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Plasma-Assisted Pretreatment of Wheat Straw for Ethanol Production

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Abstract The potential of wheat straw for ethanol production after pretreatment with O_3 generated in a plasma at atmospheric pressure and room temperature followed by fermentation was investigated. We found that cellulose and hemicellulose remained unaltered after ozonisation and a subsequent washing step, while lignin was degraded up to 95% by O_3. The loss of biomass after washing could be explained by the amount of lignin degraded. The washing water of pretreated samples (0–7 h) was analyzed for potential fermentation inhibitors. Approximately 30 lignin degradation products and a number of simple carboxylic acids and phenolic compounds were found, e.g., vanillic acid, acetic acid, and formic acid. Some components had the highest concentration at the beginning of the ozonisation process (0.5, 1 h), e.g., 4-hydroxybenzladehyde, while the concentration of others increased during the entire pretreatment (0–7 h), e.g., oxalic acid and acetovanillon. Interestingly, washing had no effect on the ethanol production with pretreatment times up to 1 h. Washing improved the glucose availability with pretreatment times of more than 2 h. One hour of ozonisation was found to be optimal for the use of washed and unwashed wheat straw for ethanol production (maximum ethanol yield, 52%). O_3 cost estimations were made for the production of ethanol at standard conditions.

Keywords Pretreatment method · Wheat straw · Lignin degradation products · Fermentation inhibitors · Enzymatic hydrolysis · Simultaneous saccharification and fermentation

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Introduction

It will be necessary to find alternative energy resources to replace mineral oil and other fossil fuels. As a substitute, lignocellulosic biomass will be important since it is renewable, does not increase the CO_2 emission, and is not competing with natural resources for food and feed production. In addition, more than 40 different chemicals, pharmaceuticals, biological active compounds, and fuels can be produced from lignocellulosic biomass as shown in different biorefinery concepts [1, 2]. Pretreatment of lignocellulosic biomass is necessary to disrupt the lignocellulosic complex to render cellulose accessible to enzymatic hydrolysis and yeast before the production of biofuels and chemicals possible. Wet oxidation, hydrothermal pretreatment, and steam explosion are commonly used methods, but they are expensive and will need improvement to allow economic production of biofuels and chemicals [1, 3]. For a pretreatment method to be cost efficient, it must be simple and the product must be highly fermentable with limited non-digestible residues. The cellulosic and hemicellulosic sugars should not be destroyed; it should work on biomass without significant mechanical cutting and in an inexpensive reactor of a reasonable size at high content of dry matter (DM) [3].

Pretreatment using plasma has recently been described [4]. O_3 generated in a plasma at atmospheric pressure and room temperature, supplied with dried air (or oxygen enriched air), is used for the pretreatment of lignocellulosic materials. Lignin was degraded specifically by O_3, and no structural effects on cellulose and hemicelluloses were observed. The procedure satisfies some of the requirements for a cost-efficient pretreatment method, e.g., it is simple, effective at high concentrations of DM, pentoses are only degraded to a limited extent, and it has a potential for up-scaling.

An important issue is the formation of compounds during pretreatment, which may inhibit the growth of the microorganisms used to produce ethanol. Fermentation inhibitors can generally be divided into four groups: products derived from (a) sugars [furfural, 5-hydroxymethylfurfural (5-HMF)], (b) lignin (aromatic, polyaromatic, phenolic, and aldehydic compounds), (c) the lignocellulose structure (e.g., acetic acid), and (d) heavy metals from the reaction vessel [5–8]. Ozonisation processes, other than plasma-assisted pretreatment, produce compounds such as citric acid, succinic acid, and oxalic acid [9, 10].

We here report an optimization of plasma-assisted pretreatment of wheat straw for ethanol production. The report focuses on reproducibility, biomass balances, fermentation inhibitors generated, and their influence on enzymatic hydrolysis and simultaneous saccharification and fermentation (SSF).

Materials and Methods

Experimental Setup The core of the reactor was a stainless steel sieve with a mesh size of 0.2 mm carrying the wheat straw. The sieve has a diameter of 30 cm corresponding to an area of 706 cm^2. O_3 containing gas was forced through the sieve from the bottom. The exhaust gas was collected from the top for analysis.

A low-temperature atmospheric pressure dielectric barrier discharge (DBD) with coaxial electrode geometry, fed with oxygen enriched air (40% O_2, 60% N_2) at a flow of 12 standard liter per minute (slm), was used for the generation of O_3. The dielectric barrier consisted of an Al_2O_3 cylinder located between the electrodes. The DBD was driven by an alternating current (AC) power supply (adjustable between 10 and 40 kHz). The frequency was set to 18.4 kHz. The power could be applied in pulses. Each pulse consisted of three

full sinusoidal periods (163 μs), followed by a pause of adjustable duration. The average power supplied to the DBD was adjusted by varying the pause between two pulses, such that the maximum O_3 concentration was obtained.

