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Steam explosion pretreatment for enhancing biogas production of late harvested hay

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Abstract

Grasslands are often abandoned due to lack of profitability. Extensively cultivating grassland for utilization in a biogas-based biorefinery concept could mend this problem. Efficient bioconversion of this lignocellulosic biomass requires a pretreatment step. In this study the effect of different steam explosion conditions on hay digestibility have been investigated. Scanning electron microscopy images showed

that steam explosion results in notable morphological changes. Increasing severity in the pretreatment induced degradation of the hemicellulose, which at the same time led to the production of inhibitors and formation of pseudo-lignin. Enzymatic hydrolysis showed that the maximum glucose yields were obtained under pretreatment at 220 °C for 15 min, while higher xylose yields were obtained at 175 °C for 10 min. Pretreatment of hay by steam explosion enhanced 15.9 % the methane yield in comparison to the untreated hay. Results indicate that hay can be effectively converted to methane after steam explosion pretreatment.

Keywords

Biogas; Methane; Hay; Biofuel; Steam explosion

List of abbreviations

ADF	Acid detergent fiber
ADL	Acid detergent lignin
DM	Dry matter
FM	Fresh matter
H-CEL	Hemicellulose
HMF	Hydroxymethylfurfural
KF	Karl Fischer
l_N	Standard liter (dry gas volume at 0°C and 1013mbar)
NDF	Neutral detergent fiber
NREL	National Renewable Energy Laboratory (United States)
ODW	Oven dried weight
SE	Steam explosion
SRS	Sugar recovery standards
VS	Volatile solids
XA	Raw ash
XP	Crude protein

1 Introduction

Among the largest habitat type in the world, grasslands are characterized by multiple functions and values; they provide forage for animals, they have a positive influence on the recharging of water tables and a protective effect on water quality. Grasslands have a big potential to sequester carbon in the soil, while safeguarding soil from erosion, as well as improving its fertility. In addition, grasslands support biodiversity and contribute significantly to rural economies (Peeters, 2009).

The grassland area in Europe is estimated at 59 million hectares, which is 34 % of the total agricultural area (FAOSTAT, 2013). In the last twenty years, the grassland area in Western Europe declined by about 15 %, incurring negative consequences for ecology, economy and society. Moreover, the situation of Alpine agriculture is further complicated, where recent warming has been roughly three times the global average (OECD, 2007). It is therefore of vital importance that alternative land use concepts are developed to help maintain Alpine agriculture. One possible strategy is the biogas-based biorefinery concept, which entails using grassland biomass to produce energy and chemical components. Among the different ways for producing energy out of grassland biomass, biogas production is currently the most common practice in Europe. Since the orographic features in Alpine areas compromise the sustainability of this type of use, extensive agricultural techniques (e.g. only one cut per year) will help mitigate this handicap. Thus, grass would be reaped slightly later than its optimum quality stage and will contain a higher lignification level than the standard feedstock.

Hay is the oldest, and still the most important, conserved fodder. Haymaking turns green, perishable, grass into a product that can be safely stored and easily transported by reducing its moisture content from approximately 70 - 90 % to 15 - 20 %. It is especially appropriate for small-scale producers since, it can be made with little equipment or costs. Natural drying of mown grass by sun and wind is still the most common way of conserving this type of biomass. For efficient methane production from grass, a pretreatment of the biomass is necessary and can lead to an important economic gain (Thamsiroj & Murphy, 2010). In order to break the lignocellulosic bonds in the biomass, autohydrolytic steam explosion has generally been accepted as one of the most effective pretreatment methods, since no addition of external catalysts is necessary.

Currently, steam explosion is one of the most intensive investigated pretreatment technology of lignocellulosic material for both ethanol and biogas production. The factors that most affect steam explosion pretreatment are temperature, residence time, particle size, and moisture content (Cara et al., 2006). The treatment is applied for a few minutes and then the pressure is abruptly reduced, which make the material suffer an explosive decompression. This produces the hydrolysis of the hemicellulose into water-soluble oligomers or to individual sugars, and also generates a good substrate for enzymatic hydrolysis by cellulases. The rupture of the union lignin-carbohydrates, together with the hemicellulose solubilization is responsible of the big increase of cellulose to enzymatic hydrolysis (Fernández-Bolaños et al., 2001). Besides, the rapid thermal expansion opens up the biomass particle structure leading to a reduction of the particle size and an increase of the pore volume.

Steam explosion is a well-documented pretreatment, tested by several researches and widely utilized nowadays since it is one of only a very limited number of cost-effective pretreatment technologies. It has been demonstrated to be an efficient pretreatment method for both ethanol and biogas production from such a different substrates as wood (Horn et al., 2011a), grasses (Prochnow et al., 2009), agricultural residues (Ballesteros et al., 2002; Bauer et al., 2009a), by-products (De Paoli et al., 2011) or municipal waste (Li et al., 2007). Moreover, commercial steam-explosion equipment is available.

Compared to alternative techniques, the advantages of steam explosion (SE) include a remarkably low environmental impact, lower capital investment and less hazardous process chemicals (Li et al., 2001). Like other physio-chemical pretreatment methods, it may create degradation products that have an inhibitory and toxic effect on the anaerobic digestion. Nelson et al., (1988) demonstrated the formation of aromatic compounds, such as furans, pyrroles, phenols, enols and carboxylic acids from carbohydrates and amino acids in slightly acidic aqueous solution under reflux or hydrothermolytic conditions. Since analogous conditions are set up in the steam explosion treatment, similar chemical substances may be produced and potentially have an inhibitory effect on the enzymatic hydrolysis and fermentation processes (Palmqvist & Hahn-Hägerdal, 2000). The composition and concentration of the inhibitors vary with the severity of the pretreatment, the raw material used, and the type and content of chemical catalyst (García-Aparicio et al., 2006).

