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Ethanol production from industrial hemp: Effect of combined dilute acid/steam pretreatment and economic aspects

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HIGHLIGHTS

- Combined dilute acid and steam treatment as an effective method of hemp pretreatment.
- Optimal hemp pretreatment conditions: 180 °C and addition of 1% H_2SO_4 as a catalyst.
- Biomass pretreated at the optimal conditions indicated positive economic results.
- Cultivation type had no significant effect on pretreatment and ethanol fermentation.
- Hydrolysis of hemp cultivated organically proceeded quicker compared to conventional type.

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ABSTRACT

In the present study, combined steam (140–180 °C) and dilute-acid pre-hydrolysis (0.0–2.0%) were applied to industrial hemp (*Cannabis sativa* L.), as pretreatment for lignocellulosic bioethanol production. The influence of the pretreatment conditions and cultivation type on the hydrolysis and ethanol yields was also evaluated. Pretreatment with 1% sulfuric acid at 180 °C resulted in the highest glucose yield (73–74%) and ethanol yield of 75–79% (0.38–0.40 g-ethanol/g-glucose). Taking into account the costs of biomass processing, from field to ethanol facility storage, the field-dried hemp pretreated at the optimal conditions showed positive economic results. The type of hemp cultivation (organic or conventional) did not influence significantly the effectiveness of the pretreatment as well as subsequent enzymatic hydrolysis and ethanol fermentation.

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1. Introduction

Bioethanol derived from biomass is considered as a promising renewable fuel. The fact that bioethanol can be easily integrated into existing fuel systems and partially substitute fossil fuels makes bioethanol of specific interest (Balat, 2011). Currently, bioethanol is produced on a large scale from the first generation substrates, including sugarcane, wheat or maize. Besides these feedstocks, lignocellulosic biomass can be used. Among these herbaceous crops are considered as particularly promising and industrial hemp (*Cannabis sativa* L.) is such a crop. Hemp is used for various applications; the fibers are used for making ropes, cloth and paper, while the seeds can be used as a protein rich food or feed. The woody core (shives) can be used as animal bedding. Additionally, new opportunities to use hemp biomass as solid fuel or feedstock in biogas and bioethanol production have been reported recently (Kreuger et al., 2011; Prade et al., 2012a; Sipos et al., 2010). The plant can produce high biomass yields even in cold climate areas, resulting in high area-efficiency, which reduces competition with food and feed crops for arable land (Prade et al., 2012b).

Biomass of lignocellulosic crops contains cellulose and hemicelluloses bound together by lignin. Pretreatments are required to loosen the lignocellulosic structure and to facilitate enzymatic hydrolysis of polysaccharides prior to ethanol fermentation. In fact, the main technological challenge in ethanol production from this type of feedstock is an effective pre-treatment before saccharification and fermentation (Hendriks and Zeeman, 2009; Talebnia et al., 2010). One of the most commonly applied pretreatment methods used for this type of feedstock is pre-hydrolysis based on dilute

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acids and steam treatment. Dilute-acid hydrolysis is usually conducted using mineral acids, most commonly sulfuric acid, which is an effective and relatively inexpensive catalyst (Talebnia et al., 2010). During the pretreatment process, the hemi-cellulose is hydrolyzed into its pentose monomers, mainly xylose as well as arabinose and galactose. This pretreatment is considered to be effective not only in hydrolyzing hemicellulose, but also in softening the lignin. However, the solubilization of hemicelluloses during thermal pretreatment results in formation of inhibitory compounds. The major toxic compounds include furfural and hydroxymethylfurfural (sugar degradation products), acetic acid (released from the hemicellulosic structure), as well as aromatic and phenolic compounds (lignin degradation products). Those compounds can cause inhibition in subsequent enzymatic hydrolysis and in ethanol production during the fermentation step (Klinke et al., 2004; Liu, 2006). Consequently, evaluation of inhibitory compounds during pre-treatment is important in order to minimize inhibition during fermentation. Utilization of new feedstock types requires an extensive evaluation of the pretreatment conditions since the optimization of those are strongly connected to the biomass nature and composition. Hemp is a limitedly examined feedstock for bioethanol production compared to other lignocellulosic biomass (e.g. wheat straw, rapeseed straw). Few reports confirm the positive influence of steam treatment (200–220 °C) after impregnation with 2% SO_2 (Kreuger et al., 2011; Sipos et al., 2010) and alkaline treatment (1% NaOH) with subsequent autoclave treatment (120 °C) (Pakarinen et al., 2012). The above mentioned studies describe only a limited range of pretreatment conditions. Furthermore, the influence of pretreatment on inhibitory compounds released during the hemp pretreatment has not been evaluated and described. According to our knowledge, the hemp cultivated in different types (e.g. conventional, organic) has never been tested as feedstock for bioethanol production.

Therefore, the aim of this study was to elucidate the potential for ethanol production from industrial hemp. Furthermore, to identify optimal pretreatment conditions for field-dried industrial hemp, applying combined steam treatment and dilute-acid (sulfuric acid) pre-hydrolysis. Moreover, the aim was to study the influence of the pretreatment and biomass cultivation type (conventional or organic) on the hydrolysis yield in the enzymatic step as well as ethanol yield from the fermentation process. Finally, it was aimed to assess the economic viability of feedstock preparation from field to biomass storage facility, including costs of hemp cultivation, harvesting, transportation and storage.