The O_3 concentration in the intake and exhaust was detected by means of ultraviolet absorption spectroscopy. A mercury lamp served as light source and a UV-photo diode in combination with an interference filter served as detector. The transmission maximum of the filter is at 254 nm where O_3 has an absorption maximum. The O_3 detector has previously been described [11]. Depending on the pretreatment progress, the O_3 concentration in the exhaust gas varied. The difference between the supplied O_3 and the O_3 in the exhaust is a measure of the O_3 consumption of the straw. A more detailed description of the process has previously been described [4].

Raw Material Wheat straw (*Triticum aestivum* L.) was grown in Denmark and stored at ambient temperature. Before humidity adjustment and pretreatment were performed, the wheat straw was milled to a final particle size of 1 mm using a technical mill (MF 10 basic, IKA-Werke GmbH & Co. KG, Germany).

Adjustment of the DM Content of the Biomass DM contents of wheat straw, cellulose, lignin, and xylan were measured with a moisture analyzer HR83 (Mettler Toledo A/S). Desired DM contents were obtained by adding water adjusted for the moisture of the wheat straw. As an example, a 50% DM sample consists of 50 g dry wheat straw and 50 g water (intrinsic moisture+added water).

Plasma-Assisted Pretreatment of Wheat Straw (Ozonisation) The ozonisation of wheat straw was performed in our in-house-build reactor under atmospheric pressure and room temperature. The wheat straw was placed into the sieve of the reactor (Fig. 1). The filling capacity of one sieve of the reactor was ~100 g wet matter (WM). After filling the reactor, the lid was sealed with an o-ring and closed. The wheat straw was ozonised between 1 and 7 h. Further details

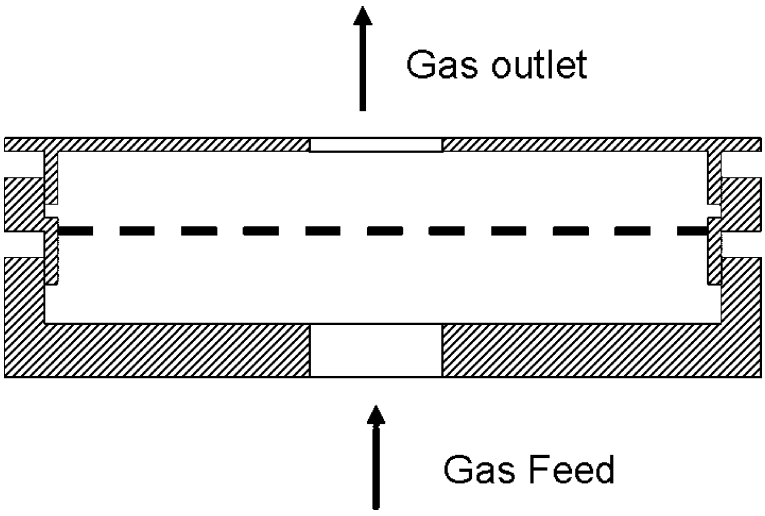

Fig. 1 Pretreatment reactor. The O_3 containing gas was fed from below. The *dashed line* depicts the sieve mesh. The outlet gas was analyzed (see text)

of plasma-assisted pretreatment (generation of O_3 by means of plasma, diagnostic of O_3 consumption, pretreatment reactor, and setup) has previously been described [4].

After pretreatment, the wheat straw was dried in an incubator cabinet (Adolf Kühner Lab-Therm LT-V, Switzerland), with air circulated heating at 40 °C. The drying time always exceeded 2 days but varied with the vessel size. Dried samples were weighed to determine the loss during the pretreatment process and stored for further analysis. pH measurement of ozonised wheat straw was performed by mixing 100 mg of dry pretreated straw with 2 ml water in a 10-ml test tube. The pH was measured 30 min after incubation.

Washing Procedure of Plasma-Pretreated Wheat Straw A series of O_3 treated wheat straw samples was extracted with water to wash out possible inhibitors. Each O_3 treatment portion (originally 49 g of DM) was washed separately in a weight ratio of 1:10 of dried ozonised wheat straw and 25 °C Millipore water ($R \geq 18$ μS cm^{-1}). The mixture was stirred (Heidolph RZR 2020 mechanical overhead stirrer) in a 1-l glass beaker at 800 rpm for 30 min. The extract was filtered with suction through a nylon membrane (pore size, 0.1 mm). The liquid fraction was frozen and stored for further experiments. The solid fraction was dried as described above.

Analysis Methods

Strong Acid Hydrolysis The composition of the raw and pretreated wheat straw was measured by strong acid hydrolysis of the carbohydrates. Dried and milled samples (160 mg) were treated with 72% (*w*/*w*) H_2SO_4 (1.5 ml) at 30 °C for 1 h. The solutions were diluted with 42 ml water and autoclaved at 121 °C for 1 h. The hydrolysates were filtered, and the Klason lignin content was determined as the weight of the insoluble residue minus the ash content. The recovery of D-glucose, D-xylose, and L-arabinose was determined by standard addition of sugars to the samples before autoclaving. The sugars were determined after separation on an high-performance liquid chromatography (HPLC) system (Shimadzu) with an Aminex HPX-87H column (BioRAD) at 63 °C using 4 mM H_2SO_4 as eluent and a flow rate of 0.6 ml/min. Detection was done by a refractive index detector (Shimadzu Corp., Japan, RID-10A) [12]. The samples were centrifuged with approximately 4,000 rpm for 10 min. The supernatant was analyzed for glucose, xylose, arabinose, and acetic acid by HPLC as described above.

