Corncoobs as Substrate for Oleaginous Yeast–Pretreatment via Steam Explosion and Hydrolysis

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Abstract: Corn cobs are a promising lignocellulosic substrate for the production of biofuels like bioethanol via conventional yeast or biodiesel via oleaginous yeast. Pretreatment of the substrate is essential for further hydrolysis and fermentation steps. This study focused on the steam explosion method as pretreatment. Therefore, different steam explosion severities were evaluated. The content of glucan, xylan and Klason lignin was examined. Xylan degraded with increasing severity from 412.7 g·kg⁻¹ (untreated) to a minimum of 127.3 g·kg⁻¹ dry matter (190 °C/30 min). Glucan concentrations increased from 315.1 g·kg⁻¹ (untreated) to a maximum of 371.6 g·kg⁻¹ dry matter (200 °C/20 min). For soluble lignin, an increase could be observed at rising severity, from 145.3 g·kg⁻¹ (untreated) to a maximum of 214.9 g·kg⁻¹ dry matter (190 °C/30 min). Furthermore, the mass recovery was calculated. At harsher pretreatment conditions, a significant mass loss was observed, estimated by the ash content in the recovered dry matter. The lowest recovery rate was observed for SF = 4.13 (190 °C/30 min) with 68.39%. The produced inhibitors were evaluated.

Key words: Corn cobs, steam explosion, lignocellulose, pretreatment.

1. Introduction

The increasing interest in replacing fossil fuels by fuels from renewable sources has led to a considerable amount of research in bio-source technology. Advanced biofuels from sustainable sources, residual and waste materials, fulfil the EU sustainability standards. Lignocellulosic residues (grasses, straw and corn cobs) have great potential as an energy source and raw material supplier. Corn cobs are an agricultural by-product and as a renewable raw material, they pose a potential feedstock for various sustainable industrial biofuel applications (bioethanol, biodiesel and biogas) [1-3]. Beside fertilisation or combustion, corncoobs have no specific usage [4]. Corn cobs can be harvested easily and are produced worldwide in large quantities [5, 6]. In 2012, a total of 2,351,370 t of grain maize was harvested in Austria [7].

Corn cobs are a lignocellulosic material composed of cellulose, hemicellulose and lignin. The polymeric

fibres of cellulose and hemicellulose consist of monomeric molecules. Cellulose is a linear polymer of glucose subunits. Hemicellulose is a branched heteropolymer built up by different monomeric saccharides, mainly by xylose, arabinose, glucose and mannose. In most plants, the predominant hemicellulose is a polymer of xylose, linked by β -1-4 glycosidic bonds [8]. These polymers are completed by short side chains of organic acids and sugars. Lignin consists of phenolic macromolecules. Cellulose, hemicellulose and lignin are embedded in a complex matrix which is very resistant to enzymatic degradation [1, 9].

Lignocellulosic substrates have to be pretreated to achieve the sugars glucose and xylose. Different pretreatment options are available, for example, the established method of steam explosion (SE) [10]. Steam explosion developed in 1928 by Mason W. H. [11] is known as one of the most efficient pretreatment methods for lignocellulosic materials [12]. The lignocellulosic structure is destroyed by the treatment with high temperature and steam in a reactor followed by a sudden pressure decrease [1].

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Hemicellulose can be hydrolysed and degraded by the treatment. The availability of the cellulose for the enzymatic saccharification is enhanced [9]. With a relative distribution of 30%-50% cellulose and 15%-25% hemicellulose, ligneous plants consist of approximately two thirds of potential fermentable sugars [1, 9]. The content of hemicellulose in corn cobs is relatively high. The fibre composition of corn cobs was described in different studies. This substrate consists of 31%-35% cellulose, 30%-35% hemicellulose and 10%-14 % lignin [13-15].

Aim of this study was to determine the relative distribution of cellulose (given as glucan), hemicellulose (described as xylan—the main hemicellulosic polymer) and lignin (as Klason lignin) in corn cobs after SE treatment. Beside the desired improved enzyme accessibility and subsequently the increased sugar yield, this process also causes a significant mass loss. This was observed in numerous studies on various substrates [16-19]. Another effect is the liberation of toxic degradation products during pretreatment and their accumulation in the hydrolysates. Some of these degradation products (acetic acid, formic acid, furfural and 5-hydroxymethylfurfural (HMF)) are reported to inhibit further steps of enzymatic hydrolysis and fermentation process [20, 21]. The aim of the pretreatment is to maintain high levels of fermentable sugars (mainly from cellulose), a high degree of degradation of hemicellulose, high mass recovery rates and low levels of potential inhibitory substances. Optimal steam explosion pretreatment conditions for the substrate corncobs were evaluated.

