JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Conversion of Carbohydrates in Herbaceous Crops during Anaerobic Digestion

Annukka Pakarinen,*[,†] Maritta Kymalainen,[§] Frederick L. Stoddard,[‡] and Liisa Viikari[†]

[†]Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27 (Latokartanonkaari 11), 00014 University of Helsinki, Finland

[§]HAMK University of Applied Sciences, P.O. Box 230, 13101 Hämeenlinna, Finland

[‡]Department of Agricultural Sciences, University of Helsinki, P.O. Box 27 (Latokartanonkaari 5), 00014 University of Helsinki, Finland

ABSTRACT: The methane yields and conversion of pentoses (xylose) and hexoses (cellulose) in hemp, maize, and white lupin were studied over 30 days of anaerobic digestion. Preservation of hemp increased the methane yield by 23% compared with the fresh hemp. The increased methane yield of hemp was verified by the enhanced conversion of C6 sugars, increasing from 48% to about 70%, whereas the conversion of C5 sugars increased from only 9% to nearly 50%. The consumption of all carbohydrates in fresh maize was almost complete in the 30 days of anaerobic digestion. Hence, there was no major difference in carbohydrate consumption between fresh and preserved maize during biogas production. Fresh white lupin produced the highest methane yield (343 ± 33 dm^3 kg^{-1} TS) in this work, mainly due to its highest amount of proteins. Conversion of C6 sugars was 80%, but that of C5 sugars was notably less at 46%.

KEYWORDS: *methane, lupin, maize, hemp, ensiling*

INTRODUCTION

Biogas or methane produced from agricultural wastes and energy crops by anaerobic digestion (AD) is an exceedingly promising potential biofuel.[1] The AD process is exceptionally omnivorous in terms of used raw materials. Besides carbohydrates, proteins, extractives, acids, and fats[2] are converted to methane from substrates varying from municipal wastes to lignocellulosic crops cultivated for energy use. AD has also been considered as a favorable method to convert organic materials and wastes into a more suitable form to be used, for example, as fertilizers.[3]

Energy crops and agricultural wastes are often rich in cellulose and lignin, which are complexly bound with hemicelluloses and pectin. The chemical composition of these raw materials, however, varies widely. Whole crop maize and sweet sorghum contain high amounts of water-soluble sugars, such as fructose and glucose, whereas fiber hemp, willow, reed canary grass, and straw are rich in cellulose.[4,5] In the hemicelluloses of these raw materials, xylose is the most abundant monosaccharide, but unlike ethanol fermentation with basic yeasts, the methane process is able to consume pentoses (C5 sugars), as well as other carbohydrates.[6] Pectin, comprising mainly galacturonic acid (gal-A) units, is most abundant in fruit wastes but is also present in fibrous crops and some woods.

Use of pretreatments to loosen the recalcitrant structure of lignocellulosic substrates is essential in biotechnical conversion processes to sugars and further in fermentation to ethanol in which the hexoses (C6 sugars), originating mostly from cellulose, are the most valuable components for the end production. Optimization of the preservation of water-soluble carbohydrates and prevention of the production of inhibitors are somewhat contradictory goals when targeting maximum glucose release from lignocellulosic raw materials and have been extensively studied, as reviewed by, for example, Mosier et al.[7] and Hendriks and Zeeman[8] during past decades. Farm-scale operations and the rather high conversion efficiency of the presently used substrates have decelerated the use of pretreatments prior to methane production. Larger, centralized biogas plants using an increased variety of raw materials have boosted the interest of pretreating substrates prior to AD. Ensiling of, for example, maize and hemp has been observed to enhance the methane yields,[9,10] so it may be sufficiently cost-effective to use ensiling as pretreatment with some raw materials.

Pretreatments targeted for enhancing the conversion of recalcitrant biomass components may degrade the more valuable substrates for AD rather than increase the use of the structural components. Lignin is not known to be utilized during the methane production process, but its removal or alteration of its structures has been observed to correlate (mostly) with increased methane yields, so these treatments are desired prior to methane production.[11] Lignin remained, whereas protein, extractives, and carbohydrates were mostly utilized, in a study investigating the separation of the hydrolytic and methanogenic stages.[12,13] Little attention has, however, been paid to individual carbohydrates and their fate in the AD process. To obtain the maximum potential of each raw material and to choose the relevant pretreatments for each substrate type, a more detailed understanding of the chemical composition and degradability of various carbohydrate components is needed.

Received: April 10, 2012
Revised: July 11, 2012
Accepted: July 12, 2012
Published: July 12, 2012

In this study, whole crop maize, fiber hemp, and white lupin were investigated as substrates for biogas production. The main emphasis was on following changes in the concentrations of monosaccharides, hemicelluloses, cellulose, and lignin, as well as total and ammonium nitrogen throughout the test period of 4 weeks. In addition to direct conversions to methane, the potential to increase energy yields with dual conversions was investigated by first hydrolyzing maize and fermenting it to ethanol and then digesting the residue to methane.