Enzymatic Hydrolysis The enzymatic convertibility assay based on commercial cellulase (Celluclast 1.5 L from *Trichoderma reesei*) and β-glucosidase (Novozym 188) from Novozymes A/S in Denmark was used to determine the efficiency of the plasma-assisted pretreatment [12]. The enzymatic activity of Celluclast was 108 filter paper units (FPU) per cubic centimeter as analyzed according to Ghose [13]. Enzymatic conversion of plasma-pretreated solids (not washed and washed) was performed at 2% DM in the presence of 0.2 M acetate buffer (pH 4.8). Enzymatic hydrolysis was carried out in triplicate in 80-ml blue capped flasks in a total volume of 50 ml, which were placed in an incubator at 50 °C and shaken at 150 rpm. Applied enzyme loadings were 25 FPU g^{-1} DM solids for all assays. The total enzymatic hydrolysis duration of the fibers was 72 h. Samples were taken regularly at 3, 6, 24, 48, and 72 h and centrifuged at 3,000 rpm for 10 min. The supernatant was filtered over a 0.45-μm filter before HPLC analysis.

Extraction of Phenols and Analysis by GC The phenols were selectively extracted from the liquid fraction (washing water of ozonised wheat straw) by solid phase extraction on

polystyrene divinylbenzene polymer columns: IST Isolute ENV+ 100 mg/l ml (International Sorbent Technology, UK) [14, 15]. The columns were conditioned with methanol, ethyl acetate, and finally Millipore-grade water and adjusted at pH 2. The columns were allowed to equilibrate 5 min after each addition of solvent and were not allowed to run dry during elution. The liquid fraction was adjusted to pH 2 with 0.1 M H_2SO_4, 1 M H_2SO_4, or 0,1 M NaOH dependent on the pH of the samples. After that, the liquid fraction was loaded and slowly percolated through the column by vacuum. Dry nitrogen was pumped through the column in order to dry it completely. The phenols were eluted with two aliquots of ethyl acetate, where the first contained the internal standard 1,2,4,5-tetrachlorobenzene. Samples (500 μl) from the extraction (pH 2) were diluted with acetronitrile (750 μl) and dried with Na_2SO_4. The supernatant (280 μl) was silylated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (70 μl) at 70 °C for 30 min (EtOAc-acetonitrile-BSTFA). Thereafter, samples were ready to be analysed by gas chromatography (GC).

Analysis of Furans in Aqueous Solutions by HPLC The washing water of pretreated wheat straw was filtered (0.45 μm), and the furans 5-HMF and furfural were separated on a HPLC-system (Shimadzu Corp., Japan) with an Aminex HPX-87H column (BioRAD) at 63 °C for 55 min, using authentic compounds as calibration standards [16]. The eluent was 4 mM H_2SO_4, and a flow rate of 0.6 ml min^{-1} was used. The furans were detected by a diode array detector (SPD-M20A) and measured at 250 nm.

Analysis of Liquid Degradation Products of the Ozonisation Process with HPLC Water-soluble products from the washing water of ozonised wheat straw were separated by HPLC and characterized using an UV absorption detector at 210 nm. The samples were run on Aminex HPX 87H column at 55 °C for 80 min. Sulfuric acid (0.012 N) at a flow rate of 0.7 ml min^{-1} was used as eluent [9].

Ethanol Fermentation Experiments

Yeast Strain Saccharomyces cerevisiae ATCC 96581 strain was obtained from the American Type Culture Collection (Manassas, VA, USA). The strain was maintained at −85 °C in the mixture of 50 vol% glycerol and medium containing 20 g of bacto peptone, 10 g of yeast extract, 20 g of l glucose per liter demineralized water.

Yeast Cultivation Starter culture of *S. cerevisiae* ATCC 96581 was grown in 250-ml cap flasks containing 100 ml of culture medium or in 750-ml Erlenmeyer flasks containing 300 ml culture medium for up-scaled batch fermentations. The medium for the growth of the yeasts was the same synthetic medium as used for the strain maintenance except for glycerol. Glyerol was not included in the growth medium. The medium was sterilized at 121 °C for 20 min. The flasks were incubated in an orbital shaker at 130 rpm for 24 h at 30 °C. After 1 day of incubation, the cultures were centrifuged at 3,000 rpm for 10 min (Sigma 4-15, SIGMA Laborzentrifugen GmbH, Germany), washed with demineralized water, and harvested in demineralized water.

