EVALUATION OF FRESH AND PRESERVED HERBACEOUS FIELD CROPS FOR BIOGAS AND ETHANOL PRODUCTION

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To my dear husband, sons and daughter:

“It’s better to know some questions than all of the answers”
~ James Thurber
ABSTRACT

In the future, various forms of bioenergy will be increasingly required to replace fossil energy. Globally, transportation uses almost one third of fossil energy resources, and it is thus of great importance to find ethically, economically, and environmentally viable biofuels in near future. Field-grown biomass, including energy crops and crop residues, are alternatives to supplement other non-food biofuel raw materials. The aim of this work was to evaluate the potential of five crops, maize (*Zea mays* L.), fiber hemp (*Cannabis sativa* L.), faba bean (*Vicia faba* L.), white lupin (*Lupinus albus* L.), and Jerusalem artichoke (*Helianthus tuberosus* L.) cultivated in boreal conditions as raw materials for methane and ethanol.

Climate, cultivation requirements, chemical composition, and recalcitrance are some of the parameters to be considered when choosing energy crops for cultivation and for efficient conversion into biofuels. Among the studied crops, protein-rich legumes (faba bean and white lupin) were attractive options for methane, while hemp and Jerusalem artichoke had high theoretical potential for ethanol. Maize was, however, equally suitable for production of both energy carriers. Preservation of crop materials is essential to preserve and supply biomass material throughout the year. Preservation can be also considered as a mild pretreatment prior to biofuel production. Ensiling was conducted on maize, hemp, and faba bean in this work and additionally hemp was preserved in alkali conditions. Ensiling was found to be most beneficial for hemp when converted to methane, increasing the methane yield by more than 50%, whereas preservation with urea increased the energy yield of hemp as ethanol by 39%. Maize, with a high content of water-soluble carbohydrates (20% of DM), required an acid additive in order to preserve the sugars. Interestingly, hydrothermal pretreatment for maize and hemp prior to methane production was less efficient than ensiling. Enzymatic hydrolysis of faba bean increased after ensiling, but methane yields were reduced.

Ensiling had a positive effect also when pectin was hydrolyzed from hemp by pectinases. It was suggested that acids, such as oxalic acid, present in crops degraded pectic compounds synergistically with polygalacturonase and weakened the lignocellulosic structure. Acids, used or formed during preservation, may also increase the access of pectinases by chelating calcium from the structure of pectins. However, the different structures, compositions, and reactions in treatments varied between crops and make it fascinating to seek deeper knowledge on all the features affecting the conversion processes and to further improve the conversion of biomass to biofuels.
ACKNOWLEDGEMENTS

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by the Roman numerals I-IV:


**Contribution of the author to papers I to IV:**

The author planned the study together with the other authors and performed most of the experimental work. She had the main responsibility of interpreting the results other than data from cultivation and she was the main and the corresponding author of the paper.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>AD</td>
<td>Anaerobic digestion</td>
</tr>
<tr>
<td>b.d.l.</td>
<td>Below detection limit</td>
</tr>
<tr>
<td>CBH</td>
<td>Cellobiohydrolase</td>
</tr>
<tr>
<td>CBP</td>
<td>Consolidated bioprocessing</td>
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<tr>
<td>DM</td>
<td>Dry matter</td>
</tr>
<tr>
<td>DNS</td>
<td>Dinitrosalisylic acid</td>
</tr>
<tr>
<td>EG</td>
<td>Endoglucanase</td>
</tr>
<tr>
<td>EU</td>
<td>European Union</td>
</tr>
<tr>
<td>FA</td>
<td>Formic acid</td>
</tr>
<tr>
<td>FOS</td>
<td>Fructo-oligosaccharide</td>
</tr>
<tr>
<td>FPU</td>
<td>Filter paper unit</td>
</tr>
<tr>
<td>Gal-A</td>
<td>Galacturonic acid</td>
</tr>
<tr>
<td>GC</td>
<td>Gas chromatography</td>
</tr>
<tr>
<td>GHG</td>
<td>Greenhouse gas</td>
</tr>
<tr>
<td>ha</td>
<td>Hectare</td>
</tr>
<tr>
<td>HHV</td>
<td>Higher heating value</td>
</tr>
<tr>
<td>HMF</td>
<td>Hydroxy-methyl-furfural</td>
</tr>
<tr>
<td>HPAEC-PAD</td>
<td>High-performance anion-exchange chromatography with pulse amperometric detection</td>
</tr>
<tr>
<td>LAP</td>
<td>Laboratory analysis procedure</td>
</tr>
<tr>
<td>LHV</td>
<td>Lower heating value</td>
</tr>
<tr>
<td>ML</td>
<td>Million liters</td>
</tr>
<tr>
<td>MWel</td>
<td>Mega Watts as electricity</td>
</tr>
<tr>
<td>n.d.</td>
<td>Not determined</td>
</tr>
<tr>
<td>Ndm³/m³</td>
<td>Normal cubic desimeter/</td>
</tr>
<tr>
<td>nkat</td>
<td>Nanokatals</td>
</tr>
<tr>
<td>NPK</td>
<td>Nitrogen-phosphorus-potassium</td>
</tr>
<tr>
<td>NREL</td>
<td>National Renewable Energy Laboratory</td>
</tr>
<tr>
<td>SE</td>
<td>Steam explosion</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SSF</td>
<td>Simultaneous saccharification and fermentation</td>
</tr>
<tr>
<td>t</td>
<td>Tonne</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>VS</td>
<td>Volatile solids</td>
</tr>
<tr>
<td>WSC</td>
<td>Water-soluble carbohydrates</td>
</tr>
</tbody>
</table>
1 INTRODUCTION

Development of biofuels in the transport sector has a strategic impact on key environmental issues, such as climate change and global warming, in compliance with the Kyoto commitment (Kyoto Protocol 1998). European union (EU) has set a target of increasing the utilization of transportation biofuels to 10% by 2020 (European Parliament 2009). Biofuels will also enhance the security of energy supply, thus reducing the fossil energy dependency and help sustainable rural economic development. Europe holds a leading position in the production of biodiesel, whereas the production of ethanol is still low compared to North America and Brazil (European biofuels 2012). Besides ethanol, methane refined from biogas is an important alternative to be used as transportation fuel. The use of crops cultivated for energy production and utilization of agricultural residues needs to be seriously considered for production of biofuels, ethanol, or methane if national self-sufficiency is required (Carr and Hettenhaus 2009). However, cultivation of energy crops and food or feed has to be in balance globally (FAO 2009).

Each country has different cultivation conditions, possibilities, and aims in their agriculture. The climate, especially, strongly dictates the alternative crops to be grown (Galbe et al. 2005). The most important issue in biofuel production is the sustainability throughout the process (European Parliament 2009). Among fulfilling effective sustainability criteria, the production costs (e.g. fertilization and management in the growing season) and biomass yields of crops for biofuels are among the most important issues when choosing the crop. However, the suitability of crops for efficient energy use depends also on the chemical structure of the feedstock at the time of harvesting (Amon et al. 2007a). To enable the use of crop raw material throughout the year, the preservation and storage conditions and their effects on the material composition are essential issues of concern (Seppälä et al. 2008, Digman et al. 2010). The processability and reactions in further treatments are important issues, as well. The biological fermentation processes of ethanol and methane favor slightly different components due to the ability of the microorganisms to convert the substrates. Thus, the same raw materials, preservation methods, or pretreatments may not be most optimal for both methane and ethanol (Petersson et al. 2007). Field biomass, its production requirements, and products are shown in Figure 1.
Figure 1  Requirements for the cultivation, biomass characteristic and biomass yields of energy field crops and potential bioenergy products from field crops.

1.1 BIOFUELS

Biofuels in this work were limited to biologically fermentable ethanol and methane. Biofuels are classified as first- and second-generation biofuels by the used raw material, namely starch and sugar-based substrates for first generation, and lignocellulosic (straws and whole crops) materials for second generation (Sims et al. 2009). Biomass often also includes municipal wastes from both groups (Gray et al. 2006). In this work studied biofuels are considered as second-generation biofuels due to the lignocellulosic field biomasses used as raw materials. Both studied energy carriers are known to be suitable transportation fuels with good properties but also having some disadvantages (Table 1) (AFDC 2012).
1.1.1 ETHANOL

Ethanol, or ethyl alcohol, C₂H₅OH is a primary alcohol used widely for beverages and as a solvent. During the last few decades, ethanol has continuously increased its role as a biofuel for transportation use (European biofuels 2012). Liquid ethanol has many advantages as 100% fuel or as an additive mixed with fossil gasoline. Partial replacement of gasoline by ethanol in mixtures up to 10% is presently used in Finland, e.g., during the transition period from fossil fuels to a larger share of biofuels (European Parliament 2009). Ethanol has high octane and heat of vaporization, low toxicity, and photochemical reactivity (Table 1) (Rutz and Janssen 2007). Additionally, ethanol reduces exhaust emissions, ozone formation, and smog, contrary to fossil fuels. Starch from wheat (Triticum aestivum L.) and maize (Zea mays L.) and sucrose from sugar cane (Saccharum officinarum L.) are substrates for most of the fuel ethanol (Hahn-Hägerdal et al. 2006). Raw materials used for first-generation ethanol are easily converted to sugars and further fermented into ethanol. Global first-generation bioethanol production in 2009 has been estimated at 73954 ML (436 MWh). The United States is the leading producer with 40130 ML (237 MWh), representing 54% of production, while Brazil produced 24900 ML (147 MWh), representing 34%. The EU-27 produced 3703 ML (22 MWh), which ranks third (with 5% of the market) behind the two major producers (European Biofuels 2012).

However, the environmental impact of first-generation bioethanol is contradictory, and the raw materials used compete with food production and have raised questions (Hahn-Hägerdal et al. 2006). Numerous calculations of greenhouse gas (GHG) emissions and other environmental impacts of biofuels from different raw materials have been published (Doornbosch and Steenblik 2007, Rutz and Janssen 2007, Mikkola and Ahokas 2009, and UNEP 2009). Figure 2 shows some promising figures for second-generation bioethanol produced from agricultural residues (UNEP 2009). However, sugar cane (mainly in Brazil) clearly has the most beneficial GHG saving measures as a substrate for bioethanol.
Second-generation lignocellulosic raw materials hold promises but depend on technological breakthroughs (Hahn-Hägerdal et al. 2006). Lignocellulose-based bioethanol is one the main future targets for development; however, the process still faces economic challenges as far as the production of a maximum amount of ethanol with a minimum energy input; environmental issues must be carefully considered as well (Hahn-Hägerdal et al. 2006). While the first-generation bioethanol substrates, such as maize, wheat, or food industry wastes, are easily converted to ethanol with traditional commercial processes (European Biofuels 2012, St1 2012), the lignocellulosic materials require pretreatment steps and more complex enzyme systems to achieve efficient conversion of raw materials (Galbe et al. 2005). Options include integrating cellulosic ethanol production with starch-based ethanol using the whole crop or developing biorefinery concepts using all the byproducts and residues from the ethanol process (Hahn-Hägerdal et al. 2006). Today in Europe and North America, some pilot or demonstration plants using e.g. wheat straw, maize stover, spruce (Picea abies), and giant reed (Arundo donax) as raw materials are running or are being commissioned, although market incentives for industrial production are still needed (Chemtex 2012, European Biofuels 2012, Inbicon 2012). In Finland the legislation of alcohol production restricts the possibilities of farm-scale ethanol plants (Finlex 2012). In Finland, the approach of distributed small-scale ethanol production units that apply a variety of biowastes as raw materials has been introduced (Heinimö and Alakangas 2011, St1 2012).
Methane is produced by microorganisms in anaerobic conditions from a range of organic materials. Favorable environmental conditions exist, e.g. in swamps, permafrost, seabed sediments, landfills, and rumen (Boyle 1990). Methane is also a valuable energy carrier that releases heat when burned. Natural gas can be nearly pure methane and is already widely used as an energy carrier for heat, electricity, and transportation fuels. The main applications for methane are in the production of combined heat and power (CHP) units or in heating by burning the gas (Weiland 2006). Methane is, however, well suited as a transportation fuel due to its high octane value and high energy potential (Table 1) (Wheeler et al. 2001, LBS 2002), although the gaseous form is a restricting feature in the highly liquid-based vehicle fuel markets. The storage and distribution of methane, being a gas, is limited without a comprehensive natural gas grid and widely available distribution. Methane is often stored and used as compressed gas, but liquefaction prior to storage and utilization is also commercially used (Deublein and Steinhauser 2008). Liquefying methane reduces its volume by 60% more than the volume reduction achieved by compressing it. Due to the energy efficiency and taxation benefits, methane is clearly a cheaper fuel option in Finland at the moment (2012). One equivalent liter of biogas costs 0.9 €, while gasoline (E95) is about 1.6 € (Gasum 2012).

Anaerobic digestion (AD) of sewage sludge is being used as a technique to degrade organic components present in the sludge. In farming, manure from domestic animals is also used as raw material for AD, from which the residue can be used as fertilizer. AD has been applied as a way to treat the manure for enriching nitrogen and other useful nutrients (in dry matter) as well as destroying pathogens and thus improving the quality of the manure as fertilizer (Arthurson 2009, Holm-Nielsen et al. 2009). Due to the increasing demand for

### Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ethanol</th>
<th>Methane (98%)</th>
<th>Gasoline</th>
<th>Diesel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical formula</td>
<td>C₂H₅OH</td>
<td>CH₄</td>
<td>C₄-C₁₂</td>
<td>C₆-C₂₅</td>
</tr>
<tr>
<td>Density kg L⁻¹ or kg m⁻³</td>
<td>0.79</td>
<td>0.72</td>
<td>0.75</td>
<td>0.83</td>
</tr>
<tr>
<td>Octane (RON)</td>
<td>108.6</td>
<td>120.0</td>
<td>95.0-99.0</td>
<td>15.0-25.0</td>
</tr>
<tr>
<td>LHV, MJ kg⁻¹</td>
<td>26.8</td>
<td>49.2</td>
<td>43.5</td>
<td>42.8</td>
</tr>
<tr>
<td>LHV, kWh dm⁻³</td>
<td>5.9</td>
<td>10.0</td>
<td>9.0</td>
<td>10.0</td>
</tr>
</tbody>
</table>

LHV = Lower heating value

### 1.1.2 METHANE

Methane gas, CH₄, is 22 times stronger as a greenhouse gas compared with CO₂ (Forster et al. 2007). Methane is produced by microorganisms in anaerobic conditions from a range of organic materials. Favorable environmental conditions exist, e.g. in swamps, permafrost, seabed sediments, landfills, and rumen (Boyle 1990). Methane is also a valuable energy carrier that releases heat when burned. Natural gas can be nearly pure methane and is already widely used as an energy carrier for heat, electricity, and transportation fuels. The main applications for methane are in the production of combined heat and power (CHP) units or in heating by burning the gas (Weiland 2006). Methane is, however, well suited as a transportation fuel due to its high octane value and high energy potential (Table 1) (Wheeler et al. 2001, LBS 2002), although the gaseous form is a restricting feature in the highly liquid-based vehicle fuel markets. The storage and distribution of methane, being a gas, is limited without a comprehensive natural gas grid and widely available distribution. Methane is often stored and used as compressed gas, but liquefaction prior to storage and utilization is also commercially used (Deublein and Steinhauser 2008). Liquefying methane reduces its volume by 60% more than the volume reduction achieved by compressing it. Due to the energy efficiency and taxation benefits, methane is clearly a cheaper fuel option in Finland at the moment (2012). One equivalent liter of biogas costs 0.9 €, while gasoline (E95) is about 1.6 € (Gasum 2012).

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biofuels, methane has become a product intended particularly as an energy carrier, instead of only an end product from waste treatments (Deublein and Steinhauser 2008). Methane also has an impact on local farm-based energy production plants, which could utilize various side streams or waste materials produced in farms or industries nearby (Weiland 2006).

Methane can be considered as a second-generation biofuel because of the range of raw materials from food waste to recalcitrant plant materials that can be used for the production (Weiland 2006). AD of biomass to methane provides a promising, alternative approach to utilize all carbohydrates, including the pentoses, as well as the proteins (Bauer et al. 2009). The main benefits of the AD process are the flexibility of the process, the ability to convert all biologically degradable components, recycling of nutrients and the lack of sensitivity for contaminations; it also doesn’t need added enzymes. On the other hand, the process is slow, and some of the recalcitrant components may not be utilized in spite of the prolonged processing time (Lehtomäki et al. 2007). The hydrolysis and fermentation time in AD is considerably longer (30 days) as compared to the hydrolysis experiments with ethanol fermentation (2 or 3 days). However, the most effective digestion time of 5 to 10 days has been considered adequate (e.g. Neureiter et al. 2005). This, however, depends on the recalcitrance properties of the raw material and the dry matter (DM) loading in the process.