MATERIALS AND METHODS

Plant Materials and Preparation of Materials for Analyses. Maize (*Zea mays* L. cv. Ronaldino), fiber hemp (*Cannabis sativa* L. cv Uso), and white lupin (*Lupinus albus* L. cv. Vesna) were grown at the Viikki Experimental Farm, University of Helsinki, Finland (60° 13′ N, 25° 00′ E) in 2008−2010. Maize samples were taken in September 2008, hemp in September 2008 and 2009, and lupin in 2010. In the year of harvest, biomass yields of maize, hemp, and white lupin were 15, 14, and 18 tons (dry matter) ha^{-1}, respectively. No prewilting was necessary for hemp in 2008, whereas maize and hemp in 2009 were wilted for 48 h to reduce the moisture content of the material. Crops were cut with a garden chopper into 1−2 cm size pieces and ensiled. Fresh material was also frozen for further use.

Preservation. Laboratory-scale ensiling for maize and hemp was done in 1.5 L glass jar "silos" in three replicates. In 2008, chopped material was ensiled with formic acid (1% w/w), dosed on a fresh material, resulting in a final concentration of 3.4% on a dry matter (DM) basis. The material was pressed tightly into the jars and sealed airtight. The density of the ensiled material simulated well full-scale ensiling systems, being 160 kg DM m^{-3} for the hemp in 2008 and 145 kg DM m^{-3} for the maize. Jars were sealed and stored at 5−10 °C for 8 months. After the preservation period, the pH of each jar was measured, the material was visually examined, and the replicates were combined and frozen for further use and chemical analyses.

Methane Production. Methane production was determined in laboratory-scale batch trials using the AMPTS (Automatic Methane Potential Test System) of Bioprocess Control AB. Each crop was studied in 500 mL bottles with 10 replicates. Digested sludge from a municipal wastewater treatment plant (Paroinen, Hämeenlinna, Finland) was used as an inoculum. The average pH of the inoculum was 7.9, the volatile solids content (VS) was 1.7%, and the total solids content (TS) was 3.6%. The VS/TS ratio for fresh and preserved (with and without formic acid (FA)) hemp was 0.92, for maize preserved with FA, 0.93, and for the rest, 0.94. The VS ratio of sample and inoculum in each bottle was 1:1. The bottles were filled to a liquid volume of 400 mL with distilled water, and $NaHCO_3$ (3 g L^{-1}) was added as buffer. The bottles were flushed with N_2 and closed and set into the water bath at 36 ± 1 °C. Mixing was carried out automatically. The formed biogas was led into 1% NaOH solution that adsorbed CO_2 from the biogas. The amount of methane was analyzed by measuring the volume of NaOH solution displaced by the volume of methane that was produced. The amount of the replaced solution was measured daily at the beginning and less frequently as biogas production decreased. All methane production trials were carried out for 30 ± 2 days. Reference experiments with only the inoculum were used as controls, and the CH_4 yield produced by the inoculum alone was subtracted from the sample yields. The methane yield was calculated as dm^3 kg^{-1} on VS and fresh material bases. The dry matter and volatile solids used for calculating the biogas yields were corrected according to the method of Huida et al.[14] That correction considers the evaporation of the organic acids in the dry matter determination causing error in the dry matter content. Samples for conversion followup were collected from the test bottles during and at the end of the incubation. Material was dried at 60 °C and milled for further analyses of carbohydrate, lignin, and nitrogen.

Ethanol Production. The raw materials were hydrolyzed with a standard commercial cellulase mixture, Celluclast (Novozymes, Denmark), containing the major cellulolytic activities (dosage 10 FPU g^{-1} DM biomass), supplemented with the β-glucosidase preparation Novozym 188 (500 nkat g^{-1} DM biomass) and fermented simultaneously to ethanol. Baker's yeast (*Saccharomyces cerevisiae*) was added (1 g/L). The dry matter content of the milled crop in the hydrolysis was 5%. The experiments, with five replicates, were carried out in 50 mM sodium citrate buffer, pH 5, in plastic bottles with a liquid volume of 400 mL and sealed with water locks. The temperature was 35 °C and the shaking speed 150 rpm. Samples were taken after 75 h of incubation. Ethanol was measured from the hydrolysate with the UV-based ethanol determination kit (Boehringer Mannheim/R-biopharm, Germany). Ethanol was evaporated by rotavapor, and the solid residues were dried at 60 °C and milled for carbohydrate and lignin analyses.

Analyses of the Fresh and Ensiled Materials. The dry matter content (total solids, TS%) was determined by drying samples at 105 °C until constant weight was reached. Dried samples were combusted in a muffle oven for 2 h at 550 °C to determine the ash content. Organic dry matter (volatile solids, VS%) was calculated by subtracting the ash content from the dry matter content. The pH was measured with a Methorm 744 pH-meter.

