

Plasma-Assisted Pretreatment of Wheat Straw

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Abstract O₃ generated in a plasma at atmospheric pressure and room temperature, fed with dried air (or oxygen-enriched dried air), has been used for the degradation of lignin in wheat straw to optimize the enzymatic hydrolysis and to get more fermentable sugars. A fixed bed reactor was used combined with a CO₂ detector and an online technique for O₃ measurement in the fed and exhaust gas allowing continuous measurement of the consumption of O₃. This rendered it possible for us to determine the progress of the pretreatment in real time (online analysis). The process time can be adjusted to produce wheat straw with desired lignin content because of the online analysis. The O₃ consumption of wheat straw and its polymeric components, i.e., cellulose, hemicellulose, and lignin, as well as a mixture of these, dry as well as with 50% water, were studied. Furthermore, the process parameters dry matter content and milled particle size (the extent to which the wheat straw was milled) were investigated and optimized. The developed methodology offered the advantage of a simple and relatively fast (0.5–2 h) pretreatment allowing a dry matter concentration of 45–60%. FTIR measurements did not suggest any structural effects on cellulose and hemicellulose by the O₃ treatment. The cost and the energy consumption for lignin degradation of 100 g of wheat straw were calculated.

Keywords Pretreatment method · Wheat straw · Lignin · Plasma · Discharge · Biofuel · Sustainable energy · Ozonisation

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Introduction

In a recently published vision, the EU plans to cover one quarter of the road transport fuel needed by renewable biofuels by 2030 [1]. It is stated that by 2030, combustion engines will continue to be the dominant transport technology; thus, organic fuels will remain essential.

Various agricultural byproducts constitute a potential source for biofuels. The three main components in such biomasses are cellulose, hemicellulose, and lignin, and among these, cellulose is of particular interest since it can be degraded to glucose which can be fermented to liquid fuels such as ethanol. Cellulose exists together with hemicellulose and lignin in a complex and highly resistant structure, often described as lignocellulose [2]. However, this structure and in particular the lignin part prevents easy depolymerization of the imbedded cellulose.

In view of the need to disrupt the lignocellulosic complex to render cellulose accessible to enzymatic hydrolysis, an efficient and cost-effective pretreatment technology needs to be developed. Presently, the most common pretreatment methods [2, 3] include milling, chopping, steam explosion, addition of hot water, addition of acid or base, organic solvents, ammonia, water, and oxygen at temperatures above 120 °C [4].

The potential of using cold plasma at atmospheric pressure and ambient temperature to assist the pretreatment of wheat straw has previously been suggested [5, 6]. The reagent in such a process is O₃ which is produced from air or pure oxygen in the plasma. O₃ is predicted to oxidatively degrade unsaturated organic compounds in lignin, whereas saturated compounds such as carbohydrates only to a minor extent should be attacked [7, 8]. The ozonolysis process takes place at room temperature and atmospheric pressure securing limited formation of degradation products which might interfere with subsequent enzymatic hydrolysis and fermentation [9, 10].

Previous results on treatment of biomass with O₃ are inconsistent. Some claim that both lignin and polysaccharides are attacked [11, 12] while others claim that primarily lignin is attacked [5, 13]. A third opinion is that O₃ has no effect on any of the major polymers [14]. However, it has to be taken into account that these experiments were carried out under conditions that are not necessarily comparable. Optimal amounts of dry matter have been reported to be in the range of 10% to 60% [14, 15]. Additional influences of the extent to which the wheat straw has been milled as well as the O₃ concentration have been reported. However, no real method based on systematic investigations has been established. This is partially due to the lack of an online technique for O₃ measurement in the exhaust gas which would allow the progress of the pretreatment to be followed in real time.

We here report a new method for online O₃ measurement allowing us to produce wheat straw with lignin degradation of 5–95%. With this information, it was possible to optimize the pretreatment with respect to lignin content such that, in the context of bioethanol production, a high rate of enzymatic hydrolysis would be possible in the subsequent step.

Material and Methods

Raw Material

The wheat straw (*Triticum aestivum* L.) was grown and harvested after a drying period in Denmark during summer 2008. The straw was baled into 500 kg bales and stored at ambient temperature. Purified lignin was obtained from Aldrich (Catalog No. 37,101–7),

cellulose was obtained from Sigma (lot: 11 K0246), and hemicellulose (birch wood xylan) was obtained from Fluka (lot: 1367783 34907270). All chemicals were of analytical grade.

Adjustment of the dry matter content of the biomass Dry matter contents of wheat straw, cellulose, lignin, and xylan were measured with a moisture analyzer HR83 (Mettler Toledo A/S). The adjustment of the dry matter content was done by calculating the approximate percentage of water needed for the dry matter content desired, e.g., for wheat straw (50% dry matter), a mixture of 50 g wheat straw and ~50 g water was prepared. The straw was soaked in water for 2 to 3 h, and the dry matter content was adjusted if necessary. The adjusted wheat straw was packed in plastic bags and stored in a refrigerator overnight.