Biogas production is already well established, comprising large centralized plants and small farm-scale digestors. The smallest biogas plants are used in family houses in less developed countries (Lebofa and Huba 2011) and do not require high investments. However, e.g. legislation increases the building costs of biogas digestors in the EU, e.g., due to strict safety regulations (Steinmüller 2011). Germany is the leading European biogas producer and alone accounts for half of European biogas-based primary energy output (50.5% in 2009) and half of biogas-sourced electricity output (49.9% in 2009) (Eurobservér 2010). The total number of biogas plants in Germany was expected to be 5700 in 2010, producing 2130 MWel (de Graaf and Fendler 2010). Other important biogas producers are the United Kingdom (mainly landfill gas) and Italy (Eurobservér 2010). Along with manure energy crops, whole crop maize and grass have been the main raw materials (41% in 2008) used for biogas e.g. in Germany (de Graaf and Fendler 2010). Mixture of Timothy and clover (Phleum pratense-Trifolium) and reed canary grass (Phalaris arundinacea), for example, have been found to be potential substrates for methane production in boreal conditions (Lehtomäki et al. 2008, Seppälä et al. 2009). Produced biogas is utilized mainly to heat and to generate electricity, but the use as a transportation fuel is a recognized alternative with increasing interest (NSCA 2006, European Biofuels 2012).
1.2 LIGNOCELLULOSIC SUBSTRATES

The use of wastes for energy production is economically and environmentally beneficial. A part of biological wastes, such as municipal food waste, contains easily degradable carbohydrates, but the available residues and wastes may also consist of more complex lignocellulosic materials, such as paper waste, leaves or maize cob (Chester and Martin 2008). Besides municipal wastes, lignocellulosic agricultural wastes, such as corn stover or straw, have already become widely used substrates for methane production (Weiland 2006). Lignocellulosic materials have for decades been studied as potential feedstocks for ethanol production, and cellulosic ethanol is soon expected to conquer the market place, mainly in Europe and North America (European Biofuels 2012).

Along with lignocellulosic wastes, selected energy crops can become a source of supplementing raw materials for biofuels. The highest energy potential of crops depends on various parameters, including growing conditions and the type of energy carrier to be produced (McKendry 2002). In this work the studied energy crops were cultivated within the Sustainable Energy program (SusEn) funded by the Academy of Finland. Crops chosen for field trials were uncommon in Finnish conditions but expected to produce high biomass yields (e.g. Stoddard et al. 2008, Stoddard et al. 2010, Santanen et al. 2011a). Due to the competition for available land used for production of food or feed, however, the crops cultivated for biofuel use should have certain critical attributes (UNEP 2009). The energy crops should have moderate requirements concerning soil and fertilization and still produce high biomass yield with a minimum need of weeding (McKendry 2002). High tolerance for pests, diseases, frost, drought, or excess of water enables cultivation in areas not suited for more demanding food crops (McKendry 2002). A maximal benefit of the land area could be obtained when the crop would be primarily used for production of food and secondarily as a source of biomass residue for biofuels.

The chemical composition of the crop would preferably be low in lignin and high in carbohydrates for sugar-platform-based biofuel (ethanol) production (as reviewed, e.g., by Mosier et al. 2005); alternatively, high protein content is essential for methane conversion (Amon et al. 2007a, Amon et al. 2007b). Chemical composition changes as the crop matures, which has an effect on methane yields as reviewed by Lehtomäki et al. (2008). Naturally, the ethanol yield is affected by the amount of fermentable sugars and the conversion of polymeric carbohydrates—i.e. lignification and proportions of various plant (anatomical) fractions which are dependent on the maturity (Pordesimo et al. 2005). The impact of harvesting time was not considered in this study, and crops were harvested at the highest biomass yield stage. Jerusalem artichoke was harvested before storage carbohydrates were assumed to be transferred to tubers (Slimestad et al. 2010).
The complex structure of lignocelluloses, the expected energy yields of various lignocellulosic materials, and the potential raw material options for either bioethanol or methane production investigated in this work are introduced below.

1.2.1 STRUCTURE OF LIGNOCELLULOSIC SUBSTRATES

The structure and the share of distinct cells with different compositions of cell walls in the plant restrict the microbial degradation differently (Raven et al. 2007), which leads to variations in conversion rates of biomass to end products and the need for optimization of pretreatments between crops. This emphasizes the importance of understanding the differences of the various crops and their dissimilar conversion efficiencies in the biofuel processes.

The recalcitrance of most lignocellulosic crops and agricultural residues is basically caused by the matrix of complex components present in the cell walls and in the middle lamellae (Cosgrove 2005). These components, mainly cellulose, hemicelluloses, pectin, and lignin, are chemically and physically interlinked to each other and together generate the recalcitrant structure of lignocelluloses, as reviewed by Taherzadeh and Karimi (2008). In addition, each individual component has its own complicated structure (e.g. CCRC 2012). Especially recalcitrant is the crystalline structure of cellulose (Bayer et al. 1998). However, the polymeric components and the cell wall structure protect and determine the rigidity of the plant. Besides structural carbohydrates and lignin, the crops contain various quantities of non-structural, water-soluble carbohydrates (WSC), such as starch, fructose, glucose, and saccharose (Chen et al. 2007a). Additionally, most crops contain low amounts of inorganic compounds, extractives, fats, and proteins varying from one substrate to another (Templeton et al. 2009). All these components and their fractions in the raw material have an effect on the potential biofuel yields. The most abundant components, cellulose, hemicelluloses, and lignin, are introduced in more detail below, along with pectin and WSC.

Cell wall structure of lignocellulosic substrates

Mature vascular plants contain several differentiated cell types, which are the building blocks of all the plant materials (Harris and Stone 2008). Cell walls surround and protect the protoplasts and give strength to the stem. A schematic picture of the plant cell wall is shown in Figure 3 (Achyuthan et al. 2010). The structure of the polysaccharide-rich cell walls varies from thin-walled parenchyma cells to thick-walled sclerenchyma cells (Dickison 2000). As the crop matures, the contents and structure of the cell wall change. In spite of primary cell wall in growing cells, mature cells often produce secondary cell walls, and their cell walls are more lignified than the immature cells (Harris and
Figure 3: Illustration of a plant cell wall. The various features of the plant cell wall described above are shown including the relative thickness of the various layers and the relative abundance and specific localization of the various cell wall components, such as pectin, cellulose, hemicellulose, lignin and protein. (Achyuthan et al. 2010).

Stone 2008). Secondary cell walls develop between the plasma membrane and primary wall and are divided into three layers (Figure 3), which account for most of the total biomass (Cosgrove 2005, CCRC 2012). The main components of the cell walls are cellulose, hemicelluloses, pectins, and lignin (Mohnen et al. 2008). The middle lamella, located between the cells, consists of mainly pectic compounds, proteins, and lignin (Dickison 2000).

**Cellulose**

Because cellulose is the most abundant compound in most lignocellulosic substrates, the structure and its capacity to be degraded by enzymes have been intensively studied by many, e.g., O’Sullivan (1996) and Brown (1999) during the last few decades. Cellulose is comprised of unbranched β-1,4-linked D-glucans, which are spontaneously bundled to form 3-5-nm-wide microfibrils (Wyman et al. 2004). These crystalline ribbons are mechanically strong, insoluble in water, and highly resistant to enzymatic attacks (Wyman 1996). Long cellulose chains are attached to each other by hydrogen bonds and Van der Waals forces, giving a structural bias to the cell wall as reviewed by Cosgrove (2005) and Perez et al. (2002). Most of cellulose is in crystalline form, while the rest is amorphous, the ratio depending on the plant material (Bayer et al. 1998).
It has been shown that cellulolytic enzymes readily degrade the more accessible amorphous parts, but the hydrolysis rate decreases dramatically when attacking crystalline cellulose (Fan et al. 1980). Several studies, reviewed by Taherzadeh and Karimi (2008) have shown that although the crystallinity is an important factor in the digestibility of cellulose and overall hydrolysis of lignocelluloses, it does not always correlate with an increasing hydrolysis rate. Another important aim when enhancing the accessibility of enzymes is to increase the surface area of the substrate, which often means, in lignocelluloses, the removal of other structural components, such as lignin or hemicelluloses, as reviewed by Mosier et al. (2005). However, it has been observed by Fan et al. (1980) that surface area is not the main limiting factor of cellulose hydrolysis; rather, the primary difficulty is in accessing and attacking the crystalline regions.

**Hemicelluloses**

Hemicelluloses are a heterogeneous group of polymers representing, in general, 15–35% of plant biomass and containing both pentoses (β-D-xylose, α-L-arabinose) and hexoses (β-D-mannose, β-D-glucose, α-D-galactose) (Wyman et al. 2004). Other sugars, such as α-L-rhamnose and α-L-fucose, may also be present in small amounts. The hydroxyl groups of sugars can be partially substituted with acetyl groups (Girio et al. 2010). Hemicelluloses are generally classified according to the main sugar residue in the backbone, e.g., xylans, mannan, and glucans, with xylans and mannan being the most prevalent (Aspinall 1970). Depending on the plant species, developmental stage, and tissue type, various subclasses of hemicellulose may be found, including glucuronoxylans, arabinoxylans, linear mannan, glucomannan, galactomannan, galactoglucomannan, b-glucan, and xyloglucan (Wyman et al. 2004). Xylose is the most common hemicelluloses-derived monosaccharide in energy crops and agricultural residues, and the term “xylan” is a catchall for polysaccharides that have a β-(1→4)-D-xylopyranose backbone with a variety of side groups (Aspinall 1980). Xylans function primarily by forming cross-links between the other cell wall components, such as cellulose, lignin, other hemicelluloses, and pectin (Cosgrove 2005). This interaction is carried out by hydrogen bonding to the other polysaccharides and by covalent linkages through the arabinofuranosyl side chains to the ferulic and coumaric acids present in lignin (Wyman et al. 2004).
Pectins

Pectin is a common constituent of fruit wastes or in other residues of the food industry, such as those from sugar beets (*Beta vulgaris* L.), but pectin may be present in fibrous herbaceous plants, as well (reviewed by Voragen et al. 2009). Pectin is composed mainly of galacturonic acid but contains side chains, probably covalently linked together (Schols and Voragen 1996). The complex pectins vary widely and are divided into three classes, homogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II as reviewed by Cosgrove (2005). The side chains of the pectin consist of L-rhamnose, arabinose, galactose, and xylose. Xylogalacturonans, for example, are modified homogalacturonans by the addition of xylose branches (Cosgrove 2005). Neutral arabinans and arabinogalactans are also linked to the acidic pectins, and it has been proposed that they promote cell wall flexibility (Jones et al. 2003) and that they bind to the surface of cellulose (Zykwinska et al. 2005). In the characteristic pectin structure—the ‘egg box-model’ introduced by Grant et al. (1973)—the calcium (Ca$^{2+}$) ions are involved in the cross-linking mechanism of polygalacturonic acids (Figure 4). Part of the pectins may be strongly bound with hemicelluloses, cellulose, and lignin (Cosgrove 2005). Pectins function as a matrix, providing cell wall porosity, water and ion retention, cell-to-cell adhesion, cell expansion, and defense, as well as glue between cells in the middle lamella (Carpita and Gibeaut 1993, Wyman et al. 2004, Cosgrove 2005).

Figure 4  Schematic picture of homogalacturonans ionically cross-linked by calcium (Vincken et al. 2003)
**Water-soluble carbohydrates**

Annual biomass crops, especially, contain variable amounts of carbohydrates, which are easily soluble in water and not bound to the solid structure (Chen et al. 2007a). These ‘water extractives’ or WSC may comprise up to 27% of the DM in sweet sorghum (*Sorghum bicolor*) or whole crop maize (Almodares et al. 2009, Chen et al., 2007a). WSC contain mostly hexoses: fructose, glucose, and disaccharides, mainly saccharose (Chen et al. 2007a). Inulin (β 2,1 fructose) and starch (α 1,4 glucose) are easily soluble storage polysaccharides (Carpita et al. 1989). The severe pretreatments required to open up the structure of recalcitrant lignocellulosic substrates may destroy the easily soluble carbohydrates. Especially, fructose is readily degraded by heat, acids, or bases into various degradation products, carboxylic acids and alcohols (Shaw et al. 1968, Nguyen et al. 2009). Optimization of pretreatments is thus necessary to avoid the loss of structural carbohydrates in raw materials containing high amounts of readily soluble components.

**Lignin**

Lignin is the least biodegradable polymer in lignocelluloses and is usually removed in the processing or left as a residue. The heat value (higher heating value) of lignin has been found to be 23-25 MJ Kg⁻¹, which is higher than cellulose (18.6 MJ Kg⁻¹), for instance; therefore, it has a higher value as a bioenergy source (Baker 1982). Contrary to polysaccharides—cellulose, hemicelluloses, and pectin—lignin is a complex water-insoluble aromatic polymer consisting of phenylpropane units linked into a three-dimensional structure. In lignocellulosic materials, the role of lignin is to confer structural support and to resist microbial attacks and oxidative stress (Perez et al. 2002). Lignin is strongly responsible for the recalcitrance of lignocellulosic materials (Forbes and Watson 1992). Eventually, linkages between cellulose, hemicelluloses, and pectin strengthen the rigid structure and may form a barrier to the access of enzymes to the carbohydrate polymers (Eriksson et al. 1980, Wyman et al. 2004).

### 1.2.2 ENERGY POTENTIALS OF FIELD CROPS

Several studies concerning ethanol and methane yields from various lignocellulosic substrates have been executed in recent years (*e.g.*, Ballesteros et al. 2006, Amon et al. 2007a, Peterson et al. 2007, Lehtomäki et al. 2008, Frigon and Quiot 2010). The ethanol yields from untreated crops are not usually available, and different pretreatments that alter the composition complicate the comparison between raw materials. Theoretical ethanol yields can, however, be calculated from the composition of crops (EERE 2012). For the widely considered cellulosic ethanol substrates corn stover, wheat straw, reed canary
grass, and switchgrass (Panicum virgatum), the ethanol yields (based on theoretical ethanol yields from all identified carbohydrates) are 428, 363, 304, and 403 (L t⁻¹ DM), respectively. In fresh maize, for instance, the real ethanol yield obtained was about 30% of the theoretical ethanol yield (Oleskowicz-Popiel et al. 2011). Comparison of methane yields is easier because the raw materials are often used as fresh or ensiled. The methane yields have been found to be 195 and 390 m³ t⁻¹ VS (volatile solids) for straw and corn stover silage, respectively (Moller et al. 2004, Amon et al. 2007a). The yield of methane from reed canary grass has been observed to vary from 253-351 m³ t⁻¹ VS (Seppälä et al. 2009), while the methane yield from grass varied from 300-430 m³ t⁻¹ VS depending on the amount of cuttings per year (Lehtomäki et al. 2008).

1.2.3 INTRODUCTION OF THE STUDIED CROPS

Crops used in this work were chosen for several reasons, but the main reason was promising biomass yields from the field experiments (Stoddard et al. 2008, Stoddard et al. 2010, Santanen et al. 2011b).

Maize

In Central Europe, the predominant crop for biogas production is maize (Zea mays L.), usually used as a whole crop. Maize is considered to produce the highest yield (20-30 t DM ha⁻¹) of the field crops grown in Europe (Amon et al. 2007a.) As maize is primarily grown for food and feed, its use as an energy source has been considered questionable both ethically and economically, as it potentially could add inflationary pressure on food prices (Kohl and Ghazouls 2008). While the use of maize grains for fuel production is ethically arguable, the use of the residue, i.e. the corn stover, attracts less criticism for energy production, as long as some residues are left in the field to return organic matter and nutrients to the soil and to prevent soil erosion (Blanco-Canqui and Lal 2007). A further option is to use the whole fresh or ensiled crop for ethanol production. Conversion of the whole crop maize to ethanol requires, however, further technological development and energy input (Sassner 2008). In this work, whole crop maize was used as a thoroughly studied European reference crop for energy production in boreal conditions.

Maize is a monocotyledonous plant in which the stem contains a large amount of vascular bundles scattered throughout the tissue. Around the vascular bundles, thick-walled sclerenchyma cells protect the vascular cells, giving strength to the stem, while the thin low-lignified parenchyma cells are the most abundant, forming the bulk of the stem (Ding and Himmel 2008). A schematic
picture of the cross section of maize stem is shown in Figure 5 (Armstrong 2012).

Figure 5  Cross section of maize (monocot) stems (Armstrong 2012).