2. Material and Methods

The corncobs (*zea mays*) investigated for this research was obtained from the Austrian Agency for Health and Food Safety (AGES) in Upper Austria (mixture of different varieties, harvested 2012 in Upper Austria). They were partially air dried (dry matter content (DM): 92.8%) at 20 ± 2 °C and

chopped up by a garden shredder (Viking GE 260, Kufstein, Austria) to a particle size of 10 mm.

Pretreatment was performed using a laboratory scale steam explosion unit (VAM GmbH Co KG, Linz, Austria), consisting of a high-voltage steam generator, a 15 L decomposition reactor and a collection and steam expansion vessel. Nine hundred grams (DM = 92.8%) of chopped corncobs and the same mass of water were added to the pressure vessel for each of the conditions. The material was treated at temperatures of 160 °C, 170 °C, 180 °C and 190 °C using 20 min and 30 min residence times and at 200 °C using a 20 min residence time. The SE sample was evacuated immediately after treatment from the collecting vessel and the inner walls were as completely as possible. The wet substrate was weighed and immediately stored in closed plastic boxes and cooled at 4 °C to avoid evaporation.

The intensity of the SE treatment was described as the severity parameter (R_0)—a semiempirical parameter [22]. The severity factor is defined by Eq. (1):

$$\log(R_0) = t^{\frac{T-100}{14.75}} \quad (1)$$

Where, upon t is the duration of the treatment in min and T the temperature in °C.

Analyses of sugars and acid insoluble lignin (Klason lignin) were conducted before and after SE treatment according to NREL/TP-510-42618 [23]. Saccharides, organic acids and additionally HMF and furfural were quantified using high performance liquid chromatography (HPLC). The HPLC system Agilent Technologies, Santa Clara, 1,200 Series with a Varian Metacarb 87 H column (300×7.8 mm) at 65 °C, H_2SO_4 ($c = 5 \text{ m mol}\cdot\text{L}^{-1}$) eluent and an isocratic flow rate of $0.8 \text{ mL}\cdot\text{min}^{-1}$ was used.

DM and ash content were determined by heating the samples at 105 °C and 550 °C overnight. Weight loss of DM and ash compared to untreated substrates was calculated. Crude protein (protein = $6.25 \times$ nitrogen) was determined according to Dumas with a

Dumatherm analyser (Gerhardt GmbH & Co. KG, Königswinter, Germany). Starch content was determined enzymatically with a test kit (Megazyme International Ireland Ltd., Bray, Ireland) according to the α Amylase/Amyloglucosidase method. Fat was determined gravimetrically according to Bligh and Dyer with chloroform and methanol [24]. The potential inhibitory substances—formic acid, acetic acid, HMF and furfural—for further process steps were determined from the wet SE products at 10% DM in water (1 hour incubation at 75 rpm).

3. Results and Discussion

Chopped corncoobs were subjected to 9 different steam explosion conditions. The temperatures ranged from 160 °C to 200 °C with residence times of 20 min and 30 min. The severity of the SE treatment, which combines temperature and time, can be described with the “severity factor”— $\log R_0$. For this study, severities from 3.07 (160 °C, 20 min) to 4.25 (200 °C, 20 min) were tested (Table 1). Higher treatment severities lead to visible changes of structure and colour of the biomass—darker products and smaller particle size. These effects are typical for SE treatment [12].

The untreated substrate was composed of 31.52 ± 0.72 % glucan, 41.27 ± 0.72 % xylan and 14.53 ± 0.05 % Klason lignin in DM. Further compounds were 4.3 ± 1.0 % crude proteins, 2.4 ± 0.2 % ash, 0.7 ± 0.1 % starch and 0.3 ± 0.0 % fat. Table 1 illustrates the

relative shares of glucan, xylan and Klason lignin in the dry matter of the untreated and SE pretreated biomass. The lowest level of the Klason lignin was measured for untreated corncoobs (14.53%) and the maximum was reached at a severity of 4.13 (21.49%). In summary, Klason lignin content in DM increased with the severity of the treatment.