Saccharification and Fermentation To evaluate the convertibility of plasma-pretreated wheat straw (with and without washing) into ethanol, SSF was performed under semi-anaerobic conditions in blue capped flasks with 3 g of dry solid content in a total volume of

50 ml, corresponding to 6% (*w*/*v*). The flasks were equipped with yeast lockers. The pH was adjusted to 4.8. The commercial cellulase (Celluclast 1.5 L and Novozym 188) was applied at 15 FPU g^{-1} dry substrate. The fermentation medium contained (per liter): 3 g pretreated sample, 5 g bacto peptone, 5 g yeast extract, 2 g NH_4Cl, 0.3 g $MgSO_4 \cdot H_2O$, and 1 g KH_2PO_4.

Prehydrolysis for 24 h at 50 °C was performed before inoculation. After the prehydrolysis, the temperature was decreased to 30 °C, and the same amount of enzymes was added as described above. The content in the flask was inoculated with harvested yeast. The initial yeast concentration was 2 g DM l^{-1}. The flasks were incubated in a rotary shaker at 30 °C for 10 days. The evolution of carbon dioxide was monitored via measuring the weight loss off the flask content. Samples were taken at the end of the fermentation and centrifuged in a laboratory desktop centrifuge at 3,000 rpm for 5 min. The supernatants were analyzed for carbohydrates and ethanol concentrations using the same HPLC system as described above.

SSF in a Bioreactor Two batch fermentations (washed and unwashed plasma-assisted pretreated wheat straw (1 mm, 45% DM, 1 h pretreatment)) were performed in a 2.5-l fermentor (Minifors, Infors HT, Switzerland). The dry solid content was 90 g in a total volume of 1.5 l (corresponding to 6% *w*/*v*). The pH was automatically adjusted to 4.8 throughout the fermentation by adding 0.1 M NaOH or 0.1 M H_2SO_4. The commercial cellulases (Celluclast 1.5 and Novozym 188) were applied at 15 FPU g^{-1} dry substrate. The composition of the fermentation medium was identical to the one described for the small scale experiments. The fermentations were run as described above (prehydrolysis for 24 h at 50 °C at 300 rpm, and after adding enzyme fermentation at 32 °C and 100 rpm). The initial yeast concentration was 2 g DM l^{-1}. Samples were taken during the fermentation, centrifuged, and analyzed for carbohydrates and ethanol concentrations by means of HPLC.

Results

Initially, the reproducibility of the plasma-assisted pretreatment method for wheat straw was investigated since, until now, such a study has not been performed. Plasma-assisted pretreated wheat straw (dry) (1,200 g) was produced, and 25 batches of 100 g were pretreated at standard conditions (1 mm particle size, 50% DM, 1 h treatment time). Out of these ten samples were investigated.

Analysis of Pretreated Wheat Straw and Washing Water

The loss of biomass during the ozonisation process (0–7 h) was investigated (Table 1). A significant loss in weight was observed for pretreatment duration of 5 h and longer. The loss in mass can be caused by volatile compounds. Condensed oily substances were found in the cover lid of the reactor. Volatile substances in the exhaust gas were CO_2 and CO (data not shown), and no quantification was performed. The contents of glucose, xylose, and arabinose in the ozonised wheat straw were determined after strong acid hydrolysis. Wheat straw, before and after washing with water (room temperature), was investigated. The results were accurate within ±5%. It was found that essentially all of the cellulose and hemicellulose remained in the fibers after pretreatment and washing with water (Table 2). In

Table 1 Investigation of the loss of biomass during the ozonisation process from 0 to 7 h of pretreated of unwashed material

	0.5 h	1 h	4 h	5 h	7 h
WS (g) at start (dry)	49.4	49.8	48.9	49.4	48.9
WS (g) after plasma, (dry)	48.6	49.5	48.1	45.8	46.6
Loss of WS (g), dry	0.8	0.3	0.8	3.6	2.3
Relative material loss (%)	1.6	0.6	1.6	7.2	4.7

WS Wheat straw

contrast, the lignin content was reduced to 20% (from 19.2 g/100 g to 4.9 g/100 g), and as a consequence, the biomass weight was reduced by 15% (Table 2). The washing water of pretreated wheat straw was analyzed subsequent to weak acid hydrolysis. Almost no glucan, and very little xylan and arabinan, was found.

The washing water obtained from the pretreatment process (0.5–7 h) was analyzed for lignin degradation products using GC and HPLC (Table 3). A number of simple carboxylic acids and phenolic compounds were found. Approximately 30 substances, partly known from other types of pretreatment, e.g., wet oxidation [5, 17], were found. Some of the components, such as ferulic acid and succinic acid, were present in the washing water of unpretreated wheat straw, but disappeared when ozonised for 0.5 h. Other components like 2-hydroxymethoxyacetophenone, palmitic acid, and coumaric acid disappeared after 1 h of pretreatment time. Compounds such as 4-hydroxybenzoic acid, acetic acid, and malonic acid varied in concentration. The concentration of a fourth group of components, e.g., acetovanillone, 4-hydroxybenzaldehyd, vanillic acid, vanillin, formic acid, and glyoxylic acid, increased to a maximum concentration and then decreased. The concentrations of a fifth group of compounds, e.g., azelaic acid, glycolic acid, levulinic acid, and, most notably, oxalic acid, increased throughout the ozonisation process.