Analysis Methods

Strong acid hydrolysis The composition of the raw and pretreated wheat straw was measured after 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 of 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 autoclavation. The sugars were determined after separation on an 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). Conversion of factors for dehydration on polymerization was 162/180 for glucose and was 132/150 for xylose and arabinose [16, 17]. Monomeric sugars and acetic acid were measured by adjusting the sample pH to 2–2.3 with H_2SO_4 . The samples were centrifuged with approx. 4,000 rpm for 10 min. The supernatant was analyzed for glucose, xylose, arabinose, and acetic acid by HPLC as described above.

Enzymatic hydrolysis An enzymatic convertibility assay based on commercial cellulase (Celluclast 1.5 l from *Trichoderma reesei*) and β -glucosidase (Novozymes 188) from Novozymes A/S in Denmark was used to determine the efficiency of the different conditions of the plasma-assisted pretreatment tested [18]. The enzyme activity of Celluclast was 108 FPU cm^{-3} as shown by Thygesen [19]. Enzymatic conversion of plasma pretreated solids (not washed) was performed at 2% dry matter in the presence of 0.2 M acetate buffer (pH=4.8). Enzymatic hydrolysis was carried out in triplicate in 10-cm³ test tubes, which were placed in an incubator at 50 °C and were shaken at 150 rpm. Applied enzyme loadings were 25 FPU/g dry matter solids for all assays. The duration of the enzymatic hydrolysis of the fibers was 24 h. The reaction was stopped after 24 h and centrifuged (4,000 rpm, 10 min). From the supernatant, 350 μl were withdrawn and mixed with 800 μl 0.01 M H_2SO_4 in Eppendorf tubes. The Eppendorf tubes were centrifuged (10,000 rpm, 10 min) and the supernatant was filtered over a 0.45- μm filter before HPLC analysis.

Fourier transform infrared spectroscopy analysis Cellulose and xylan were measured by means of Fourier transform infrared spectroscopy (FTIR) as described by Griffith [20]. Briefly, a small quantity of matter (a few milligrams, e.g., 2 mg) was ground, then mixed with 100 mg KBr, and pressed to a pellet and scanned with a PerkinElmer Spectrum GX FTIR apparatus (Massachusetts, USA). Infrared spectra of different samples were recorded and analyzed.

Generation of O_3 by Means of Plasma

For the generation of O_3 , a low temperature atmospheric pressure dielectric barrier discharge (DBD) with a coaxial electrode geometry was used. An Al_2O_3 cylinder between the electrodes served as a dielectric barrier. A scheme of the DBD is shown in Figs. 1 and 2. The DBD has been introduced by Leipold [21]. The DBD was driven by an alternating current power supply. For continuous operation, the power supplied to the DBD was adjusted by changing the frequency in the range between 10 and 40 kHz corresponding to a maximum power of 1,500 W. In order to apply lower powers, the power supply was operated in pulsed mode. The pulse consisted of three full sinusoidal periods (163 μ s) of high voltage followed by a pause of adjustable duration. The power supplied to the DBD was the average power. This is the energy provided in one pulse divided by the repetition time. The average power delivered to the discharge could be read on the power supply. It was adjusted to 230 W, providing the maximum O_3 concentration. In order to generate the O_3 , the DBD was fed with air, oxygen-enriched air, or pure oxygen. The O_3 concentration depends on the power supplied to the DBD, the gas composition, and the gas flow rate.

Diagnostic of O_3 Consumption

O_3 was detected by means of ultraviolet absorption spectroscopy using a mercury lamp as light source and a UV-detector diode in combination with an interference filter with a transmission maximum at 254 nm where O_3 had an absorption maximum. The relevant cross-sections were described by Orphal [22]. The O_3 concentration was between 0.6% and 1%. The air- O_3 mixture was fed through a pipe into a reaction chamber. The reaction chamber was a sealed metal vessel which was horizontally separated by a wire mesh with a mesh size of 0.2 mm. The straw was placed on the mesh. The air-ozone mixture was fed from below through the mesh. The O_3 concentration of the exhaust gas was measured. The difference between the supplied O_3 and the O_3 concentration in the exhaust yielded the O_3 consumption of the straw.

Pretreatment Reactor

The laboratory scale reactor and a bench scale reactor were made from aluminum and had a diameter of 7 and 30 cm corresponding to an area of 38 and 706 cm², respectively. The bottom part of the round, fixed bed reactors were equipped with a channel that provided the best O_3 distribution to the biomass sample. The O_3 flow direction through the reactor was upwards. The wheat straw was placed on the mesh with a layer height of approximately 1 cm. The bench scale reactor was provided with removable sieves of 0.2 mm mesh size (Retsch test sieves, Retsch GmbH, Germany) to support the wheat straw.

