Refining Hemp Hurds into Fermentable Sugars or Ethanol

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Steam pretreatment is one of the most efficient pretreatment technologies employed prior to enzymatic hydrolysis of lignocellulosics to obtain high polysaccharide conversion. In this study, steam pretreatment of non-impregnated hemp hurds was investigated at two reactor scales (2 and 10 L) by varying the temperature from 200–230 °C. Glucan recoveries were relatively high (> 82 % of original content), while xylan recoveries ranged from 18–66 % of original. Conversions of glucan and xylan in enzymatic hydrolysis varied between X = 62–83 % and 46–96 %, respectively, based on glucan and xylan contents of the substrate. Ethanol yields in simultaneous saccharification and fermentation ranged from Y = 38–70 %, based on glucan and mannan contents of the substrate. The highest overall glucose yield was 336 g kg^{-1} dry hurds obtained at 210 °C, whereas the maximum sugar yield (glucose + xylose), 414 g kg^{-1} dry hurds, was achieved at 200 °C. The highest ethanol yield, 141 g kg^{-1} dry hurds, was obtained at 210 °C.

Key words:
Hemp hurds, steam pretreatment, enzymatic hydrolysis, SSF, ethanol production

Introduction

Ethanol has a rapidly expanding market primarily due to utilisation in vehicular fuel.[1,2] Currently, more than 96 % is produced from renewable resources, referred to as bioethanol.[3] First- and second-generation production technologies can be distinguished according to maturity and feedstocks.[4] For the former, sugar substances and grains serve as feedstocks and these technologies already exist on a large scale, while the latter utilise lignocellulosic materials and involve more complex technologies that have not yet been introduced at an industrial scale. Whereas sugar and starch-containing feedstocks are limited, relatively expensive and supply the food industry as well, lignocellulosic biomass is abundant, available at low cost and largely unused.[5,6]

Cellulose can be broken down into fermentable glucose using an acid catalyst or cellulase enzyme complex.[7] In order to obtain appropriate polysaccharide conversion in enzymatic hydrolysis, pretreatment of the raw material is required. Steam pretreatment has been found to be one of the most efficient pretreatment technologies.[8,9] In this process, chopped biomass is subjected to high-pressure steam in a reactor for a given time, and then the pressure is released instantaneously. Since hemicellulose contains acetyl groups that are cleaved during the reaction forming acetic acid, catalysis occurs, which is referred to as autohydrolysis.[10] Nevertheless, impregnation with other acids (catalyst addition) prior to pretreatment has been proven to have a positive effect on the sugar recovery through steam pretreatment by enabling lower temperatures and shorter residence times.[11] Moreover, impregnation improves enzymatic hydrolysis as well as ethanol fermentation by reducing the formation of inhibitory compounds from sugar degradation.[12,13] Drawbacks of steam pretreatment are the partial degradation of hemicellulose, production of enzymes and fermentation inhibitors, and incomplete separation of lignin and cellulose.[8,14]

Hemp hurds are the woody core of industrial hemp (non-drug varieties of *Cannabis sativa* L.) which is one of the raw materials of the fibre industry, where bast fibres are required. Conventionally, they are removed after dew-retting the hemp stalks in the field[15] or after water-retting. In these processes, the pectinases produced by bacteria present on the material digest the pectin and enable fibre separation. The residue, containing less cellulose but more hemicellulose than the fibre fraction, is referred to as hemp hurds and has had only minor applications so far, such as animal bedding due to its high water-absorbing ability, garden mulch or a component of light-weight concrete. Hemp hurds constitute 70 % of the stalk dry matter.[16]

Industrial hemp is an attractive crop, because it can reach high biomass yield, possesses low sus-

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ceptibility to pests and diseases, and suppresses competing weeds.[17] In the 1960s, Hungary produced huge amounts of industrial hemp, which has since dropped significantly; nevertheless the conditions (climate, soil etc.) are still appropriate for cultivation of large quantities. Since hemp hurds are an agro-industrial by-product with a high carbohydrate content, they are a potential candidate for second-generation fuel-ethanol production. Vignon *et al.*[19] investigated impregnated hemp woody core in a steam explosion treatment to optimise fibre separation and delignification, primarily by means of optical and scanning electron microscopy and compositional analysis. Moxley *et al.*[17] performed cellulose-solvent-based lignocellulose fractionation of hemp hurds and subsequent enzymatic hydrolysis of the residual cellulose fraction.