While biogas production from permanent grassland has been widely investigated (Prochnow et al., 2009), the use of steam exploded hay has hardly been investigated. Thus, the objective of this work was to evaluate the effect of temperature and time of steam explosion pretreatment on the biogas production and enzymatic hydrolysis of Alpine hay. Steam explosion was tested for a range of different temperature and time combinations- the two most important parameters for optimizing methane production from biomass (Ballesteros et al., 2002). For every pretreatment, detailed chemical analyses of the substrate were carried out for a better understanding of the effect of the pretreatment severity on the degradation process.

2 Materials and Methods

2.1 Raw material and steam explosion pretreatment

The hay used in the experiments was harvested in Purgstall an der Erlauf (Lower Austria) in June 2010. The biomass was dried on the field and the samples were stored in a sheltered area for nine months until the start of the tests. The water content of the untreated hay determined by Karl Fischer (KF) titrator was 12.8 % fresh matter (FM).

The harvested biomass was pretreated with a steam explosion unit at the University of Life Sciences (UMB) in Ås, Norway (see Horn et al., (2011b)). For this process, 300 g of hay was filled into the 20 L pre-heated reactor and the steam explosion pretreatments were performed at temperatures ranging from 160 °C to 220 °C, using intervals of 15 °C. Each temperature was maintained for 5, 10 or 15 min. The steam-exploded material was vacuum stored at 4 °C for one to two weeks until the biogas trials and laboratory analysis commenced.

2.2 Chemical Analysis

The composition of native and steam exploded hay was determined by analyzing the following parameters: oven dry weight (ODW), water content, raw ash (XA), volatile solids (VS), crude protein (XP), cellulose, hemicellulose and acid detergent lignin (ADL). The ODW was analyzed by drying the biomass in an oven at 105 °C until constant weight was reached. The water content was determined with the KF titrator Mettler

Toledo V20 (Columbus, Ohio, USA) using Hydranal Composite 5 and Hydranal Methanol dry from Sigma Aldrich (St. Louis, Missouri, USA). The XA was analyzed by determining the residue left after dry oxidation of the oven dried material in a muffle furnace at 550 °C (Sluiter et al., 2004). The volatile solids (VS) were calculated by subtracting the raw ash content from the total solids (Naumann & Bassler, 1993). Nitrogen composition was determined by combustion using a Leco CHN-1000 instrument (St. Joseph, Michigan, USA) and the total crude protein was calculated by multiplying the amount of nitrogen by the factor 6.25.

Cellulose and hemicellulose were determined by using two different standard procedures, the Van Soest method (Van Soest & Wine, 1967) and the sulphuric acid hydrolysis procedure provided by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2011). This second method is able to provide additional information about the composition of the different hemicelluloses, which is not included in the Van Soest procedures. The ADL content was determined by using the Van Soest method.

According to Van Soest, samples were previously dried and grinded to pass through a 1 mm sieve. For determining the content of neutral detergent fiber (NDF), a neutral detergent solution, decahydronaphthalene and sodium sulphite was added to the sample material and boiled for 60 minutes. Afterwards, the remains were filtered using sintered glass crucibles and the filtrate was washed with deionized water and acetone. Filters with their respective filtrates were dried at 105 °C to constant weight. NDF measures most of the structural components in plant cells, including cellulose, hemicellulose and lignin. The acid detergent fiber (ADF) was determined by adding a

mixture of an acid detergent solution and 0.5 M sulphuric acid to the samples, followed by decahydronaphthalene. The samples were boiled for 60 minutes, filtered, washed with deionized water and acetone and dried at 105 °C to constant weight. ADF is mainly composed of cellulose and lignin. Therefore, hemicellulose was calculated as the difference between NDF and ADF. The acid detergent lignin (ADL) was determined from the filtrates remaining in the glass filters after ADF determination. For this, 72 % sulphuric acid was added to the remains and they were periodically stirred for 3 hours and washed with deionized water until a pH of 7 was reached. Filters with their respective filtrates were dried at 105 °C to constant weight and placed afterwards in a muffle furnace at 500 °C for 3 hours. Cellulose was calculated by determining the difference between ADF and ADL.

In the acid hydrolysis method, the structural carbohydrates analyses were carried out for native and pretreated samples in triplicate, following the Laboratory Analytical Procedure presented in the technical Report of NREL no. TP-510-42618 (Sluiter et al., 2011). The dried biomass was milled until the entire sample passes through the 1 mm screen. First, 150 mg +/- 5 mg of the samples were weighted into pressure tubes and then incubated at 30 °C for 60 min after the addition of 1.5 ml 72 % sulphuric acid. In a second stage, the samples were incubated for one hour at 121 °C in an autoclave after a dilution of the sulfuric acid to 4 % using 42 ml of deionized water. In addition to the samples, a set of sugar recovery standards (SRS) was prepared, which are taken through the remaining hydrolysis with 4 % sulfuric acid in order to correct losses due to destruction of sugars during the dilute acid hydrolysis. SRS included D-(+)glucose, D-(+)xylose, D-(+)galactose, -L-(+)arabinose. The structural carbohydrates were analyzed

in the hydrolysis liquor. After the dilute acid step was filtered with glass filters, the remaining insoluble residue was washed and dried at 105 °C overnight and weighed. Glucose concentrations correspond with the cellulose content in the hay sample. Xylose, arabinose and galactose are the major components of hemicelluloses.

The content of soluble sugars (glucose, xylose, galactose, arabinose) as well as inhibitors (furfural and HMF) were analyzed by isocratic HPLC run on a Dionex UltiMate 3000 HPLC system (Dionex, Sunnyvale, CA, USA) set up with a 7.8x100 mm Rezex RFQ-Fast Fruit H+ column (Phenomenex) heated to 82 °C. The mobile phase consisted of 5 mM sulfuric acid and the flow rate used was 1.0 ml/min. The HPLC samples were prepared by diluting samples from the reactions 5-fold with the mobile phase followed by centrifugation and filtration (0.2 µm Sarstedt Filtropur S). Eluted glucose, xylose, arabinose and galactose were monitored by recording refractive index. Furfural and HMF concentrations were monitored using an UV detector at 280 nm. Analytes were identified and quantified by running standards. Chromatograms were recorded, integrated and analyzed using the Chromeleon 6.8 chromatography software (Dionex).