2. Methods

2.1. Raw material

Industrial hemp (*C. sativa* L.) of *Felina* 32 variety was cultivated on a loamy clay soil, with 15% clay and 3% organic matter, both conventionally and organically at Lönnstorp experimental farm, at the Swedish University of Agricultural Sciences, in southern Sweden (55°40′N 13°06′E). The hemp was sown in late April and harvested in October 2011. After harvesting the whole-crop, biomass was dried indoors for 4 months at approx. 18 °C to simulate field-drying. Then the dry hemp was chopped in a garden shredder to a length of 2–3 cm and ground (<1 mm) to particle size by using a cutting mill. Characteristics of the hemp biomass was analyzed using methods described below and is presented in Table 1.

2.2. Pre-treatment of hemp biomass

The pre-treatment procedure in the present study was based on temperature (140 and 180 °C) and sulfuric acid addition (0.0, 0.5, 1.0 and 2.0% w/v). The process was conducted at solid content of 10% (w/w) feedstock/water. After acid addition, the mixture was steam treated in a batch reactor at 140 °C for 20 min or at 180 °C for 10 min. Each biomass pretreatment (8 temperature/acid combinations) was replicated four times. After pretreatments, the slurry was separated into solid fraction (water insoluble fraction, WIS) and liquid fraction (hydrolysate). The separation was performed in a commercial filtration unit (Buchner unit) with a filtrating cloth pore of 15 μm. The filter cake (solid fraction) was dried in a forced air oven at 55 °C for 24 h, and stored in sealed plastic bags at 4 °C for further enzymatic hydrolysis and fermentation. The separated liquid fraction was stored at −18 °C for further analyses.

Table 1
Characteristics of hemp biomass (% of dry matter, ±standard deviations, numbers in the same row followed by the same letter are not significantly different $p > 0.05$).

Parameter	*Felina* 32 strain	
	Conventional cultivation	Organic cultivation
VS	93.9 ± 0.4a	93.8 ± 0.5a
Glucan	39.8 ± 0.9b	42.0 ± 1.2a
Xylan	14.4 ± 0.5a	14.8 ± 0.7a
Arabinian	0.98 ± 0.1a	0.87 ± 0.1a
Protein	3.1 ± 0.4a	3.8 ± 0.3a
Lipids	0.6 ± 0.1b	0.8 ± 0.1a
Ash	5.80 ± 0.2a	4.70 ± 0.3b
Lignin	15.0 ± 1.0a	13.2 ± 1.2b

2.3. Enzymatic hydrolysis and fermentation

After the pre-treatment, enzymatic hydrolysis was conducted at a solid loading of 5% (w/v) in a 50 mM sodium citrate buffer, pH 4.8. Hydrolysis was performed at 50 °C for 48 h. Celluclast 1.5 L® (Celluclast) derived from *Trichoderma ressei* and Novozyme 188 (Novozyme) from *Aspergillus niger* were used for enzymatic hydrolysis. Enzyme loadings of Celluclast (cellulose) and Novozyme 188 (β-glucosidase) ware 30 FPU/g glucan and 20 IU/g glucan, respectively. The fermentation was carried out at 37 °C for 48 h in 300 ml Pyrex flasks equipped with air locks. Pure nitrogen gas was sparged into the media at the beginning of the fermentation to keep anaerobic conditions.

Furthermore, all assays undergoing fermentation were supplemented with the following amounts of minerals (g/l): $(NH_4)_2SO_4$, 3.75; K_2HPO_4, 2.11; $MgSO_4{\cdot}7H_2O$, 0.375 and $CaCl_2{\cdot}2H_2O$, 0.5. For fermentation, 30 ml/l (3% v/v) inoculum of *Sachharomyces cerevisiae* was added. The enzymatic hydrolysis and subsequent fermentation were replicated four times for both untreated and pretreated biomass (all temperature/acid combinations). Samples of one milliliter were taken periodically (after 0, 3, 6, 12, 24, 36 and 48 h) and immediately centrifuged at 10,000×*g* for 10 min. The supernatants were filtered through 0.2 μm pore size filters before sugars and ethanol determination.

2.4. Calculations

2.4.1. Pretreatment

The WIS (water insoluble) recovery was calculated according to Eq. (1):

$$\mathrm{WIS_{Recovery}}(\%) = \frac{\mathrm{Solid\ fraction_{Dry}}}{\mathrm{Feedstock_{Dry}}} \cdot 100 \quad (1)$$

where, $\mathrm{Solid\ fraction_{Dry}}$ – mass of solid material recovered after pretreatment and drying at 55 °C for 24 h, g; $\mathrm{Feedstock_{Dry}}$ – mass of material used for pretreatment after drying at 55 °C for 24 h, g.

Distribution of cellulose and hemicellulose after the pretreatments between solid and liquid fractions was determined. The loss

(volatile or/and further degraded fraction) of cellulose and hemicellulose in the mass balances was calculated on the basis of their difference between untreated biomass and recovered after pretreatment.