In this study, the hemp hurds upstream-processing lines, consisting of steam pretreatment and subsequent enzymatic hydrolysis or simultaneous saccharification and fermentation, were investigated to produce fermentable sugars or ethanol, respectively. The lines differed in pretreatment conditions. The efficiency of pretreatment was assessed in terms of sugar recoveries, polysaccharide conversions in enzymatic hydrolysis using commercial cellulases, as well as ethanol yields obtained in simultaneous saccharification and fermentation (SSF) employing ordinary baker's yeast.

Materials and methods

Raw material and chemicals

Hemp hurds were kindly provided by Hungarohemp (Nagylak, Hungary). The 1.5–2.5 m long hemp stalks were retted, dried, and then milled by means of finned rolls. The fibres remained more or less intact, whereas the hemp hurds were broken into 1–2 cm long pieces with a width of 1–5 mm. Hemp hurds were used for steam pretreatment at that size. All chemicals were obtained from Merck (Darmstadt, Germany), otherwise it is given below.

Steam pretreatment

Five pretreatments were performed for 10 min without impregnation at 220 and 230 °C in a 2 L reactor as well as at 200, 210 and 220 °C in a 10 L reactor. Steam pretreatment units have been described elsewhere.[20] Dry matter loading was 200 g in the 2 L reactor and 500 g in the 10 L reactor. The resulting slurry was weighed and separated into liquid and solid fractions. The solids were washed and analysed for carbohydrate and lignin contents. Monomeric sugars and degradation products in the liquid fraction were measured by high-performance liquid chromatography (HPLC). Post-hydrolysis of oligomeric sugars in the liquid fraction was also performed.

Estimation of furan derivatives and acids in flash vapour

During expansion to ambient pressure, the volatile compounds partially vapourise. Since the steam pretreatment unit is not equipped with a flash vapour condenser, these compounds were measured only in the slurry. A flash calculation was performed with Aspen Plus (Aspen Tech Inc. Cambridge, MA, USA) using NRTL-2 method to estimate the total amount of furfural, hydroxymethylfurfural (HMF), and acetic and formic acids formed in the reaction. Before expansion, the vapour fraction in the reactor was set to zero, i.e., all steam injected was assumed to condense completely.

Compositional analysis

Oven-dried samples of raw hurds were ground in a laboratory hammer mill (type: SM 2000, Retsch, Haan, Germany) and separated with a sieve set (type: RP 20, CISA, Barcelona, Spain) into two fractions in accordance with the procedure of National Renewable Energy Laboratory (NREL, Golden, CO).[21] Samples of oven-dried pretreated solid fractions were milled and sieved to a mesh size of 1 mm. Non-volatile water and ethanol extractives of the raw hurds were determined by means of an accelerated solvent extraction (ASE) according to the NREL protocol.[22] ASE was performed on the 0.18–0.85 mm fraction. Non-volatile extractives remaining in the flask after evaporation were measured by weight after drying at 105 °C. The carbohydrate and lignin contents were determined according to the NREL standardised method.[23] Extracted material as well as pretreated solid fractions were treated in a two-step acid hydrolysis with 72 % H_2SO_4 for 1 h at 30 °C and then with 4 % H_2SO_4 for 1 h at 121 °C. Sugar contents of the supernatants were analysed with HPLC. The dried residue consisted of acid-insoluble lignin and acid-insoluble ash. Total ash, structural inorganics and acid-insoluble ash contents were measured by ashing the sample at 575 °C for at least 4 h until the sample weight was constant.[24] Total ash refers to the inorganic part of the raw material or solid fraction after pretreatment, whereas structural inorganics are measured as ash of the extracted material. To determine the total sugar content in the liquid fraction of the pretreated material, samples of 25 mL were mixed with 0.72 mL of 98 % H_2SO_4, and autoclaved for 30 min at 121 °C.[25] Sugar contents were determined by HPLC.