The Microanalytical Laboratory of the University of Vienna performed the elemental analysis of the untreated hay sample. For the analysis, an Element-Analyzer of Perkin Elmer (EA 1108 CHNS-O, Carlo Erba) was used, according to standard procedures (Theiner, 2008).

2.3 Enzymatic Hydrolysis (EH)

Enzymatic hydrolysis was performed on both untreated and steam exploded hay samples using Cellic CTec2, consisting on a blend of cellulases, β -glucosidases and hemicellulases (from Novozymes, Bagsvaerd, Denmark), dosed at 20 FPU g^{-1} DM of substrate. Hydrolysis was carried out in triplicate using 30 ml reaction volumes in 50 ml screw-capped centrifuge tubes, which were preheated at 50 °C before the enzymes were added. The tubes were horizontally shaken at 130 rpm and maintained at 50 °C. The pH in the hydrolysis reactions was adjusted by adding succinate buffer, pH 5.0, to a final concentration of 100 mM. The substrate concentration in the tubes was 50 g VS l^{-1} and reactions were started by adding 200 μl of the enzyme preparation.

2.4 Specific Methane Yield according to VDI 4630

Anaerobic digestion batch trials were carried out in triplicate in accordance with VDI 4630 (VDI, 2006), employing eudiometer batch digesters of 0.25 l capacity. The substrates and the inoculum were weighed out in a ratio of 1:3 (based on volatile solids content). The inoculum utilized was taken from a biogas plant in Utzenaich, Upper Austria (detailed information about the biogas plant is published in (Bauer et al., 2009b)). The digesters, incubated at 37.5°C, were continuously stirred and the biogas yields were monitored on a daily basis during the whole digestion process. Biogas and methane production were measured in norm liters (273 K and 1013 mbar) per kg of volatile solids ($\text{l}_N \text{kg}^{-1}$ VS). The portable gas analyzer Dräger X-AM 7000 was used to determine the biogas composition (CH_4 and CO_2). The results from the elemental

analyses of untreated hay (section 3.4) can be used to calculate the theoretical biogas and methane yields as well as the concentrations of the trace gases ammonia and hydrogen sulphide (Boyle, 1976). Hence, the theoretical potential can be calculated and fixed as the maximum achievable yields by the native and pretreated samples. Measured and potential yields can be compared in order to estimate the remaining capacity for process improvement.

2.6 Calculations and statistical analysis

2.6.1. Mass loss

During the steam explosion pretreatment some of the volatile solids of the biomass are lost. The amount of loss can be estimated through the ash content of both steam exploded and untreated biomass. This mass has been calculated in relation to the total mass as stated in the formula 1.

$$[\text{Mass loss}/M \text{ total}] = \frac{XA_{SE} - XA_{\text{untreated}}}{XA_{SE}} \quad (1)$$

Mass loss	loss of volatile solids
M total	total mass of the untreated biomass
XA_{SE}	ash content of the steam exploded biomass
$XA_{\text{untreated}}$	ash content of the untreated biomass

2.6.2. Statistical analysis

Data provided in tables and figures present means and standard deviations of performed experiments. Statistical analysis was carried out using SPSS Version 18. Data was analyzed by one-way ANOVA, followed by Dunet-T3 test for post-hoc comparison and t-test for paired samples. The level of significance was set at $p < 0.05$.

3 Results and discussion

3.1 Chemical composition

The chemical composition of untreated and pretreated samples is shown in Table 1.

The native biomass had an ODW content (% FM) of 87.1 %. This value decreased strongly in all treated samples since the steam used in the pretreatment added water to the samples. The ODW content of the pretreated samples ranged between 22.8 % (220 °C for 15 minutes) and 40.6 % (175 °C for 5 minutes). In general terms, the ODW content appeared to be lower in samples treated for longer durations, since the biomass was exposed to the steam for a longer time. Moreover, higher temperatures also resulted in lower ODW values. The DM (% FM) determined by KF titration followed a similar trend to that formed by the ODW content. While the native biomass had a DM (KF) content of 87.2 %, the values for the pretreated samples ranged from 26.3 % (220 °C for 15 minutes) to 40.3 % (175 °C for 5 minutes). When comparing the values obtained by the two methods for pretreated samples, lower values were obtained by the oven drying than the KF titrator. These results are in accordance with those obtained by Agger et al. (2013). The untreated hay sample had a VS content (% ODW) of 94.1 %. This value decreased in pretreated samples, ranging from 94.1 to 90.6 %, the latter corresponding to the sample with the second strongest pretreatment (220 °C for 10 minutes). Noteworthy is also a clear drop in the pH produced by the pretreatment, decreasing from 5.75 in the native sample up to 3.65 (205°C for 5 minutes). This phenomenon is probably caused by acetate release from the xylan.

The calculated mass losses are shown in Table 1. The highest mass loss was 39.2 % ODW, which corresponded to the most intense pretreatment (220 °C and 15 minutes). Therefore, an increment of first the temperatures and secondly the pretreatment time lead to a significant decrease in biogas yield caused by the loss of biomass. No exhaust gas, which contains different volatile components, was collected during the pretreatment step. However, it is known that the water dissolved in the process biomass mainly contains acetic acid, levulinic acid, formic acid, furfural and HMF (Parawira & Tekere, 2011) and microorganisms can convert these components into biogas. According to Boyle (1976), the theoretical specific methane yields of the compounds cited above are 373, 531, 122, 583 and 533 l_N kg⁻¹ VS, respectively. Therefore, in a full-scale biogas plant all the steam has to be condensed using adapted technologies.