2.4.2. Glucose, xylose and ethanol yield

The glucose and xylose yields were based on the sugars released during enzymatic hydrolysis and calculated according to Eqs. (2) and (3), respectively.

$$\text{Glucose}_{\text{Yield}}(\%) = \frac{\text{Glucose}_{\text{Released}}}{\text{Glucan}_{\text{Feedstock}} \cdot \left(\frac{180}{162}\right)} \cdot 100 \quad (2)$$

$$\text{Xylose}_{\text{Yield}}(\%) = \frac{\text{Xylose}_{\text{Released}}}{\text{Xylose}_{\text{Feedstock}} \cdot \left(\frac{150}{132}\right)} \cdot 100 \quad (3)$$

where, $\text{Glucose}_{\text{Released}}$ and $\text{Xylose}_{\text{Released}}$ – the amount of glucose and xylose released during enzymatic hydrolysis, g; $\text{Glucan}_{\text{Feedstock}}$ and $\text{Xylan}_{\text{Feedstock}}$ – total amount of glucan and xylan in raw hemp or solid fraction after pretreatment, respectively, g; 180/162 and 150/132 – stoichiometric conversion factors of glucan to glucose and xylan to xylose, respectively.

Ethanol yield (Y_{Ethanol}) was calculated as the amount of ethanol produced during fermentation from unit mass of glucose and was based on the assumption that all glucose found in the feedstock could be converted by *S. cerevisiae* into ethanol, with a yield of 0.51 g EtOH/g of glucose.

2.5. Analytical methods

Volatile solids (VS), ash content and protein content were determined according to standards methods (APHA, 1995). Lipid content was analyzed after extraction using the Soxhlet method. pH was measured using a standard pH meter. Klason lignin in the solid fraction was determined from the weight of the filter cake generated during the strong acid hydrolysis minus the ash content.

Concentrations of sugars (glucose, xylose, arabinose) and ethanol were measured by high performance liquid chromatography HPLC (Agilent 1100) equipped with a BioRad Aminex HPX-87 H at 63 °C and refractive index (RI) detector (RID 1362A) using 0.6 ml/min of 4 mM H_2SO_4 as eluent. Sugar contents in solid fractions (raw and pretreated feedstock) was analyzed using strong acid (72% w/w H_2SO_4) hydrolysis. Furfural and 5-hydroxymethyl-2-furaldehyde (HMF) in liquid fractions were detected by HPLC fitted with ultraviolet (UV) detector.

2.6. Statistical analysis

MS Excel and the statistical package SAS (2004) were used for data analysis. For comparison of significant variation of untreated and pretreated characteristics of hemp as well as data obtained during enzymatic hydrolysis and fermentation, analysis of variance was carried out followed by Duncan's post hoc test between means. Student's *t*-test was used for analyzing the differences between raw biomass characteristics (hemp grown conventionally and organically).

2.7. Economic analysis

A basic economic analysis performed was based on the following assumptions: hemp was sown in late April and harvested in October. For harvest, the hemp was cut into 80 cm long pieces and windrowed using a forage harvester with a HempCut 4500 header. The windrows were field-dried for approx. 4 days and then chopped using a forage harvester with a pick-up header at a moisture content of approx. 35%. The hemp biomass was transported 8 km to the processing plant and stored until further processing. For storage, air tight compression in a plastic tube normally used for ensiling was assumed.

Costs of cultivation, harvesting as well as transportation and storage of the field-dried hemp were calculated for both the conventional and the organic cultivation system using literature data on recommended machinery costs. In order to evaluate economic viability of field-dried hemp as an ethanol feedstock, wheat grain was used as reference feedstock. Using typical wheat grain ethanol yield, actual feedstock price [€/L_{ethanol}] and assuming the same price even for hemp, the revenue and gross margin for hemp feedstock production and supply were calculated (Table 2). Overhead costs were not included in the economic analysis. Due to the lack and uncertainty of data on a production-scale process, the economic analysis was based on the potential hemp ethanol yield compared to the yield and concurrent prices of the reference feedstock (wheat grain). Due to this limitation, results on economic viability of hemp as ethanol feedstock can only be seen as indications.

3. Results and discussion

3.1. Hemp composition

The main polysaccharide components of the industrial hemp used as a feedstock in this study ware glucan (40–42%) and xylan (14–15%) (Table 1). Thus, the content of sugars in hemp is similar to what has been reported for *Salix* (Sassner et al., 2008), sweet sorghum bagasse (Goshadrou et al., 2011) and straws (Horn et al., 2011; Kaparaju et al., 2009; Lu et al., 2011). In the present study, glucan content as percentage of dry matter was significantly higher in hemp grown organically than in the one grown conventionally. Also, significantly higher amounts of lipids were found in the organically grown hemp compared to the conventionally grown one, while lower amounts of ash and lignin were found (Table 1). Contents of various components in organic versus conventional cultivation can vary due to biomass yield. As yield is increased, components are diluted. Nevertheless, the same yields in both cultivations were achieved (Table 2). The differences in chemical composition between analyzed cultivation types might have been a result of N availability as well as other nutrients in various stages during hemp growth, which have been observed previously for various crops, e.g. wheat (Nitika et al., 2008; Ryan et al., 2004).

3.2. Feedstock pretreatment

3.2.1. Characteristics of solid residues

The recovery of the water insoluble fraction (WIS) ranged from 53% to 82%, depending on the pretreatment conditions. The type of hemp cultivation did not have a significant influence on the WIS fraction recovery (Table 3). As a rule, an increased severity of pretreatment (i.e. higher temperature and/or higher acid content; Table 3) resulted in lower solid fraction recovery. This is in agreement with findings from previous studies on other crops, e.g. Lu et al. (2009). Higher concentrations of acid used in the pretreatment, resulted in darker color of the solid fraction and a less visible structure of residual fibers, which has also been observed in other studies using various crops, e.g. Horn et al. (2011).