Fig. 1 Schematic drawing of the dielectric barrier charge (front view). Electrodes (dark gray), dielectric barrier (gray), and plasma volume (white)

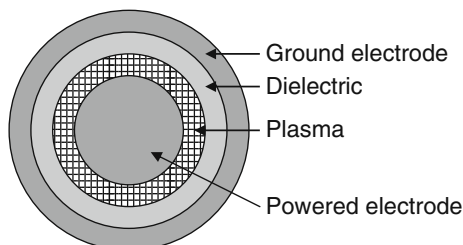
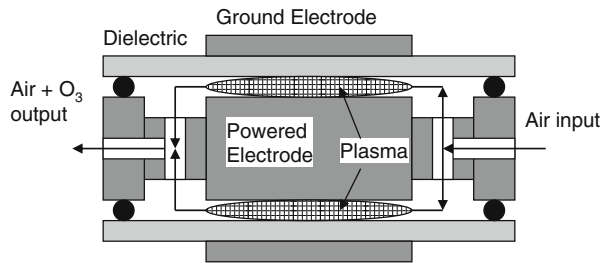


Fig. 2 Coaxial dielectric barrier charge. Gas enters in the center pipe, is guided through the discharge volume, and exits through the center pipe on the opposite side (side view)



Safety

A custom-made exhaust system covers the pretreatment reactor, and an encapsulated plasma source was built to protect the operator and the environment from gaseous hazards, e.g., O_3 or degradation products of lignocellulosic biomaterials and electrical shock.

Plasma-Assisted Pretreatment Unit and System Setup

The pretreatment unit for lignocellulosic biomaterials is depicted in Fig. 3. It consists of a DBD responsible for the production of O_3 and a UV-absorption spectroscopy unit to measure the O_3 concentration as described in “[Generation of \$O_3\$ by Means of Plasma](#)” and “[Diagnostic of \$O_3\$ Consumption](#).” Furthermore, the apparatus is equipped with a detector that measured the gas concentration of CO and CO_2 in the exhaust gas from the reactor. The reactors described under “[Pretreatment Reactor](#)” can easily be interchanged.

Plasma-Assisted Pretreatment of Wheat Straw (Ozonization)

The ozonization of wheat straw was performed in our in-house-built reactors (see “[Pretreatment Reactor](#)”) under atmospheric pressure at room temperature. Before pretreatment, the wheat straw was adjusted to the required dry matter content and milled to the desired particle size (0.5 to 4 mm) with the help of a technical mill (MF 10 basic, IKA-Werke GmbH & Co. KG, Germany). The wheat straw (with defined dry matter content) was placed into the sieve of the reactor. The filling capacity of one sieve of the bench scale reactor (\varnothing 30 cm) was ~100 g wet matter (WM). The filling capacity of the laboratory scale reactor (\varnothing 7 cm) was ~5 g WM. A

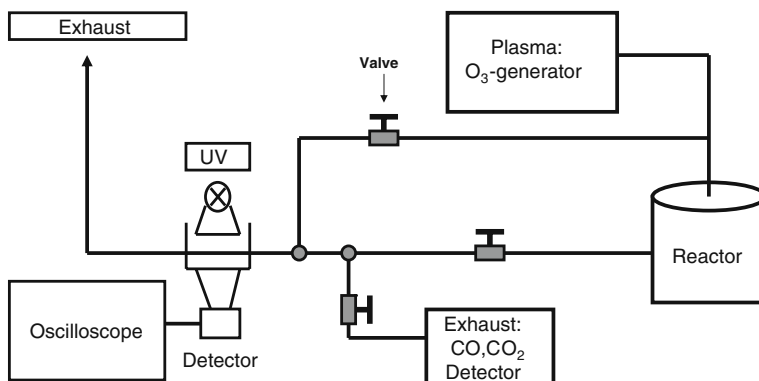


Fig. 3 Pretreatment unit

removable filter paper was placed on top of the mesh of the laboratory scale reactor to avoid loss of biomass. The filter was exposed to O_3 beforehand to ensure that it did not consume O_3 during the experiment. After filling the reactors, their lids were closed and sealed with and o-ring. To start the pretreatment process, air (laboratory scale reactor) or oxygen-enriched air (bench scale reactor) was fed into the DBD. The O_3 production was controlled by the electrical power supplied and the oxygen concentration in the gas supply. Kinetic experiments were carried out to determine the reaction time for full lignin degradation under different dry matter contents of wheat straw, particle sizes, and gas flow rates. Standard conditions for most pretreatments of wheat straw were defined and set to an O_3 concentration of 0.6% to 1% O_3 at a gas flow rate of 0.6 l/min for the laboratory scale and a gas flow rate of 12 l/min for the bench scale reactor (40% oxygen admixture) at standard conditions. Wheat straw was ozonized for 1 to 2 h as a standard. Mechanical stirring by a spatula in case of long-term ozonization (more than 2 h) was required. After stopping the pretreatment, the wheat straw was removed from the reactor and stored in a plastic bag with closure until further use or dried for 24 h at 60 °C. Dried samples were weighed to determine weight loss during the pretreatment process and stored for further analysis.