Lignin and carbohydrates were analyzed according to the NREL LAP method (Determination of Structural Carbohydrates and Lignin),[15] in which acid hydrolysis is used to hydrolyze the lignocellulosic material into monosaccharides. Samples were not extracted, which differed from the standard procedure. The amount of total sugars formed after acid and enzymatic hydrolysis was determined as reducing sugars by the dinitrosalicylic acid (DNS) method[16] at 540 nm. Monosaccharides (glucose, xylose, mannose, arabinose, and galactose) were determined by HPAEC-PAD.[5] Klason lignin was determined gravimetrically as the acid-insoluble residue from acid hydrolysis. Acid-insoluble ash and nitrogenous compounds (mainly protein) were not subtracted from the residue but are present in the lignin values. Acid-soluble lignin was determined from the filtrate from the acid hydrolysis with a spectrophotometer at 320 nm. The uronic acids and noncellulosic glucose were determined by acid methanolysis[17] in which the depolymerized carbohydrates were silylated and noncellulosic glucose and uronic acids were determined by gas chromatography using an Agilent 6890N (Agilent Technology, Palo Alto, CA, USA) equipped with a FID. The column used was a DB-1 (30 m × 0.32 mm × 0.25 mm). The conditions were as follows: oven temperature, 150 °C (5 min), 2 °C min^{-1} to 186 °C, followed by 1 °C min^{-1} to 200 °C and 20 °C min^{-1} to 325 °C; injector temperature, 225 °C; and FID temperature, 280 °C. The injection volume was 2 μL with a split ratio of 1:30, and the flow rate of the helium carrier gas was 1 mL min^{-1}.

The ammonium (NH_4^+) and ammonia (NH_3, aq) contents were determined by titration; the ammonia is first liberated from an alkalized sample by steam distillation and then absorbed into an acid solution followed by titration for the ammonia. Total nitrogen was determined according to the Kjeldahl method with three replicates.

Statistical Analysis. The effect of ensiling on methane yields and sugar conversions was tested with the *t* test using PASW (v. 18.0, SPSS Inc., Chicago, IL, USA). Statistical significance was recognized for $p < 0.05$.

RESULTS AND DISCUSSION

Composition of Fresh Crops. The original content and structure of carbohydrates varied between the three different crops; lupin had the lowest carbohydrate content of 44.2% of DM and hemp the highest, 62.3% of DM (Table 1). The content of hexoses (C6), mainly originating from cellulose, was highest in hemp, partially explaining the recalcitrance toward biological conversion. The content of pentoses (C5) derived from hemicelluloses was also highest in hemp. In lupin the cellulose content was relatively low, 14.3% of DM (Table 1), and part of the glucans originated from hemicelluloses and water-soluble carbohydrates. Galactans increased the C6 sugar content by 4.7% (DM basis) (data not shown). The impact of

Table 1. Chemical Composition (Percent Dry Matter) of Studied Crops[a]

	maize	hemp	lupin
fructose	6.0	2.3	4.8
C6 sugars (total)	38.7	43.9	30.4
cellulose	23.6	33.8	14.3
C5 sugars	13.2	16.1	9.0
galacturonic acid	1.7	6.9	5.9
protein	10.6	9.1	16.9
lignin	19.3	21.4	16.2

[a]The C6 sugars contain the total in anhydrosugars (soluble and insoluble). Fructose was not included in C6 sugars. Standard deviation of each analysis was below 0.5%.

water-soluble carbohydrates, such as fructose, was remarkable in the total carbohydrate amount of maize (Table 1). The conversion of fructose was not determined but was assumed to be complete. Lupin produced a high quantity of seeds that were not ripe but which are known to contain a high amount of protein and nonstarch polysaccharides. The protein content of lupin was 16.9% of DM, whereas it was 10.6 and 9.1% in maize and hemp, respectively (Table 1). Fiber hemp was clearly divided into bast fibers and xylem (woody layers), whereas lupin was divisible into seeds (about one-third), stems, and leaves.

Methane Potential and Carbohydrate Conversion of Fresh Crops. Fresh white lupin produced the highest methane yield (343 ± 33 dm^3 kg^{-1} TS) in the small-scale tests and fresh maize somewhat less, 274 ± 8 dm^3 kg^{-1} TS (Table 2). The

Table 2. Methane Yields and TS/VS Ratios of Maize, Lupin, and Hemp

	methane yield, dm^3 kg^{-1} TS $^{-1}$	methane energy yield, kWh kg^{-1} TS^{-1}
maize fresh	274 ± 8	2.7
maize preserved	259 ± 2	2.6
maize preserved with FA	230 ± 5	2.3
lupine fresh	343 ± 33	3.4
hemp fresh	184 ± 2	1.9
hemp preserved	225 ± 3	2.3
hemp preserved with FA	209 ± 14	2.1

structure of the fresh fiber hemp obviously was resistant to the bacterial digestion, producing clearly less, 184 ± 2 dm^3 kg^{-1} TS of methane (Table 2). The consumption of carbohydrates in fresh maize was almost complete after 30 days of AD. There was only about 5% of the original hexose content (Figure 1) and 3% of pentose (Figure 1) left in the dry residual material after AD. The enzymatic hydrolyzability of fresh and preserved hemp has been previously studied,[18−20] and when there was no pretreatment, the conversion of total glucans was about 30% and of total xylans, only 4%. In this study, after 30 days of biogas production, 48% of the original C6 sugar content in fresh hemp was converted to methane (Figure 1), but only 9% of the original C5 sugars, mostly from xylans. Although the highest methane yield of the studied crops was produced from lupin, the C5 sugar conversion followed the same pattern as in ensiled hemp, with 46% conversion of C5 sugars and 80% of C6 sugars (Figure 1). The higher amount of protein in lupin (Table 1) compared with the hemp was associated with increased methane production. The structural differences of the glucans in the raw materials may also explain the better methane yields and C6 conversion of lupin. The relatively high amount of glucans in lupin originated from noncellulosic compounds (Table 1), whereas glucans in hemp were from more recalcitrant cellulose fibers.