The pretreatment methods used in this study had a significant effect on the chemical composition of the pretreated hemp. The main components of the solid fraction after pretreatment were glucan, xylan and lignin (Table 3). All pretreatments applied resulted in a positive effect on the chemical composition i.e. an increase of the cellulose fraction (glucan) in the solid residue (Table 3). An increase in glucan content was associated with solubilization of the hemicellulose fraction through pretreatment, as has also been

Table 2
Parameters used for economic calculations.

Parameter	Unit	Value	Reference
Hemp biomass dry matter yield, autumn harvest, organic	[Mg/ha]	12.4	Own unpublished data
Hemp biomass dry matter yield, autumn harvest, conventional	[Mg/ha]	12.4	Own unpublished data
Exchange rate	[SEK/€]	9.0	
Average transport distance field - ethanol plant	[km]	8.0	
Economic lifetime storage facilities	[a]	25	
Assumed ethanol yield from wheat grain feedstock	[L/t]	370	
Substrate market price for wheat kernals for ethanol production	[€/L]	0.486	AgroEtanol (2013)
	[€/t][a]	180	AgroEtanol (2013)

[a] Weight at 14.5–17.0% moisture content, depending on the delivery date.

Table 3
Characteristics of solid fraction after pretreatment (±standard deviations, values with the same letters are not significantly different $p > 0.05$).

Temp., °C	Acid, % (w/v)	Conventional cultivation				Organic cultivation			
		Glucan, %	Xylan, %	Lignin, %	WIS, %	Glucan, %	Xylan, %	Lignin, %	WIS, %
		% of dry matter							
140	0.0	47.9 ± 1.2i	12.4 ± 0.6a	15.0 ± 0.5de	79 ± 2ab	49.5 ± 0.9hi	12.3 ± 0.4a	13.2 ± 0.5f	82 ± 2a
140	0.5	49.8 ± 1.1h	11.4 ± 0.8ab	16.1 ± 0.5bc	76 ± 2bcd	52.4 ± 0.9g	11.3 ± 0.8ab	13.3 ± 0.5f	77 ± 2bc
140	1.0	52.3 ± 1.2g	10.0 ± 0.7c	17.3 ± 0.5bc	72 ± 2def	54.2 ± 0.7efg	9.40 ± 0.6cd	14.7 ± 0.3e	75 ± 1cd
140	2.0	53.4 ± 1.5fg	10.4 ± 1.1bc	17.8 ± 0.6b	65 ± 2hi	54.9 ± 1.1ef	9.80 ± 0.5c	15.2 ± 0.5de	67 ± 2gh
180	0.0	55.2 ± 0.9ef	9.76 ± 0.7c	16.1 ± 0.4bc	70 ± 2efg	56.0 ± 1.0e	9.83 ± 0.8c	15.6 ± 0.7de	73 ± 1de
180	0.5	58.3 ± 0.7d	7.80 ± 0.6e	17.2 ± 0.8b	67 ± 3gh	59.7 ± 1.1cd	8.45 ± 0.8de	15.8 ± 0.6de	69 ± 3ghi
180	1.0	61.1 ± 1.0bc	5.94 ± 0.6f	20.1 ± 0.6a	62 ± 4j	62.8 ± 1.2ab	6.27 ± 0.6f	16.7 ± 0.7bc	65 ± 2ij
180	2.0	63.7 ± 1.3a	5.77 ± 0.3f	20.5 ± 0.8a	53 ± 4k	64.7 ± 1.3a	6.20 ± 0.4f	16.8 ± 0.7bc	55 ± 2k

shown in previous studies, e.g. Barta et al. (2010). The hemicelluloses in hemp were constituted of xylose and its content in the solid fraction decreased as the severity of the pretreatment increased (Table 3). However, there was in general no significant variations in glucan and xylan contents between the two cultivation types, besides the one involving 140 °C and 0.5% of acid. The hemp grown organically and pretreated in the latter conditions resulted in significantly higher glucan content compared to biomass produced in conventional cultivation (Table 3). Arabinan was also found in hemicellulose of pretreated hemp although the content was very low, less than 0.5% (data not shown).

The lignin content increased with severity of the pretreatment method (Table 3), which is consistent with the increased hemicelluloses degradation (Lim et al., 2013). Similarly to raw biomass, conventionally cultivated hemp after pretreatment exhibited significantly higher lignin content compared to the one cultivated organically. The increase in lignin content after pretreatment might have also been caused by generation of pseudo-lignin during polysaccharide degradation products, which could increase the Klason lignin content. Similar observations were noticed in previous studies, e.g. by Hu and Ragauskas (2012) and Vivekanand et al. (2013).

3.2.2. Cellulose and hemicellulose distributions after pretreatment

The effectiveness of pretreatment methods applied was also evaluated by estimating the glucan distribution between fractions and the glucan loss (Fig. 1a and b). For all treatments (both cultivation types), except when 2% acid was used, more than 95% of the glucan was retained in the solid fraction. About 1–3% was released into the liquid fraction, while the remaining part was lost during pretreatment. When the pretreatment involved an acid concentration level of 2% (independent of other factors), the glucose content in the hydrolysate was significantly increased to about 5%, while the loss reached about 7–11% of the total cellulose (Fig. 1a and b). This negative effect of higher acid concentration applied during pretreatment is in agreement with previous studies, e.g. Xu et al. (2011).

