KXA 7251	408±7	441±8	331±32
Valdez	349±7	357±8	360±8

[*]Pyykkönen 2009

TS losses occur during the ensiling. TS losses were 5-29 %, which, in case of some varieties, are higher than in previous studies (16.1-24.7 %) (McDonald et al. 1990). TS losses during ensiling can be divided to field losses, fermentation losses, effluent losses and oxidation losses (caused by oxygen presence in silo) (McDonald et al. 1990). In this study, field losses are not taking into account and effluent losses are improbable because of silo type, so losses are probably caused by fermentation losses and oxidation losses. Big differences between TS losses of the different varieties can be caused by inaccuracies in measurements.

It seems that ensiling was successful. pH of all the maize silage varieties were between 4.2 and 4.4. When dry matter content of silage is 20-25 %, the critical ph value is 4.20-4.35 (Buxton et al. 2003), so the pHs were near to pH of the successful ensiling. Molds are involved in aerobic deterioration of silages (Buxton et al. 2003). Moldiness in top of maize silages is caused by air between top and maize in silos. There was no mold in other parts of the silages except in some varieties, so anaerobic conditions probably remained well inside the silos.

Ensiling seems to increase NH$_4$-N content of maize. NH$_4$-N results (0.46-0.51 mg/gTS) were bigger than results from previous studies for fresh maize (0.15-0.25 mg/gTS) (Pyykkönen 2009), but smaller than NH$_4$-N content of typical maize silage (0.95 mg/gTS) (McDonald et al. 1990). N$_{tot}$ contents of Amadeo (16.7 mg/gTS) and KXA 7251 (16.1 mg/gTS) corresponds to results from previous studies, 12-19 mg/gTS for fresh maizes

(Pyykkönen 2009) and 15 mg/gTS for typical maize silage (McDonald et al. 1990). Protein contents (98-102 mg/gTS) corresponds to protein contents about 10 % of TS and are near to results from previous studies for fresh maize (7.7-8.2 % of TS) (Bruni et al. 2010a).

Harvesting yields of maize varieties were bigger (15.8-21.5 tTS) in year 2009 than in year 2008 (13.4-18.1 tTS) (Pyykkönen 2009) (Table 7). That affect methane yields per cultivation area, and yields in 2009 (in this study) were bigger than in 2008 (Pyykkönen 2009) (Table 10).

Table 10. Harvesting yields and methane yields per cultivation area (±standard error) 2009 (maizes used in this study) and 2008 (Pyykkönen 2009).

Variety	Harvesting yield (tTS) 2009	Harvesting yield (tTS) 2008 *	CH_4 per cult. area 2009 (silage) (m^3 CH_4/ha)	CH_4 per cult. area 2008 * (fresh maize) (m^3 CH_4/ha)
Ronaldino	15.8	13.4	5200±80	4146
Cannavaro	18.1	-	6680±610	-
Amara	20.1	17.8	6300±60	3887
KXA 7211	17.9	-	5740±300	-
Amadeo	16.2	11.6	4350±910	4001
KXA 7254	18.2	18.1	4260±100	4911
KXA 7251	20.5	15.8	7780±130	4832
Valdez	21.5	16.7	6930±140	5581

*Pyykkönen 2009

4.2 Hydrothermally pretreated silages

Hydrothermally pretreated maizes produced less methane than untreated maizes, which is probably due to inhibitory compounds formed during the pretreatment, appearing especially in the liquid fraction. Hydrolysates produced no methane, and the untreated maizes and solid fraction of the pretreated maizes started produce methane faster than the pretreated maizes (including both solid fraction and hydrolysate). Steam pretreatments include a risk on production of compounds, which are inhibiting methane production (Hendriks & Zeeman 2008). Bauer et al. (2009) found out, that steam pretreatment conditions time 15 min and temperature 180 °C increased methane potential of wheat straw, but conditions time 20 min and temperature 180 °C did not.

Furans are more probably the main reason of the inhibition than phenols. Total furans were between 0.21 and 0.37 g/100 g crop, which is more than Klinke et al. (2002) determined from wet-oxidized wheat straw: when treatment conditions were temperature 195 ℃, time 15 min and pressure 12 bar, total furans were 0.179 g/100 g crop. Total phenols analyzed in this study vary between 0.125 and 0.120 g/100 g crop. Klinke et al. (2002) determined the highest total phenol concentrations, 0.515 g/100 g crop, when the treatment conditions were temperature 195 ℃, 15 min and pressure 12 bar. The lowest total phenol concentrations, 0.148 g/100 g crop, they got when the conditions were temperature 185 ℃, time 10 min and pressure 6 bar. Then the total phenols analyzed in this study were lower than Klinke et al. (2002) got from any pretreatment conditions.