Results

Effect of O_3 on Pure Cellulose, Xylan, and Lignin Compared to a Mixture of the Polymeric Constituents of Wheat Straw

Initially, the effect of O_3 on the pure cellulose, xylan, and lignin, which constitute 40%, 20%, and 20% of wheat straw, respectively, was determined. Cellulose, hemicellulose, and lignin were used in form of powder. Samples of cellulose (2 g), xylan (1 g), and lignin (1 g), either dry or at dry matter content of 50%, were ozonized for a period of 2 h using the laboratory scale reactor at a flow rate of 0.6 l/min and with an O_3 concentration of approximately 1%. With lignin, dry or at 50% dry matter, 350 and 325 mg O_3 was consumed during the pretreatment, respectively (Table 1, Fig. 4). With xylan the corresponding consumptions of O_3 were 25 and 37 mg, respectively. For cellulose no O_3 was consumed. With a mixture of 1 g lignin, 1 g xylan, and 2 g cellulose, the O_3 consumption was 275 mg which is similar to the value obtained with 1 g of lignin (see above). With 4 g wheat of straw, the O_3 consumption was 225 mg using a particle size of 0.5 mm and only 50 mg using a particle size of 1 mm. Particle size means that the largest dimension of a straw particle does not exceed the specified number.

Table 1 Effect of O_3 on pure cellulose, xylan, and lignin compared to a mixture of the polymeric constituents of wheat straw after a reaction time of 2 h

	Dry, total O_3 consumption (mg)	50% Dry matter, total O_3 consumption (mg)
Cellulose (powder)	0	0
Xylan (powder)	25	37
Lignin (powder)	350	325
Mixture of the polymeric constituents of wheat straw	275	
Wheat straw 0.5 mm	225	
Wheat straw 1 mm	50	

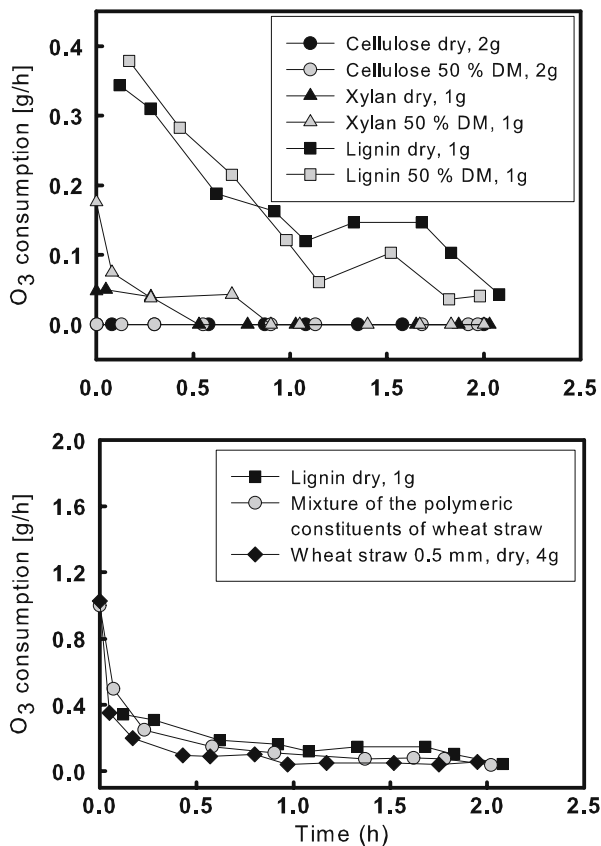


Fig. 4 Effect of O₃ on pure cellulose (2 g), xylan (1 g), and lignin (1 g) (a) compared to artificially composed wheat straw (b)

FTIR Investigation of Pretreated and Unpretreated Cellulose and Xylan

Carbonyl groups ($\sim 1,700\text{ cm}^{-1}$) would be expected in cellulose and xylan if they were to be oxidized by O₃ [23, 24]. However, treated and untreated cellulose were identical in this respect (Fig. 5a). Xylan shows a slight difference at $\sim 1,700\text{ cm}^{-1}$ in the FTIR spectrum (Fig. 5b). The O₃ consumption of xylan is small (approximately 10%) compared to the O₃ consumption of lignin (Table 1).

Influence of the Particle Size of Wheat Straw on the Efficiency of the Pretreatment and the O₃ Consumption

In contrast to the O₃ consumption for a 2-h pretreatment of dry samples (“Effect of O₃ on Pure Cellulose, Xylan and Lignin Compared to a Mixture of the Polymeric Constituents of Wheat Straw”), the particle size had only a minor effect on the O₃ consumption for a 7-h treatment of samples with a dry matter content of 50%. The pretreatment of wheat straw of three different particle sizes (2, 1, 0.5 mm), each with a dry matter content of 45%, was investigated (Fig. 6). With an ozonization time of 7 h, approximately the same O₃

consumption was found for the different particle sizes: 24 g (2 mm), 23 g (1 mm), and 21 g (0.5 mm). The particle size, however, influences the release of sugars (glucose and xylose). With increasing pretreatment time, the glucose concentration (following enzymatic hydrolysis) increases while the O_3 consumption decreases (Fig. 6) for all particle sizes. The steepest decrease of O_3 is concomitant with the sharpest increase of glucose availability in wheat straw of unwashed material; 0.5 mm wheat straw reaches a maximum of ~23 g glucose per 100 g wheat straw after only 1 h pretreatment. However, with 2 h of pretreatment of 100 g of wheat straw, 26 g glucose is obtained with 1 mm particles whereas with 2 mm particles only 12 g glucose is obtained. The hemicellulose accessibility (also measured by enzymatic hydrolysis) also increases with time, but longer time is required, and the released amounts are lower than with cellulose, consistent with the fact that the content of cellulose exceeds that of xylan. This is illustrated by the fact that 4 h of