The original content of protein raised progressively as the intensity of treatment increased, ranging from 7.5 % up to 10.6 %. Table 1 also shows the results of ADL, cellulose and hemicellulose concentrations, based on the Van Soest method. The ADL content was strongly affected by the steam-explosion pretreatment. Untreated hay had an ADL concentration of 5.8 % ODW, rising to 24 % ODW under the pretreatment (220 °C for 15 minutes), which represents an increase of up to four-fold the original value. The cellulose content in the untreated hay sample was 34.9 % ODW and its content after steam-explosion pretreatment ranged from 26.7 to 36.9 % ODW. Its content did not follow any trend according to the pretreatment severity. Moreover, steam explosion led to a reduction in the content of hemicellulose as the pretreatment intensity increased, since it starts to solubilize at a temperature of 150 °C (Garrote et

al., 1999). The untreated hay sample had a hemicellulose (H-CEL) content of 18.9 % ODW. The pretreatment at 220 °C for 15 min reduced this content by about 99 % compared to the untreated sample. It is also apparent that not only the temperature, but also the pretreatment duration plays an important role. At pretreatment durations of 5 or 10 minutes, the content of hemicellulose for each temperature level was markedly higher than at pretreatment durations of 15 minutes.

Some researchers have reported ADL increases and hemicellulose reductions for different steam-exploded biomasses (Ballesteros et al., 2004; Vivekanand et al., 2013). However, in the present study, variations of the cited components are exceptionally higher. These changes increase as a function of pretreatment intensity and are caused by different reactions occurring during this process. The ADL increase could be related to the degradation of hemicellulose sugars since their monosaccharides, under the acid conditions of steam explosion, lead to the formation of different degradation products (Nelson et al., 1988), which may be transformed to acidic insoluble pseudo-lignin (Sannigrahi et al., 2011) and then measured in the Van Soest analysis as ADL. Ballesteros (2004) reported slight increases in the acid insoluble lignin content of herbaceous biomass caused by condensation and repolymerization reactions.

As showed in Table 1, there is a relation between the increase in the percentage of ADL and the mass loss. This relation was expected as no loss of lignin is assumed in the pretreatment and the loss of volatile compounds reduces the quantity of remaining sample, thereby increasing its percentage in ADL. If the increase in ADL content were only due to mass loss, the 39 % mass loss reported in the pretreatment at 220 °C for 15

min would have increased the ADL content from 5.8 to 9.5 %. However, the observed ADL content is 24.0 %, clearly showing the contribution of pseudo-lignin formation to the total amount of analyzed ADL. Similar observations have been done for steam exploded birch (Vivekanand et al., 2013).

The combined effects of temperature and time on cellulose and hemicellulose concentrations were also determined by the sulphuric acid hydrolysis procedure according to NREL (Sluiter et al., 2011). The sugar composition data for the pretreated samples showed similar trends when compared to the results obtained with the Van Soest method. No correlation was observed between the severity of the pretreatment and the glucan content, which ranged between 29.7 and 40.3 % ODW. Hemicellulose sugars were extensively solubilized during steam explosion; xylose content decreased nearly 95 % compared to the raw material, arabinose and galactose decreased from 3.9 % and 1.8 % respectively to non-detectable concentrations. As stated in the former method, the remaining compounds constituting the biomass are ash, protein, lignin, lipids, organic acids and other carbohydrates.

Comparing the results obtained by the acid hydrolysis and the Van Soest method, no differences could be detected regarding the cellulose composition. However, some differences were observed regarding the hemicellulose contents; when increasing the first the temperature and secondly the time of the pretreatment, the decrease in the hemicellulose content is more pronounced under the Van Soest method. This lower content is probably caused by a higher hemicellulose removed during the washing caused by the acid detergent solution and the neutral detergent solution used.

Therefore, it is expected that a small part of hemicellulose is quantified as the organic rest.

Furfural and hydroxymethylfurfural (HMF) are furan compounds generated from pentoses and hexoses, respectively. Their concentration was determined from the 50 g VS l⁻¹ hydrolysates after 4, 24 and 48 hours hydrolysis (data not shown). Analyses revealed that there were no significant differences in the concentration of inhibitors after 4 and 24 hours of hydrolysis. However, significant decreases in their concentration were detected after 48 hours of hydrolysis. Therefore, 4 hours of hydrolysis was chosen for the graphical representation of the concentration of HMF and furfural in the pretreated hay (see Figure 1). The HMF content increased as a function of the pretreatment severity, starting at 211 mg kg⁻¹ ODW (160°C for 5 min) and raising up to 2884 mg kg⁻¹ ODW (205°C for 10 min). A temperature increase beyond this intensity reversed the trend and resulted in a reduction of the HMF content to 926 mg kg⁻¹ ODW (220°C for 10 min). A similar progression can be seen for furfural, which started to be detected under the pretreatment at 160 °C for 10 min, with a concentration of 23.3 mg kg⁻¹ ODW, peaked at 2995 mg kg⁻¹ ODW (190°C for 15 min) and decreased up to 1348 mg kg⁻¹ ODW (220°C for 10 min). This decrease in the contents of HMF and furfural under severe steam explosion conditions can be explained by their volatility, degradation to other substances and formation of pseudo-lignin (Bösch et al., 2010). When compared to other test results from steam exploded *Salix* (Horn et al., 2011a) or wheat straw (Horn et al., 2011b), the HMF and furfural contents reached very high concentrations of inhibitors at relatively low temperatures. This is probably caused because hay is a premature agricultural product

containing easily digestible components, which furthers the enzymatic susceptibility of their cellulose and hemicellulose components (Chen et al., 2007), leading to the formation of inhibitors.

3.2 Enzymatic Hydrolysis

The hay samples were enzymatically hydrolyzed in order to evaluate the effect of the different thermal pretreatments on the glucose and xylose release. Figure 2 shows the glucose yields after 4, 24 and 48 hours of enzymatic hydrolysis in a solution with 50 g VS l⁻¹. The results indicate that the release of glucose is strongly affected by the severity of the pretreatment. After 4 hours of enzymatic hydrolysis, the glucose yields reached their maximum value at 7 g l⁻¹ for the samples pretreated at 175 °C for 15 min). The 24-hour hydrolysis increased the glucose yields up to 16.6 g l⁻¹ (pretreatment at 205 °C for 15 min), which corresponds to a yield of 332 g kg⁻¹ VS. A comparison of the glucose yield with the glucan content of the untreated sample (368.7 g kg⁻¹ VS) shows that 90 % of the maximum potential is converted. The glucose content after 24 hours is not markedly different to the content obtained after 48 hours, except for the pretreatment at 220 °C for 15 min.