In addition to the stem, leaves form a large part of maize biomass. The maturity of the plant determines the amount of biomass in the cobs. The chemical composition of the overall maize feedstock depends on whether the cob is separated from the residue (corn stover) or whether maize is used as a whole crop. Also, the size and the maturity of the cob, as well as the species and the harvesting time of the whole crop, have an impact on the chemical composition. The amounts of the main components in maize are listed in Table 2 (Thammasouk et al. 1997, Chen et al. 2007a, Templeton et al. 2009).

Table 2  Chemical composition of maize species reported in previous studies expressed as % of DM. (Thammasouk et al. 1997, Chen et al. 2007a, Templeton et al. 2009).

<table>
<thead>
<tr>
<th>Glucan</th>
<th>Xylan</th>
<th>Galactan</th>
<th>Arabinan</th>
<th>Mannan</th>
<th>Lignin$^1$</th>
<th>Protein</th>
<th>WSC$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td>31.8</td>
<td>17.5</td>
<td>1.0</td>
<td>2.0</td>
<td>0.0</td>
<td>13.8</td>
<td>1.3</td>
</tr>
<tr>
<td>max.</td>
<td>45.1</td>
<td>25.6</td>
<td>2.3</td>
<td>4.4</td>
<td>0.8</td>
<td>19.7</td>
<td>7.3</td>
</tr>
</tbody>
</table>

$^1$Acid insoluble protein subtracted (Sluiter et al. 2010)
$^2$WSC=Water-soluble carbohydrates
**Fiber hemp**

Hemp (*Cannabis sativa* L.) is considered to be one of the oldest crops cultivated for non-food use (Cole and Zurbo 2008). The main interest has been in fibers, which have been used for the manufacture of ropes, paper, and fabrics, but also for medical purposes and production of hemp seed oil (Van Der Werf et al. 1996). Lately, new opportunities to use hemp for various applications, including thermal insulation (Kymäläinen and Sjöberg 2008), composite manufacturing (Hautala et al. 2004), and bioethanol production (Zatta and Venturi 2009, Sipos et al. 2010) have been intensively studied. Hemp is not widely grown in Europe on account of the illicit uses of cultivars with high-tetrahydrocannabinol (THC) content. Drug-free fiber and oilseed cultivars may, however, be grown under permit in most European countries. Although the conditions (soil and growth conditions) were not optimal, promising cultivars were identified and fair yields (quality and amount) were obtained from 1995 to 1997 in Finland, where hemp benefits from the long-day growth conditions (Sankari 2000). Field trials in Sweden from 1999 to 2001 showed biomass yields of hemp from 7.8 to 14.5 t DM ha⁻¹ (Svennersted and Svensson 2006).

Fiber hemp consists of stems, leaves, and inflorescence. The stem consists of epidermis, which covers and protects the single cells or elementary bast fibers in the bark right under the epidermis. Fibers are attached to each other by pectin, forming fiber bundles (Haudek and Viti 1978). Each bundle (0.5-5 mm) contains from two to over 40 elementary fibers or single cells (0.015-0.050 mm), as reviewed by Kymäläinen (2004). Mature bast fibers are formed of supportive sclerenchyma cells that have thick cell walls. The inner part of the hollow stem is xylem (wood layer), with thick and strong-walled wood cells giving strength to the crop (Haudek and Viti 1978). In this thesis, the term “fiber” is used for the bast fiber around the stem, and “xylem” is used for the wood layer. A cross section of a hemp stem is shown in Figure 6 (Härkäsalmi 2008).

The growing interest in using fiber hemp as a raw material for biofuels has increased knowledge on the chemical properties of hemp (Barta et al. 2010, Kreuger et al. 2010). The main carbohydrates are glucans, including cellulose (about 44% of DM) and xylans (about 10% of DM). Hemicelluloses form altogether about 15% of the DM, most of which are xylans (Sipos et al. 2010).
In many studies on hemp, the major interest has been in the bast fibers in which the content of cellulose has been determined to be about 60%, hemicelluloses 14%, and pectin 7% (of DM) (Nykter et al. 2008). A notable difference has been observed in the amount of acid-insoluble lignin, which was reported to be only 3% in the fiber but 15% in the whole crop (Nykter et al. 2008, Kreuger et al. 2010). This indicates a remarkable variation between the compositions of the fiber and wood layer parts of the crop. WSC comprise approximately 10% and 13% of the DM in the fiber and the whole crop, respectively (Nykter et al. 2008, Kreuger et al. 2010). The high carbohydrate content reported in fiber hemp indicates the potential of hemp as a substrate for bioethanol or methane production.

**Faba bean**

The cultivation and use of faba bean (*Vicia faba* L.) has a long history in Finland, where it has been cultivated mainly for livestock feed on a relatively small scale (10 000 ha in 2011) (Stoddard et al. 2009, Agricultural statistics in Finland 2012). Biomass yield of 10.6 t DM ha⁻¹ have been obtained in earlier cultivation studies in Finland (Stoddard et al. 2009). It is widely used as a feed
in some other countries and as human food in the Mediterranean region (Duc 1997). Some of the cultivars of faba bean have been suggested for use as a raw material for bioenergy mainly because of their ability to supply nitrogen via symbiotic N₂ fixation with *Rhizobium* bacteria. Intercropping with even higher yielding perennial monocots has also been suggested (Jensen et al. 2010). As a nitrogen-fixing legume, it has potential to contribute to sustainability in energy cropping, and it is a robust crop that produces high biomass yields (Stoddard et al. 2008). It also has been found to be a positive precrop, mainly due to nitrogen fixation. It can decrease tillage intensity and provide reduced energy requirements and GHG emissions after introduction into cereal-rich, intensive crop rotations (Köpke and Nemecek 2010). The high content of protein would benefit especially methane production, if the whole crop would be used for energy production (Amon et al. 2007b). Protein rich faba bean seeds comprise half of the biomass, while stems and leaves cover the rest (Stoddard et al. 2010). Faba bean straw has been found to contain 28% of glucans and 12% of xylans as the major carbohydrates in the stem (Petersson et al. 2007).

**White lupin**

As a faba bean, white lupin (*Lupinus albus* L.) is a legume with the ability to fix nitrogen in a symbiotic relationship with *Rhizobium* bacteria. The roots of lupin are particularly large and long reaching, which accomplish an efficient use of elements from the ground, leading also to extensive nitrogen fertilizer (Stoddard et al. 2011). Lupin seeds have a high content of galactan, referred to as insoluble dietary fiber (Carre et al. 1985). A low content of oil (5-8%) in the seeds has been reported, whereas a high amount, up to 50% of protein was observed (Kurlocvich et al. 2002). White lupin has been regarded rich in nutrients and has been used as food and feed since ancient times (Gross 1988). The anatomy of the upper and lower parts differs in white lupin stem. The most abundant cells are comprised of thin-walled parenchyma cells located under the epidermis. Above the parenchyma cells and on the side of the stems, thin layers of thick-walled collenchyma cells strengthen the lupin stem (Petrova 2002).

**Jerusalem artichoke**

The Jerusalem artichoke (*Helianthus tuberosus* L.) has been cultivated widely in North America and Europe since the seventeenth century to produce inulin-rich tubers for food or feed (Cosgrove et al. 1991). Jerusalem artichoke has shown good frost tolerance and is resistant to pests and diseases (Caserta and Cervini 1991). Subsequently, Jerusalem artichoke has raised renewed interest, not only as food and feed, but also as a raw material for the production of fructose (Caserta and Cervigni 1991). Besides tubers, Jerusalem artichoke produces a high above-ground stem, 3 m high, with a biomass 16 t ha⁻¹ (Gunnarson et al. 1985). The stems contain—in addition to cellulose (17-20%),
hemicelluloses (21%), and lignin (12-14%)—inulin, which consists of fructooligosaccharides (FOS) (Gunnarson et al. 1985, Slimestad et al. 2010). The amount of FOS and the degree of polymerization of inulin depend on the stage of maturity (Slimestad et al. 2010). It has been observed that WSC are stored in the stem until they are rapidly transferred to the tubers in late autumn (Slimestad et al. 2010, Caserta and Cervini 1991). The harvesting time is therefore optimized based on the size and sugar content of the tubers and the easily fermentable sugars in the stem.

1.3 BIOMASS CONVERSION PROCESSES

The conversion processes of lignocellulosic raw materials into ethanol or methane consist of the basic stages of preservation, pretreatment, hydrolysis, and fermentation (Hahn-Hägerdal 2006) (Figure 7). Compared with raw materials, such as grains used in first-generation biofuel production, the crops used for second-generation biofuels may need different and prolonged preservation methods as well as more severe pretreatment (McDonald et al. 1991, Gray et al. 2006). The pretreatment step is essential, especially to speed up the enzymatic hydrolysis of lignocellulose in the ethanol production process (reviewed by Mosier et al. 2005). The stages in the processes of converting the raw materials to ethanol and methane are introduced in more detail in the next sections, i.e., preservation methods (acid and alkali), pretreatments studied in this work (milling, hydrothermal, and alkali treatments), as well as hydrolysis and fermentation stages of the conversion process.

Figure 7 Process scheme of ethanol and methane production from the raw material (modified from Weiland 2003 and Deublein and Steinhauser 2008).


1.3.1 PRESERVATION OF HERBACEOUS CROPS

Storing of crops for supplying raw material for biofuels throughout the whole year is an important issue. The traditional practice of storing is drying of grain or hay for food and feed use (Shinners et al. 2007). Drying of the material for biogas or bioethanol production may not be an economically viable storing method and may be even harmful for the utilization of the substrate. Drying of fibers can result in irreversible collapse and shrinking of the capillaries and thus reduce the accessible surface area (Fan et al. 1980, Taherzadeh and Karimi 2008). This feature hampers the hydrolysis of lignocellulosic substrates in both processes: in methane production (Egg et al. 1993) and enzymatic hydrolysis prior to ethanol production (Wada et al. 2010). In addition, the energy consumption (Mikkola and Ahokas 2010) and biomass losses may be high during drying (Shinners et al. 2007). Another traditional storing method, adapted from the feed sector, is acidic anaerobic storing, i.e., ensiling of fresh crop material (McDonald et al. 1991). As the term ensiling has been generally used for acidic preservation with or without acidic additives, in this work, preservation in alkaline conditions is referred to as alkali preservation.

Ensiling

The basic aim of ensiling is to induce anaerobic conditions in which the lactic acid bacteria, which is present in plants, can convert mainly WSC into organic acids. The decreased pH (about 4) prevents the growth of mold and other unwanted microorganisms, and structural carbohydrates and proteins are thereby preserved (McDonald et al. 1991). Another important aim is to prevent the conversion of biomass to unwanted products (biomass losses). Typical reported figures for biomass losses (DM) in ensiling have been between 1% and 10% (McDonald 1991, Plöchl et al. 2009), which are lower than observed for drying of, e.g., corn stover (Shinners et al. 2007). Ensiling has been successfully used for animal feed preservation for almost 100 years. Additionally, ensiling has been discovered to be suitable for treating raw materials for AD. Ensiled corn stover and grasses are commonly used raw materials in present methane production plants (Weiland 2006, Amon et al. 2007a, de Graaf and Fendler 2010). Due to the increased formation of lactic and acetic acids in ensiling, higher methane yields have been obtained (Neureiter et al. 2005, Amon et al. 2007a). Ensiling prior to AD has been found to even enhance the methane yields of horse manure mixed with high amounts of wood chips or peat (Danner 2011). In general, more severe pretreatment conditions of lignocelluloses are used for bioethanol production than for ensiling due to the need to increase the conversion rate in ethanol production. However, ensiling has been considered a promising method primarily to store the raw material and secondarily to enhance the hydrolyzability (Chen et al. 2007b, Thomsen et al. 2008).
Various additives have been found to improve the efficiency of the ensiling processes. The amount of lactic acid bacteria can be increased in order to ensure efficient bacterial fermentation (Chen et al. 2007b). The conversion of whole crop maize, rye (*Secale cereale*), and clover, ensiled with the addition of lactic acid bacteria, was observed to be improved by ensiling prior to the hydrolysis and ethanol production processes (Oleskowicz-Popiel et al. 2011). Additional carbon sources, such as soluble sugars or molasses, have been added for the bacteria in ensiling, *e.g.*, wild sunflower (*Tithonia diversifolia*) (Fasuyi et al. 2010). Besides additives promoting the natural ensiling process, acidification can be improved by externally added acids. The main aim of external acidification is to preserve most of the valuable WSC along with the structural carbohydrates (McDonald et al. 1991). Formic acid has been found efficient for improving the quality of feed and for increasing the nutritional value (Jaakkola et al. 2006a). Sulfuric acid (H$_2$SO$_4$) has been successfully used to optimize the pretreatment of switchgrass and reed canary grass for fuel ethanol process (Digman et al. 2010).

Acid and enzymatic hydrolysis has been found to solubilize saccharose, inulin, and hemicelluloses as part of the structural components during ensiling (McDonald et al. 1991). The conditions at pH 4-5 are, however, relatively mild as compared to lower pH values (*e.g.* pH 1) used for acidic pretreatments. In addition to mild acid pretreatments, ensiled materials such as hemp and maize have been successfully treated with stronger methods, such as hydrothermal pretreatments, for further conversion to ethanol (Sipos et al. 2010, Oleskowicz-Popiel et al. 2011).

**Alkaline preservation**

In addition to acidic conditions, alkaline conditions have been used in ensiling to preserve herbaceous plants for feed use. Alkaline preservation requires base addition to increase the pH (7.7 to 8.7) (Guedes et al. 2006). Urea is a common additive used for alkaline preservation since along with preservation, it increases the nutritional value of feed due to the added ammonium (Huber and Thomas 1970). In alkaline pretreatments, mild conditions have already been demonstrated to remove or alter lignin chemically. In addition, partial degradation of lignin in corn ensiled with urea in anaerobic conditions by the rumen bacteria has been observed (Akin 1980, Huber et al. 1968). Alkali-preserved crops have not been traditionally used for biogas production or ethanol fermentation, but some positive indications of enhanced glucose conversion in enzymatic hydrolysis have been observed after treating reed canary grass and switchgrass with lime prior to anaerobic preservation (Digman et al. 2010).
1.3.2 PRETREATMENTS OF LIGNOCELLULOSIC MATERIALS

Pretreatments, in general, aim to increase the availability of carbohydrates, especially cellulose, to be converted into platform sugars and further to, e.g., ethanol or methane. Optimization of different additives and process parameters has been carried out to obtain easily hydrolyzable substrates, satisfying both environmental and economical feasibility. Numerous studies reviewed by Hsu (1996), Sun and Zheng (2002), Mosier et al. (2005), Hendriks and Zeeman (2009), and Taherzadeh and Karimi (2009) on pretreatments of various lignocellulosic materials have been published during last decades. Some pretreatments are already used in demonstration scale in companies aiming at commercialization of ethanol production (Galbe et al. 2005, Inbicon 2012)). However, large-scale pretreatment facilities have not yet shored into crop utilizing biogas processes due to the already relatively efficient conversion of materials during the AD process and the fairly small scale plants operating in the field.

The most frequently studied pretreatments can be divided into the following categories: physical (e.g., milling, irradiation, steaming, extrusion, and pyrolysis), chemical (e.g., acidic and alkaline thermal treatments, oxidative treatments, and extraction with solvents or ionic liquids) or biological treatments, as well as their combinations as reviewed by Hendriks and Zeeman (2009). The commonly used and efficient combinations are the steam pretreatment combined with either alkali or acids (McMillan 1994). The optimal processing time, temperature, and concentrations of added chemicals vary from one substrate to the other, depending on the recalcitrance of the raw material (Sipos et al. 2010, Goshadrou et al. 2011). The major objectives of pretreatments are increasing the surface area for enzymes, reducing the particle size, separating the complex polymers from each other, or decreasing the crystallinity of cellulose (Mosier et al. 2005). The impact of pretreatments on ethanol and AD processes are summarized in Table 3.

In pretreatments aiming at improved enzymatic hydrolysis and ethanol production, the main objective has been to remove hemicelluloses or lignin with maximum glucose recovery. Preferably, the crystallinity of cellulose is simultaneously decreased and the surface area increased (Hsu 1996). In addition to these, avoiding the formation of inhibitors, such as acetic acid or furfural, is important. In biogas production, formation of inhibitors or removal of hemicelluloses is not as essential. Pentoses, acetic acid, furfural, and even degradation products of lignin may be utilized during the process (Barakat et al. 2011). However, the same recalcitrant structures of cellulose and other polymers in lignocellulosic materials also limit the AD process, resulting in incomplete hydrolysis (Carrère et al. 2011).
Table 3  Impacts of common pretreatments on ethanol and methane production from lignocellulosic raw materials (Adapted from Carrere et al., 2011, and modified from Mosier et al. 2005).