Enzymatic hydrolysis

Enzymatic hydrolysis (EH) of the washed solid fraction was carried out in shake flasks in a reaction volume of 25 mL at a water-insoluble solid (WIS) content of 5 % at 50 °C using commercial enzyme preparations (NS50013 – cellulase complex, NS50010 – beta-glucosidase preparation, both from Novozymes A/S, Bagsværd, Denmark). Enzyme loading of NS50013 was 15 FPU (filter paper unit) g^{-1} WIS and the volumetric ratio (ψ) of NS50010 to NS50013 was 0.1. The rotary shaker (Certomat-R, B-Braun, Germany) was adjusted to 150 rpm. Sodium citrate buffer ($c = 0.05$ mol dm^{-3}) was used to maintain pH at 5.0. Triplicates were incubated for 72 h. Released sugars were analysed by HPLC.

Simultaneous saccharification and fermentation

Non-sterile simultaneous saccharification and fermentation of washed fibres was performed in shake flasks in a volume of 50 mL at a WIS content of $w = 10$ % by applying commercial enzyme preparations (NS50013, NS50010) and *Saccharomyces cerevisiae* (DER-CIEMAT Culture Collection No. 1701) at 32 °C. Enzyme loading of NS 50013 was 25 FPU g^{-1} glucan. Volumetric ratio of NS50010 to NS50013 and agitation speed were the same as for the EH experiments. Yeast extract, NH_4Cl, KH_2PO_4 (Sigma-Aldrich, Munich, Germany) and $MgSO_4 \cdot 7H_2O$ were added in final concentrations of $\gamma = 5$, 2, 1 and 0.3 g L^{-1}, respectively. Flasks were inoculated with a 4 % (v/w) *S. cerevisiae* culture grown for 16 h at 32 °C at 150 rpm in the same growth medium supplemented with glucose ($\gamma = 30$ g L^{-1}). Sodium citrate buffer ($c = 0.05$ mol dm^{-3}) was used to adjust the initial pH to 5.0. Triplicates were incubated for 72 h. Ethanol was measured by gas chromatography.

Chromatography

Sugar contents (glucose, xylose, arabinose, mannose, and galactose) were determined by a Waters liquid chromatograph with a refractive index detector using an Aminex HPX-87P column (Bio-Rad, Hercules, CA) operating at 85 °C. Ultrapure water was used as eluent at a flow rate of $Q = 0.6$ mL min^{-1}. Furfural and HMF contents were analysed by a Hewlett Packard (HP) liquid chromatograph, equipped with an UV-diode-array detector, and the separation was performed with an Aminex HPX-87H column at 55 °C with an eluent of $c = 5$ mmol dm^{-3} H_2SO_4 (89 %) and acetonitrile (11 %) at a flow rate of $Q = 0.7$ mL min^{-1}. Quantification of acetic and formic acids was carried out with a Waters liquid chromatograph with a refractive index detector using an Aminex HPX-87H column at 65 °C. The mobile phase was $c = 5$ mmol dm^{-3} H_2SO_4 at a flow rate of $Q = 0.6$ mL min^{-1}. Ethanol content was measured by gas chromatography, using an HP apparatus equipped with a flame ionisation detector and a Carbowax column operating at 85 °C. The 20M injector and detector temperatures were maintained at 150 °C.

Statistical analysis

Statistical evaluation was carried out using Statistica 8.0 software (Statsoft Inc., Tulsa, OK). Independent *t*-tests of glucan and xylan conversions in EH and yields in SSF experiments were performed for some pairs of conditions to ascertain whether they differed significantly, where the significance level was ≤ 0.05. The two-tailed *t*-test probability is denoted here by *p*.

Results and discussion

Compositions

Results of the compositional analysis are presented in Table 1. The most abundant polysaccharides in the raw hurds were glucan and xylan, together comprising $w = 59$ % of the dry matter. The hemp-hurd composition reported by Moxley *et al.*[17] is in line with that obtained here, except that their raw material consisted of 4.7 % arabinan. The composition of hemp hurds was compared to that of other lignocellulosic raw materials such as wheat straw,[26,27] corn stover,[28,29] softwoods (spruce,[30] pine[26]) and hardwoods (salix,[11] poplar,[31] aspen, birch[26]). The glucan content of hemp hurds was slightly higher than that of wheat and barley straw, and it was in the range of corn stover, softwoods and hardwoods. Regarding the xylan, mannan and lignin contents, major differences were obtained between softwoods and hemp hurds. The hemicellulose of softwoods consisted of more mannan, but less xylan, and the lignin content of softwoods was also higher. Galactan and arabinan are minor constituents of each raw material, and none of their contents varied significantly among the materials surveyed.