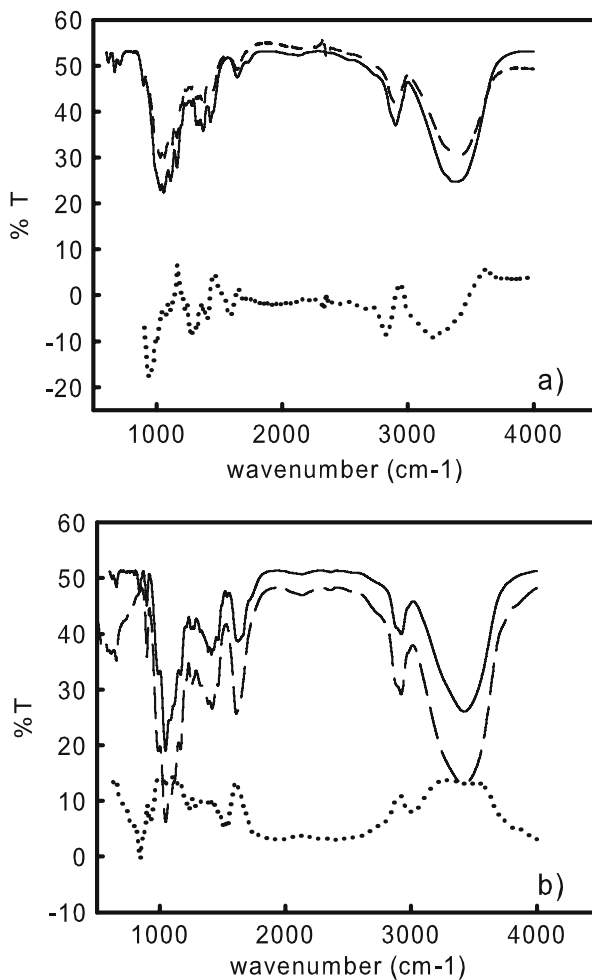


Fig. 5 FTIR investigation of pretreated and untreated cellulose (a) and xylan (b). The dotted line is the difference between the transmission of untreated samples (solid line) and treated samples (dashed lines)

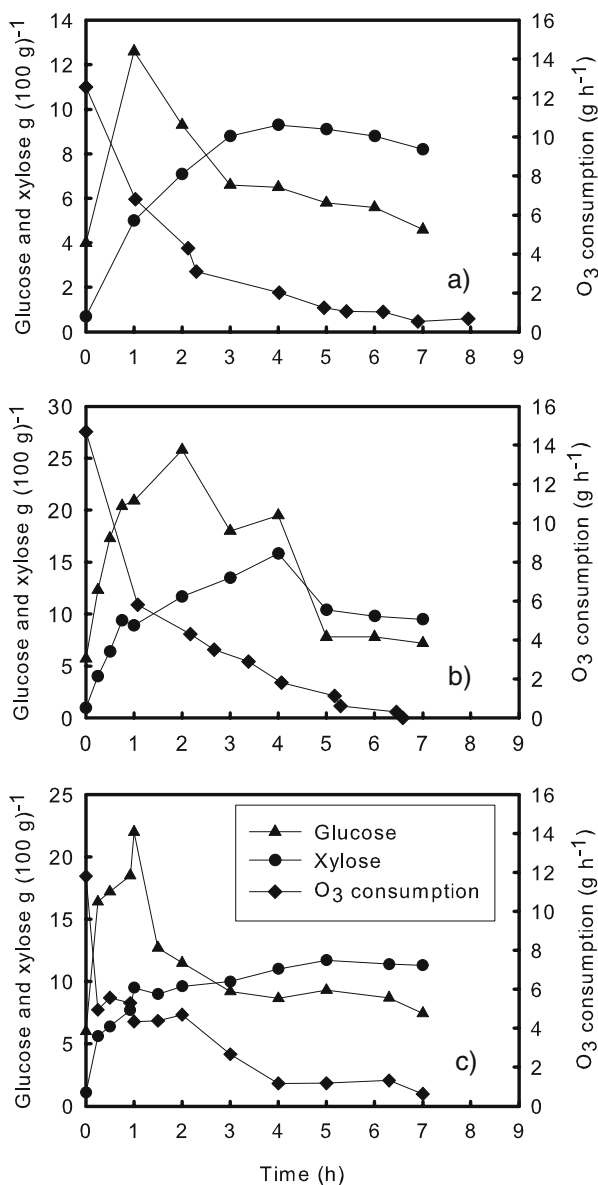


Fig. 6 O₃ consumption for wheat straw and the development of relative glucose and xylan versus treatment time for three different sizes of wheat straw (2 mm (a); 1 mm (b); 0.5 mm (c))

ozonization is required to reach the maximum of 10 g xylose per 100 g straw with 2 mm particles and 15 g xylose with 1 mm particles. For maximum yield of glucose, the O₃ consumption with 1 mm particles is approximately 5 g/h whereas it is 7.4 g/h with 2 mm particles. The conversion of glucan and xylan after 4 h pretreatment for all three particle sizes is shown in Table 2. The conversion efficiency is higher for xylan.