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Ethanol</th>
<th>Methane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lignin solubilization</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Lignin structure alteration</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Surface area increase</td>
<td>++</td>
<td>+/++</td>
</tr>
<tr>
<td>Hemicellulose solubilization</td>
<td>++</td>
<td>o/+</td>
</tr>
<tr>
<td>Cellulose decrystallization</td>
<td>++</td>
<td>o/+</td>
</tr>
<tr>
<td>Cellulose degradation</td>
<td>--</td>
<td>o/+</td>
</tr>
<tr>
<td>Furfural, hydroxymethylfurfural formation</td>
<td>--</td>
<td>0</td>
</tr>
</tbody>
</table>

++ major positive impact, - - major negative impact, o no impact
+ minor positive impact, - minor negative impact

The methods used in this work—milling, steam explosion, alkaline extraction, and enzymatic pretreatment—are introduced in more detail. The traditional retting treatment of hemp fibers is also reviewed because of the question of pectin hydrolysis in this work.

**Milling**

Milling and other grinding techniques to reduce the particle size of the substrate have been considered as environmentally friendly pretreatment because chemicals are not required (Ana da Silva et al. 2009). Among other benefits, milling does not form inhibitors, such as furfural, which is beneficial especially for ethanol production. Wet disk milling, for instance, has recently been described as a potentially feasible mechanical technique to treat rice straw prior to hydrolysis and ethanol production (Hideno et al. 2009). However, the energy consumption of milling is considerable at 3.2-20 kWh t⁻¹ DM (maize stover), depending on final size and mill type, as reviewed by Sun and Cheng (2002).

The main aim of milling is to increase the surface area by decreasing the particle size of the material. Extensive grinding reduces crystallinity of cellulose, as well (Mosier et al. 2005). It is, however, believed that recrystallization taking place during, e.g., water swelling may even increase the crystallinity of highly ball-milled cellulose. However, increased surface area for better accessibility of enzymes has been obtained (Fan et al. 1980). Expectedly, both crystallinity and surface area have an effect on ethanol and biogas processes (Mosier et al. 2005). However, reduction of the degree of crystallinity has been observed to have less effect in biogas production compared with enzymatic hydrolysis (Carrère 2011). No delignification or removal of hemicelluloses takes place in mechanical pretreatments (Mosier et al. 2005). Therefore, combinations of more severe
treatments and milling have been found to enhance both the enzymatic accessibility and the methane yield of rice straw (Zhang 1999, Jin and Chen 2006).

**Thermochemical pretreatments**

In the most extensively studied thermochemical pretreatment, steam explosion, water in the biomass is exploded by a rapid decrease of pressure at temperatures of 160°C to 260°C (Sun and Zheng 2002). The severity of the conditions needed depends strongly on the chemical composition and the recalcitrance of the raw material used (Kreuger et al. 2010, Goshadrou et al. 2011). Harsh conditions may destroy valuable components and form inhibitors by, e.g., degrading xylose into furfural or glucose to HMF (Hydroxy-methyl-furfural) (Mosier et al. 2005). In general, steam explosion removes most of hemicelluloses, increases the surface area, and alters the lignin structure, as reviewed by Mosier et al. (2005). Steam pretreatment, with or without explosion, has received attention as a potential pretreatment for both ethanol and methane production (Horn et al. 2011).

With recalcitrant substrates, acid is often used to enhance the effect of the thermochemical treatment. Addition of H₂SO₄ can decrease the required time and temperature, effectively improve hydrolysis, decrease the production of inhibitory compounds, and lead to complete removal of hemicelluloses (Stenberg et al. 1998, Ballesteros et al. 2006). Impregnation with 2% SO₂ followed by steam pretreatment at 219 °C increased the enzymatic conversion of fresh and ensiled fiber hemp (Sipos et al. 2010). Lignin has been observed to be removed only to a limited extent during the pretreatment but has been observed to become relocated on fiber surfaces as a result of melting and depolymerization and repolymerization reactions (Li et al. 2007).

**Alkaline pretreatments**

Delignification has been found to be one of the most efficient structural changes to improve enzymatic hydrolysis and biogas production (Öhgren et al. 2007, Carrère et al. 2011, Monlau et al. 2011). Almost theoretical (95%) saccharification yields were reported for alkali pretreated sorghum straw (McIntosh and Vancov 2011). Sunflower stalks were treated similarly prior to AD, accomplishing a significant increase in methane yield (Monlau et al. 2011). A strong correlation between lignin removal and enhanced conversion was observed in both studies.

The fundamental effects of alkaline treatments are lignin removal and swelling of cellulose fibers, which tends to decrease crystallinity. In delignification, the β-aryl linkages, the primary linkages between the phenylpropane units, are cleaved by alkaline chemicals at high temperatures (Gierer 1985). This causes
the formation of free phenolic hydroxyl groups that increase the hydrophilic characteristics of lignin, resulting in increased solubility (Chakar and Ragauskas 2004). Alkaline pretreatments do not only affect lignin and cellulose but can also remove hemicelluloses, pectin, and acetyl groups (Chang and Holtzapple 2000). It has been observed that pectin in hemp was completely removed in pretreatments by NaOH for textile applications, a characteristic that could be favorable in hydrolysis and ethanol production, as well (Wang et al. 2003).

**Retting**

Retting of fibers is an old method in textile processing to increase the quality of fibers. The main aim of retting is to remove bast fiber bundles from the surrounding elements, i.e., the epidermis and wood layer (Easson and Molloy 1996), or to separate the bundles further to elementary fibers by removing the gluing components between individual fibers (Carpita and Gibeaut 1993). Pectic compounds are the main cementing components between individual fibers and fiber bundles. Different techniques for retting have been developed during the past years, with the oldest water retting technique still being in use (Sultana 1992). Besides using different chemicals, such as NaOH, pectinolytic enzymes have been successful used for fiber retting (Kymäläinen 2004, Nykter et al. 2008).

1.3.3 HYDROLYSIS

The hydrolysis step is the major bottleneck when utilizing the more recalcitrant polysaccharides for both ethanol production and AD (Claassen et al. 1999). Enhancement of the hydrolysis of lignocellulosic substrates has been one of the major concerns in studies of the conversion process (Hahn-Hägerdal et al. 2006). In general, hydrolysis is a reaction in which the glucosidic bonds between single sugar molecules in polymers are cleaved by the addition of a water molecule, forming shorter oligosaccharides or monosaccharides (Chemistry Encyclopedia 2012). In AD, a versatile mixture of enzymes produced by microorganisms catalyzes the hydrolysis of polymers present (Lynd et al. 2002). In the saccharification for platform sugars and ethanol production, selected externally added hydrolytic enzymes are used, and various glycoside hydrolases (glycosidases) catalyze the cleavage of polysaccharides (Wyman et al. 2004). In the dilute acid hydrolysis of cellulose or hemicelluloses, a hydrogen ion is added to form a conjugated acid, leading to the cleavage of the glycosidic bond. The hydrogen ion thus acts as catalyst that facilitates hydrolysis without net consumption of these species (Wyman et al. 2004). While hydrogen ions (H⁺) catalyze hemicellulose hydrolysis and removal at low pH, operation at high pH (above 10) can solubilize and remove lignin and result in improved cellulose digestibility (Yang and Wyman 2004). High temperatures, e.g., > 160° C in steam explosion, is required to complete hydrolysis of polysaccharides into
monomers, but degradation of hemicelluloses and cellulose occurs already at lower temperatures, as reviewed by e.g. Pedersen and Meyer (2010). Mildly acidic or alkaline conditions in anaerobic preservation have revealed partial scission of structural components (McDonald et al. 1991, Digman et al. 2010). The effects of pH and temperature on cellulose, hemicelluloses, and lignin are shown in Figure 8 (Pedersen and Meyer 2010).

![Figure 8](image)

Figure 8 Sketch of pretreatment of lignocellulose as affected by temperature and final pH. Gray ‘veil’ indicates lignin sheath; orange and red tubes illustrate cellulosic fibrils and microfibrils, respectively; black curved lines illustrate hemicellulose (xylan); the gray dots on the cellulose microfibrils in the low pH region illustrate redeposited lignin (Pedersen and Meyer 2010). Figure is presented courtesy of Elsevier.

**Enzymatic hydrolysis**

Bioethanol is produced from carbohydrates by fermenting with yeast or bacteria (Hahn-Hägerdal et al. 2006). Prior to the fermentation step, complex polysaccharides are saccharified in hydrolysis catalyzed by enzyme mixtures (Wright 1988, Gray et al. 2006). The hydrolysis is performed either before fermentation (SHF) or simultaneously (SSF) (Wright et al. 1987). Enzymes needed for the hydrolysis are dependent on the raw material, and usually, mixtures rich in various enzyme components are used (Hahn-Hägerdal et al. 2006). First-generation substrates, like starch, are hydrolyzed with amylases,
while invertase is used to hydrolyze saccharose. Hydrolysis of lignocelluloses is a significantly more challenging step as compared with, e.g., starch. Various hemicellulolytic and cellulolytic enzymes are required to hydrolyze different components of the cell wall. Hydrolysis of lignocelluloses materials represents a special case of enzymology since the recalcitrant substrate is solid and the substrate is changed after each reaction. Extensive research during last few decades has led to the development of efficient enzyme mixtures, which are already commercially available (Gray et al. 2006). The most thoroughly studied mesophilic-fungus-producing cellulases is *Trichoderma reesei* (Kirk and Cullen 1998).

As cellulose forms the major carbohydrate share in lignocellulosic plant materials, it is the most important substrate for the conversion of the raw material (Lynd et al. 2002). Despite the complexity of the cellulose structure, it can be almost completely hydrolyzed by enzymes over time (as reviewed by Schwarz 2001). Traditionally, two classes of cellulases are needed for hydrolysis of cellulose, endoglucanases (EG) (endo-1,4-β-glucanases) and cellobiohydrolases (CBH) (exo-1,4-β-glucanases) (Xu et al. 2007). EGs can hydrolyze internal bonds of cellulose chains, preferring amorphous parts and releasing new terminal ends. The chain ends are attacked by the CBHs. CBHs are the only enzymes that efficiently hydrolyze and crystallize cellulose. They are divided into two types: CBH I and CBH II. CBH I acts on the reducing ends and CBH II on the non-reducing ends of the chain (e.g. Bayer et al. 1998). EGs and CBHs release cello-oligosaccharides and cellobiose from cellulose, which are further cleaved into glucose by β-glucosidase (Gray et al. 2006).

The complexity of native hemicellulose requires a high degree of coordination between the enzymes involved (Viikari et al. 1993). Most enzymes have very specific requirements for substrate binding and precise transition state formation, which usually leads to high catalytic turnover rates (Viikari et al. 1999). Each backbone and side group requires a special type of hemicellulase to cleave the polymer into single sugar molecules (Shallom and Shoham 2003). The major backbone cleaving hemicellulolytic enzymes are xylanases and mannanases, and the side group cleaving enzymes include arabinosidases, galactosidases, glucuronidases, and acetyl esterases (Viikari et al. 1999). It has been observed that the amount of xylans, especially, seems often to restrict the overall enzymatic hydrolysis of celluloses by, e.g., covering the surface of cellulose and preventing the access of cellulases to the cellulose surface (Berlin et al. 2007, Várnai et al. 2010).

Analogous to hemicellulases, a number of enzymes are needed for cleaving pectin polymers (Kashyap et al. 2001). Pectinases catalyze the random hydrolysis of 1,4-α-D-galactosiduronic linkages in pectin and other galacturonans. Polygalacturonase attacking the galacturonic acid polymer, and forming galacturonic acid as hydrolysis products, are the major pectin
depolymerizing enzymes in common pectinase mixtures (Gummadi et al. 2007). Other enzymes hydrolyzing α-1,4-glycosidic linkages of pectin are (poly)methylgalacturonases, polymethyl- and polygalacturonate lyases, cleaving the α-1,4-glycosidic linkages by trans-elimination (Kashyap et al. 2001). Enzymatic hydrolysis of pectin is commonly used in food industries as well as for textile applications, utilizing pectin-rich flax or hemp fibers. Hydrolysis of pectin enhances the fiber properties that are required for textile, composite, or paper applications by releasing fiber bundles from each other (Wang et al. 2003).

Gilligan and Reese (1954) first showed that the amount of reducing sugars released from cellulose by the combined fractions of fungal culture filtrate was higher than the sum of the amounts released by the individual fractions. Since the initial report, the synergistic action of exo- and endo-acting cellulase components has been demonstrated by many investigators (Wood and McCrae 1979, Baker et al. 1994). The synergism between cellulolytic and pectinolytic enzymes has previously been observed in highly pectin-rich sugar beet pulp (Spagnuolo et al. 1997). The synergistic action of xylanolytic and mannanolytic enzymes with cellulases has also been observed enhancing the hydrolysis rate of xylan-containing substrates (Banerjee et al. 2010, Várnai et al. 2011).

Consolidated bioprocessing

Consolidated bioprocessing (CBP) is a potential process under development in which cellulase production, substrate hydrolysis, and fermentation are accomplished by cellulolytic and ethanologenic microorganisms (Carere et al. 2008). Although no natural microorganism found exhibits all the features for efficient CBP, several bacteria and fungi possess some of the desired properties (Zyl et al. 2007). However, engineering of the metabolic and enzyme systems is required to enhance the ethanol yields produced by native cellulolytic microorganisms. Conversely, recombinant cellulolytic microorganisms naturally give high product yields, but the ethanol production systems need to be engineered (Lynd et al. 2002). Effective examples of native cellulolytic microorganisms having high production yields and potential hydrolysis systems are found among anaerobic bacteria or fungi (Lynd et al. 2005). During the last few years, the development of organisms for CBP has advanced, although some remaining barriers are still to be resolved (Olson et al. 2011).

The CBP, in principle, is also involved in the production system of biogas, although the number of micro-organisms acting in concert comprises more than one organism (Doi and Kosugi 2004, Cirne et al. 2007). Mixtures of microorganisms are able to simultaneously hydrolyze the substrate and ferment the hydrolyzed sugars to methane and CO₂ without added enzymes. In nature,
cellulose is slowly degraded in anaerobic conditions, such as in soil and rumen as well as in constructed anaerobic digestors, by various anaerobic microorganisms (Leschine 1995, Doi and Kosugi 2004). Cattle manure or digested sludge from waste water treatment plants can be used as an inoculum when starting continuous methane production processes or testing methane production potentials in batch tests. These inocula contain a wide microbial flora, which is able to produce enzymes and accomplish all stages of the methane production process. The microorganisms in the inocula produce multiple enzymes to degrade the plant cell components: cellulose, hemicelluloses, and pectins (Warren 1996, Lynd et al. 2002). These large extracellular multi-enzyme complexes, called cellulosomes, consist of cellulases and hemicellulases and are commonly produced by anaerobic bacteria of the genera including Clostridium, Acetivibrio, Bacteroides, and Ruminococcus. The cellulosome system has been described as the principle mechanism by which some anaerobic cellulolytic microorganisms accomplish efficient breakdown of the recalcitrant polysaccharides present in plant cells. The cellulosome system has been claimed to be more efficient than the free enzyme system because it collects and positions enzymes onto the substrate surface (Wyman et al. 2004).

The in situ bacteria and other microorganisms as enzyme factories have some benefits compared with the externally produced enzymes. It has been proposed that the lack of the ability of anaerobic bacteria to effectively penetrate cellulosic material has probably led to the development of complexed cellulase systems, which position cellulase-producing cells at the site of hydrolysis as observed in ruminal bacteria (Lynd et al. 2002). The problems of inactivation of enzymes or adsorption on the surface of lignin or cellulose during the enzymatic hydrolysis can be overcome by the production of new hydrolytic enzymes in situ by the bacteria. Generally, the hydrolysis of recalcitrant polysaccharides to sugars is also still considered to be the rate-limiting step in AD, and therefore, the slowest and the most uncompleted step in the total process (Veeken and Hamelers 1999).