The glucan contents of the pretreated solid fraction varied between 57–63 %. The increase in glucan and lignin contents, compared to the original material, was due to solubilisation of the hemicellulose fraction through pretreatment. Indeed, mannan and galactan present in the raw hurds disappeared entirely from the solid fraction, except for the case of the 200 °C treatment; a significant drop in xylan content was observed as well. Vignon *et al.*[19] reported the composition of two kinds of

Table 1 – *Composition of raw hemp hurds and pretreated separated, washed solid fractions expressed as % of dry matter. Standard deviations were calculated from triplicates.*

	Raw hemp hurds	2 L		10 L		
		220 °C	230 °C	200 °C	210 °C	220 °C
glucan	40.1 ± 1.1	62.8 ± 0.5	60.3 ± 1.8	57.4 ± 0.8	57.5 ± 1.1	58.6 ± 3.0
mannan	0.9 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
galactan	0.3 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
xylan	18.4 ± 0.3	2.6 ± 0.2	2.1 ± 0.1	6.9 ± 0.9	2.9 ± 0.0	3.1 ± 0.1
arabinan	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
acid-insoluble lignin	21.7 ± 0.2	36.2 ± 0.3	38.8 ± 0.2	34.7 ± 1.1	37.9 ± 0.1	40.9 ± 1.4
water extractives	3.3 ± 0.2	– –	– –	– –	– –	– –
ethanol extractives	1.5 ± 0.4	– –	– –	– –	– –	– –
total ash	2.3 ± 0.0	1.2 ± 0.1	1.5 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	1.3 ± 0.0
structural inorganics	1.2 ± 0.0	– –	– –	– –	– –	– –
acid-insoluble ash	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

steam-pretreated hemp hurds (220 and 240 °C, 3 min, 0.1 % H_2SO_4). A major difference was found in the glucan contents (48 and 53 %, respectively) compared to those in Table 1, however, the authors suggested that their glucan contents might be underestimated.

Steam pretreatment

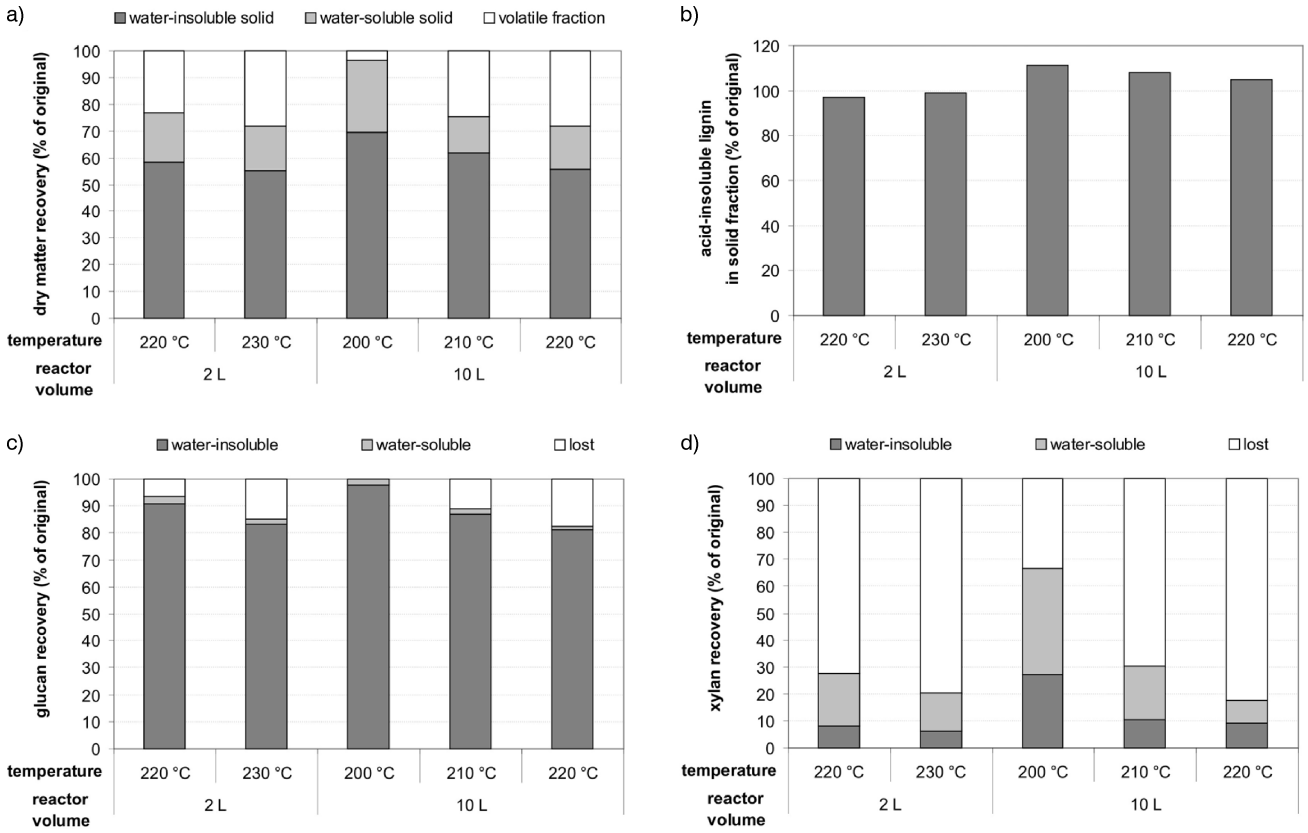
In steam pretreatment, as the steam heats the material, it simultaneously condenses. Depending on the heat flux between the environment and the reactor, i.e., on the heat losses to ambient, further condensation can occur. Increased steam condensation results in a more diluted slurry. Table 2 shows that higher solid contents were obtained in the 10 L reactor than in the smaller one, which could be due to different equipment designs, and concomitant differences in heat loss. As acid concentrations in the liquid fraction obtained in the large reactor were only twice that in the small one, the pH values did not differ significantly.