Coumaric and ferulic acid resemble the basic unit of lignin in their chemical structure. Those compounds are no longer detectable in the washing water after 0.5 h ozonisation of wheat straw. Smaller compounds with a related aromatic substitution pattern arise such as

Table 2 Biomass balances of washed and plasma-assisted pretreated wheat straw

Fibers (g/100 g)							Washing water (g/100 g)			
Treatment time (h)	Klason lignin	Glucan	Xylan	Arabinan	Ash	Mass balance	Washing Glucan	Washing Xylan	Washing Arabinan	Mass balance
Unpretreated	19.2	41.6	23	3.5	5.5	93	0.07	0.07	0.02	93.2
0.5	16.5	40.5	22	3	6	88	0.07	0.2	0.06	88.3
1	14.9	41	22	3	6	87	0.09	0.3	0.16	87.6
2	9.5	47	18	2	6	83	0.12	0.1	0.25	83.5
3	7.7	45	18	2.0	6	79	0.13	0.11	0.26	79.5
4	6.2	47	17	2	6	78	0.13	0.12	0.25	78.5
5	5.4	46	18	2	6	77	0.13	0.1	0.25	77.5
7	4.9	44.3	18	2	6.5	76	0.13	0.97	0.23	77.3

Wheat straw was milled to 1 mm size and adjusted to 50% DM

Table 3 Analysis of the washing water of ozonised wheat straw (0–7 h)

	Name of component	Percent					
		0 h	0.5 h	1 h	3 h	4 h	7 h
GC	Acetovanillone	0	0.09	0.04	1.17	0.04	0.04
	Acetoveratrone	0	0	0	0.04	0	0
	Azelaic acid	0.26	0.62	1.13	1.53	1.15	1.66
	Caproic acid	0.04	0.09	0.17	0.09	0.08	0.13
	Coumaric acid	0.18	0.04	0	0	0	0
	2,6-di-tert-butylphenone	0	0	0	0	0	0
	Ferulic acid	0.26	0	0	0	0	0
	Guaiacol	0	0	0	0	0	0
	4-hydroxybenzaldehyde	0	0.89	0.74	0.58	0.31	0.35
	4-hydroxybenzoic acid	0.09	0.45	0.39	0.36	0.73	0.57
	2-hydroxymethoxyacetophenone	0	0.04	0.04	0	0	0
	Palmitic acid	0.13	0.04	0.04	0	0	0
	Syringol	0	0	0	0	0	0
	Vanillic acid	0.22	0.22	0.31	0.22	0.08	0.13
	Vanillin	0	0.31	0.35	0.27	0.12	0.13
	Veratrol	0	0	0	0	0	0
HPLC	Acetic acid	0.26	0.14	0.46	0.48	0.36	0.70
	Citric acid	0.03	0.02	0.05	0.08	0.09	0.16
	Formic acid	0.12	0.12	0.34	0.17	0.09	0.20
	Glycolic acid	0	0.04	0.07	0.17	0.18	0.19
	Glyoxylic acid	0.20	0.15	0.23	0.67	0.82	0.56
	Lactic acid	0.06	0	0	0	0	0
	Levulinic acid	0	0.01	0	0.06	0.06	0.08
	Malic acid	0	0	0	0	0	0
	Malonic acid	0	0	0.10	0	0	0.33
	Oxalic acid	0.01	0.73	1.49	3.13	3.31	3.44
	Propionic acid	0	0.01	0.01	0.01	0.01	0.01
	Succinic acid	0.09	0	0	0	0	0
	Furfural	0	0	0	0	0	0
	5 HMF	0	0	0	0	0	0

Twenty-six different compounds could be identified. Measurements were done with GC and HPLC analysis

vanillin and vanillic acid or 4-OH-benzaldehyd. 4-hydroxybenzaldehyd had a maximum concentration after 0.5 h pretreatment. It was noteworthy that the corresponding carboxylic acid increased in concentration when the aldehyde decreased.

Enzymatic Hydrolysis of Ozonised Wheat Straw

The development of toxic compounds during pretreatment and their effects on the enzymatic hydrolysis were investigated. Therefore, the enzymatic hydrolysis using washed and unwashed ozonised wheat straw was performed for 72 h, and the amounts of released, fermentable sugars (glucose and xylose) were compared (Figs. 2 and 3). The influence of