**Lignin degradation**

The biological degradation of the complex structure of lignin is still a challenge for scientists (e.g. Hatakka and Hammel 2010). Two families of enzymes—peroxidases and phenoloxidases (laccases) produced mainly by white-rot fungi—are known to participate in biodegradation of lignin (Hatakka 1994). Lignin biodegradation, however, is considered to be an aerobic process, although some authors have reported that anaerobic microorganisms in the rumen may alter, or even partially degrade, lignin in plant cells (Akin 1980, Benner et al. 1984).
1.3.4 FERMENTATION AND METHANE PRODUCTION

The fermentation of hydrolyzed carbohydrates to ethanol is an ancient process harnessed into industrial use (Forbes 1970). Hexoses (C6 sugars)—glucose, fructose, mannose, and galactose—are easily fermented to ethanol by common baker’s yeast (*Saccharomyces cerevisiae*) (Picataggio and Zhang 1996). Therefore, these are the most applicable substrates for ethanol fermentation. Pentoses (C5 sugars), xylose, and arabinose from hemicelluloses require tailored yeasts, filamentous fungi, or bacteria (Olsson and Hahn-Hägerdal 1996). Galacturonic acid, originating from pectin, may also be a valuable substrate for ethanol production. Some bacteria (*e.g.* *Escherichia coli*) could be also used for fermentation of pectin-rich substrates because of their ability to convert pure galacturonic acid to ethanol (Doran et al. 2000). The economic motivation of developing microorganisms capable of also utilizing pentoses for ethanol production has increased research efforts for developing more efficient systems, including those using CBP organisms (Olson et al. 2011).

Compared with ethanol fermentation, methane production is distinctly a more robust system, especially with respect to the spectrum of convertible substrates (Weiland 2006, Barakat et al. 2011). While ethanol can be fermented only from carbohydrates, methane can also be produced from proteins, fats, extractives, acids, and even degradation products of carbohydrates and lignin (Barakat et al. 2011). The monomeric sugars from the hydrolysis stage are converted into methane in three stages (Figure 7). The detailed process and principles of the anaerobic degradation process is reported in numerous publications, *e.g.*, in Deublein and Steinhauser (2008). Acidogenic bacteria convert hydrolyzed sugars, amino acids, and fatty acids into alcohols and smaller acids, and to CO$_2$ and H$_2$. During the acetogenic stage, the formed products are converted to acetic acid and H$_2$. In the final stage, methane is formed from two separate routes, from acetic acid and H$_2$, and from CO$_2$ and H$_2$ by methanogenesis (Deublein and Steinhauser 2008).
2 AIMS OF THE WORK

The main aim of this work was to evaluate the convertibility of fresh and preserved herbaceous field crops for biogas and bioethanol production. Five different crops, which have not been studied extensively as raw materials for bioenergy production in the boreal climate, were studied in this work. Cultivation was conducted in a separate project funded by the Academy of Finland. Maize represented a common crop for food, feed, and energy production, cultivated especially in southern climates. Fiber hemp was chosen because of its potential high carbohydrate yield and also because growth is favored by the long days of the growth period. Faba bean and white lupin, representing legumes in this work, were expected to produce high amounts of protein and therefore would be potentially viable, especially for methane production. Jerusalem artichoke, with its high inulin content, was the fifth energy crop studied. Jerusalem artichoke was the only crop in which the edible parts were not utilized for energy production experiments. All other raw materials were used as whole crops.

Preservation of fresh, easily biodegradable crops throughout the year is an important question. Thus, this work evaluated anaerobic preservation of these crops prior to enzymatic hydrolysis and/or conversion to ethanol, as well as methane production. The effect of different additives on the preservation and conversion of carbohydrates in both processes was investigated. The main emphasis was on studying the role of the chemical and physical structure and the conversion of plant cell components in AD to methane and enzymatic conversion to platform sugars before and after storing. In addition to preservation, the effect of steam explosion, alkali extraction, and pectinase treatment for fiber hemp were studied in this work.

The detailed objectives were to:

1. Evaluate the suitability of maize, hemp, faba bean, white lupin, and Jerusalem artichoke as biomass feedstock in boreal conditions by comparing the energy yields of the two energy carriers, ethanol and methane (I, IV).
2. Evaluate the effects of ensiling and anaerobic-preservation on carbohydrate conversion in enzymatic hydrolysis and methane production (II, III, IV).
3. Examine the effects of commonly used pretreatments (milling, hydrothermal, and alkali) on convertibility of fresh and ensiled hemp and maize to sugars, ethanol and methane (III).
4. Study the effect of pectin in enzymatic hydrolysis and methane production of fresh and preserved hemp (III, IV).
3 MATERIALS AND METHODS

Preparation of crop materials

Crops were grown in separate field trials at the University of Helsinki campus at Viikki as part of a program funded by the Academy of Finland, aiming at identifying suitable energy crop species and developing sustainable energy cropping systems for the boreal zone (Stoddard et al. 2010, Santanen et al. 2011a). On 30th September 2008, at the end of the growing season, all above-ground biomass of maize, hemp and faba bean, was collected manually from 1-2 m² for further analysis. Hemp was also harvested in October 2009, and white lupin and Jerusalem artichoke at the beginning of September 2010. Hectare yields of crops used for energy calculations per ha were 15 t ha⁻¹, 14 t ha⁻¹, 10 t ha⁻¹, 18 t ha⁻¹, and 18 t ha⁻¹ for maize, hemp, faba bean, white lupin, and above ground material of Jerusalem artichoke, respectively (Stoddard et al. 2010, Santanen et al. 2011b).

After harvesting, maize and faba bean were prewilted in a greenhouse for 48 h and 20 h, respectively, to reduce the moisture content of the materials. No prewilting was necessary for white lupin, Jerusalem artichoke, and hemp in 2008, while in 2009 hemp was wilted for 48 h (I, II). Crops were cut with a garden chopper into 1-2-cm-size pieces and preserved anaerobically. Samples of fresh materials were frozen for further use. The chopped material was used as such for methane production assays. For enzymatic hydrolysis, the raw material was milled with an IKA M20 universal mill, resulting in a maximum particle size of 7 mm (I, II, III). For enzymatic hydrolysis and for chemical analyses, the raw materials were freeze dried or dried at 60 °C for 72 h and milled with an IKA A10 basic analytical grinder to an average particle size of 1 mm (I-IV). Dried material was also used for enzymatic hydrolysis performed at a small scale (III). Bast fibers and xylem (woody stems) were separated manually for chemical analyses and microscopical examinations after washing and freeze drying (III). Prior to enzymatic hydrolysis or chemical analyses, the materials were washed with warm ultra-pure water (III).

Preservation, pretreatment and conversion processes and characterization of materials

Processes used in this study included the typical steps required when field crops are converted to biofuels (Figure 7). The processes used in this work were preservation of the crop material and a pretreatment step to enhance the further conversion step. The yield of enzymatic hydrolysis of biomass to fermentable sugars was studied in hydrolysis tests. The actual conversion of biomass to methane was tested in AD batch experiments using digested sludge from the
municipal wastewater treatment plant as inoculum. Ethanol fermentation was conducted only with maize (IV). Theoretical ethanol yields for other studied crops were calculated from the total sugars determined and potential ethanol yields from the fermentable sugars obtained in enzymatic hydrolysis (EERE 2012). Methods are summarized in Table 4, and different processes used for each crop are listed in Table 5. All the parameters used in each enzymatic hydrolysis in different publications are listed in Table 6. Detailed descriptions of materials and methods can be found in the original publications’ I-IV.

Table 4  The methods used in this work.

<table>
<thead>
<tr>
<th>Method</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PROCESSES</strong></td>
<td></td>
</tr>
<tr>
<td>Ensiling (formic acid)</td>
<td>II</td>
</tr>
<tr>
<td>Alkaline preservation (urea)</td>
<td>II</td>
</tr>
<tr>
<td>Steam explosion</td>
<td>III</td>
</tr>
<tr>
<td>Alkaline pretreatment</td>
<td>III</td>
</tr>
<tr>
<td>Methane production</td>
<td>I, II, IV</td>
</tr>
<tr>
<td>Enzymatic conversion</td>
<td>I, II, III</td>
</tr>
<tr>
<td>Simultaneous saccharification and ethanol fermentation</td>
<td>IV</td>
</tr>
<tr>
<td><strong>CHARACTERIZATION OF CROP MATERIALS and PRODUCTS</strong></td>
<td></td>
</tr>
<tr>
<td>DM content</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>Lignin and carbyhydrates (acid hydrolysis)</td>
<td>I, II, III, IV</td>
</tr>
<tr>
<td>Non-cellulosic glucan and uronic acid (from solids)</td>
<td>I, II, II, IV</td>
</tr>
<tr>
<td>Organic acids</td>
<td>II</td>
</tr>
<tr>
<td>Minerals</td>
<td>I</td>
</tr>
<tr>
<td>Total C and N (Dumas and Kjeldahl)</td>
<td>I, IV</td>
</tr>
<tr>
<td>Ammonia content</td>
<td>IV</td>
</tr>
<tr>
<td>pH</td>
<td>I</td>
</tr>
<tr>
<td>Enzyme activity</td>
<td>III</td>
</tr>
<tr>
<td>Scanning electron microscopy (SEM)</td>
<td>III</td>
</tr>
</tbody>
</table>

Additionally, fructose amount of Jerusalem artichoke was determined from the hydrolysate obtained from the standard hydrolysis experiments. Uronic acid from the liquids was analysed by HPAEC-PAD with the modified method by Rantanen et al. (2007). SE of hemp was conducted with and without additional acid (H₂SO₄). In acid pretreatment prior to SE hemp was soaked in 2,5% H₂SO₄ solution at room temperature for 0.5 h. Solid residue was filtrated and steam exploded.
Table 5  Processes studied for maize, hemp, faba bean, white lupin, and Jerusalem artichoke (I, II, III, IV).

<table>
<thead>
<tr>
<th></th>
<th>Preservation</th>
<th>Pre-treatment</th>
<th>Enzymes in hydrolysis</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>- Ensiling</td>
<td>- Milling</td>
<td>- Cellulases</td>
<td>- Methane (AD)</td>
</tr>
<tr>
<td></td>
<td>- Ensiling with FA</td>
<td>- SE</td>
<td>- Pectinases</td>
<td>- Ethanol (SSF)</td>
</tr>
<tr>
<td>Hemp</td>
<td>- Ensiling</td>
<td>- Milling</td>
<td>- Cellulases</td>
<td>- Methane (AD)</td>
</tr>
<tr>
<td></td>
<td>- Ensiling with FA</td>
<td>- SE + SE with acid</td>
<td>- Pectinases</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Alkaline preservation</td>
<td>- Alkali treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faba bean</td>
<td>- Ensiling</td>
<td>- Not conducted</td>
<td>- Cellulases</td>
<td>- Methane (AD)</td>
</tr>
<tr>
<td></td>
<td>- Ensiling with FA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White lupin</td>
<td>- Not conducted</td>
<td>- Not conducted</td>
<td>- Cellulases</td>
<td>- Methane (AD)</td>
</tr>
<tr>
<td>Jerusalem artichoke</td>
<td>- Not conducted</td>
<td></td>
<td>- Pectinases</td>
<td></td>
</tr>
</tbody>
</table>

FA = formic acid, SE= steam explosion, AD = anaerobic digestion, SSF = simultaneous saccharification and fermentation

Table 6  Parameters for the enzymatic hydrolysis experiments in each article

<table>
<thead>
<tr>
<th>Article/parameter</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Material</td>
<td>Hemp, maize, faba bean, J. artichoke, white lupin</td>
<td>Hemp, maize, faba bean</td>
<td>Hemp</td>
<td>Maize (SSF)</td>
</tr>
<tr>
<td>Preparation</td>
<td>Wet, milled max 7 and aver. 1 mm</td>
<td>Wet, milled max 7 mm</td>
<td>Washed, freeze dried and milled aver.1 mm</td>
<td>Wet, cut into 10 - 20 mm</td>
</tr>
<tr>
<td>Incubation volume, ml</td>
<td>50</td>
<td>5</td>
<td>3</td>
<td>400</td>
</tr>
<tr>
<td>DM, %</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

SSF = Simultaneous saccharification and fermentation, DM = dry matter
Statistical evaluation

Statistical evaluation of the results on the chemical composition, methane yield, and release of sugars in enzymatic hydrolysis was tested with paired-samples t-tests using the PASW (ver. 18.0, SPSS Inc., Chicago, USA). Statistical significance was recognized for p <0.05. Chemical analyses and enzymatic hydrolysis were conducted in three replicates. In methane trials, eight to ten replicates were used. Results are expressed as average value of replicates. Standard deviations between replicates in chemical analyses, enzymatic hydrolysis, and methane yields were calculated and expressed in the results as error bars.
4 RESULTS

4.1 MAIZE, HEMP, FABA BEAN, WHITE LUPIN, AND JERUSALEM ARTICHOKE – POTENTIAL ENERGY CROPS?

4.1.1 CHEMICAL COMPOSITION OF FRESH CROPS (I, II, III, IV)

**Carbohydrates (I, II, III, IV)**

Carbohydrates were analyzed in detail using different methods. The carbohydrate composition of the studied crops (I: Table 3; II: Tables 1, 2, and 3; and III: Table 1) are presented in Table 7. The fresh (frozen) untreated material was always used as a control and therefore analyzed several times in different trials, resulting in a slight variation between the determinations (I, II, III, IV, Table 7).

Table 7 The amounts of carbohydrates, lignin and protein in maize, hemp, faba bean, white lupin, and Jerusalem artichoke, expressed as polymers % of DM. Standard deviation is in parenthesis (n= 3).

<table>
<thead>
<tr>
<th>Components</th>
<th>Maize</th>
<th>Hemp</th>
<th>Faba bean</th>
<th>White lupine</th>
<th>Jerusalem artichoke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucans</td>
<td>36.9±0.6</td>
<td>45.6±0.7</td>
<td>42.0±0.7</td>
<td>24.6±0.6</td>
<td>22.0±0.5</td>
</tr>
<tr>
<td>Cellulose</td>
<td>23.6</td>
<td>38.1</td>
<td>16.8</td>
<td>14.3</td>
<td>n.d.</td>
</tr>
<tr>
<td>Non-cellulosic glucan</td>
<td>13.3±1.1</td>
<td>7.5±0.7</td>
<td>29.1±0.9</td>
<td>10.2±0.8</td>
<td>n.d.</td>
</tr>
<tr>
<td>Water soluble glucose</td>
<td>5.7±0.2</td>
<td>3.2±0.2</td>
<td>3.6±0.1</td>
<td>4.9±0.1</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>Xylans</td>
<td>14.8±0.4</td>
<td>10.1±0.5</td>
<td>6.4±0.5</td>
<td>8.2±0.7</td>
<td>9.5±0.2</td>
</tr>
<tr>
<td>Arabinans</td>
<td>1.8±0.2</td>
<td>1.3±0.1</td>
<td>1.7±0.3</td>
<td>2.4±0.2</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>Mannans</td>
<td>b.d.l.</td>
<td>1.5±0.2</td>
<td>b.d.l.</td>
<td>1.0±0.1</td>
<td>1.1±0.0</td>
</tr>
<tr>
<td>Galactans</td>
<td>0.5±0.1</td>
<td>1.8±0.2</td>
<td>1.0±0.1</td>
<td>4.1±0.2</td>
<td>1.8±0.1</td>
</tr>
<tr>
<td>Fructose</td>
<td>8.0±0.5</td>
<td>2.4±0.2</td>
<td>1.8±0.1</td>
<td>4.8±0.8</td>
<td>24.5±1.1</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>1.7±0.2</td>
<td>6.4±0.5</td>
<td>4.3±0.4</td>
<td>5.9±0.4</td>
<td>n.d.</td>
</tr>
<tr>
<td>Sucrose</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>2.7±0.2</td>
</tr>
<tr>
<td>Lignin</td>
<td>14.1±0.1</td>
<td>16.9±0.1</td>
<td>12.5±0.2</td>
<td>16.5±0.3</td>
<td>20.9±0.2</td>
</tr>
<tr>
<td>Protein</td>
<td>10.6±0.3</td>
<td>7.5±0.4</td>
<td>18.5±0.3</td>
<td>16.9±0.3</td>
<td>6.2±0.5</td>
</tr>
<tr>
<td>C:N</td>
<td>25:1</td>
<td>37:1</td>
<td>15:1</td>
<td>23:1</td>
<td>41:1</td>
</tr>
</tbody>
</table>

n.d. = not determined, b.d.l. = below detection limit (< 0.5% of DM).
Hemp was clearly the richest in cellulose, while the cellulose content of faba bean was the lowest. Non-cellulosic glucans, determined by acid methanolysis, consist of starch and glucans from hemicelluloses, and were especially high in faba bean. Thus, the total glucans in faba bean were almost nearly equal to glucans in hemp, although the cellulose content was low (Table 7). White lupin and Jerusalem artichoke contained fairly low amounts of glucans, but the high amount of fructans in artichoke increased the hexose amount to a relatively high level. Hemicelluloses were determined by HPAEC-PAD after acid hydrolysis and by GC-DB1 after depolymerization by acid methanolysis, resulting nearly to the same values. The values are presented as the main sugar in the polymer, not describing the composition of individual polymers (Table 7). Xylans were the most abundant sugars of the hemicelluloses, as expected. The maximum amount was observed in maize, approximately 15% of DM, whereas hemp and Jerusalem artichoke contained approximately 10.0%, and faba bean and lupin less than 7% (Table 7). Other hemicellulosic polymers, arabinans, mannans, and galactans, were low: from zero up to 4.1% of DM. Arabinans were mainly found in white lupin and Jerusalem artichoke, while galactans were clearly most abundant in white lupin due to the β-galactan-rich seeds. Mannan was most abundant in hemp, while practically no mannans were observed in maize (Table 7). The amount of galacturonic acid, which represents the amount of pectin, was highest in hemp and white lupin approximately 6% of DM) and 4% in faba bean, while it was a minor component in maize. Galacturonic acid was not determined from the Jerusalem artichoke.