Fig. 1 shows the dry matter and component recoveries after pretreatment as % of original. Similar trends were observed for galactan and mannan as well (data not shown). The recovered portion decreased when the pretreatment severity was enhanced, i.e., by elevating the temperature. Dry matter loss can be referred to as the volatile fraction, since this represents the dry mass difference between the initial material and the non-volatile fraction (slurry).

At least 82 % of the initial glucan was recovered in the slurry, mainly in the water-insoluble fraction. Lost glucan was the portion hydrolysed to glucose, however, later it was further degraded to HMF. The latter compound can decompose to levulinic acid and formic acid, or condense with the lignin.[10] Recoveries of galactan and mannan fluctuated between 17–92 % and 17–98 % of original, respectively. These compounds were recovered almost exclusively in the liquid phase; however, at 200 °C a remarkable amount of mannan (54 %

Table 2 – *Solid contents of the pretreated slurry and acidity, and acid concentrations in liquid fractions. Standard deviations were calculated from duplicates.*

	2 L		10 L		
	220 °C	230 °C	200 °C	210 °C	220 °C
total solids, *w*/%	5.9 ± 0.1	5.5 ± 0.1	21.2 ± 0.8	13.0 ± 0.5	13.3 ± 0.1
water-insoluble solids, *w*/%	4.4 ± 0.1	4.2 ± 0.1	15.3 ± 0.4	10.7 ± 0.3	10.3 ± 0.1
pH	3.6	3.6	3.7	3.7	3.6
acetic acid, γ/g L^{-1}	2.6	2.4	4.7	5.2	5.5
formic acid, γ/g L^{-1}	0.8	0.8	1.9	1.8	1.8



F i g . 1 – *(a) Dry matter, (b) acid-insoluble lignin, (c) glucan and (d) xylan recoveries after pretreatment as % of original*

of original) remained in the fibre fraction. Losses of galactan and mannan were similar to xylan at a given pretreatment condition, except at 200 °C, where they were significantly lower. Galactose and mannose can also be degraded to HMF.