Table 2 The conversion of glucan and xylan after 4 h pretreatment for all three particle sizes

Conversion (4 h)	0.5 mm (%)	1 mm (%)	2 mm (%)
Glucan	23	50	19.4
Xylan	57.5	75	45

Influence of the Dry Matter Content of Wheat Straw on the Degradation of Lignin

Wheat straw, milled to a particle size of 1 mm, was adjusted to dry matter contents of 94%, 85%, 64%, 45%, 29%, and 20% and ozonized for 2 h. The samples were analyzed for their glucan, xylan, arabinan, and lignin contents. The highest lignin degradation and O₃ consumption was seen at 45% dry matter. The relation between the O₃ consumption, the dry matter content of wheat straw, and the lignin degradation is shown in Table 3. The sugars, however, were not affected (Table 3).

Comparison of Different Flow Rates and the O₃ Consumption of Wheat Straw as a Function of Time

The influence of airflow on pretreatment time (1–7 h) was investigated with the laboratory scale reactor. The ozonization of wheat straw was highly reproducible with pretreatment periods, ranging from 1 to 7 h (Fig. 7a and b). With an airflow of 0.6 l/min, the O₃ consumption decreases drastically within the 1 h of pretreatment. With 0.125 l/min, the O₃ consumption decreases constantly and slowly over 7 h of pretreatment (Fig. 7b). However, both curves reached a level of O₃ consumption of 0–0.1 g/h after approximately 7.5 h of pretreatment. The required time for the maximum sugar accessibility as determined after enzymatic hydrolysis was obtained at 1 to 2 h at an airflow of 0.6 l/min and at 3–4 h with a flow of 0.125 l/min (Table 4).

Comparison of the Pretreatment Process with the Laboratory Scale and with the Bench Scale Reactor

The pretreatment results of the laboratory scale and the bench scale reactor were compared to assure that the plasma-assisted pretreatment had the same impact on wheat straw in both reactor types. Therefore, proportional amounts of wheat straw (1 mm, 45% dry matter) were

Table 3 Influence of the dry matter content of wheat straw on the degradation of lignin for a pretreatment time of 2 h

Dry matter% at start (1 mm wheat straw)	O ₃ consumption (g)	g O ₃ /100 g biomass (wet)	Klason lignin (g/100 g)	Glucan (g/100 g)	Xylan (g/100 g)	Arabinan (g/100 g)
94	1	1.1	17	38	21	2.5
85	2	2.4	17	39	22	2.5
64	6.5	10.0	8	38	21	2.7
45	6.5	14.0	5	39	21	2.8
29	4	14.0	10	37	20	2.7
20	1.75	8.8	9.5	38	20	2.7

Results measured with the strong acid hydrolysis. The total weight of each sample was 100 g

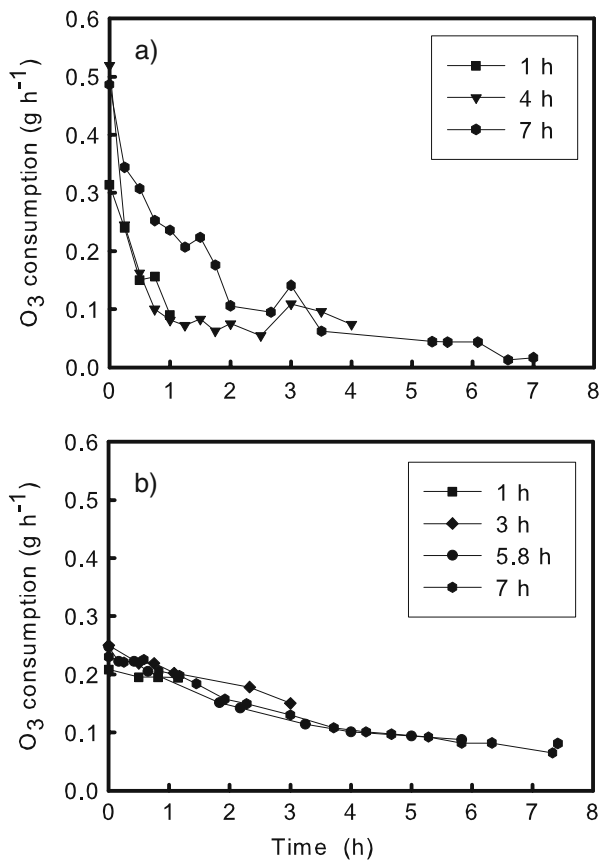


Fig. 7 Comparison of different air-flow rates (**a** 0.6 l/min, **b** 0.125 l/min) and the O_3 consumption of wheat straw (1 mm, 45% dry matter) versus time