**Lignin (I, II, III, IV)**

The lignin content in studied fresh materials varied from 12.5% of DM to 20.9% of DM (Table 7, II: Tables 2, 3, and 4). Acid soluble ash and protein are included in all lignin results if not otherwise mentioned.

**Acids, proteins, and inorganic compounds, (I, II, IV)**

In addition to the main components, carbohydrates and lignin, the studied feedstocks contained minor amounts of minerals (I: Table 4). The fairly high amount of oxalic acid in maize (9% of DM) and in late harvested hemp (5% of DM) and minor amounts of malic acid were interesting observations. The protein content was clearly lowest in hemp and highest in the legumes; faba bean and white lupin (Table 7, I: Table 2, II: Table 4, IV: Figure 4).
4.1.2 ENZYMATIC CONVERSION TO SUGARS OF FRESH CROPS (I, II, III, IV)

Enzymatic hydrolysis was studied by applying a commonly used commercial enzyme mixture at a standard dosage and conditions explained in materials and method section. Additionally, the effect of pectin removal on the hydrolysis of maize, hemp, and lupin by standard cellulolytic and hemicellulolytic enzymes was studied by the addition of a commercial pectinase preparation, rich in polygalacturonase activity. Fermentable sugars liberated in hydrolysis experiments were used to calculate the potential ethanol yields from all studied crops.

Hydrolysis tests (I, II, III, IV)

The conversion yields of carbohydrates into sugars in all five untreated fresh crops were relatively low, from 16% to 32% of DM (Figure 9). The conversion of the whole maize DM into sugars was the lowest among the studied crops. Maize contained a high original amount of soluble carbohydrates, thus still providing a high amount of fermentable sugars after 48 h enzymatic hydrolysis. A similar result was obtained with white lupin; the conversion of structured polysaccharides remained low, and a significant part of sugars in the hydrolyzate originated from WSC.

Figure 9 WSC in the original material and carbohydrates hydrolyzed enzymatically to neutral sugars and galacturonic acid are expressed as total carbohydrates (by HPAEC-PAD) of the DM. Hydrolysis was conducted for 48 hours with Celluclast and Novozyme (I-III), with and without addition of Pectinex (*) (III). Bar indicates ± one standard deviation of mean, n = 3.
Jerusalem artichoke showed the highest conversion of insoluble carbohydrates in the standard enzymatic hydrolysis of the studied crops. Fructose was the main hydrolysis product (35% of DM), while glucose represented only a minor fraction of monosaccharides (7% of DM).

**Hydrolysis of pectin in fresh maize, hemp, and lupin (III)**

The standard commercial enzyme complemented with pectinase increased the conversion of maize by 13%, hemp by 24%, and lupin by 31% of dry matter (Figure 9, III Figure 4). When the released galacturonic acid was calculated as part of the total carbohydrates, the conversion was 28% higher in hemp and lupin and 14% in maize compared to hydrolysis without pectinase addition (Figure 9). Additionally, the effect of removing pectin by pectinases from hemp was visually examined by SEM (Figure 10). The separation of fibers from larger bundles of hemp fiber was clearly seen (Figure 10C). In Figure 10B the fiber bundles were hydrolyzed with cellulases and hemicellulases, which were capable of utilizing carbohydrates from the surface of the fiber but not capable of separating them, whereas after the hydrolysis with pectinases, separation of individual bast fibers within fiber bundles was clearly seen.

![Electron microscopy images of hemp before and after enzymatic treatments with pectinase (III). A: Fresh hemp bast fibers; B: Hemp bast fibers hydrolyzed with cellulases and hemicellulases; C: Hemp bast fibers hydrolyzed with pectinase. The magnification was 2000 in A and 400 in B and C.](image)

**4.1.3 METHANE PRODUCTION OF FRESH CROPS (I, II, IV)**

The methane yields of the studied crops in 30 days of anaerobic batch digestion varied from 218 to 355 Ndm³ kg⁻¹ TS⁻¹ (Figure 11, I: Figure 1, II: Figure 2, IV: Table 1). Faba bean, maize, and lupin produced the highest yields, while the amount of methane produced from hemp and artichoke remained lower.
The conversion of pentoses (C5-sugars) and hexoses (C6-sugars) in the standard 30 days AD of maize, hemp, and lupin was studied, showing interesting variations between crops and different sugars. The C5-sugars, mainly xylose (analyzed by HPLC) (Table 7, I: Table 3, II: Tables 1, 2, and 3, III: Table 1), originated mainly from xylan-based hemicelluloses, and the C6 sugars, mainly glucose and fructose, originated from WSC (mainly starch) and cellulose. Conversion of C5- and C6-based carbohydrates (monomeric and polymeric sugars) was nearly complete in maize during the digestion (IV: Figure 1). Also C6-sugars in lupin were consumed in a similar way, whereas the conversion of C5-sugars was clearly lower, 46% of the total amount of C5-sugars. In hemp, the conversion of C5-sugars was almost undetectable; only 9% of total C5-sugars were consumed. The conversion of C6-sugars in hemp was 48%, which is clearly less compared with the C6-conversion in maize and lupin (IV: Figure 1).

4.1.4 ENERGY YIELD OF FRESH CROPS AS METHANE AND ETHANOL (I, IV)

The energy yields per hectare of the two energy carriers, ethanol and methane, and the lower heating value of the whole biomass when combusted are shown in Table 8.
Energy contents of maize, hemp, faba bean, white lupin, and Jerusalem artichoke are expressed as MWh ha$^{-1}$ as methane, theoretical ethanol yield from all determined carbohydrates, potential ethanol yield from hydrolyzed carbohydrates, and lower heating value (Santanen et al. 2011b).

<table>
<thead>
<tr>
<th>Methane$^1$</th>
<th>Ethanol $^2$</th>
<th>Ethanol $^3$</th>
<th>LHV$^4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MWh ha$^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maize</td>
<td>47.1</td>
<td>46.3</td>
<td>21.9</td>
</tr>
<tr>
<td>Hemp</td>
<td>30.6</td>
<td>45.3</td>
<td>20.4</td>
</tr>
<tr>
<td>Faba bean</td>
<td>35.5</td>
<td>28.3</td>
<td>14.1</td>
</tr>
<tr>
<td>Lupin</td>
<td>55.8</td>
<td>38.8</td>
<td>23.7</td>
</tr>
<tr>
<td>Artichoke</td>
<td>46.2</td>
<td>54.5</td>
<td>30.7</td>
</tr>
</tbody>
</table>

$^1$ Methane potential from the batch tests.
$^2$ Theoretical ethanol yield calculated from all carbohydrates.
$^3$ Potential ethanol yield calculated from hydrolyzed carbohydrates.
$^4$ Lower heating value (Santanen et al. 2011b).

Because of the high hectare yields of artichoke and lupin, the corresponding heating values were also the highest. The energy output of methane produced from lupin was highest, while the energy from calculated ethanol yields was relatively low. The energy output of ethanol calculated from the water-soluble or enzymatically hydrolyzed carbohydrates was only about half of the ethanol calculated from the total carbohydrates in the substrates. The incomplete utilization of biomass in methane and ethanol production, however, did not produce energy values as high as the combustion. Obviously, the combustion of the residue would increase the energy output.

### 4.2 EFFECT OF PRESERVATION ON HEMP, MAIZE, AND FABA BEAN

Maize, hemp and faba bean were ensiled with and without formic acid, and the hemp was additionally preserved with urea. Effects of preservation for four and eight months on chemical composition, enzymatic hydrolysis, and methane production were examined. In addition, the effect of pectin hydrolysis by pectinases on the conversion of fresh and preserved crops to fermentable sugars was studied in this work.
4.2.1 EFFECT OF ANAEROBIC PRESERVATION ON CHEMICAL COMPOSITION (II)

The traditional preservation methods are based on the natural production of acids (without additives) or on the addition of acids or alkali. The main effect of the preservation with and without additives is based on the change of the pH and aims at prevention of unwanted microbial growth, which causes nutritional losses and material spoilage. Formation or addition of acids induced the reduction of pH to the lowest value or 3.7 in maize, while addition of urea increased the pH of hemp up to 8.7 (II: Table 4).

The formation and influence of acids on the WSC during preservation were followed in the preserved crops (Figure 12, II: Figure 1 and Table 4). Fresh crops contained WSC, mainly fructose and glucose, from 2.8% to 20.8% (of DM). The major part of these sugars present in the fresh samples was converted to lactic and acetic acids by bacteria present in the plant material during the preservation (Figure 12, II: Figure 1). When the acidification was assisted with the added formic acid, WSC were well preserved, and only minor amounts or no acids were formed. The addition of the formic acid not only preserved the original WSC, but increased their amount compared with the fresh crop (II: Figure 1).

Figure 12 Water-soluble carbohydrates, expressed as total reducing sugars, and acids formed during the storing of various crops (% of DM). FA = formic acid, 4 = four months, and 8 = eight months. Bar indicates ± one standard deviation of mean, n = 3.
In 2009, prewilted hemp was treated with urea-water solution prior to the preservation process. The dry hemp contained only a low amount of WSC, and thus there were hardly any changes in WSC (Figure 12, II: Figure 1). Dry conditions also prevented the activity of microbes leading to low formation of acids. The most remarkable change in WSC was observed in faba bean preserved for eight months; the amount of WSC was 2.5 times higher compared with the fresh crop (Figure 12, II: Figure 1).

Besides the increase or decrease of WSC, there were no major changes in carbohydrates during preservation in acidic or alkaline conditions (Figure 12, II: Tables 1-3, Figure 1). Biological degradation of lignin is generally considered an aerobic process. A small increase in ammonium nitrogen was observed in prolonged preservation of all crops, especially when no acid was added (II: Table 4). Other organic acids found originally in the fresh crops included mainly malic and oxalic acids. The content of oxalic acid was especially high in fresh maize and hemp used for alkali preservation and remained throughout the preservation time. The only exception was the clear decrease of oxalic acid after eight months of storage with the formic acid as an additive.

### 4.2.2 EFFECT OF ANAEROBIC PRESERVATION ON ENZYMATIC CONVERSION TO SUGARS (II, III)

The monosaccharides after enzymatic hydrolysis consisted of sugars that were water soluble already at the beginning of the hydrolysis and of sugars converted from the polymers by enzymes. The conversion of almost all studied crops was increased slightly as a consequence of preservation (II: Figure 3). Part of the hydrolysis of structural carbohydrates occurred during the preservation, and the enzymatic conversion of actual polymers remained the same or was even reduced (II: Figures 1 and 3). Hydrolysis of structural carbohydrates, mainly starch (which is abundant in beans), was most clearly seen in faba beans after preservation for eight months with added formic acid. The conversion of the preserved material in enzymatic hydrolysis was only 3% of DM, and thus the sugar yields in the hydrolyzate increased by 42% compared with the fresh faba bean (II: Figure 3). On the other hand, the conversion of WSC into acids in maize, preserved without additives, decreased the sugar yield in hydrolysis, although the actual conversion of polymers in enzymatic hydrolysis was increased from 8% to 11% of DM (II: Figure 3). The most remarkable increase in conversion of polymers into monosaccharides was observed in enzymatic hydrolysis of alkali preserved hemp. The addition of urea doubled the hydrolysis yield of the fairly recalcitrant fresh hemp after four and eight month’s preservation (II: Figure 3).

Complementation of the conventional cellulose preparation with pectinases in the hydrolysis of fresh materials, especially on hemp, increased the conversion
of neutral sugars in the enzymatic hydrolysis (Table 9, **III**: Figure 4). Addition of pectinase on preserved maize had no effect (data not shown), but in hemp preserved in acidic or alkali conditions, the enzymatic conversion was remarkably improved as compared with fresh hemp (Table 9, **III**: Figures 3 and 4). As expected, the conversion increased in correlation with the pectinase dosage in the hydrolysis.

Table 9  Impact of pectinase addition on the conversion of sugars from the polymeric carbohydrates in enzymatic hydrolysis using commercial cellulases supplemented with pectinases at dosages of 0.2, 2.5, and 10 mg g⁻¹ substrate.

<table>
<thead>
<tr>
<th>Pectinase, mg/g dry substrate</th>
<th>Impact of pectinase addition, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemp fresh</td>
<td>-2  +16  +32</td>
</tr>
<tr>
<td>Hemp acid preserved</td>
<td>+14  +35  +38</td>
</tr>
<tr>
<td>Hemp alkali preserved</td>
<td>+36  +52  +75</td>
</tr>
<tr>
<td>Lupin fresh</td>
<td>n.d. +19 n.d.</td>
</tr>
</tbody>
</table>

n.d. = not determined

Hydrolysis with pectinase alone showed negligible liberation of neutral sugars, while the amount of galacturonic acid released was about the same when hydrolytic enzymes were also applied at the same time. SEM pictures of preserved materials treated with pectinases showed similar separation of bast fiber bundles into individual fiber cells in fresh hemp hydrolyzed with pectinases (Figure 10).

**4.2.3 EFFECT OF ANAEROBIC PRESERVATION ON METHANE YIELDS (II, IV)**

The conversion of soluble and polymeric C6 and C5 sugars in preserved hemp increased significantly during the AD as compared with fresh hemp (Figure 13, **IV**: Figure 1). The most notable decrease in the content of C5 sugars was observed in ensiled hemp after 30 days of AD. The consumption rates of C6 and C5 sugars were 48% and 9% of DM, respectively, in fresh hemp. The consumption of C6 sugars increased to 70% of DM, irrespective of formic acid addition in the preservation, whereas the consumption of C5 sugars increased to 36% with formic acid and to 45% of DM without additives. Galacturonic acid was completely consumed in formic acid-ensiled hemp; however, the conversion was already high in fresh hemp and ensiled hemp without formic acid.
The carbohydrates in fresh maize were converted to methane almost completely, and no major increase was observed after the material was preserved with or without formic acid for eight months. The detailed conversion of carbohydrates in ensiled faba bean was not studied. Analogous to the increased conversion of carbohydrates, the methane yields were clearly higher for ensiled and acid-preserved hemp compared to fresh hemp (**: Figure 2, ***: Table 1). Instead, only a minor increase in methane yield was observed in alkali-preserved hemp (**: Figure 2).

Increased methane yield after acidic preservation was observed also in maize. Considering the reproducibility of the experiments, *i.e.* the deviations of the eight replicates, the increased effect of ensiling on methane yield of maize was not as high as in hemp. Since the conversion of sugars during AD of fresh maize was already almost complete (**: Figure 1), the enhancement effect of ensiling on conversion was clearly notable, however statistically significant. The maize ensiled for eight months that was chosen for detailed conversion experiments showed, however, decreased yields. Preservation of faba bean, especially after prolonged storing time and without additional acid, reduced methane yields significantly (**: Figure 2).
4.2.4 EFFECT OF ANAEROBIC PRESERVATION ON ENERGY YIELDS AS METHANE AND ETHANOL (II, IV)

The effect of preservation on conversion of carbohydrates to fermentable sugars and methane differed. The effects of various preservation methods and periods on the energy yield as methane (measured) and ethanol (calculated) are shown in Table 10.

Table 10  Impacts of preservation on energy yields ha⁻¹ DM as methane and ethanol compared to energy yields of fresh raw materials (II).

<table>
<thead>
<tr>
<th>Impact on energy yield, %</th>
<th>Methane</th>
<th>Ethanol</th>
<th>Methane</th>
<th>Ethanol</th>
<th>Methane</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preserved 4M</td>
<td>+15</td>
<td>-52</td>
<td>+54</td>
<td>+0</td>
<td>-17</td>
<td>+5</td>
</tr>
<tr>
<td>Preserved 8M</td>
<td>+25</td>
<td>-26</td>
<td>+26</td>
<td>+11</td>
<td>-36</td>
<td>+0</td>
</tr>
<tr>
<td>Preserved with FA 4M</td>
<td>+35</td>
<td>+18</td>
<td>+48</td>
<td>+5</td>
<td>-27</td>
<td>+11</td>
</tr>
<tr>
<td>Preserved with FA 8M</td>
<td>+8</td>
<td>+30</td>
<td>+33</td>
<td>+17</td>
<td>-10</td>
<td>+39</td>
</tr>
<tr>
<td>Preserved with urea 4M</td>
<td>n.d.</td>
<td>n.d.</td>
<td>+21</td>
<td>+39</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

*Calculated from WSC and carbohydrates hydrolyzed during the preservation or in enzymatic hydrolysis.