Figure 3 displays the xylose content during hydrolysis of the pretreated samples. The results differ greatly from those obtained for glucose: after 4 hours of hydrolysis, the released xylose reached a maximum of 4.4 g l⁻¹ for the samples pretreated at 175 °C for 15 min. As the pretreatment severity increases (pretreatment at 220 °C for 10 min),

the xylose yields decrease down to 0.2 g l⁻¹. After the 24-hour hydrolysis, the released xylose reached a maximum of 6.1 g l⁻¹ in samples pretreated at 175 °C for 10 min and decreased considerably down to 0.6 g, corresponding again to the pretreatment performed at 220 °C for 10 min. The released xylose after 48 hours followed a similar pattern than that reported after 24 hours, with the only difference of slightly higher maximum yields after 48 hours.

Similar trends regarding the effect of the pretreatment severity on enzymatically released glucose and xylose have been reported for different biomasses (Horn et al., 2011a; Horn et al., 2011b; Vivekanand et al., 2013). However, in the present study the maximum xylose yields are reached at lower temperatures. Since high concentrations of HMF and furfural were also detected in the lower temperature range, this is in accordance with the theory presented above stating that hay contains high quantities of easily degradable polysaccharides, resulting in the formation of inhibitors.

3.3 Specific Biogas and Methane Yields

Figure 4 shows measured specific biogas and methane yields for untreated and steam exploded hay. The specific biogas yield from untreated biomass was 420 l_N kg⁻¹ VS. The highest yield was obtained from the material pretreated at 175 °C for 10 min, which increased the production up to 469 l_N kg⁻¹ VS. Higher temperatures resulted in substantially lower specific biogas yields, ranging from 436 to 306 l_N kg⁻¹ VS. The specific methane yield of untreated hay resulted in 243 l_N kg⁻¹ VS. When the biomass was pretreated at 175 °C for 10 min, the methane yield increased 16 %, up to

281 I_N kg⁻¹ VS. The figure shows that an additional increase in the temperature markedly decreased the methane yield. The reason for this reduction under severe pretreatment conditions can mainly be attributed to the formation of substances inhibiting the microorganisms responsible for the anaerobic digestion process (e.g. phenolic compounds or furan derivatives) as well as to the loss of sugars due to pseudo-lignin formation. These results are in keeping with previous studies, which reported similar trends in the biogas yields after the steam explosion of other biomass such as wheat straw, sugarcane straw, *Salix*, miscanthus and rape straw. (Bauer et al., 2009a; De Paoli et al., 2011; Horn et al., 2011a; Menardo et al., 2012; Vivekanand et al., 2012). Nevertheless, the yield improvements recorded in the present study are lower than those obtained in the cited studies. Figure 4 also shows the share of methane in the biogas, which varied from 50 to 64 %, with the softer pretreatments being those that obtained the highest methane percentages.

The elemental analysis of the untreated hay showed that it is composed (% of volatile solids) of 46.15 % carbon, 6.45 % hydrogen, 1.55 % nitrogen, 0.09 % sulfur and 45.76 % oxygen. The calculated methane potential of the untreated biomass according to Boyle (Boyle, 1976) was 440 I_N kg⁻¹ VS. This is a theoretical value, which indicates the maximum methane potential that can be achieved with the native biomass, assuming that the organic matter is degraded and transformed completely into biogas. Hence, the highest methane yield obtained (281 I_N kg⁻¹ VS) shows that 64 % of the theoretical potential is achieved, which indicates that there is still scope for improvement in future investigations.

4. Conclusion

Results show a slight increase in the methane yield of hay after pretreatment with steam explosion. There is a remarkable decrease in the hemicellulose content and a raise in the ADL content, increasing the harshness of pretreatment. The degradation of the hemicellulose leads to the production of aromatic compounds. Compared to other biomasses, hay needs milder pretreatment conditions to achieve a high degradation, requiring less energy. The utilization of hay as a feedstock in biogas-based biorefinery concepts could help making the maintenance of grasslands in Alpine regions profitable. Further attempts to optimize the pretreatment conditions should be undertaken.