Carboxylic acids were not analyzed in this study, but they are also the probable reason of the inhibition ((Palmquist & Hahn-Hägerdal 2000). It is possible that some furans and phenols have degraded into carboxylic acids. Klinke et al. (2002) found out, that in their study carboxylic acids were the main degradation products apart from carbon dioxide and water.

Hemicellulose degradation occurred during the hydrothermal pretreatment. There was more cellulose, Klason lignin (insoluble lignin) and ash and less hemicellulose in solid fraction of pretreated maize silage than in solid fraction of original maize silage. This is probably caused by partial removal of the hemicellulose: according to Taherzadeh & Karimi (2008), steam pretreatment removes most of the hemicellulose. This causes increase of proportion of the cellulose, the Klason lignin and the ash. Klinke et al. (2002) also found out that wet-oxidation of wheat straw resulted in solid fraction enriched in cellulose

5 CONCLUSIONS

Ensiling seems to be a good preservation method for energy (biogas) maize. Maize silages produced even 25-50 % more methane than fresh maizes in previous studies and even if losses during the ensiling are taking into account, the maize silages produced methane as well as the fresh maizes. Amara and KXA 7251 produced most methane among the maize silage varieties used in this study, but all varieties could be suitable for biogas production. Hydrothermal pretreatment, with conditions used in this study, caused inhibition and decreased the methane yield of the maize silages.

Methane potential of fresh maizes and maize silages from same cultivation should be analyzed to get more reliable results. To get better methane yield from silages, things like preservation additives could be studied. Varieties suited especially to Finland and to biogas production could be developed more, so as energy maize cultivation would be more profitable in Finland. Hydrothermal pretreatment conditions should be optimized: shorter pretreatment time and probably base addition to pretreatment reactor could reduce formation of inhibitory compounds. Carboxylic acids could be significant cause of inhibition and their analysis is needed to get more information from the process.

ACKNOWLEGMENTS

REFERENCES

Bauer A., Bösch P., Friedl A. and Amon T. 2009: Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methanol production. –Journal of Biotechnology 142: 50-55

Bruni E., Jensen A.P., Pedersen E.S. & Angelidaki I. 2010: Anaerobic digestion of maize focusing on variety, harvest time and pretreatment. –Applied energy, in press

Bruni E. Jensen A.P. & Angelidaki I. 2010: Comparative study of mechanical, hydrothermal, chemical and enzymatic treatments of digested biofibers to improve biogas production. –Bioresource Technology, in press

Buxton D.R., Muck R.E. & Harrison J.H. 2003: Silage Science and Technology.

Chen Y., Cheng J. & Creamer K. 2008: Inhibition of anaerobic digestion process: A review. –Bioresource Technology 99: 4044–4064

Egg R.P., Coble C.G. Engler C.R. & Lewis D.H. 1993: Feedstock storage, handling and processing. –Biomass and Bioenergy 5:71-94

Fernandes T.V., Klaasse Bos G.J., Zeeman G., Sanders J.P.M. & van Lier J.B. 2009: Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass. –Bioresource Technology 100: 2575–2579

Hendriks A.T.W.M. & Zeeman G. 2009: Pretreatments to enhance the digestibility of lignocellulosic biomass. Review. –Bioresource Technology 100: 10–18

Kaparaju P. & Felby C. 2010: Characterization of lignin during oxidative and hydrothermal pre-treatment process of wheat and corn stover. –Bioresource Technology 101: 3175–3181

Klinke H.B., Ahring B.K., Schmidt A.S. & Thomsen A.B. 2002: Characterization of degradation products from alkaline wet oxidation of wheat straw. Bioresource Technology 82: 15–26

McDonald P., Henderson A.R. & Heron S.J.E. 1990: The Biochemistry of Silage. Second Edition.

Palmqvist E. & Hahn-Hägerdal B. 2000: Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanism of inhibition. –Bioresource Technology 74: 25–33

Perstorp analytical/ Tecator AB 2005: Determination of nitrogen according to Kjeldahl using block digestion and steam distillation. Tecator application note.

Pyykkönen V. 2009: Biokaasua maissista – lajikkeen vaikutusenergiasaantoon sekä maissin ja lehmän lietelannan yhteiskäsittely biokaasureaktorissa. Master of Science Thesis.

Seppälä M., Pyykkönen V., Laine A. & Rintala J. 2010: Methane production from maize in Finland – screening for different maize varieties and plant parts. Submitted for publication

Zheng M., Li X., Li L., Yang X. & He Y. 2009: Enchancing anaerobic biogasification of corn stover through wet state NaOH pretreatment. –Bioresource Technology 100: 5140–5145