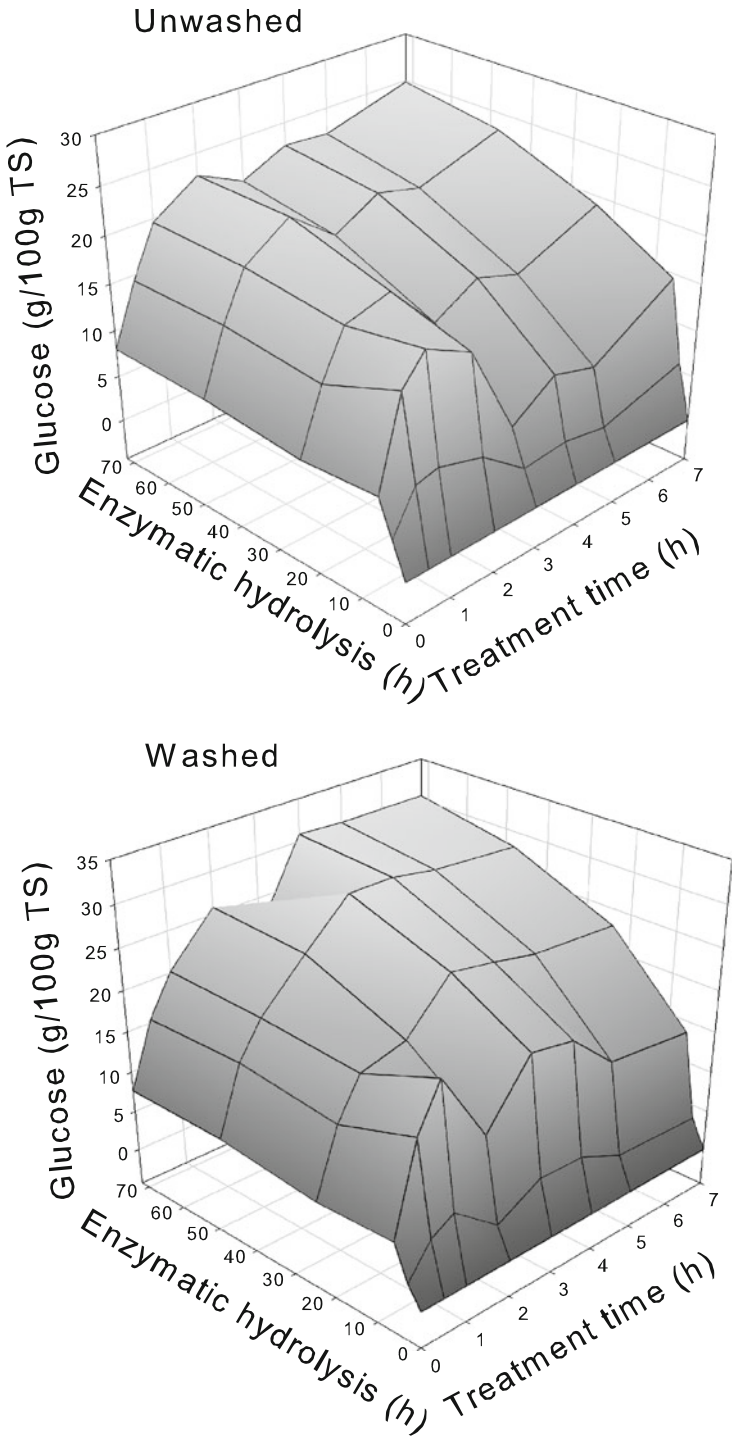

Fig. 2 Released glucose during enzymatic hydrolysis of washed and unwashed plasma-assisted pretreated wheat straw