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References

- Agger, J.W., Nilsen, P.J., Eijsink, V.G.H., Horn, S.J. 2013. On the Determination of Water Content in Biomass Processing. *Bioenergy Research*, 1-8.
- Ballesteros, I., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, M. 2002. Enzymic hydrolysis of steam exploded herbaceous agricultural waste (*Brassica carinata*) at different particule sizes. *Process Biochemistry*, 38, 187-192.
- Ballesteros, M., Oliva, J.M., Negro, M.J., Manzanares, P., Ballesteros, I. 2004. Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with *Kluyveromyces marxianus* CECT 10875. *Process Biochemistry*, 39, 1843-1848.
- Bauer, A., Bösch, P., Friedl, A., Amon, T. 2009a. Analysis of methane potentials of steam-exploded wheat straw and estimation of energy yields of combined ethanol and methane production. *Journal of Biotechnology*, 142, 50-55.
- Bauer, A., Mayr, H., Hopfner-Sixt, K., Amon, T. 2009b. Detailed monitoring of two biogas plants and mechanical solid-liquid separation of fermentation residues. *Journal of Biotechnology*, 142, 56-63.
- Boyle, W.C. 1976. Energy recovery from sanitary landfills - a review, Pergamon Press. Oxford, pp. 119-138.
- Bösch, P., Wallberg, O., Joelsson, E., Galbe, M., Zacchi, G. 2010. Research impact of dual temperature profile in dilute acid hydrolysis of spruce for ethanol production. *Biotechnology for Biofuels*, 3.
- Cara, C., Ruiz, E., Ballesteros, I., Negro, M.J., Castro, E. 2006. Enhanced enzymatic hydrolysis of olive tree wood by steam explosion and alkaline peroxide delignification. *Process Biochemistry*, 41, 423-429.
- Chen, Y., Sharma-Shivappa, R.R., Keshwani, D., Chen, C. 2007. Potential of agricultural residues and hay for bioethanol production. *Applied Biochemistry and Biotechnology*, 142, 276-290.
- De Paoli, F., Bauer, A., Leonhartsberger, C., Amon, B., Amon, T. 2011. Utilization of by-products from ethanol production as substrate for biogas production. *Bioresource Technology*, 102, 6621-6624.
- FAOSTAT. 2013. Agriculture, forestry and fishery statistics - 2013 edition., European Comission, pp. 249.
- Fernández-Bolaños, J., Felizón, B., Heredia, A., Rodríguez, R., Guillén, R., Jiménez, A. 2001. Steam-explosion of olive stones: Hemicellulose solubilization and enhancement of enzymatic hydrolysis of cellulose. *Bioresource Technology*, 79, 53-61.
- García-Aparicio, M.P., Ballesteros, I., González, A., Oliva, J.M., Ballesteros, M., Negro, M.J. 2006. Effect of inhibitors released during steam-explosion pretreatment of barley straw on enzymatic hydrolysis. *Applied Biochemistry and Biotechnology*, 129, 278-288.
- Garrote, G., Domínguez, H., Parajó, J.C. 1999. Hydrothermal processing of lignocellulosic materials. *Holz als Roh - und Werkstoff*, 57, 191-202.
- Horn, S.J., Estevez, M.M., Nielsen, H.K., Linjordet, R., Eijsink, V.G.H. 2011a. Biogas production and saccharification of *Salix* pretreated at different steam explosion conditions. *Bioresource Technology*, 102, 7932-7936.
- Horn, S.J., Nguyen, Q.D., Westereng, B., Nilsen, P.J., Eijsink, V.G.H. 2011b. Screening of steam explosion conditions for glucose production from non-impregnated wheat straw. *Biomass and Bioenergy*, 35, 4879-4886.
- Li, A., Antizar-Ladislao, B., Khraisheh, M. 2007. Bioconversion of municipal solid waste to glucose for bio-ethanol production. *Bioprocess and Biosystems Engineering*, 30(3), 189-196.

- Li, J., Lennholm, H., Henriksson, G., Gellerstedt, G. 2001. Bio-refinery of lignocellulosic materials for ethanol production. II. Fundamentals and strategic of steam explosion. *First World Conference on Biomass for Energy and Industry*. pp. 767-770.
- Menardo, S., Bauer, A., Theuretzbacher, F., Piringner, G., Nilsen, P.J., Balsari, P., Pavliska, O., Amon, T. 2012. Biogas Production from Steam-Exploded Miscanthus and Utilization of Biogas Energy and CO₂ in Greenhouses. *Bioenergy Research*, 1-11.
- Naumann, C., Bassler, R. 1993. Die chemische Untersuchung von Futtermittel, Vol. 3th ed., VDLUFA-Verlag. Darmstadt.
- Nelson, D.A., Hallen, R.T., Theander, O. 1988. Formation of aromatic compounds from carbohydrates: Reaction of xylose, glucose, and glucuronic acid in acidic solution at 300°C. *ACS Symposium Series*, Denver, CO, USA. Publ by ACS. 113-118.
- OECD. 2007. Climate change in the European Alps, Organisation for Economic CO-operation and Development.
- Palmqvist, E., Hahn-Hägerdal, B. 2000. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. *Bioresource Technology*, 74, 25-33.
- Parawira, W., Tekere, M. 2011. Biotechnological strategies to overcome inhibitors in lignocellulose hydrolysates for ethanol production: Review. *Critical Reviews in Biotechnology*, 31, 20-31.
- Peeters, A. 2009. Importance, evolution, environmental impact and future challenges of grasslands and grassland-based systems in Europe. *Grassland Science*, 55(3), 113-125.
- Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., Hobbs, P.J. 2009. Bioenergy from permanent grassland - A review: 1. Biogas. *Bioresource Technology*, 100, 4931-4944.
- Sannigrahi, P., Kim, D.H., Jung, S., Ragauskas, A. 2011. Pseudo-lignin and pretreatment chemistry. *Energy and Environmental Science*, 4, 1306-1310.
- Sluiter, A., Hames, B., Riuz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D. 2011. Determination of Structural Carbohydrates and Lignin in Biomass. Laboratory Analytical Procedure (LAP).
- Sluiter, A., Hames, B., Ruiz, R.O., Scarlata, C., Sluiter, J., Templeton, D., Energy, D.o. 2004. Determination of Ash in Biomass. National Renewable Energy Laboratory; National Bioenergy Center; Department of Energy.
- Thamsiriroj, T., Murphy, J.D. 2010. Difficulties associated with monodigestion of grass as exemplified by commissioning a pilot-scale digester. *Energy and Fuels*, 24, 4459-4469.
- Theiner, J. 2008. Elemental C/H/N/S Analysis, Microanalytical Laboratory, University of Vienna, Austria. Vienna.
- Van Soest, P.J., Wine, R.H. 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *Journal of the Association of Official Analytical Chemists*, 50, 50-55.
- VDI. 2006. VDI 4630 - Fermentation of organic materials. Characterisation of substrate, sampling, collection of material data, fermentation tests.
- Vivekanand, V., Olsen, E.F., Eijsink, V.G.H., Horn, S.J. 2013. Effect of different steam explosion conditions on methane potential and enzymatic saccharification of birch. *Bioresource Technology*, 127, 343-349.
- Vivekanand, V., Ryden, P., Horn, S.J., Tapp, H.S., Wellner, N., Eijsink, V.G.H., Waldron, K.W. 2012. Impact of steam explosion on biogas production from rape straw in relation to changes in chemical composition. *Bioresource Technology*, 123, 608-615.