The hemicellulose content in solid fraction was gradually decreasing with increasing severity of the pre-treatment, which indicates that high temperature and sulfuric acid are powerful catalysts for removing hemicellulose (Fig. 1c and d). The highest recovery of hydrolyzed hemicellulose (54–56%) was achieved by pretreatment at 180 °C and with addition of 1% sulfuric acid. When the acid concentration increased from 1% to 2%, a slight decrease in hemicellulose recovery was observed. This was most probably connected with sugar degradation. High sugar solubilization at harsh pretreatment conditions does not always correlate with good sugar recoveries in pre-hydrolysate, due to sugar losses by degradation at acidic conditions (Boussaid et al., 1999). Decrease in hemicellulose content with increasing severity of the pretreatment was also observed in previous studies, e.g. Barta et al. (2010).

Release of sugars during pretreatment, at high temperatures, is connected with generation of degradation products, such as furfural and HMF, which are toxic to most microorganisms (Thomsen et al., 2006). Relatively low concentrations of furfural (0.02–0.1 g/l) and HMF (0.04–0.25 g/l) were recorded during the experiment. With higher sulfuric concentrations, the increase of hemicellulose degradation products was more noticeable, but their concentrations were still negligible (furfural = 0.10 g/l, HMF = 0.21–0.25 g/l). This is in agreement with a study showing that relatively low concentration of furfural and HMF is generated during pretreatment at temperatures below 190 °C (Horn et al., 2011). However, other degradation products of hemicellulose, like formic acid or pseudolignin, can be generated (Barta et al., 2010), which have not been analyzed for in this study.

3.3. Enzymatic hydrolysis

The glucose yield of untreated feedstock amounted to 30–32% after 48 h of enzymatic hydrolysis, which is in the range previously reported (20–42%) (Pakarinen et al., 2011, 2012). All tested pretreatment methods influenced glucose yield in a positive way (Table 4). Higher glucose yields were achieved when applying higher temperature (180 °C) compared to lower temperature (140 °C). Moreover,

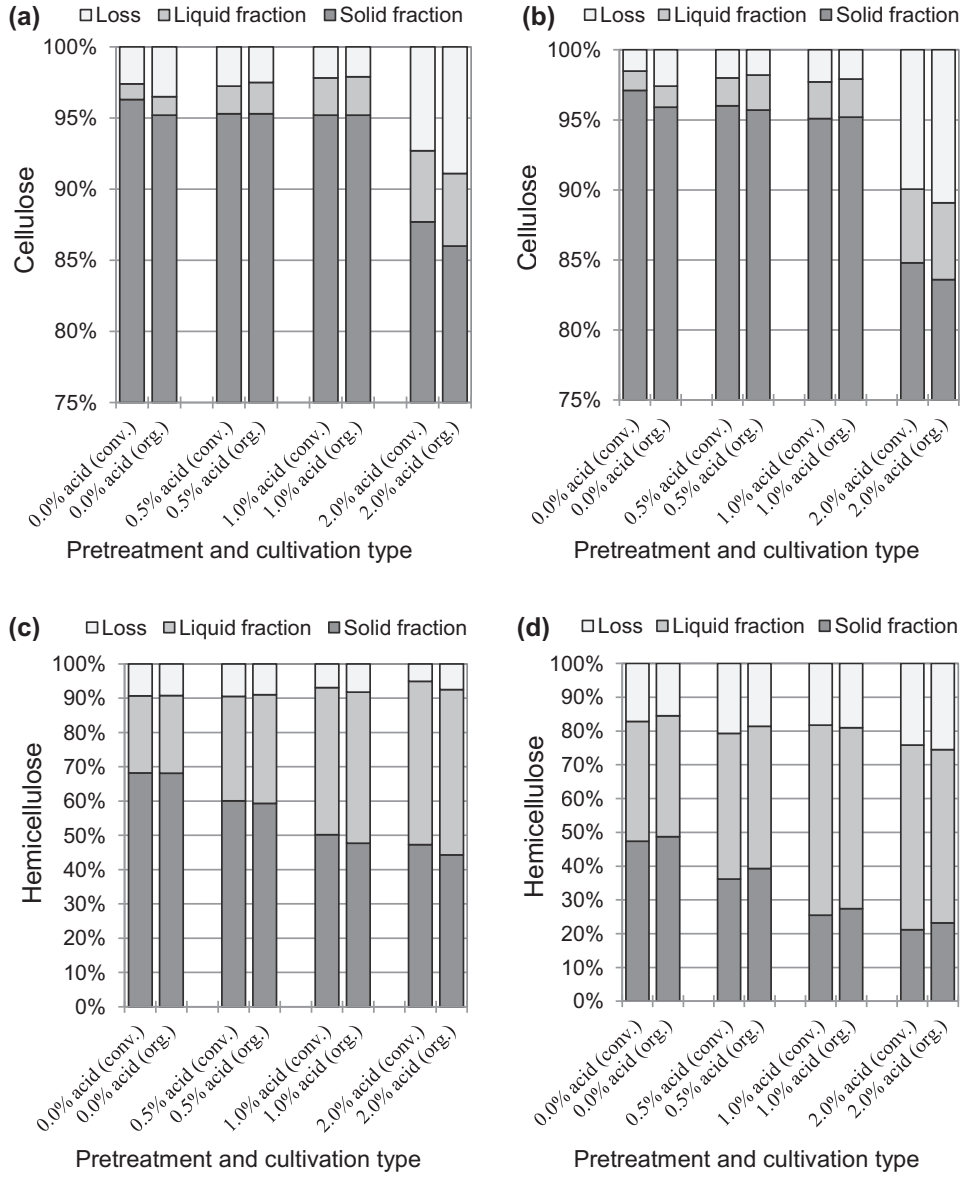

Fig. 1. Carbohydrates distribution after pretreatment (a – cellulose at 140 °C, b – cellulose at 180 °C, c – hemicellulose at 140 °C, d – hemicellulose at 180 °C; conv. – conventional cultivation, org. – organic cultivation).