Increasing pretreatment severity led to an accumulation of glucan. The maximum content (37.16%) was observed at the highest severity (4.25) evaluated. The increase of the amount of soluble lignin measured for higher severity indicates the formation of pseudo-lignin from xylan degradation at higher temperatures [25–27]. The xylan is degraded at higher severity to 13.72% at 4.13 in comparison to the xylan value—34.59%—of the corn coobs pretreated at soft condition (160 °C, 20 minutes). Xylan showed a significant dependence on the pretreatment severity, which is in accordance with the literature reviewed [26, 28]. The relative shares of the three main components glucan, xylan and Klason lignin changed due to the loss of xylan at harsh conditions. None detailed paper about the effects on the composition of the corn coobs after SE exists but the composition of steam exploded wheat straw showed the same trend [29].

In Table 2, the mass balances of the products obtained after the SE treatment are shown. The mass loss (compared to untreated substrate) was estimated

Table 1 Fibre composition of untreated and pretreated corn coobs at different severities. compositional data are expressed as percentage of dry matter (absolute standard deviation in brackets).

Sample	Severity factor $\log(R_0)$	Glucan (%)	Xylan (%)	Klason lignin (%)
Untreated	-	31.51 (0.72)	41.27 (0.72)	14.53 (0.05)
160 °C/20 min	3.07	32.97 (0.13)	34.59 (0.57)	15.02 (0.16)
160 °C/30 min	3.24	33.65 (0.43)	31.63 (0.12)	14.57 (0.07)
170 °C/20 min	3.36	33.26 (0.38)	31.11 (0.87)	15.01 (0.08)
170 °C/30 min	3.54	32.77 (0.76)	33.22 (0.07)	17.07 (0.08)
180 °C/20 min	3.66	35.49 (0.96)	23.31 (0.94)	16.28 (0.72)
180 °C/30 min	3.83	32.01 (0.06)	21.47 (0.44)	19.74 (0.23)
190 °C/20 min	3.95	36.88 (0.26)	20.83 (0.08)	16.37 (0.58)
190 °C/30 min	4.13	36.35 (0.00)	12.73 (0.25)	21.49 (0.03)
200 °C/20 min	4.25	37.16 (0.37)	13.72 (0.96)	17.19 (0.13)

Table 2 Mass balance of steam explosion pretreatment of corncobs dry matter and mass loss calculation from ash are referred to untreated corncobs (absolute standard deviation in brackets).

Sample	Severity factor $\log(R_0)$	DM loss in %	Mass loss calc. from ash %	Ash content in DM in %
Untreated	-	-	-	2.26 (0.06)
160 °C/20 min	3.07	5.18 (0.18)	-1.13 (1.75)	2.41 (0.04)
160 °C/30 min	3.24	15.51 (0.13)	5.77 (0.04)	2.52 (0.00)
170 °C/20 min	3.36	16.00 (0.11)	4.31 (0.05)	2.58 (0.11)
170 °C/30 min	3.54	18.56 (0.30)	6.98 (1.32)	2.59 (0.04)
180 °C/20 min	3.66	24.73 (0.05)	11.79 (2.03)	2.65 (0.06)
180 °C/30 min	3.83	24.84 (0.09)	4.84 (1.81)	2.87 (0.05)
190 °C/20 min	3.95	26.59 (0.14)	1.49 (0.63)	3.04 (0.02)
190 °C/30 min	4.13	31.61 (0.07)	8.19 (0.70)	3.04 (0.02)
200 °C/20 min	4.25	36.34 (0.07)	14.12 (0.04)	3.05 (0.00)

by the ash content in the recovered dry matter. The data obtained shows that mass loss increased with the severity of the SE pretreatment. The ash content conformed to other data [30]. The achieved data (mass loss and increase of the ash content) were consistent with the characteristics from steam exploded wheat straw at different pretreatment conditions [31].