Thus, the main obstacles for the degradation of carbohydrates in the biogas process of fresh hemp and lupin seemed to be the presence and slow removal of xylan, as well as the inaccessibility of cellulose in hemp to the enzyme systems of the microorganisms involved. The presence of microorganisms in the inoculum capable of the hydrolysis of cellulose and xylans was demonstrated by the utilization of these polymers in maize. The microorganisms in the inocula produce multiple enzymes to degrade plant cell materials, which are active on cellulose, hemicelluloses, and pectin.[8,21] The role of bacteria and other microorganisms in the inocula as enzyme "factories" may have some benefits as compared with exogenous enzymes. Thus, the inability of anaerobic bacteria to effectively penetrate cellulosic materials probably led to the development of complex enzyme systems that localize cellulose production at the site of hydrolysis, as observed in ruminant bacteria. Therefore, the problems of, for example, inactivation of enzymes or adsorption on the surface of lignin or cellulose in the enzymatic hydrolysis could be managed by production of new hydrolytic enzymes at the site by the bacteria during the fairly long methane production process.[8] In addition, the number of different microorganisms present may also provide a richer source of various enzymes needed, as compared with the commercial enzyme preparations. However, especially the structure of hemp was shown to be recalcitrant even for the effective anaerobic microorganisms. This may be due to the macroscale structure of hemp fibers and, on the other hand, the structure of the woody stem, as compared to the somewhat softer structure of maize. This argument was supported by the positive effect obtained by reducing the particle size of the raw materials by milling, which increased methane yields from hemp while having no effect on the already high yield from maize.[5]

The role of pectin, which was more abundant in hemp and lupin, may be the partial reason for lower conversions of sugars. The content of galacturonic acid (gal-A), the major carbohydrate unit in pectin, was fairly low in maize, and it was utilized completely during the 30 days OF biogas production (Figure 2). In fresh hemp and lupin, 72 and 77% of gal-A was consumed, respectively. Pectin is considered as glue material between bast fiber cells and present also in the cell wall,[22] so its removal could enhance the hydrolysis of the lignocellulosic material as it does in pectin-rich citrus processing waste.[23] The effect of pectin removal by polygalacturonase was recently shown to increase the liberation of neutral sugars,[19] and the use of pectinases increased methane yields from switchgrass without chemical pretreatment.[24] Horn et al.[25] found a positive correlation between the yields of enzymatic hydrolysis and biogas production from *Salix* chips. These observations encouraged the assumption that removal of pectin by enzymes would enhance also the biogas yield of fresh hemp.

Effect of Preservation on Methane Potential and Carbohydrate Conversion of Maize and Hemp. Preservation of hemp for 8 months enhanced the conversion of pectin, glucans, and xylan and increased the methane yields by 14 and

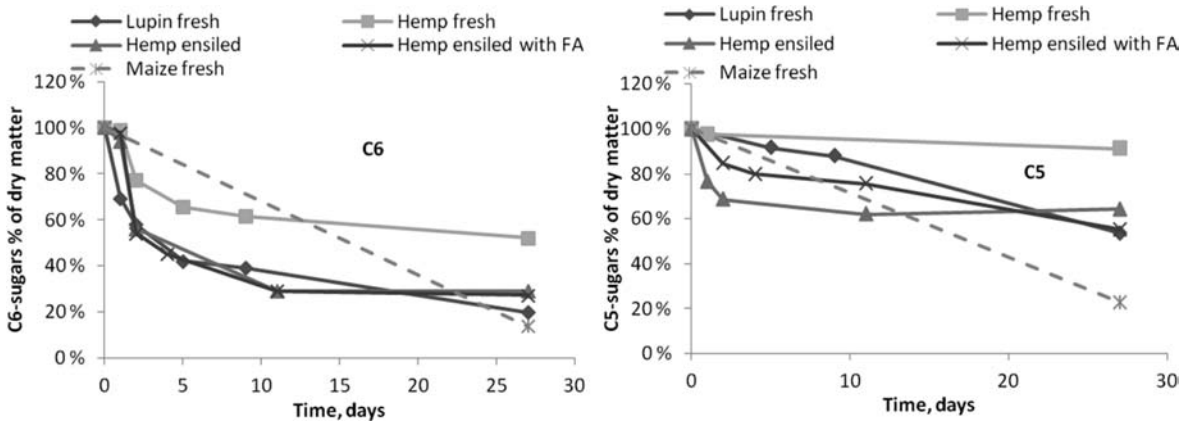

Figure 1. Consumption of C6 (mainly from starch and cellulose) and C5 sugars (from hemicelluloses), expressed as percent of original, during biogas production from fresh lupin, maize, and hemp as well as from ensiled hemp (with and without formic acid). Standard deviation for triplicate analyses was <1.