the acid-facilitated pretreatment allowed to achieve higher glucose yields during subsequent enzymatic hydrolysis compared to samples which underwent pretreatment based only on temperature. The highest concentration of glucose released (24.9–25.9 g of glucose/l) and glucose yield (73–74%) were observed for the sample pretreated at 180 °C with addition of 1.0% acid as a catalyst. This pretreatment more than doubled the glucose yield of the hydrolysis compared to hydrolysis of untreated hemp (30–32%). Application of a higher acid concentration (2%) did not allow to achieve higher glucose yield compared to the sample pretreated at 180 °C with addition of 1.0%. This trend was also observed in previous studies (Ferreira et al., 2011; Xu et al., 2011). Depending on the pretreatment conditions, the xylose released during enzymatic hydrolysis amounted to 15–32% of hemicellulose present in the solid fraction after pretreatment, regardless of the cultivation type. Almost all xylose released occurred during the first of 3–6 h of the process and then remained unchanged (data not shown). Similar observations have been made by Lu et al. (2009). The arabinose was only detected in case of samples pretreated at 140 °C in low concentration (<0.3 g/l, data not shown).

No significant differences of glucose yields were observed between batches containing biomass coming from conventional and organic type of cultivation. The hydrolysis rate in all samples of organically cultivated hemp was faster compared to the samples containing conventionally growth biomass, which can be beneficial from an economical point of view (Fig. 2). For example, after 12 h of the enzymatic hydrolysis of "organic" hemp, about 90–95% of total glucose (released during 48 h of the process) was noticed, compared to 72–79% released during hydrolysis of "conventional" hemp. Fig. 2 presents the time course of enzymatic hydrolysis of selected assays as examples. Since the process conditions and the amount of enzymes used were the same, the difference in the hydrolysis efficiency may be associated with about 12% lower lignin content of hemp cultivated in an organic way (Table 3).

3.4. Fermentation

In all batches, ethanol production started immediately without any lag phase and no inhibition was found during the

Table 4
Effectiveness of enzymatic hydrolysis and ethanol fermentation (±standard deviations, values with the same letters are not significantly different $p > 0.05$).

Temp., °C	Acid, % (w/v)	Conventional cultivation				Organic cultivation			
		Enzymatic hydrolysis		Ethanol fermentation		Enzymatic hydrolysis		Ethanol fermentation	
		Glucose yield[a], %	Xylose yield[a], %	Etanol conc.[b], g/l	$Y_{Ethanol}$[b], %	Glucose yield[a], %	Xylose yield[a], %	Etanol conc.[b], g/l	$Y_{Ethanol}$[b], %
Untreated		29.6 ± 0.9k	13.2 ± 1.4fg	2.89 ± 0.1f	89 ± 5ab	32.2 ± 2.0jk	13.3 ± 0.6fg	3.12 ± 0.1f	86 ± 3ab
140	0.0	37.7 ± 0.5ij	15.4 ± 1.6de	4.62 ± 0.2e	90 ± 6ab	38.7 ± 2.9hi	15.7 ± 0.7e	4.60 ± 0.3e	85 ± 8ab
140	0.5	43.7 ± 2.0hi	13.8 ± 0.9ef	5.17 ± 0.2d	92 ± 3a	44.5 ± 3.4gh	15.6 ± 0.8de	5.45 ± 0.3d	83 ± 2bcd
140	1.0	49.9 ± 2.2fg	13.4 ± 0.8ef	6.35 ± 0.3c	86 ± 7ab	50.3 ± 1.5fg	14.2 ± 0.8ef	6.34 ± 0.3c	82 ± 5bcd
140	2.0	53.2 ± 1.5ef	13.6 ± 0.7ef	6.68 ± 0.3c	83 ± 3bcd	55.1 ± 2.9ef	14.2 ± 0.9ef	6.45 ± 0.3c	75 ± 4de
180	0.0	56.6 ± 1.1de	24.4 ± 0.7bc	7.55 ± 0.1b	85 ± 3ab	55.7 ± 2.9ef	25.2 ± 0.7b	7.56 ± 0.1b	86 ± 5ab
180	0.5	68.3 ± 2.2bc	25.8 ± 0.9b	9.43 ± 0.3a	84 ± 3bc	67.2 ± 1.8bc	25.8 ± 1.0b	9.45 ± 0.1a	83 ± 2bcd
180	1.0	73.6 ± 2.3a	30.4 ± 0.8a	10.0 ± 0.1a	79 ± 3ce	74.3 ± 2.5a	31.7 ± 1.2a	9.95 ± 0.4a	75 ± 4de
180	2.0	54.8 ± 1.7de	22.5 ± 1.2c	7.78 ± 0.2b	79 ± 1ce	61.9 ± 2.3cd	23.7 ± 1.1bc	8.15 ± 0.5b	74 ± 4e

[a] Results after 48 h.
[b] Results after 24 h.