M = months, FA = formic acid, and n.d. = not determined

4.3 EFFECT OF PRETREATMENTS ON METHANE AND ENZYMATIC HYDROLYSIS YIELDS (I,III)

All the studied pretreatments led to clear changes in the chemical composition of hemp. (Table 11, III: Table 1). Steam explosion solubilized xylan but had less effect on lignin, although the lignin structure was likely modified. Presoaking in acid prior to steam explosion increased the solubilization of hemicelluloses. The alkaline treatment significantly increased the share of glucans by releasing lignin, hemicelluloses, and pectin. Xylan was solubilized to some extent. The amount of solubilized fractions during the pretreatments varied from 8% to 40% (Table 11). The amount of cellulose decreased in acid-presoaked material, while the amount remained intact in other treatments. The enzymatic conversion of hemp showed strong correlation with the amount of pectin (III: Figure 2).
Table 11  Chemical composition of the studied hemp materials (fresh and pretreated) expressed as % of DM. Standard deviation is in parenthesis (n= 3).

<table>
<thead>
<tr>
<th></th>
<th>Fresh</th>
<th>SE</th>
<th>SE with acid</th>
<th>Alkali treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of dry matter</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucan</td>
<td>46.1 (1.3)</td>
<td>55.4 (0.4)</td>
<td>64.6 (1.6)</td>
<td>83.6 (1.2)</td>
</tr>
<tr>
<td>Xylan</td>
<td>9.5 (0.1)</td>
<td>4.4 (0.1)</td>
<td>1.8 (0.1)</td>
<td>8.4 (0.2)</td>
</tr>
<tr>
<td>Arabinan</td>
<td>1.2 (0.1)</td>
<td>b.l.d.</td>
<td>b.l.d.</td>
<td>b.l.d.</td>
</tr>
<tr>
<td>Mannan</td>
<td>2.2 (0.1)</td>
<td>1.7 (0.1)</td>
<td>b.l.d.</td>
<td>b.l.d.</td>
</tr>
<tr>
<td>Galactan</td>
<td>1.6 (0.1)</td>
<td>b.l.d.</td>
<td>b.l.d.</td>
<td>b.l.d.</td>
</tr>
<tr>
<td>Galacturonic acid</td>
<td>5.9 (0.6)</td>
<td>1.3 (0.1)</td>
<td>b.l.d.</td>
<td>b.l.d.</td>
</tr>
<tr>
<td>Lignin</td>
<td>18.0 (0.3)</td>
<td>19.8 (0.4)</td>
<td>29.6 (1.4)</td>
<td>7.2 (0.4)</td>
</tr>
<tr>
<td>Total identified components</td>
<td>84.6</td>
<td>82.7</td>
<td>95.4</td>
<td>99.3</td>
</tr>
<tr>
<td>Loss of organic material</td>
<td>7.9</td>
<td>18.6</td>
<td>39.7</td>
<td>35.0</td>
</tr>
</tbody>
</table>

SE = steam explosion, b.l.d. = below detection limit (< 0.5% of DM).

The changes in the hemp structure were most significant after alkali treatment (Figure 14B) (III), visualized by SEM, and were comparable to modifications observed after pectin removal in enzymatic hydrolysis (Figure 10C).

Figure 14  Electron microscopy images of fresh hemp after extraction with water or with 1% NaOH. A: Bast fibers extracted with water; B: Bast fibers extracted with 1% NaOH; C: Woody layers extracted with water; and D: Woody layers extracted with 1% NaOH. The magnification of A = 2000, B = 400, and C and D = 100.
The visualized release of fiber bundles was efficient, and the effect on the wood layer part was remarkable. The exposed wood layer structures, spiral tracheids, became clearly visible after treating with NaOH (Figure 14D). Such clear visible changes were not observed in steam-exploded materials (III).

Steam explosion and alkaline treatment had a desired effect on the conversion of carbohydrates in hemp. The conversion of both glucan and xylan in standard enzymatic hydrolysis increased after all pretreatments (Figure 15). The steam explosion, however, was improved after soaking in 2.5% H₂SO₄ prior to the treatment and resulted almost to a theoretical yield in the enzymatic conversion. However, the loss of carbohydrates was the highest as well.

Figure 15 Conversion of glucan and xylan to sugars in enzymatic hydrolysis of fresh, steam-exploded with and without H₂SO₄, and alkali-treated hemp, expressed as % of DM. Bar indicates ± one standard deviation of mean, n = 3.
Steam explosion was conducted also on fresh hemp and ensiled hemp as well as on fresh and ensiled maize. These samples were not washed prior to methane production or enzymatic hydrolysis. The results showed that the steam explosion improved the methane conversion of fresh crops (Figure 16), but impaired the conversion of ensiled crops (Figure 16), at least during the early stages of the AD. It could also be clearly observed that ensiling increased the methane yields of both crops more than the steam pretreatment (Figure 16). The effect of steam explosion and ensiling was clearer for hemp than maize, indicating the more recalcitrant structure of this material, requiring pretreatment.

Steam explosion resulted in increased hydrolysis yields, especially on hemp, and only the steam explosion of fresh maize decreased the amount of WSC, leading to the reduction of total fermentable sugars. Steam explosion decreased the amount of WSC in formic acid-ensiled maize as well, but the yield was compensated with the increased conversion of the structural carbohydrates. The effects of steam explosion on maize, however, were less remarkable than on hemp due to the softer, more accessible structure of the raw material, whereas the steam explosion increased the yields of all hemp samples, fresh and ensiled (Figure 17).

Figure 16  Methane yields of fresh and ensiled maize and hemp, without or after steam explosion (SE) after 30 days of AD at 35°C. Results are expressed as Ndm³ kg⁻¹ TS. CH₄ yield of inoculum is subtracted from the sample yields. Bar indicates ± one standard deviation of mean, n = 8.
Figure 17  Conversion of carbohydrates as reducing sugars in fresh and ensiled maize and hemp, without or after steam explosion, to sugars in standard enzymatic hydrolysis, expressed as % of DM. The WSC indicates the amount of water-soluble carbohydrates in the samples; SE = steam exploded. Bar indicates ± one standard deviation of mean, n = 3.

In addition to steam explosion and alkaline pretreatment, the effect of milling as a physical pretreatment method was examined to enhance the accessibility of enzymes in the hydrolysis and AD and thus to increase the conversion of carbohydrates to sugars. Milling of fresh hemp and maize to fine particles of the size of about 1 mm (instead of chopped 10-20 mm) was carried out. The reduction of particle size, however, had no effect on the hydrolysis yield of maize, whereas milling significantly increased the conversion of polymers into sugars in hemp from 25% to 35% of DM (I: Figure 2) and the methane yield from 218 Ndm$^3$ kg$^{-1}$ TS$^{-1}$ to 263 Ndm$^3$ kg$^{-1}$ TS$^{-1}$ (Figure 16, I).
5 DISCUSSION

5.1 FRESH MAIZE, HEMP, FABA BEAN, WHITE LUPIN, AND JERUSALEM ARTICHOKE AS RAW MATERIALS FOR FERMENTABLE SUGARS AND METHANE

Each of the studied crops has potential as an energy crop based on the chemical composition, although all components of the raw material cannot always be easily converted into biofuels. The high content of protein in legumes, faba bean and white lupin, increased their ability as raw materials for methane as earlier observed for faba bean (Peterson et al. 2007). The total energy content as methane per hectare was especially high for white lupin due to its promising biomass yield (Santanen et al. 2011b). In this work, the best conversion of carbohydrates to sugars in the enzymatic hydrolysis along with the high biomass hectare yield (Santanen et al. 2011b) made the Jerusalem artichoke a highly productive crop for ethanol production, which is in agreement with earlier studies by Caserta and Cervigni (1991).

The recalcitrance of lignocellulosic materials prevents the accessibility of enzymes to cleave the polysaccharides to sugars (Wyman et al. 2004). Hemp represented most clearly the material with the highest cellulose content, but the crystalline, inaccessible structure hindered the efficient conversion, requiring pretreatment to enhance the methane and ethanol yields (Kreuger et al. 2010). Maize, the only monocot among the studied crops, has a different structure compared to, e.g., hemp and white lupin. Parenchyma cells, most abundant in maize (Ding and Himmel 2008), generally have only primary cell walls, which make them easier to be degraded, while sclerenchyma cells, present, e.g., in hemp fiber (Haudek and Viti 1978), act as supporting elements in stems that have finished elongations. These cells have thick, often lignified, secondary walls that help to provide mechanical strength to the plant but additionally make it more resistant to enzymatic and microbial degradation (Raven et al. 2007). This structural dissimilarity may have an effect on the distinct detachment of the linkages between components within the tissues. Xylans, especially, have previously been observed to limit the hydrolysis of cellulose due to the proximity or overlapping layers of these polysaccharides in the raw materials. Thus, the addition of xylanases significantly enhanced the hydrolysis of maize when using pure cellulases (CBH, BG), while xylanases had no effect on hemp, expectedly due to the recalcitrance of cellulose, primarily limiting the hydrolysis (Zhang, unpublished).

Pectic compounds seem also to have an essential role in the conversion of materials rich in pectins (Wang et al. 2003, Cosgrove 2005). The enzymatic conversion, especially of hemp and white lupin, was increased when
supplementing cellulases and hemicellulases with polygalacturonase activity. The result indicated that pectic compounds may also hinder the conversion in AD, or the used inoculum lacked microorganisms capable of utilizing pectin efficiently. In a continuous process, the microbial flora could eventually be enriched with pectin-consuming organisms if the raw material is rich in pectin (Lynd et al. 2002). Obviously, due to the low content of pectin in maize, the addition of pectinases had only a minor impact in the hydrolysis.

The enzymatic hydrolysis was conducted with a standard commercial mixture and dosage of enzymes, which may have been less efficient on some of the crops. The inulin-rich Jerusalem artichoke would have most probably benefited from an additional supplementation of fructanase or inulinase (Buyn and Nahm 1978). The dosing of enzyme mixture per DM was also somewhat unfair to crops containing a higher share of structural carbohydrates, especially of cellulose. However, it was estimated that the amount of enzymes used was relatively high and thus sufficient for reaching a representative hydrolysis level.

5.2 EFFECT OF PRESERVATION

5.2.1 CHEMICAL COMPOSITION

The most notable change in chemical composition of crops during the preservation was noticed in WSC. The free sugars are utilized by the anaerobic bacteria to produce organic acids as was observed especially in maize and is the main aim in ensiling (McDonald et al. 1991). In contrast to the consumption of sugars in untreated ensiling, the formic acid addition preserved carbohydrates well, and no or only minor acid formation was observed. The addition of \(H_2SO_4\) prior to ensiling has been observed to be as efficient as formic acid (Digman et al. 2010), and both were found to be more beneficial than, e.g., addition of sugars as substrates for \textit{in situ} production of acids (Thompson et al. 2005). Addition of lactic acid bacteria to the natural flora present in the raw materials was found to be successful in ensiling of sugar beet pulp, rich in WSC, which made it particularly vulnerable for deterioration in storing (Zheng et al. 2011). Concurrently with the preservation of carbohydrates, the amount of WSC increased during ensiling with formic acid as observed also in ensiling of kenaf treated with enzymes (Murphy et al. 2007).

An increased amount of WSC, in especially maize and faba bean, indicates partial (mild) acid hydrolysis during the preservation in anaerobic conditions, as observed also by Jaakkola et al. (2006b). Only minor conversion of sugars to acids was observed in alkali-preserved hemp due to the low amount of WSC and high DM concentration (Tetlow 1992). The concentration of acetate increased with the addition of either acid or urea in all studied crops, which agrees with
earlier observations by Digman et al. (2010). The authors suggested that acetate most likely originated from chemical deacetylation of arabinoxylans, as it is doubtful that fermentative activity was responsible for the formation of acetate at the extreme pH values. Previously, the alkaline pretreatment releasing acetyl groups from hemicelluloses have been shown to improve the digestibility of crop residues in the rumen of sheep (Chesson 1981).

A small decrease of acid hydrolysis residue (mainly lignin) amount in especially hemp was observed during ensiling. The same minor degradation of the residue was observed after 30 days of AD, which showed presumable alterations of other components such as protein rather than partial degradation or modification of lignin structure. Similarly, neutral detergent fiber content measurement was interfered by the released structural nitrogen during ensiling of grass in previous studies (Rinne et al. 1997).

5.2.2 YIELDS OF ENERGY CARRIERS

Methane

The effect of preservation on methane yields varied among different crops. A maximum increase of methane yield by 54% was observed in hemp ensiled for four months, while the yield decreased in each ensiling experiment of faba bean. Preservation time and used additives also influenced the methane production of each crop. Previously, in several studies, e.g., by Amon et al. (2007a) and Plöchl et al. (2009), ensiling has been observed to improve the methane yields of maize and alfalfa grass (Medicago sativa). However, the calculation methods based on methane yield per total solids or volatile solids have recently aroused discussion (Kreuger et al. 2011). Acids and alcohols added or formed during the preservation process evaporate in the determination of the DM content, resulting in underestimations of TS and VS. The incorrectly estimated solid content leads to excessively high methane yields. Correction of the yield for the TS content has been a regularly used procedure in studies concerning ensiling for feed production (Huida et al. 1986), and it was used in this work, as well, for calculations of acids and methane yields of preserved materials. Therefore, the values obtained for the improvement of methane yields, observed especially on hemp, can be considered reliable. Only the material loss to formation of gases other than methane or other side products during lab scale ensiling experiments was not determined and thus not considered in the total methane yield. Nevertheless, losses of energy are often lower than the losses of DM since the formed fermentation products during ensiling have a higher gross energy (GE) value than the original substrates (McDonald et al. 1991).

Improvements of methane yields of ensiled hemp were observed also by increased conversion efficiency of both hexoses- and pentoses-based
polysaccharides to methane. These results suggest that although the preservation led to only small visible changes in the structure and to minor degradation of pectin, lignin (including acid insoluble protein and ash), cellulose, and hemicelluloses, it increased remarkably the conversion of especially xylan with consequently improved methane yields. Pectin is considered as a glue material between bast fiber cells and is present also in the cell wall (Carpita and Gibeaut, 1993), whereas xyloglucans are suggested to be covalently attached to pectic polysaccharides, forming a macromolecule that anchors the microfibrils by sticking xyloglucans to cellulose surfaces (Cosgrove 2005). This could explain part of the increased pentose consumption together with the increased pectin release during the AD. Similarly to hemp, in fresh white lupin (which also has a high content of pectin), the xylan was not as efficiently converted to methane as it was in maize. Although there was poor conversion of C5 carbohydrates in hemp and lupin, the efficient conversion of fresh maize to methane showed that the inoculum contained microorganisms that were able to produce hemicellulases with adequate xylanolytic activity. Conversion of both C6- and C5-based carbohydrates was almost complete in fresh maize, and no major difference in the consumption of carbohydrates between fresh and preserved maize during biogas production was observed.

**Ethanol**

The conversion in enzymatic hydrolysis after preservation (with the enzyme dosages used) was most significantly improved in hemp preserved with urea. The positive effect on digestibility of straw after alkali ensiling has been observed earlier in feed used for buffalos (Wanapat et al. 1985) and in conversion of switchgrass to glucose (Digman et al. 2010). The conversion increased also in acidic conditions, but the effect was less notable. However, such a clear increase could not be verified when preserved hemp was washed and freeze dried, which may reflect the sensitivity of the raw materials to treatment conditions. Drying of fibers can result in irreversible collapse and shrinking of the capillary structure, thus reducing the accessible surface area, as reviewed earlier by Hubbe et al. (2007) and Taherzadeh and Karimi (2008).