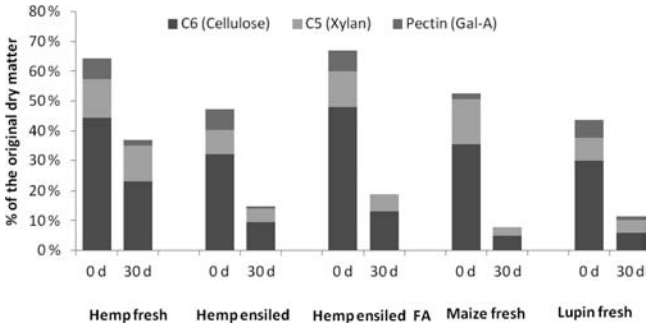

Figure 2. Cellulose, xylan, and galacturonic acid (pectin) content (counted as polymers) of fresh and ensiled hemp and fresh maize and lupin in the starting material and after 30 days of AD.

23%, with or without addition of fFA, respectively, whereas preservation of maize reduced methane yields (Table 1). This positive effect of anaerobic preservation of hemp on methane yields has been previously observed.[18]

Although there was no significant difference in carbohydrate consumption between fresh and preserved maize during biogas production (data not shown), the preservation had a positive effect on the carbohydrate digestibility of fiber hemp. The increased methane yield of hemp was verified by the enhanced conversion of C6 sugars, increasing from 48% to about 70% (Figure 1). The conversion of C5 sugars increased from only 9% in fresh hemp to 36 and 45% in hemp preserved without and with FA, respectively (Figure 1). The slight decreases of lignin (containing acid-insoluble protein) and hemicelluloses (with formation of lactic acid) were the most notable changes in the chemical content of preserved hemp compared to the fresh hemp.[18] These results suggest that the structural modifications due to the preservation led to only small changes in the relative amounts of pectin, lignin, cellulose, and hemicelluloses but increased significantly the conversion of especially xylan with consequently improved methane yields.

The role of pectin, hindering the hydrolysis of neutral carbohydrates (cellulose and hemicelluloses) in pectin-rich crops, was recently studied in preserved hemp.[19] The pectin content correlated negatively with the hydrolysis rate, and enzymatic pectin removal had a more pronounced effect on preserved materials than on fresh hemp. In this study, the utilization of galacturonic acid during 30 days of biogas production increased from 72 to 92% due to the preservation of hemp and to 100% due to preservation by FA (Figure 2). Although the conversion of pectic compounds was relatively high already in the fresh hemp, the more complete removal of pectin could be one reason for the increased degradation of preserved hemp in biogas production by releasing structural obstacles between cellulose, hemicelluloses, and lignin.

Losses of biomass during preservation are to be considered when judging the real effect of the method on energy yields. The DM loss during ensiling was not measured, but it is assumable that the total mass loss in laboratory conditions was relatively low. Mass loss in a closed fermentation system has been observed to be around 4%, whereas the energy loss was only 0.5%.[26,27] If the DM loss in this study was 4%, the increase of methane yields would have been 9 and 17% for initial hemp preserved with or without FA, respectively. However, the preservation of herbaceous crops is often necessary to provide feedstock throughout the year. Biomass losses during another common preservation method, drying, are not avoidable and have been observed to be even higher than in ensiling.[27] Drying of fibers can also result in irreversible collapse and shrinking of the capillaries and thus reduce the accessible surface area.[28] This feature hampers the hydrolysis of lignocellulosic feedstocks and causes decreased methane production.[29]

Conversion of Lignin and Formation of Ammonium. Degradation of Klason, the solid residue after acid hydrolysis (including acid insoluble lignin, protein, and ash), during the 30 days of biogas production was lowest (22% of the original amount) in fresh hemp, whereas in preserved and formic acid preserved hemp 35 and 47% of "lignin" was degraded, respectively (Figure 3). In comparison, 38% of compounds quantified as lignin in maize and 47% in lupin were utilized. However, lignin has not been shown to be degraded during the biogas production processes, so the conversion shown may be due to the degradation of protein present in the Klason lignin. The removal of lignin-bound protein during the preservation and AD may also have affected the analysis of Klason lignin. It has been reported that anaerobic microorganisms present in sediments or rumen fluids may alter, if not partially degrade, portions of lignified plant cells,[30,31] even though biodegradation of lignin is widely accepted to be an aerobic process.

The formation of ammonium during AD was clearly highest in white lupin, which originally contained the highest amount of total nitrogen (Figure 4). The original amounts of total nitrogen in the hemp and lupin were 0.45 and 0.32 mg N mL^{-1}.

dx.doi.org/10.1021/jf301549b | J. Agric. Food Chem. 2012, 60, 7934−7940

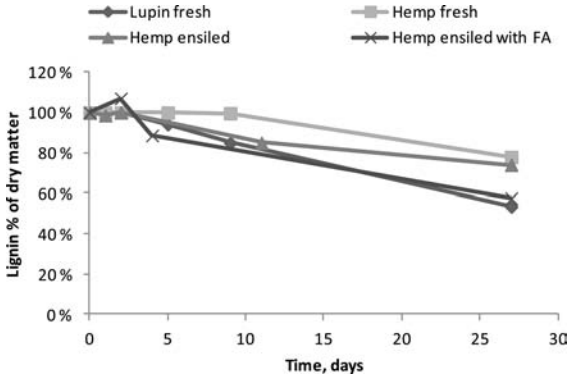

Figure 3. Degradation of Klason lignin (acid-soluble ash and protein included) during 30 days of AD in fresh lupin, maize, and hemp as well as in ensiled hemp samples. Standard deviation for triplicate analyses was <0.5.