Table 4 Dependence between O_3 consumption and sugar accessibility of ozonized wheat straw (1 mm, 45% dry matter) for different flow rates (0.6 l/min and 0.125 l/min)

Flow rate (l/min)	Glucose (g/100 g)		Xylose (g/100 g)	
	0.6	0.125	0.6	0.125
Pretreatment time (h)				
Untreated	6.5	6.1	1.1	1
0.5	24	15	10	5.3
1	25	18.5	11	6.8
2	26	25.5	13	12
3	25	27	14.8	14.6
4	17	—	15	—
5.9	—	21	—	15.4
7	3.5	23.6	7.2	13

The sugar accessibility was measured by means of enzymatic hydrolysis

pretreated in the laboratory scale reactor (5 g) and in the bench scale reactor (100 g) for 7 h (Fig. 8). It was found that the total amount of O_3 consumed by the wheat straw, i.e., 1 and 22 g, respectively, were proportional to the amount of straw used. Furthermore, lignin was degraded to the same extent after the same amount of time, e.g., 95% after 7 h pretreatment (Table 5). The measurements with two different reactors sizes indicate that up-scaling is possible which is required for industrial application.

Online Analysis of Lignin Degradation

O_3 consumption was the highest initially and declined during ozonization. After 4 h, a total of 20 g O_3 was consumed (Table 6). The consumption of O_3 with 1 and 2 mm particles was compared with the degradation of lignin and the amount of accessible sugars during the pretreatment (Fig. 6). The O_3 consumption was clearly related to the lignin degradation and the accessibility of the sugars. Similarly, the amount of CO_2 emitted during the process was related to the lignin degradation, and the O_3 consumption. CO_2 emission reaches its maximum in the beginning and declines to a level corresponding to the ambient CO_2 content when lignin degradation was completed (~95% degradation). Almost complete degradation of lignin was observed with 1-mm particles, but with 2-mm particles, the degradation did not exceed 80%. The overall loss of weight was 10% of the dry mass content for both 1 and 2 mm particles. In conclusion, O_3 consumption and CO_2 emission were reliable parameters for the measurement of the lignin degradation in real time. Those measurements were used for online monitoring of the progress of the process on a display.

Cost Calculation

It was found that 8 g of O_3 is required to degrade 50% of the lignin in 100 g wheat straw and 22.5 g to degrade 95% of the lignin. The power requirement for O_3 generation depends on the discharge parameters such as the distance between electrodes, O_3 concentration, and feed gas composition as described by Sung [25]. According to his finding, with air as feed gas, the O_3 content is 0.8% at 150 g/kWh, but this energy consumption is only for the discharge. It does not take the losses within the power supply into account, and this depends on the model. The energy requirement for lignin degradation is consequently 0.053 kWh for 50% degradation of 100 g wheat straw and 0.15 kWh for 95% degradation (Table 7). This requires that the O_3 in the exhaust gas can be recycled. If this is not the case, the O_3 production must be considered,

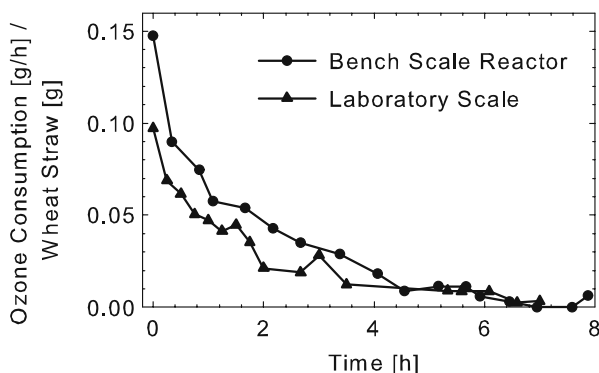


Fig. 8 Comparison of the pretreatment process with the laboratory scale and the bench scale reactor

Table 5 Comparison of the laboratory scale and the bench scale reactor

	Sample, dry weight (g)	O ₃ Total consumpt. (g)	g O ₃ /g wheat straw	Klason lignin (g/100 g)	Glucan (g/100 g)	Xylan (g/100 g)
Bench scale reactor	44	22	46%	1	41	20
Laboratory scale reactor	2.4	0.8–1	36%	1	40	20

not only the O₃ consumption. With an O₃ concentration of 0.8%, 50% lignin degradation can be achieved with an O₃ production of 11.5 g, and 95% degradation can be achieved with 80.6 g. The corresponding energy consumption, for 100 g wheat straw is 0.077 kWh (1 h) and 0.53 kWh (7 h). In these experiments, we used oxygen enriched air, but similar productivities can be obtained with the less expensive pure air.