Xylan recovery varied between 18–66 % of original. The conditions investigated were more severe than the optimum for xylan, hence xylan recovery decreased drastically by increasing the temperature. The water-soluble xylan made up a significant share of the recovered part. These poor recoveries might be due to the rather long residence time in addition to the moisture content of the raw hurds fed to the reactor, which was low as well (around 10 %). These conditions have been reported to have unfavourable effects on xylan recoveries.[11] Released pentoses can degrade into furfural that can then decompose to formic acid or condense with the lignin.[10] Since no arabinan was detected in the raw hemp hurds, the furfural present must have originated from xylan. Concerning the stoichiometric reaction of xylose into furfural and the estimated total amount of furfural, see Table 3, the % of xylan that were degraded to furfural were calculated as follows: 26 and 25 % for the 2 L reactor at 220 and 230 °C, respectively, and 6, 14, and 14 % for the 10 L reactor at 200, 210, and 220 °C, respectively. By subtracting these values from the xylan losses, it can be concluded that a significant amount of the original xylan was lost to the formation of formic acid or pseudolignin detailed below.

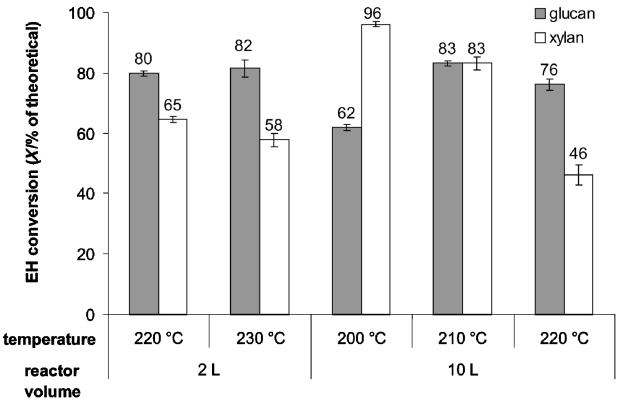
The aim of using two different reactors was to investigate whether the same temperature and residence time resulted in the same severity. It was observed that, at the same temperature (220 °C), larger losses were obtained in case of the 10 L reactor. Dry matter, glucan, or xylan losses obtained in the large reactor at 210 and 220 °C were similar to those obtained in the small one at 220 and 230 °C, respectively. These indicate that a given treatment temperature resulted in more severe pretreatment in the 10 L reactor. This might be due to differences in the heating and cooling profiles of the two reactors. The 2 L reactor takes ~1 min to reach the pretreatment temperature, whereas the larger one requires ~3 min. Opening the slurry receiver also takes more time with the larger one, due to differing equipment designs. Since the pH values were nearly the same in both reactors at each temperature, the severity was therefore not much affected by the acidity.

Acid-soluble lignin recoveries were measured from the solid fraction as the residue after a two-step acid hydrolysis. All the lignin recoveries were around 100 %, i.e., the extent of lignin solubilisation was low; additionally, in the 10 L reactor, the values were higher than 100 %. This ap-

parent contradiction can be resolved by taking into account the fact that during pretreatment the solubilised lignin partially undergoes repolymerisation, and reactions between lignin and sugars or sugar degradation products also occur, forming pseudolignin, which contributes to the amount of acid-insoluble lignin.[32]

Enzymatic hydrolysis

Fig. 2 shows the conversions of glucan and xylan in enzymatic hydrolysis. Glucan conversion after pretreatment in the 10 L reactor at 210 °C was 83 % of theoretical, which was significantly higher than that in case of the 200 °C ($p = 10^{-5}$) and 220 °C ($p = 0.0038$) pretreatments. In the 2 L reactor, there was no significant difference between the two temperatures ($p = 0.3876$).

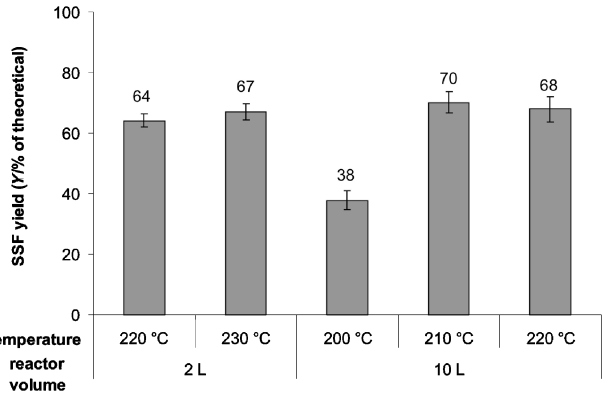


F i g . 2 – *Conversions of glucan and xylan in enzymatic hydrolysis (EH) as % of theoretical*