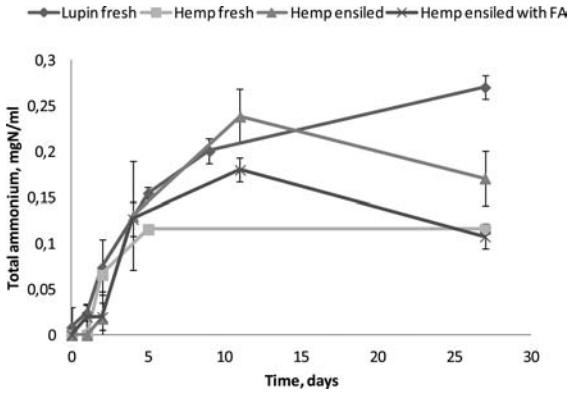

Figure 4. Formation of ammonium from the raw materials (expressed as mg N mL^{-1}) during the 30 days of AD. The original amounts of total nitrogen in the hemp and lupin were 0.45 and 0.32 mg N mL^{-1}, respectively. The ammonium formed corresponded to conversions of 37 and 60% of the total nitrogen in hemp and lupin, respectively.

The conversion of organic nitrogen to mainly ammonium in white lupin reached 63%, indicating the potential value of the biogas residue as fertilizer. The preservation of hemp slightly enhanced the ammonium formation compared with the fresh hemp, showing, however, a relatively low conversion of about 37%.

Ethanol Fermentation followed by AD. When enzymatic hydrolysis and ethanol production from fresh and preserved maize was tested prior to anaerobic digestion, ethanol yield was clearly lowest, 42.4 g kg^{-1} DM for maize preserved without added acid, and highest for the fresh maize, 84.7 g kg^{-1} DM (Table 3). Maize preserved with formic acid reached the yield of fresh maize, but the fermentation was somewhat slower, probably due to the inhibition of fermentation by the added FA.[32] The main effect of the preservation on the chemical composition is the disappearance of water-soluble carbohydrates consumed for the formation of preserving acids, when no additives are added.[26] Therefore, the amount of soluble carbohydrates, mainly fructose and glucose, was lower in preserved maize, whereas the amount was increased when FA was added. Nevertheless, the ethanol yields were about 30–40% of the theoretical amount of ethanol, in agreement with previous findings.[33]

Table 3. Ethanol and Methane Yields and TS/VS Ratios after Ethanol Fermentation of Fresh and Preserved Maize

	ethanol yield, g kg^{-1}	methane yield (after ethanol fermentation), dm^3 kg^{-1} original TS $^{-1}$
maize fresh	85 ± 0.3	239 ± 15
maize preserved	42 ± 0.2	270 ± 5
maize preserved with FA	82 ± 0.2	307 ± 24

Methane production from the prefermented maize was even higher than that from its nonfermented counterparts, on a VS basis. This increase was caused by the increased share of higher methane-productive components, such as protein, because part of the carbohydrate fraction had already been consumed in the ethanol process. Maize preserved with FA gave the highest yield of 307 ± 24 dm^3 TS^{-1} (Table 3).

The fermentation into ethanol consumed only about half of the available C6 sugars (glucans + fructans) in fresh and formic acid preserved substrates (Figure 5) and only 26% in maize preserved without formic acid, which had lost most of its easily fermentable sugars. From 7 to 19% of the C5 sugars in the solid residue were converted during the enzymatic hydrolysis, and the rest presumably remained unfermented in the slurry (Figure 5). Hydrolysis and fermentation of maize without a stronger pretreatment was expectedly not efficient enough, and ensiling alone was not able to enhance the hydrolysis yield by loosening the lignocellulosic structure, as also demonstrated by Oleskowicz-Popiel et al.[33]

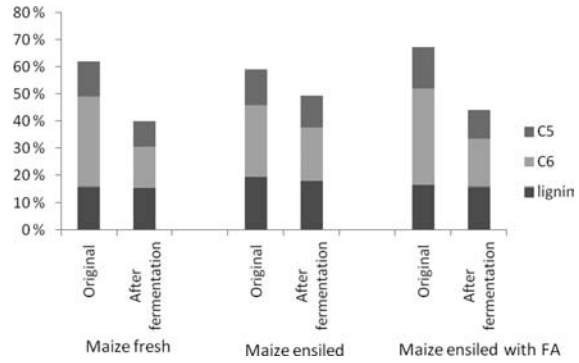

Figure 5. Lignin and carbohydrate contents of fresh maize before and after ethanol fermentation.

It was interesting to study whether the hydrolysis of maize with specific hydrolytic enzymes would have an effect on the sugar and consequent methane yields. In the 30 day AD process, even without the hydrolysis and ethanol fermentation step, the consumption of carbohydrates in fresh and preserved maize was already almost complete, and only about 5% of hexoses and 3% of pentoses remained. Hence, there was no major enhancement when the material was hydrolyzed and fermented prior to AD.