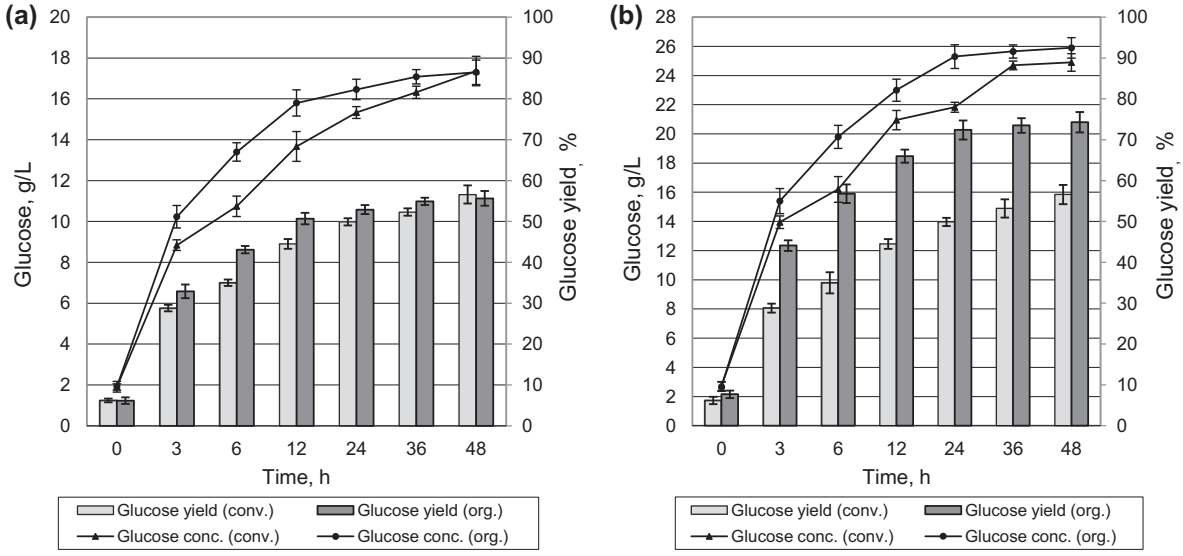

Fig. 2. The course of enzymatic hydrolysis (a – hemp pretreated at 180 °C + 0% acid, b – hemp pretreated at 180 °C + 1% acid; conv. – conventional cultivation, org. – organic cultivation; selected assays, conc. – concentration).

fermentation. Ethanol yield was 74–92% (0.37–0.47 g-ethanol/g-glucose released during enzymatic hydrolysis) of theoretical yield (0.51 g-ethanol/g-glucose) after 24 h of fermentation (Table 4). Regardless of the cultivation type, bioethanol production more than tripled (9.4–10.0 g/l) after applying pretreatment involving the temperature of 180 °C and acid concentration of 0.5–1.0% compared to fermentation of untreated hemp (2.89–3.12 g/l). The slight decrease in the ethanol yield between 24 h and 48 h of the process was probably due to catabolic ethanol oxidation at low sugar concentration and/or evaporation of the ethanol (data not shown) (Lu et al., 2011; Ruiz et al., 2008). The xylose concentration did not change its concentration significantly during the fermentation (data not shown), which showed that *S. cerevisiae* was not able to use xylose (Lu et al., 2009).

3.5. Economic analysis

Costs for hemp production, harvesting, transportation and storage were similar for conventional and organic production systems (Table 5). Due to the similar yields between the production systems, biomass production costs per liter ethanol were comparable for the two cultivation types. It should be noted that the present study is a simplified analysis and does not contain energy requirements for biomass pretreatment. The results, however, can be used as an input for detailed life cycle assessments.

Based on the assumed biomass yield per hectare, only two of the substrate treatments (180 °C + 1% H_2SO_4, both conventional and organic cultivation) showed a positive economic result (Fig. 3). The break-even point for the costs at the assumed price situation was 11.6 and 11.7 t/ha of hemp dry matter from the conventional and organic production system, respectively (Fig. 4). The sensitivity analysis showed that a ±10% change of the market price for wheat at 180 €/t dry matter results in required hemp biomass yields varying from 11.7% to +17.0% for conventional production and 11.8% to +17.3% for organic production (Fig. 5).

Ethanol production generates large amounts of stillage as a by-product, which depending on the raw material can be sold as feed. Another option to increase the economic profit of applying hemp as a substrate for bioethanol production is connected to the high content of lignin in stillage. In the current study, the lignin content in the stillage amounted to between 14.5% and 20.5% (data not shown). Since lignin is not degraded under anaerobic conditions at all or degraded very poorly (2–7% of total lignin) (Barakat et al., 2012), it can be separated by a decanter centrifuge or a filter press from stillage. Lignin residues can be used for heat or heat and

Table 5
Costs for operations and material for the cultivation, transportation and storage of hemp biomass from field to ethanol plant storage.