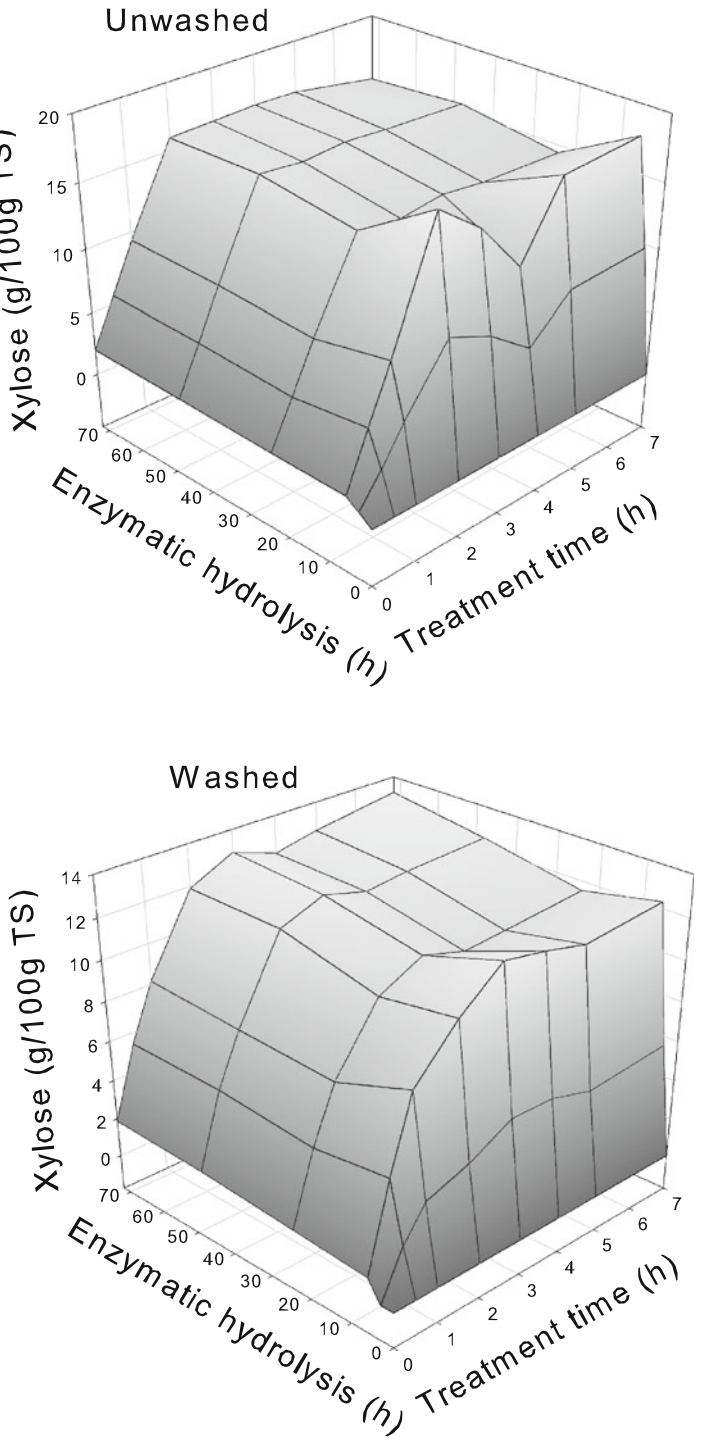

Fig. 3 Released xylose during enzymatic hydrolysis of washed and unwashed plasma-assisted pretreated wheat straw

the pretreatment time was the same. In the case of unwashed wheat straw, the duration of pretreatment influenced the amount of glucose released due to enzymatic hydrolysis (Fig. 2). The maximum amount of fermentable glucose with samples pretreated for 1 h was reached after 6 h of enzymatic hydrolysis, while 72 h was needed with samples pretreated for longer time (4, 5, 7 h). The maximum amount of glucose 24.8 g/100 g was released after 72 h of enzymatic hydrolysis of samples that had been pretreated for 7 h. In comparison, with unpretreated samples, 8 g/100 g was released under the same conditions (Fig. 2).

With samples pretreated for more than 3 h, the amount of released fermentable sugars increased significantly compared with unwashed wheat straw. With samples pretreated for 1 h and washed, 20 g/100 g glucose was released after 6 h, while with samples pretreated for 3, 4, 5, and 7 h 30 g/100 g glucose was reached after 48 h enzymatic hydrolysis. Seventy-eight percent of the theoretically available glucose was released with washed samples (Fig. 2). Interestingly, the amount of xylose released enzymatically from washed and unwashed samples were very similar. In both cases, the release of xylose was the highest in samples pretreated for longer than 2 h. For both sample types (washed and unwashed), the maximum amount of xylose was approximately 14–16 g/100 g. This corresponds to 75% of the theoretically available xylose (Fig. 3).

SSF of Ozonised Wheat Straw

Washed and unwashed pretreated wheat straw was used for SSF. Ethanol yields are calculated based on to the potential glucose content. With samples pretreated for 1 h, the ethanol yield reached approximately 45% for both washed and unwashed samples, i.e., the influence of washing was negligible. With pretreatment for 3 h, washed samples reached 52%, while unwashed samples could not be fermented (Fig. 4).

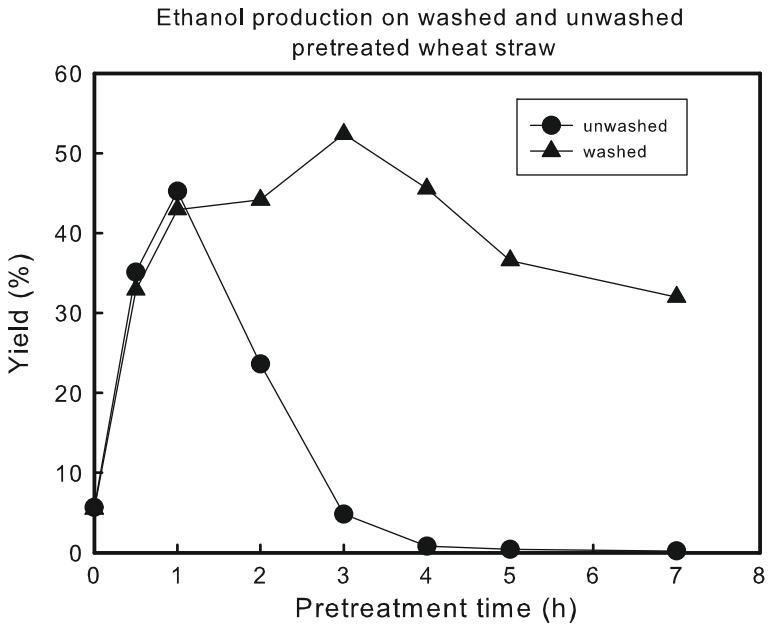

Fig. 4 Final ethanol yields of SSF experiments on washed and unwashed plasma-pretreated wheat straw

Fermentation in a Bioreactor

Based on the results of the SSF, wheat straw pretreated for 1 h was found to be the best suited for ethanol production. Therefore, 1-l scale fermentations of both washed and unwashed materials were run in order to compare their ethanol yields under pH-controlled conditions. The maximum ethanol yield of washed wheat straw was ~53%, while the maximum ethanol yield of unwashed wheat straw was ~44%. Both results were comparable with those obtained in small-scale experiments. Both fermentations reached the maximum amount of ethanol after about 7 days of fermentation. In the case of washed wheat straw, it was possible to produce about 10 g ethanol/100 g pretreated wheat straw.