The xylan content of the pretreated solid fractions was low (see Table 1), therefore the accuracy of xylan conversion was low as well. Considering the two reactor sizes separately, it can be seen that xylan conversion decreased monotonically with the elevation of temperature. In other studies, the same trend has been observed, i.e., xylan conversions decreased when pretreatment severity was enhanced.[11,33] Xyloglucan, one of the major hemicellulosic polysaccharides, can be present in three macromolecular domains in lignocellulosics.[34] These domains differ in resistance and accessibility. More severe pretreatment solubilises the less recalcitrant xyloglucan to a greater extent; hence the proportion of resistant, less-accessible regions increases in the pretreated solid fraction, which might interpret the declining xylan conversions in EH. Pretreating hemp hurds in the small reactor at 220 °C resulted in significantly higher glucan and xylan conversion than in the large one at the same temperature ($p = 0.0343$ and 0.0008, respectively).

Simultaneous saccharification and fermentation

Fig. 3 shows the SSF yields based on glucan and mannan contents of the substrate. The highest ethanol yield from washed fibres, 70 % of theoretical, was obtained from the pretreatment at 210 °C. This was significantly higher than that at 200 °C ($p = 0.0003$); nevertheless, the difference was not significant compared to that at 220 °C ($p = 0.4925$). No significant differences were obtained either in the 2 L reactor between the two investigated temperatures ($p = 0.2081$) or between the two reactor sizes at 220 °C ($p = 0.2360$).



F i g . 3 – *Ethanol yields in simultaneous saccharification and fermentation (SSF) expressed as % of theoretical, based on glucan and mannan contents of the substrate*

Overall product yields

Table 3 shows the product masses obtained from 100 g of dry hemp hurds. After pretreatment, solubilised sugars were present mainly in their oligomeric forms. The results of the Aspen Plus flash calculations for furan derivatives and acids showed that, for a given volatile compound, elevating the pretreatment temperature resulted in a decreased mass recovery (amount in slurry/estimated total amount produced). At a given condition mass recoveries increased in the following order: furfural < formic acid < acetic acid < HMF, the last remaining entirely in the liquid phase. Generally, in the 2 L reactor, more furfural and HMF were obtained in the slurry than in the 10 L reactor, hence the total amounts of these compounds were also higher in the small reactor. At 220 °C, less sugar was lost in the 2 L reactor compared to the 10 L one; however, more furfural and HMF were obtained. Consequently, more furan derivatives were further degraded or formed pseudolignin at 220 °C in the 10 L reactor.

Overall sugar yields were calculated from Table 3 values by the following equation:

Table 3 – *Product masses expressed in g obtained from 100 g hemp hurds dry matter. Oligo-sugars are given as monomer equivalents.*

hemp hurds dry matter				100.0	
glucose potential				44.6	
xylose potential				20.9	
	2 L		10 L		
	220 °C	230 °C	200 °C	210 °C	220 °C
Steam pretreatment					
oligo-glucose	0.9	0.9	0.9	0.7	0.4
glucose	0.2	0.1	0.0	0.1	0.1
oligo-xylose	3.3	2.8	7.4	3.3	1.5
xylose	0.7	0.3	0.5	0.7	0.2
furfural in slurry	0.9	0.8	0.2	0.5	0.5
furfural in flash vapour[a]	2.6	2.6	0.6	1.3	1.4
HMF[b]	0.2	0.2	0.0	0.1	0.1
HMF[b] in flash vapour[a]	t.a.[c]	t.a.[c]	t.a.[c]	t.a.[c]	t.a.[c]
acetic acid	3.3	3.0	1.8	2.7	2.6
acetic acid in flash vapour[a]	0.7	0.8	0.3	0.5	0.6
formic acid	1.0	1.0	0.7	0.9	0.9
formic acid in flash vapour[a]	0.4	0.4	0.2	0.3	0.3
Enzymatic hydrolysis					
glucose	32.3	31.7	27.3	32.8	27.5
xylose	1.1	0.8	5.2	1.7	0.9
SSF[d]					
ethanol	13.3	12.7	8.5	14.1	12.5