Figure captions

Figure 1: Concentration of HMF and furfural after 4 hours hydrolysis in the untreated and pretreated hay.

Figure 2: Glucose released during enzymatic hydrolysis of pretreated hay after 0, 4 and 24h.

Figure 3: Xylose released during enzymatic hydrolysis of pretreated hay after 0, 4 and 24h.

Figure 4: Specific biogas and methane yields of untreated and steam exploded hay.

Tables

Table 1

Chemical composition of untreated and steam exploded hay using the Van Soest method.

Sample	ODW	Water	DM (KF)	XA	VS	Mass loss	pH	XP	CEL	H-CEL	ADL	Organic rest ^a
	[% FM]											
Untreated	87.1	12.8	87.2	5.9	94.1	-	5.75	7.5	34.9	18.9	5.8	27.0
160°C, 5 min	25.4	72.7	27.3	6.3	93.7	6.3	4.91	7.5	34.8	22.7	13.5	15.2
160°C, 10 min	25.5	73.9	26.1	5.9	94.1	< 5.0	4.60	8.1	36.9	22.3	12.9	13.9
160°C, 15 min	34.5	67.3	32.7	7.4	92.6	20.3	4.34	7.5	28.9	15.6	15.6	25.0
175°C, 5 min	40.6	59.7	40.3	6.5	93.5	9.2	4.32	7.5	31.9	15.6	13.5	25.0
175°C, 10 min	35.1	64.9	35.1	7.1	92.9	16.9	4.24	8.1	31.6	8.6	15.1	29.5
175°C, 15 min	27.4	71.5	28.5	7.7	92.3	23.4	3.91	8.8	32.0	2.2	16.9	32.4
190°C, 5 min	32.6	65.7	34.3	7.5	92.5	21.3	3.95	8.1	30.3	2.1	15.4	36.6
190°C, 10 min	30.8	66.4	33.6	7.8	92.2	19.2	3.72	9.4	33.5	0.1	14.5	34.7
190°C, 15 min	26.4	69.8	30.2	8.2	91.8	25.3	3.70	10.6	30.2	0.1	18.2	32.7
205°C, 5 min	31.7	64.4	35.6	8.2	91.8	28.0	3.65	9.4	34.4	0.1	14.4	33.5
205°C, 10 min	30.4	67.4	32.6	9.1	90.9	35.2	3.76	10.0	32.4	0.2	19.3	29.0
205°C, 15 min	28.5	70.5	29.5	9.0	91.0	34.4	3.71	10.0	30.4	0.1	20.5	30.0
220°C, 5 min	30.3	68.3	31.7	9.3	90.7	33.7	3.76	10.6	26.7	0.1	21.9	31.4
220°C, 10 min	24.1	73.2	26.8	9.4	90.6	31.4	3.86	10.6	30.4	2.9	23.2	23.5
220°C, 15 min	22.8	73.7	26.3	8.6	91.4	39.2	3.78	10.6	32.6	0.1	24.0	24.1

ODW: oven dry weight; FM: fresh matter (mass of material in its original state with its natural water content); DM (KF): dry matter (Karl Fischer); VS: volatile solids; XP: crude protein; CEL: cellulose; H-CEL: hemicellulose; ADL: Acid detergent lignin.

^a Organic rest was calculated by subtracting XA, XP, CEL, H-CEL and ADL values from 100 %, mainly composed by lipids, organic acids and different carbohydrates such as starch, soluble sugars (e.g. monosaccharides) and pectin.

Table 2

Results of the carbohydrate analysis of untreated and steam exploded hay using the acid hydrolysis method.

Sample	Glucan		Xylan		Arabinan		Galactan	
	% ODW	SD	% ODW	SD	% ODW	SD	% ODW	SD
Untreated	36.9	4.50	17.3	0.48	3.9	0.13	1.8	0.09
160°C, 5 min	35.1	4.07	20.1	1.43	4.3	0.27	1.8	0.08
160°C, 10 min	29.7	1.42	17.8	0.81	3.6	0.18	1.7	0.07
160°C, 15 min	32.7	1.65	18.3	0.68	2.9	0.08	1.7	0.07
175°C, 5 min	33.0	0.28	17.5	0.25	2.9	0.06	1.5	0.06
175°C, 10 min	32.9	0.07	15.9	1.26	2.2	0.21	1.4	0.13
175°C, 15 min	31.9	-	11.6	2.09	1.1	0.19	1.1	0.18
190°C, 5 min	31.7	5.46	14.0	2.54	1.4	0.33	1.5	0.09
190°C, 10 min	35.9	0.75	9.6	0.26	0.6	0.00	1.0	0.07
190°C, 15 min	40.3	0.17	6.6	0.18	0.4	0.00	0.7	0.00
205°C, 5 min	37.0	0.26	7.1	0.11	0.5	0.00	0.8	0.01
205°C, 10 min	33.0	0.30	2.2	0.06	-	-	0.3	0.07
205°C, 15 min	37.2	1.14	1.9	0.08	0.1	0.00	0.2	0.00
220°C, 5 min	33.4	0.67	1.5	0.02	0.1	0.00	0.2	0.00
220°C, 10 min	34.8	1.01	0.6	0.00	-	-	-	-
220°C, 15 min	32.1	1.28	0.8	0.07	-	-	-	-