Discussion

A system using UV absorption that allows continuous O₃ measurement in the feed and exhaust gas during a pretreatment process has been developed. This method has a number of advantages over previous methods described. As an example, the consumption of O₃ can be measured continuously, and this renders it possible to determine the progress of the pretreatment in real time. By the measurement of O₃ consumption, the extent to which lignin has been degraded can be estimated, thus abolishing the necessity to perform time consuming chemical analysis after acid hydrolysis. Others have used an iodometric titration method to detect the remaining O₃ in the exhaust gas, but this method is a more time consuming and does not allow following the process [10, 26]. Measurements limited to the amount of O₃ in the contacting gas at the start of the reaction have also been reported [27, 28]. Spectrophotometric determinations of the O₃ concentration at the inlet and at the outlet of the treatment chamber have been described [12] but only at low dry matter content. For industrial applications, this is not advantageous due to capacity and cost issues.

Table 6 Comparison of lignin degradation O₃ consumption and milled particle size of wheat straw (1, 2 mm)

WS in mm	Klason Lignin (g/100 g)		Glucan (g/100 g)		Xylan (g/100 g)		Arabinan (g/100 g)		Ash (g/100 g)		O ₃ consumption total (g)		CO ₂ emission (ppm)
	1	2	1	2	1	2	1	2	1	2	1	2	
Pretreatment time (h)													
Untreated	21	20	38	37	22	21	2.5	3	4	5	—	—	—
1	11	14	38	37	21	21	3	3	4	5	8	8	1640
2	7	9	40	36	22	20	3	3	4	5	12.5	14	1260
3	5	6	39	35	22	19	3	3	4	5	16.5	18	750
4	4	5	40	36	21	20	3	3	4	5	20	20.5	680
5	4	5	38	37	20	20	3	3	4	5	21	22.5	460
6	3	3	38	36	20	19	3	3	3	5	22	23.5	350
7	1	3.5	40	36	20	19	3	3	4	5	22.5	24	280

The total weight of each sample was 100 g, containing 50% dry matter

Table 7 The O₃ and energy requirement for a pretreatment time of 1 and 7 h are shown

Treatment time [h]	Lignin degradation [%]	O ₃ requirement [g/100 g WS]	Energy requirement [kWh/100 g WS]	O ₃ production [g/100 g WS]	Energy consumption [kWh/100 g WS]
1	50	8	0.053	11.5	0.077
7	95	22.5	0.15	80.6	0.53

The O₃ production rather than the O₃ consumption must be used for energy consumption calculations, when the O₃ in the exhaust gas cannot be recycled

A pretreatment process using O₃ to degrade lignin over a relatively short time at up to 65% dry matter of biomass was established. Consistent with previous observations [5, 10], it was found out that O₃ did not attack sugars. However, as shown by Binder [12], under reaction conditions highly different from ours, carbohydrates can be oxidized by O₃. In their study, acidic ozonization and more than 20 h of pretreatment was used, and the degradation of carbohydrates is probably due to the formation of carboxylic acids following the degradation of lignin. For all treatment processes of lignocellulosic biomass using O₃, acetic acid is a byproduct. This does not need to be a problem since the acetic acid can be converted into biogas which may be used for production of heat and electricity.

Earlier pretreatment studies carried out with 50% dry matter showed that the reaction time to reach 80% digestibility of the biomass, increased fourfold with an increase in particle size from 0.5 to 1 mm [29]. This is advantageous since such shavings are much less expensive to produce than finely milled material. At this particle size (0.5 mm) and dry matter contents of 90–95%, we found an O₃ consumption only slightly reduced compared to a mixture of the polymeric constituents of wheat straw, i.e., cellulose, hemicellulose, and lignin. This demonstrates that pretreatment can be carried out at high dry matter contents. At higher particle size (1 mm), the O₃ consumption was significantly reduced, signifying lower reaction rate. We found that plasma-assisted pretreatment with 1-mm particles can be performed at dry matter contents of 45–65% with an optimum at 50%. Consistent with our observations, Silverstein [14] reported no effect of O₃ on lignin at approximately 10% dry matter. The results of our investigation suggested that water was needed to ensure that O₃ could diffuse into the plant material and attack lignin from the inside of the plant.

The described method could be the basis for separation of lignocellulosic material into cellulose, hemicellulose, and lignin with the aim to facilitate production of high-value byproducts derived from cellulose-based bioethanol production. Oxygen permeability of biopolymer films are comparable to values found for traditional films [30], making them potentially useful for food packaging. This can become important since the majority of plastic materials for packaging such as ethylene vinyl alcohol and polyvinyl alcohol [31] are still based on fossil resources. Hemicelluloses are particularly interesting in this context. The degradation products of lignin could potentially also be recovered and reused. The most common adhesives used in the particle board industry are urea formaldehyde and phenol formaldehyde resins, the latter of which being the more popular due to their high water resistance securing their suitability for outdoor applications. The phenolic portion of phenol-formaldehyde resins could possibly be replaced with lignin, based on the chemical similarity between lignin degradation products and phenolics [32]. A successful replacement of phenol would also imply a substantial cost reduction since lignin is considerably cheaper than phenol [33].

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