Operation/material	Unit	Conventional	Organic	Frequency
Fertilizer	[€/ha]	277	228	
Seeds	[€/ha]	191	191	
Stubble treatment	[€/ha]	29	29	1
Ploughing	[€/ha]	90	90	1
Seedbed preparation	[€/ha]	22	22	1
Conventional only				
Combiseeding	[€/ha]	91		1
Mineral fertilizer spreading	[€/ha]	13		1
Organic only				
Seeding	[€/ha]		91	1
Fertilizer spreading	[€/ha]		25	2
Biofertilizer spreading	[€/ha]		105	1
Conventional and organic				
Rolling	[€/ha]	26	26	1
Cutting and windrowing	[€/ha]	81	81	1
Pick-up and chopping	[€/ha]	157	157	1
Collecting, transport to plant	[€/ha]	277	277	3
Compaction in silo	[€/ha]	96	96	1
Feed in plant	[€/ha]	75	75	1
Plastic sheet for tube storage	[€/ha]	77	77	
Total	[€/ha]	1501	1569	
Cultivation and harvest	[€/ha]	975	975	
Transport	[€/ha]	353	421	
Storage	[€/ha]	173	173	
Total	[€/ha]	1501	1569	

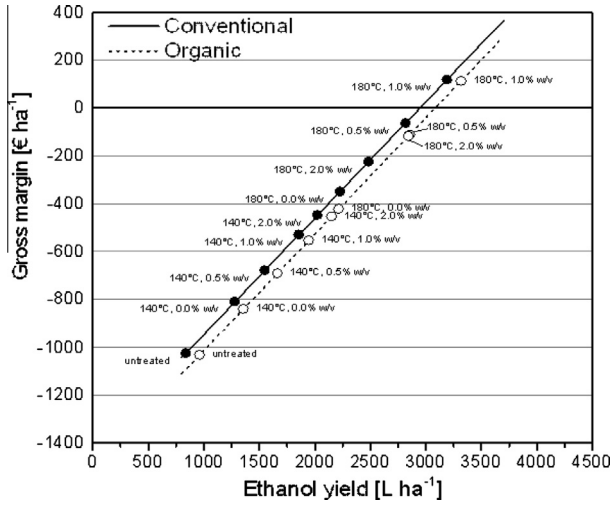

Fig. 3. Gross margin for hemp feedstock production and supply according to yield results of the pretreatment experiments.

power generation, e.g. through combustion in a CHP (combined heat and power) plant (Larsen et al., 2008). Another alternative for further increase of energy output, is using both stillage and hydrolysate after hemp pretreatment for biogas production. Multiple biofuels production "biorefinery concept" from other crops and wastes has been successfully presented in previous studies (Kaparaju et al., 2009; Oleskowicz-Popiel et al., 2012; Rabelo et al., 2011).

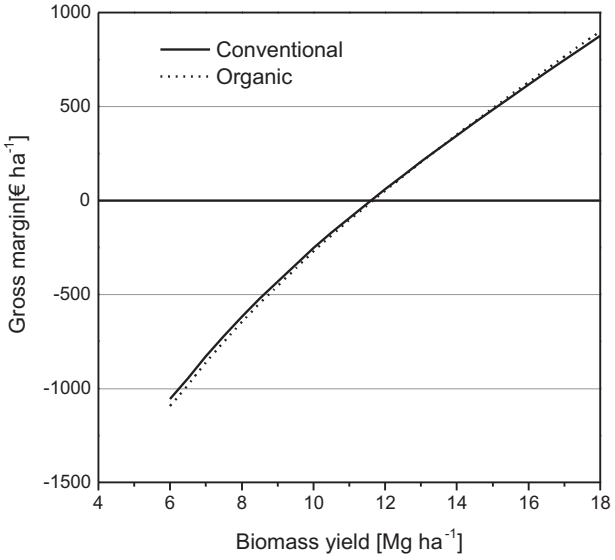

Fig. 4. Effect of hemp dry matter yield on the gross margin of feedstock production and supply.

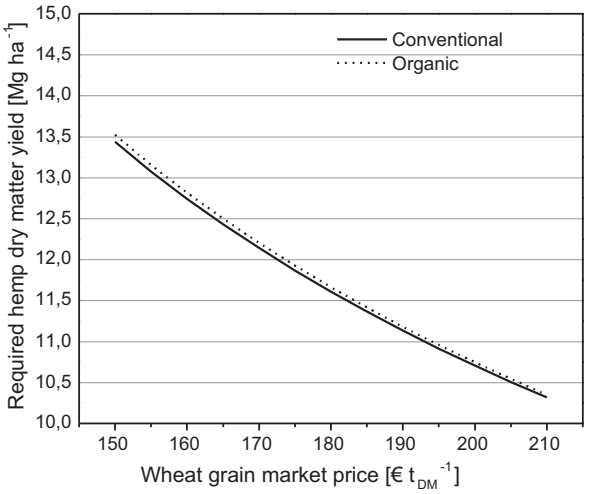

Fig. 5. Sensitivity analysis for the hemp biomass yield required for economic break even as affected by a changing wheat grain market price.

4. Conclusions

The presented results revealed that dry hemp, can be treated as a promising feedstock for lignocellulosic ethanol production. Regardless of the cultivation type (conventional or organic), pretreatment at 180 °C during 10 min and addition of 1% of sulfuric acid resulted in the highest sugar yields, the highest effectiveness of the enzymatic hydrolysis and fermentation as well as indicated positive economic results. The enzymatic hydrolysis of hemp cultivated organically proceeded quicker, which can be beneficial from an economical point of view.

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