The energy yield in the combined ethanol and methane production was compared with the energy content gained from AD alone. Although the ethanol yields of nonpretreated maize were low, the methane yields from the preserved materials were enhanced (Figure 6). This suggests that although there is no major increase in carbohydrate conversion, simultaneous saccharification and fermentation (SSF) may have released some protein to be converted to methane. Removal of ethanol by evaporation after fermentation preserved also the organic acids as raw materials for methane production. To reach a

dx.doi.org/10.1021/jf301549b | J. Agric. Food Chem. 2012, 60, 7934–7940

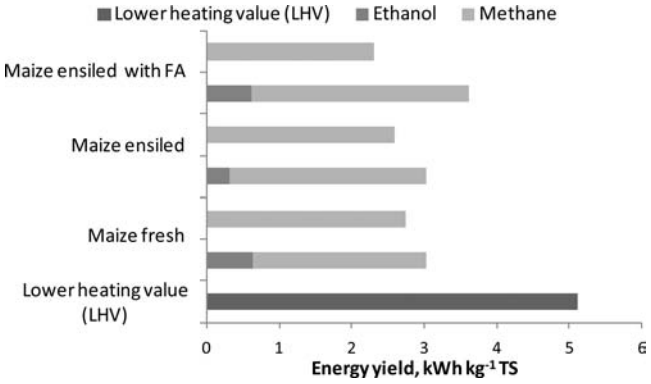

Figure 6. Energy yields of fresh and preserved maize for direct AD and for combined ethanol fermentation and AD. Theoretical heating value was taken from http://www.afdc.energy.gov/biomass/progs/search1.cgi.

maximal energy yield, the residual biomass from the AD can also be incinerated, but it has a greater value as a nitrogen-rich fertilizer. The residual lignin in the digestate in that case is a useful addition to soil organic matter.

AUTHOR INFORMATION

Corresponding Author

*Phone: +358 40 7353747. Fax +358-09-191 58475. E-mail: annukka.z.pakarinen@helsinki.fi.

Funding

Funding of the field trials was supported by the Sustainable Energy Programme, SusEn, of the Academy of Finland under the grant "Carbon-sequestering Species Mixtures for Sustainable Energy Cropping", a part of the consortium "Bioenergy, Electricity, and Emission Trading Markets". The work was also supported by the University of Helsinki, and A.P. was supported by the Graduate School for Biomass Refining funded by the Academy of Finland.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Panu Mölsä for assisting in the ethanol fermentation experiments and Laura Huikko and Laura Kannisto for excellent technical assistance.

ABBREVIATIONS USED

TS, total solids; DM, dry matter, dry weight; VS, volatile solids; FW, fresh weight; FA, formic acid.

REFERENCES

(1) Börjesson, P.; Mattiasson, B. Biogas as a resource − efficient vehicle fuel. *Trends Biotechnol.* **2007**, *26*, 7−13.

(2) VDI 4630, Fermentation of organic materials. Characterization of the substrates, sampling, collection of material data, fermentation tests. *VDI Handbuch Energietechnik*; Verein Deutscher Ingenieure: Dusseldorf, Germany, 2006.

(3) Weiland, P. Biomass digestion in agriculture: a successful pathway for the energy production and waste treatment in Germany. *Eng. Life Sci.* **2006**, *6*, 302−309.

(4) Bridgeman, T. G.; Jones, J. M.; Shield, I.; Williams, P. T. Torrefaction of reed canary grass, wheat straw and willow to enhance solid fuel qualities and combustion properties. *Fuel* **2007**, *87*, 844−856.

(5) Pakarinen, A.; Maijala, P.; Stoddard, F.; Santanen, A.; Kymäläinen, M.; Tuomainen, P.; Viikari, L. Evaluation of annual bioenergy crops in the Boreal zone for biogas and ethanol production. *Biomass Bioenerg.* **2011**, *35*, 3071−3078.

(6) Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.; Holtzappe, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96*, 673−686.

(7) Hendriks, A. T. W.; Zeeman, G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* **2009**, *100*, 10−18.

(8) Lynd, L. R.; Weimer, P. J.; van Zyl, W. H.; Pretorius, I. S. Microbial cellulose utilization: fundamentals and biotechnology. *Microb. Mol. Biol. Rev.* **2002**, *66*, 506−577.

(9) Neureiter, M.; dos Santos, J. T. P.; Lopez, C. P.; Pichler, H.; Kirchmayr, R.; Braun, R. Effect of silage preparation on methane yields from whole crop maize silages. In Proceedings of the 4th International Symposium on Anaerobic Digestion of Solid Waste, Aug 31−Sept 2, 2005; Copenhagen, Denmark;Ahring, B. K., Hartmann, H., Eds. *Water Sci. Technol.* **2005**, *53*, 109−115.

(10) Amon, T.; Amon, B.; Kyvoruchko, V.; Zollitsch, W.; Mayer, K.; Gruber, L. Biogas production from maize and dairy cattle manure − influence of biomass composition on the methane yield. *Agric. Ecosyst. Environ.* **2007**, *118*, 173−182.