In Fig. 1, the recovered masses of glucan, xylan and lignin in $\text{g}\cdot\text{kg}^{-1}$ dry matter input for the different SE conditions are shown (combining mass recovery and relative masses in the DM). Xylan is degraded with increasing pretreatment severity from $412.68 \text{ g}\cdot\text{kg}^{-1}$ DM (untreated) to a minimum of $120.73 \text{ g}\cdot\text{kg}^{-1}$ DM (SF = 4.13). Fig. 1 indicates that the degradation of xylan increases at temperatures above 170 °C (from $311.63 \text{ g}\cdot\text{kg}^{-1}$ DM at SF = 3.36 to $214.69 \text{ g}\cdot\text{kg}^{-1}$ DM at SF = 3.83). Higher severities lead to further degradation of xylan. The total mass of glucan recovered after steam explosion treatment decreased from $315.16 \text{ g}\cdot\text{kg}^{-1}$ DM (untreated) to a minimum of $236.56 \text{ g}\cdot\text{kg}^{-1}$ DM (SF = 4.25). For soluble lignin, a slight increase could be observed at rising treatment severity ($145.25 \text{ g}\cdot\text{kg}^{-1}$ DM (untreated) to a maximum of $214.91 \text{ g}\cdot\text{kg}^{-1}$ DM at SF = 4.13). Similar results were achieved from wheat straw and rice straw after steam explosion [31, 32].

Table 3 illustrates the presence of potentially inhibitory substances for further process steps (formic acid, acetic acid, HMF and furfural) in the

hydrolysates of the SE products (10% DM in water, 1 hour incubation at 75 rpm). The quantities of the inhibitors increased with the severity of the SE treatment and reached their highest levels (formic acid, HMF and furfural) at SF = 4.13. The maximum value for acetic acid was observed at SF = 4.25. Also the inhibitor quantities from other lignocellulose (rice straw, sunflower stalks and poplar wood) increased at harsher steam explosion condition, altogether the inhibitor quantities were lower—a phenomenon depends on the composition of the lignocellulose itself [28, 32, 33].

The increased levels of these substances can be explained by the formation of degradation products during thermal treatment of lignocellulosic plants [34–36]. Low amounts of inhibitors are favourable for hydrolysis and fermentation [20, 21]. At high inhibitor concentration, detoxification measures (drying) must be applied [37].

4. Conclusions

This study has shown that corncobs are a potential feedstock for uncatalysed steam explosion pretreatment and in consequence, a substrate for biofuel production by fermentation (bioethanol via conventional yeast or biodiesel via oleaginous yeast). A harsh pretreatment led to an extensive decomposition of the lignocellulosic fibre matrix and reduced contents of xylan. This ensures an excellent enzyme-to-substrate accessibility

Fig. 1 The recovered masses of glucan, xylan and lignin (in g·kg⁻¹ dry matter input).

Table 3 Contents of potential inhibitory substances at different pretreatment conditions.

Sample	Formic acid in g/L	Acetic acid in g/L	HMF in g/L	Furfural in g/L
Untreated	0.00	0.32	0.00	0.00
160 °C/20 min	0.11	1.39	0.09	0.00
160 °C/30 min	0.07	1.83	0.16	0.00
170 °C/20 min	0.19	2.75	0.21	0.16
170 °C/30 min	0.47	3.44	0.20	0.43
180 °C/20 min	0.36	3.26	0.20	0.53
180 °C/30 min	0.73	4.15	0.35	1.47
190 °C/20 min	0.92	3.84	0.36	1.44
190 °C/30 min	1.04	4.28	0.58	1.98
200 °C/20 min	1.47	4.62	0.31	1.76

and subsequently leads to high glucose yields after enzymatic hydrolysis [38, 39].

However, corncobs were subject to mass loss during SE pretreatment. This effect increased with pre-treatment severities tested and therefore, decreased the recoverable amount of cellulose.

After comprehensive analysis, the most promising pretreatment condition is 190 °C reaction temperature with 20 min retention time (SF = 3.95). This setting causes a favourable intermediate of necessary fibre decomposition, unavoidable mass loss (degradation, evaporation and remaining solids in the SE apparatus) and inhibitor formation respectively.

For a more detailed analysis of an entire biofuel process—a detailed examination of enzymatic hydrolysis, the microbial cell growth, fermentative capacity and an economic and energetic evaluation of every pretreatment condition tested should be done to evaluate the potential of this promising substrate for biofuel applications.

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