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

Mariusz Kuglarz [a], Ingólfur B. Gunnarsson [b], Sven-Erik Svensson [c], Thomas Prade [c], Eva Johansson [d], Irini Angelidaki [b,*]

[a] Faculty of Materials and Environmental Sciences, University of Bielsko-Biala, Willowa 2, 43-309 Bielsko-Biala, Poland
[b] Department of Environmental Engineering, Technical University of Denmark, Building 113, DK-2800 Lyngby, Denmark
[c] Department of Biosystems and Technology, Swedish University of Agricultural Sciences, Box 103, SE-230 53 Alnarp, Sweden
[d] Department of Plant Breeding, Swedish University of Agricultural Sciences, Box 101, SE-230 53 Alnarp, Sweden

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.

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

* Corresponding author. Tel.: +45 4525 1429; fax: +45 4593 2850.
E-mail address: iria@env.dtu.dk (I. Angelidaki).

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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