Ozone Cost Estimation

After 1 or 3 h pretreatment, 1 kg of wheat straw can produce 180 or 208 g of ethanol, respectively. The O_3 requirement was 160 or 330 g, respectively [4]. The generation of 160 or 330 g of ozone requires an electrical energy of 3.8 or 7.87 MJ, respectively [18]. Combustion of 180 or 208 g ethanol releases 4.82 or 5.56 MJ (lower heating values of ethanol), respectively. The released energy is higher than the electrical energy consumption for a 1-h pretreatment. However, for a 3-h pretreatment, this is not the case.

Discussion

Plasma-assisted pretreatment of wheat straw has been optimized such that up to 78% of the glucose found in the cellulose can be released enzymatically and subsequently converted into ethanol. The ethanol yield was 52%, close to the highest possible value.

Enzymatic saccharification of ozonised lignocellulosic material has previously been reported [4, 19–23]. Sugimoto and coworkers [24] was the first to publish results on the subsequent ethanol production. However, the results presented here represent progress in a number of ways. As an example, Sugimoto observed a 3% decrease in the amount of polysaccharides during ozonisation of cedar sawdust, while we observed an 11% increase in glucose in ozonised and washed wheat straw samples (Table 2). Especially, hemicelluloses were highly conserved [4], which potentially can lead to a higher ethanol yield or alternatively, to C-5 derived packaging materials [25]. Sugimoto et al. [24] achieved only 20% of the theoretically possible amount of ethanol, while we found an ethanol yield of 52%. A yield of 52% equals the theoretically expected yield from glucose taking the consumption of sugars for the metabolism of the yeast into account [26].

Our investigation clearly shows the influence of lignin degradation products on the yield of the enzymatic hydrolysis and on the ethanol fermentation. This was demonstrated by fermenting washed and unwashed ozonised wheat straw in parallel (Fig. 4), showing that the inhibiting effect increases in samples pretreated for longer than 1 h. Some of the components, e.g., 4-OH-benzaldehyde, ferulic acid, or coumaric acid, oxalic acid, acetic acid, etc., are known as fermentation inhibitors from other pretreatment processes, e.g., wet oxidation or hydrothermal pretreatment [27]. The inhibitors generated explain why the ethanol fermentation of unwashed material, pretreated for longer than 2 h, was inhibited.

Among the compounds investigated, significant increases in concentrations (between pretreatment hour 1 and 3) are mainly observed for oxalic acid and acetovanillone (Table 3). Oxalic acid and acetovanillone could therefore be a reason for the inhibition of yeast fermentation or a synergistic effect of different inhibiting compounds including formic or

acetic acid [17]. This observation is consistent with the fact that unwashed wheat straw, ozonised for longer than 2–3 h, no longer can be fermented to ethanol.

Another group [9] has investigated ozonisation products from lignocellulosic material, and we can confirm the compounds they found, and the range of the concentrations. Similar to us, they identified oxalic acid as the component with the highest concentration [9]. Sugimoto et al. [24] has suggested that fermentation inhibitors are not produced during ozonisation, since O_3 only attacks C–C double bonds. Different from Sugimoto, we found fermentation inhibitors, such as vanillin, vanillic acid, *p*-hydroxybenzaldehyde or *p*-hydroxybenzoic acid; however, we can confirm that the most relevant inhibitors furfural and 5-HMF were not found [5].

In our studies, most of the fermentation inhibitors derived from the lignin degradation were removed simply by washing with water (at room temperature), and 52% ethanol yield could be achieved. Temperature and pH are assumed to play an important role for the washing process; however, this has not been investigated here.

Lignin is the only renewable source of aromatics that is an important and high-volume class of compounds [28]. The described pattern of lignin degradation products (Table 3) inspires the thought of converting lignin to molecules or classes of high-volume, low-molecular weight aromatic molecules, e.g., phenols, terephthalic acids, and benzene, toluene, and xylene chemicals. Generally, exploring lignin for value products is a challenging but attractive and viable long-term goal [28].

Conclusion

Plasma-assisted pretreatment of wheat straw has been optimized such that up to 78% of the glucose found in the cellulose can be released enzymatically and subsequently converted into ethanol. Despite the fact that the ethanol yield was 52%, according to our cost estimate, the production of ethanol alone does not appear economic using the plasma-assisted pretreatment process in the present form. However, if the degradation products of lignin as well as the hemicelluloses could be recovered as value added products on top of the ethanol production, this technology may become attractive.

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