Figures

Figure 1

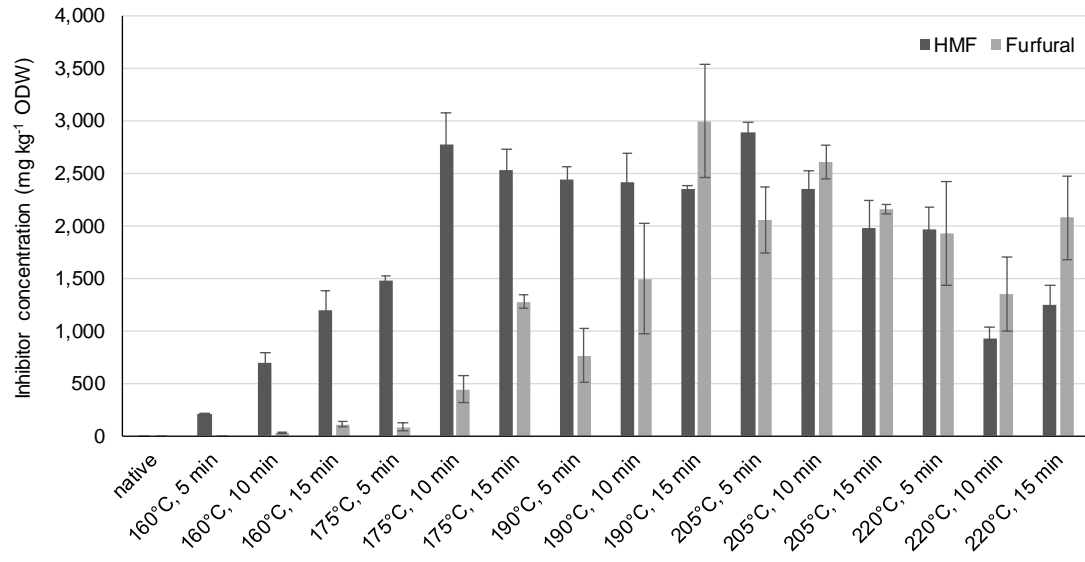


Figure 2

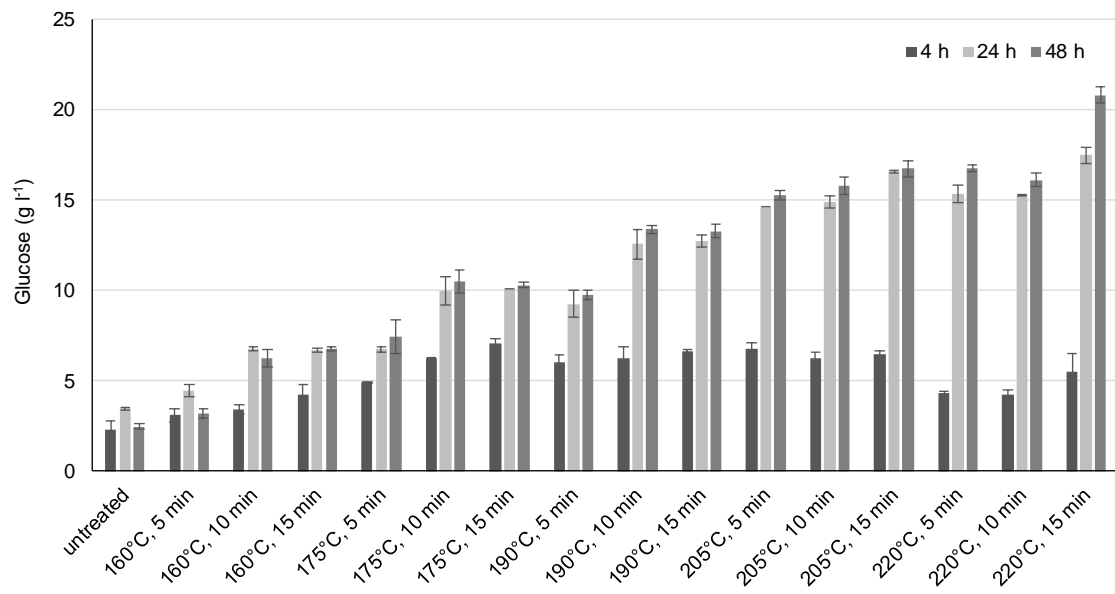


Figure 3

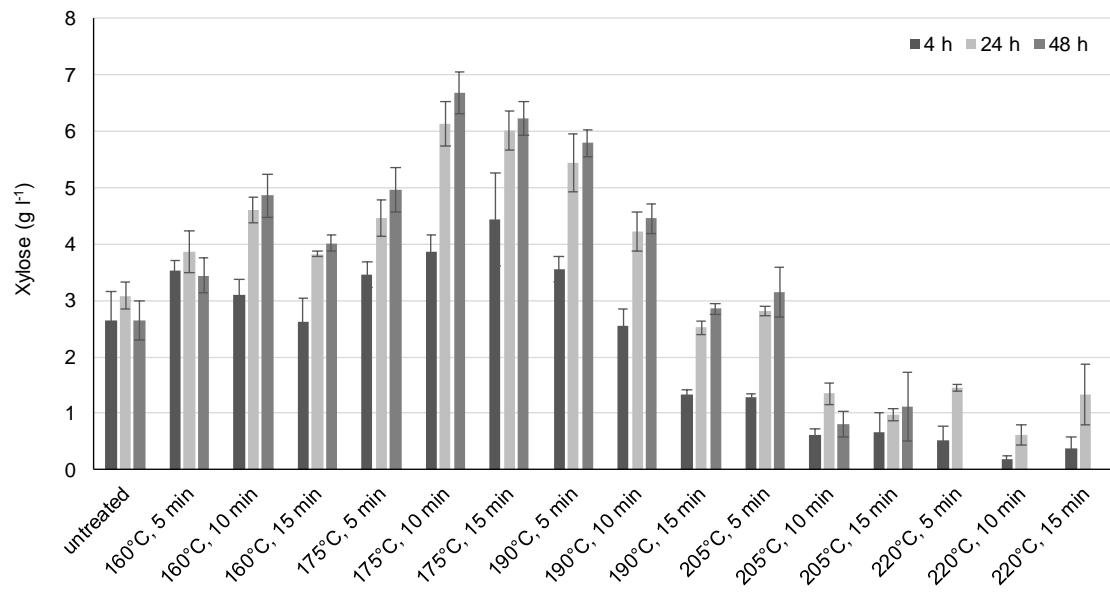


Figure 4