[a]estimated with Aspen Plus
[b]hydroxymethylfurfural
[c]trace amounts
[d]simultaneous saccharification and fermentation

$$\text{yield}_{\text{sugar}} = \frac{\text{mass}_{\text{sugar}}\text{ in liquid fraction} + \text{mass}_{\text{sugar}}\text{ obtained in EH}}{\text{mass}_{\text{sugar}}\text{ in raw material}}$$

where sugar denotes either glucose or xylose. The oligomeric sugars are also included in the liquid fraction term, expressed as their monomer equivalents. Overall glucose yields varied between 63–75 % of theoretical and they showed the same pattern as the EH glucan conversions, since the amounts of monomeric and oligomeric glucose in the liquid fraction after pretreatment were rather small. Overall xylose yields ranged between 13 and 63 % of theoretical and decreased monotonically with higher temperatures in both reactors. Beside the xylose released in EH, however, xylose solubilised during pretreatment made up a significant share.

In terms of overall glucose yields, the best pretreatment condition was that of 210 °C, where 336 g glucose was obtained from 1 kg of dry raw material, while with regard to total sugar yield, i.e., glucose + xylose overall yields, it was shifted to 200 °C. At this temperature, 414 g sugar was recovered from 1 kg of dry raw material. In regard to the highest overall ethanol yield, with the pretreatment at 210 °C, 141 g ethanol could be produced from 1 kg of dry raw material.

Ordinary baker's yeast, the robust full-scale ethanol fermenting microorganism, is unable to convert pentoses to ethanol, hence in a process applying this organism, these sugars can form co-products such as solid fuel or are burnt on-site.[35] Furthermore, they can also be utilized as carbon source of on-site cellulase enzyme fermentation.[36] A lot of effort has been made to develop pentose-fermenting yeast strains, however the

co-fermentation of hexoses and pentoses has not been solved on an industrial scale yet.[37]

If a pentose-fermenting yeast were used, assuming the same ethanol yield from xylose as from glucose, an addition of 0.8 g ethanol (per 1 kg of dry raw material) could be produced from the xylan of the solid fraction obtained at 210 °C. Raw hemp hurds contain a remarkable amount of xylan; however, its recovery through steam pretreatment was poor at this temperature. If the ultimate aim is sugar or ethanol production, xylan recovery must be improved. A two-step steam pretreatment is a possible alternative for preventing the less-recalcitrant hemicellulose fraction from further degradation, thus improving its recovery.[30] Nevertheless, the additional pretreatment step increases both the capital investment and operational costs. Sassner *et al.*[11] reported that impregnating the raw material with acid before steam pretreatment resulted in closer-to-optimum conditions for glucan and xylan recoveries. As demonstrated in this study, the optimum temperatures for glucan and xylan recoveries differed greatly, therefore this alternative should be considered as well.

Conclusions

Hemp hurds, being an agro-industrial by-product with high carbohydrate content, are a potential candidate for second-generation fuel-ethanol production. Steam pretreatment of non-impregnated hemp hurds was investigated for sugar and ethanol production by enzymatic hydrolysis and SSF, respectively. Dry matter and sugar recoveries throughout the pretreatments decreased monotonically with increasing temperature. Regarding enzymatic digestibility of the fibre fraction and ethanol production, no significant difference was observed between the two investigated temperatures in the 2 L reactor. In the 10 L reactor, the most favourable condition was at 210 °C in terms of overall glucose and ethanol yield; however, the maximum sugar yield was obtained at 200 °C. These yields were in the same size order as the highest yields reported in other studies,[11,27,30,33] where a similar experimental procedure (steam pretreatment, EH and SSF) was applied on various lignocellulosic raw materials. To improve xylan recovery, further investigation of impregnation and two-step steam pretreatment is required.

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List of symbols

c – concentration, mol dm^{-3}
p – corresponding level of significance
Q – volume flow rate, mL min^{-1}
w – mass fraction, %
X – conversion, %
Y – yield, %, g kg^{-1}
γ – mass concentration, g L^{-1}
ψ – volume ratio

Abbreviations

ASE – accelerated solvent extraction
DM – dry matter
EH – enzymatic hydrolysis
FPU – filter paper unit
HMF – hydroxymethylfurfural
HP – Hewlett Packard
HPLC – high-performance liquid chromatography
NREL – National Renewable Energy Laboratory
SSF – simultaneous saccharification and fermentation
WIS – water-insoluble solids

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