The addition of formic acid in ensiling was most essential on maize due to its high WSC, which was lost during ensiling without additives. This leads to the conclusion that prevention of the natural formation of lactic acid by supplementation of acid or base (Digman et al. 2010) is essential for crops containing high amounts of WSC in order to prevent the loss of WSC or easily hydrolyzed carbohydrates (Zheng et al. 2011). When using crops containing relatively higher amounts of structural polysaccharides, additives are not as important. During the preservation, there are usually fewer available carbohydrates fermented easily to acids that lead to loss of carbohydrates in ethanol fermentation. However, additives are important for preservation of the material if the natural acid formation is limited (McDonald 1991). The slower
formation of ethanol from ensiled maize, however, similar to the ethanol yield of fresh maize, indicates that formic acid may have inhibited the yeast used for fermentation as observed earlier (Klinke et al. 2004). In contrast, in earlier ensiling studies it has been observed that yeasts were more active in formic-acid-treated herbaceous plants (Henderson et al. 1972). It has been reported earlier that acetate levels as low as 0.5 g L⁻¹ can cause stress on some yeasts (Almeida et al. 2007). In this work the acetate load in the fermentation of ensiled maize (without formic acid) was 1.6 g L⁻¹. However, at a buffered pH of 5, yeast can tolerate considerably higher levels of both acetic (pKa 4.74) and lactic acid (pKa 3.86), as the acids will be mostly in the dissociated form, which is not inhibitory to growth (Graves et al. 2006). However, this has been a concern in some other approaches, such as in fermentations at higher solids loading or at lower pH (Digman et al. 2010). Ensiling of faba bean also increased the amount of fermentable sugars but resulted in a lower overall energy level as an ethanol due to its somewhat lower biomass yield per hectare compared to all the other crops studied in this work.

5.2.3 ENHANCEMENT OF ENZYMATIC HYDROLYSIS OF ENSILED CROPS BY HYDROLYSINGPECTIN

The addition of pectinases in the enzymatic hydrolysis of hemp removed part of the pectic compounds located between the single bast fiber cells. The separation of fibers within the fiber bundle was clearly seen in the SEM images, which agree with earlier observation in the retting process (Zhang et al. 2000). The increased availability of the surface area of the substrate (cell walls) thus led to an improved accessibility of enzymes and about 20% higher conversion compared to hemp hydrolyzed without pectinases. Interestingly, the conversion of pectin to galacturonic acid and glucans to glucose was clearly improved more in the preserved material. Although no clear decrease in the amount of pectin was observed after preservation, the acidic conditions could have affected the interactions of the compounds in the lignocellulosic matrix. The positive effect of ensiling has also been observed in pectinase-aided retting of flax. Preservation for two months with sulphur dioxide prior to enzymatic retting enhanced the separation of flax fibers, compared to the pectinase retting of dried flax (Easson and Molloy 1998). It has been suggested that the more complete removal of pectin and separation of bast fibers is caused by the chelation of Ca²⁺ by acids. Removal of Ca²⁺ ions has been observed to improve the hydrolysis of pectic acids by polygalacturonases (Voragen et al. 1995). In this work, ensiling was not observed to solubilize pectins, but the results suggested that the acids (added or formed) may have contributed to the access of pectinases. The positive effect of pectin hydrolysis on the hydrolysis of hemp was even more remarkable when hemp was preserved with urea. In alkaline conditions, scissions of polysaccharides caused by peeling reactions may partly explain the enhanced conversion.
These results, however, may also be caused by the composition of the raw material itself. Hemp was cultivated in two different years, and the hemp from the 2009 cultivar that was used for alkaline-preservation tests contained remarkably higher amounts of oxalic acid than the hemp from 2008. Oxalate is a common constituent of plants (Libert and Franceschi 1987) and may significantly vary within the same species in different years of cultivation because of, e.g., soil moisture, temperature, and hours of sunlight during the growing period (Rahman and Kawamura 2011). Oxalic acid and polygalaturonases have been found to function in concert to degrade pectic compounds, thus partially explaining the enhanced galacturonic acid release from hemp containing more oxalate (Green et al. 1996). The synergistic action of oxalate and pectinases has been observed to weaken the lignocellulosic structure, thus increasing the pore size to permit penetration of lignocellulolytic enzymes (Dutton et al. 1993). The alkali-preserved hemp produced a higher yield of galacturonic acid in hydrolysis supplemented with pectinases, and the fresh hemp from 2009 with higher amount of oxalate also resulted in a higher hydrolysis yield compared to the hemp from the 2008 cultivar. Oxalic acid was recently observed to depolymerize cotton cellulose, which suggests another potential role of oxalate in addition to being a chelating agent (Hastrup et al. 2011). Oxalate could act as a reducing agent for the conversion of Fe$^{3+}$ to Fe$^{2+}$ or Cu$^{2+}$ to Cu$^{+}$ in the crop, thus depolymerizing polysaccharides by the Fenton chemistry (Fenton 1989). However, if this assumption is correct, maize with the highest oxalate content would be affected by the free radicals formed by the Fenton reaction during ensiling.

5.3 EFFECT OF PRETREATMENTS ON METHANE AND ETHANOL PRODUCTION

Steam explosion and alkali treatment had an expected impact on the chemical structure of hemp. Steam explosion removed a major amount of xylan and H$_2$SO$_4$ soaking enhanced the solubilization of hemicelluloses, as also observed recently by Sipos et al. (2010). The severity of the treatment seemed to even cause some degradation of cellulose to glucose, as reported earlier by Jorgensen et al. (2007). Washing of the materials prior to enzymatic hydrolysis leads to partial losses of the valuable sugars. However, the possible inhibitors were removed as well. Lignin was not solubilized but most probably altered, as reviewed by Mosier et al. (2005). Pretreatments in alkaline conditions have been observed to solubilize lignin; however, it depends on the alkali concentration used (Wang et al. 2003). Steam explosion and NaOH treatment have also been used to remove pectin from the middle lamella of fibrous crop cells (Nykter et al. 2008). In this work, the NaOH treatment at 120°C showed a similar effect, and consequently, the pretreated material had less than half of the lignin than the untreated hemp, and no pectin was detected in the untreated
hemp. Total carbohydrates determined from steam-exploded maize did not remarkably increase due to the partial loss of WSC during the hydrothermal pretreatment; individual sugars were not determined. The increased hydrolysis yield of xylans, however, indicated that xylans had either been partially removed or altered.

Chemical changes in hemp caused by the steam explosion and alkali pretreatments were studied in more detail, and the improved conversion of glucan and xylan was clearly observed in both steam- and alkali-pretreated hemp. The reduced amounts of xylans and lignin are usually emphasized as the main factors that strongly correlate with an increased yield of enzymatic hydrolysis (Öhgren et al. 2007). The removal of these components allows swelling of the material, increases the surface area, and eventually cleaves some lignin–carbohydrate linkages and removes other hindering components, thereby enhancing the action of enzymes (Fan et al. 1987). The fact that a relatively small amount of released xylose could increase the cellulose hydrolysis, as observed by Várnaí et al. (2010), indicates that the relative amount of hemicelluloses and the location of these carbohydrates with respect to cellulose (or lignin) play important roles in the efficiency of the hydrolysis. In this work, a positive correlation between the reduced amount of xylan (or lignin) and the increased enzymatic hydrolysis, however, was not clearly observed. If the steam-exploded samples with the high lignin content but increased conversion were removed, the correlation between the lignin amount and the degree of hydrolysis became more evident. This result is in agreement with previous results in which even the modification of lignin structure without reducing the amount has been suggested to affect the enzymatic digestibility of corn stover (Yang and Wyman 2004). The amount of pectin, however, seemed to have a clear correlation with the degree of enzymatic conversion of hemp biomass into sugars. The removal of pectin between the single bast fiber cells was clearly seen after steam explosion and alkali treatment, thus leading to an increased surface area available for enzymes. The effects of pectin removal are consistent with results obtained earlier in retting studies (Wang et al. 2003, Nykter et al. 2008).

Although steam explosion of fresh maize and hemp had a minor increasing effect on methane yields, the improvement by ensiling was more significant than by thermal pretreatment. Conditions of the steam pretreatment were not optimized in this work, and obviously, the obtained benefits were limited for both. Therefore, in light of these results, the steam explosion was not necessary if the crop was preserved prior to methane production. However, ethanol production from the recalcitrant hemp, fresh or ensiled, was enhanced by the hydrothermal treatment, as observed recently by Sipos et al. (2010). The hydrothermal treatment has been found to improve the yield of enzymatic hydrolysis when the amount of WSC in maize was low or was completely lost in earlier stages (Varga et al. 2003).
5.4 EVALUATION OF THE SUITABILITY OF STUDIED CROPS AS ENERGY CROPS

Several parameters require consideration when choosing the best energy crop for cultivation; therefore, some of the advantages and disadvantages of studied crops are collected in Table 12 (Stoddard 2008, Stoddard 2010). Cultivation properties differed among crops; however, the biomass yields were encouraging for all crops (Santanen et al. 2011b). Energy yields per hectare in this work are calculated based on the biomass yield on harvesting years. However, crops were cultivated for several years, giving similar or even higher yields of biomass. Maize, for instance, yielded over 25 t ha⁻¹ in 2007 (Santanen et al. 2011b).

A favorable alternative would be to combine the production of food and bioenergy from the same crop. In this work, Jerusalem artichoke represented this combined approach. Maize, white lupin, and faba bean were used as whole crops, only for energy production. The edible cobs in maize or seeds of lupin for feed were not mature enough at the time of harvesting. Faba bean is presently cultivated for harvesting of the seeds for feed use, and the residue would be available for production of bioenergy. The energy yields would thus be lower if cobs and seeds were used for food or feed production instead. The use of the bast fibers of hemp for textile or composite industry would also be a valuable alternative (Wang et al. 2003). In this case the woody stem part could be used as raw material for bioenergy (Barta et al. 2010).

<table>
<thead>
<tr>
<th>Crop/attribute</th>
<th>Maize</th>
<th>Hemp</th>
<th>Jerusalem artichoke</th>
<th>White lupin</th>
<th>Faba bean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fertilizer requirement</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weed control requirement</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Pest and disease control requirement</td>
<td>-</td>
<td>--</td>
<td>--</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Frost/cold sensitivity</td>
<td>++</td>
<td>-</td>
<td>--</td>
<td>-</td>
<td>--</td>
</tr>
<tr>
<td>Ease of termination of production</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Potential biomass production</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>

+++high, +moderate, −low, −−very low
Besides cultivation suitability, the total environmental impacts of the whole conversion processes from field to products are essential when choosing energy crops. This thesis did not focus on GHG or energy balance calculations, but these would be required before establishing production on an industrial scale. Based on current research results of greenhouse gas assessments, both cellulose-based ethanol and biogas, however, are regarded to belong to the category of biofuels that reduce more than 70% of greenhouse gas emissions when substituting fuels based on fossil raw materials (Nylund and Aakko-Saksa 2007). Energy consumption is dependent on various parameters, including, e.g., transportation of the raw material and nitrogen fertilizer used (Börjesson 1996, Mikkola and Ahokas 2009).

In 2009, the total energy consumption in Finland was 368 TWh (1326 PJ); about 20% of the energy was used for transportation (Center of Statistics, Finland 2012). The Ministry of Agriculture and Forestry in Finland has proposed that by 2012 up to 500 000 ha could be dedicated to energy crop production (Vainio-Mattila et al. 2005). Biogas production and recovery in Finland in 2010 was about 421 GWh, and 75% of this gas was utilized for heat production and the rest for electricity (Huttunen and Kuittinen 2011). Utilization of methane as a transportation fuel has been negligible, but a positive increase has been assumed for 2011, as reported by Huttunen and Kuittinen (2011). Over half of the biogas was recovered from landfills, and the rest was produced at several waste water treatment plants, i.e. in sewage sludge digesters, co-digestion plants using various raw materials, and in 10 farm-scale biogas plants. If the biomass produced on this available area, 500 000 ha, would be converted to methane, the theoretical energy yield from crops studied in this work could be about 27 TWh from white lupin, 23 TWh from maize and artichoke, 18TWh from faba bean, and 15 TWh from hemp. Preservation or pretreatment of hemp, especially, would increase the yields even further.

Bioethanol from second-generation feedstocks is not yet produced in Finland, although presently, some of the ethanol mixed with gasoline is produced from different wastes (Autoalan Tiedotuskeskus 2012) or imported. If all theoretically available carbohydrates in these crops would be utilized for ethanol, only hemp and Jerusalem artichoke would produce more energy than when converted to methane. However, according to the enzymatic hydrolysis results in this work, the energy obtained as ethanol from all five crops without pretreatments would be significantly less—30-50% of the methane values. It would be expected that optimization of the preservation and pretreatments, however, would increase the ethanol yields. The utilization of different wastes, agricultural residues, and energy crops would together strengthen the supply of raw materials for second-generation transportation biofuels in Finland as well as in other countries.
The future will show which of these biofuel alternatives will dominate, however, there will be the need as well as the room for both. The recognized bottlenecks of enzymatic hydrolysis—including a fairly high amount of added enzymes, non-specific enzyme adsorption on the surfaces of the substrate, eventual inactivation, and possible recycling—are to be solved. The efficiency of methane production could eventually be improved by selecting more efficient “enzyme factories,” i.e., strains to be added to the inocula used. These improvements will lead to greater feasibility of both processes. If the political decisions lead to methane as the preferred choice for energy conversion to electricity, the importance of ethanol as transportation fuel would increase. Today, the unquestionable need is to produce fuels only from nonfood biomasses. On the other hand, it is expected that in the future, ethanol will be replaced by other, more efficient solutions, not requiring the hydrolysis of biomass. However, also in the future, the main aim will be to produce the maximum amount of biofuels with the minimum environmental consumption.
6 CONCLUSIONS

The present work evaluated the suitability of five yet uncommonly cultivated field crops, namely maize, fiber hemp, faba bean, white lupin, and Jerusalem artichoke to be cultivated as raw materials of methane and ethanol production in boreal conditions. The energy yields of the two energy carriers were also compared. Because of the ability to utilize proteins in AD, it would be justified to use white lupin and faba bean, among the studied whole crops, primarily for methane production due to their high protein content remaining unutilized in ethanol production. Hemp and Jerusalem artichoke showed potential as raw materials for bioethanol and methane. Maize seemed to be suitable for production of both energy carrier products. In order to gain the best benefit of all constituents in crops, the high amount of WSC and the utilization of pentoses need to be considered in ethanol production and pretreatments.

The work also introduced acidic and alkaline preservation methods to enable year-around use of wet biomass. Preservation was observed to have several effects on crops in enhancing the conversion of biomass to fermentable sugars or methane. Hemp ensiled with or without formic acid showed the highest enhancement in methane production, while the increase of the hydrolysis yield was most remarkable in hemp preserved in alkaline conditions. Formic-acid addition was essential in preservation of maize for ethanol production to prevent the high loss of water-soluble sugars into fermented acids. Especially in maize and faba bean, structural carbohydrates were already hydrolyzed during ensiling. Improvement of conversion in enzymatic hydrolysis, however, remained low or the same compared with the conversion of fresh crop.

Interestingly, methane production from fresh maize and hemp was more beneficial after ensiling than after hydrothermal pretreatment. Hydrolysis of polymers in hemp to sugars for ethanol production required more harsh treatment conditions to increase the accessibility of enzymes and to achieve more efficient conversion of biomass. In maize, rich in WSC, the balance between pretreatment severity and the loss of fructose, e.g., needs optimization and further consideration.

The amount of pectic compounds seemed to strongly correlate with the yield of enzymatic hydrolysis in hemp. Pectin, consisting of galacturonic acid, is known to glue hemp bast fibers to bundles and to the surrounding tissues. Supplementation of standard cellulases with pectinases enhanced not only the yield of galacturonic acid but also of glucose in the hydrolysis of hemp. Acids in ensiling have been suggested to have a chelating effect on Ca^{2+} ions, thus increasing the hydrolysis with pectinases even further by loosening the structure of pectin. Interestingly, the effect of hydrolysis of pectin was highest in alkal-
preserved hemp. The reason was suggested to be due to the high amount of oxalic acid, which has been observed to act synergistically with polygalacturonase by weakening the lignocellulosic structure and thus increasing the accessibility of enzymes. Pectin removal by pretreatments correlated strongly with enzymatic hydrolysis and seemed to have a positive effect on methane production as well.

In the future, it would be interesting to study the effect of preservation of the highest yielding biomass crops, Jerusalem artichoke and white lupin. Also, the effect of preservation on the structure of various crops is still partially unknown and a fascinating topic. Also, the role of oxalic or other acids in hydrolysis of pectin and the degradation mechanism of pectin together with other polymers during the preservation of recalcitrant lignocellulosic materials would need further investigation.

As a final conclusion of this work, the importance of understanding the chemical composition and structure of crops used as raw materials for bioenergy production cannot be overestimated. To achieve the best possible conversion efficiency of different crops from field to fuel, knowledge on the effects of preservation, pretreatments, and used parameters in enzymatic hydrolysis, e.g., is essential. The selection of suitable crops in the existing climate conditions as well as how to convert the raw materials into the most convenient energy carriers are nationally and internationally important issues.
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