(11) Monlau, F.; Barakat, A.; Steyer, J. P.; Carrere, H. Comparison of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. *Bioresour. Technol.* **2012**, *120*, 241−247.

(12) Lehtomäki, A.; Huttunen, S.; Lehtinen, T. M.; Rintala, J. A. Anaerobic digestion of grass silage in batch leach bed processes for methane production. *Bioresour. Technol.* **2007**, *99*, 3267−3278.

(13) Cirne, D. G.; Lehtomäki, A.; Björnsson, L.; Blackall, L. L. Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *J. Appl. Microbiol.* **2007**, *103*, 516−527.

(14) Huida, L.; Väätäinen, H.; Lampila, M. Comparison of dry matter contents in grass silages as determined by oven drying and gas chromatographic water analysis. *Ann. Agric. Fenn.* **1986**, *25*, 215−230.

(15) Sluiter, A.; Hamnes, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of structural carbohydrates and lignin in biomass. Laboratory analytical procedure, 2010; (http://www.nrel.gov/biomass/analytical_procedures.html) (accessed Feb 3, 2012).

(16) Miller, G. L. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal. Chem.* **1959**, *31*, 426−428.

(17) Sundberg, A.; Sundberg, K.; Lillandt, C.; Holmbom, B Determination of hemicelluloses and pectins in wood and pulp fibres by acid methanolysis and gas chromatography. *Nord. Pulp Pap. Res. J.* **1996**, *11*, 216−219.

(18) Pakarinen, A.; Maijala, P.; Jaakkola, S.; Stoddard, F. L.; Kymäläinen, M.; Viikari, L. Evaluation of preservation methods for improving biogas production and enzymatic conversion yields of annual crops. *Biotechnol. Biofuels* **2011**, *4*, 1−13.

(19) Pakarinen, A.; Zhang, J.; Brock, T.; Maijala, P.; Viikari, L. Enzymatic accessibility of fiber hemp is enhanced by enzymatic or chemical removal of pectin. *Bioresour. Technol.* **2012**, *107*, 275−281.

(20) Sipos, B.; Kreuger, E.; Svensson, S. E.; Réczey, K.; Björnsson, L.; Zacchi, G. Steam pretreatment of dry and ensiled industrial hemp for ethanol production. *Biomass Bioenerg.* **2010**, *34*, 1721−1731.

(21) Warren, R. A. J. Microbial hydrolysis of polysaccharides. *Annu. Rev. Microbiol.* **1996**, *50*, 183−212.

(22) Carpita, N. C.; Gibeaut, D. M. Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. *Plant J.* **1993**, *3*, 1−30.

(23) Widmer, W.; Zhou, W.; Grohmann, K. Pretreatment effects on orange processing waste for making ethanol by simultaneous saccharification and fermentation. *Bioresour. Technol.* **2010**, *101*, 5242−5249.

(24) Frigon, J. C.; Mehta, P.; Guiot, S. R. Impact of mechanical, chemical and enzymatic pre-treatments on the methane yield from the anaerobic digestion of switch grass. *Biomass Bioenerg.* **2012**, *36*, 1−11.

(25) Horn, S.; Estevez, M.; Nielsen, H.; Linjordet, R.; Eijsink, V. G. H. Biogas production and saccharification of *Salix* pretreated at different steam explosion conditions. *Bioresour. Technol.* **2011**, *102*, 7932−7936.
(26) McDonald, P.; Henderson, A. R.; Heron, S. J. E. *The Biochemistry of Silage*, 2nd ed.; Cambrian Printers: Aberystwyth, U.K., 1991.
(27) Shinners, K.; Binversie, B.; Muck, R.; Weimer, P. Comparison of wet and dry corn stover harvest and storage. *Biomass Bioenerg.* **2007**, *31*, 211−221.
(28) Fan, L. T.; Lee, Y.; Beardmore, D. H. Mechanism of the enzymatic hydrolysis of cellulose: effects of major structural features of cellulose on enzymatic hydrolysis. *Biotechnol. Bioeng.* **1980**, *22*, 177−199.
(29) Egg, R.; Coble, C.; Engler, C.; Lewis, D. Feedstock storage, handling and processing. *Biomass Bioenerg.* **1993**, *5*, 71−94.
(30) Akin, D. E. Attack on lignified cell walls by facultatively anaerobic bacterium. *Appl. Environ. Microbiol.* **1980**, *40*, 809−820.
(31) Benner, R.; Maccubbin, A. E.; Hodson, R. Anaerobic biodegradation of the lignin and polysaccharide components of lignocellulose and synthetic lignin by sediment microflora. *Appl. Environ. Microbiol.* **1984**, *47*, 998−1004.
(32) Klinke, H. B.; Thomsen, A. B.; Ahring, B. K. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl. Microbiol. Biotechnol.* **2004**, *66*, 10−26.
(33) Oleskowicz-Popiel, P.; Thomsen, A. B.; Schmidt, J. E. Ensiling − wet-storage method for lignocellulosic biomass for bioethanol production. *Biomass Bioenerg.* **2011**, *35*, 2087−2092.