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Pretreatment of Reed by Wet Oxidation and Subsequent Utilization of the Pretreated Fibers for Ethanol Production

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Abstract Common reed (*Phragmites australis*) is often recognized as a promising source of renewable energy. However, it is among the least characterized crops from the bioethanol perspective. Although one third of reed dry matter is cellulose, without pretreatment, it resists enzymatic hydrolysis like lignocelluloses usually do. In the present study, wet oxidation was investigated as the pretreatment method to enhance the enzymatic digestibility of reed cellulose to soluble sugars and thus improve the convertibility of reed to ethanol. The most effective treatment increased the digestibility of reed cellulose by cellulases more than three times compared to the untreated control. During this wet oxidation, 51.7% of the hemicellulose and 58.3% of the lignin were solubilized, whereas 87.1% of the cellulose remained in the solids. After enzymatic hydrolysis of pretreated fibers from the same treatment, the conversion of cellulose to glucose was 82.4%. Simultaneous saccharification and fermentation of pretreated solids resulted in a final ethanol concentration as high as 8.7 g/L, yielding 73% of the theoretical.

Keywords Lignocellulose · *Phragmites australis* · Pretreatment · Wet oxidation · Fermentation · SSF · Ethanol

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Abbreviations

DM	dry matter
ECC	enzymatically converted cellulose
FPU	filter paper unit
HPLC	high performance liquid chromatography
IU	international unit
SSF	simultaneous saccharification and fermentation
WO	wet oxidation

Introduction

Common reed, *Phragmites australis*, is a large perennial grass native to wetland habitats at temperate and tropical latitudes [1]. It usually forms vast belts along shores of freshwater lakes, streams, as well as other wetlands. Reed beds may have important ecological functions [2, 3] which has led some authors to argue for its protection [4–6]. However, the rapid expansion of reed [7] may just as well represent a serious threat to natural ecosystems; due to its aggressive and persistent survival strategies, reed can easily dominate over other plant species and thereby destroy the biodiversity of wetland habitats [8, 9].

Reed is one of the most widely distributed plant species on Earth covering over ten million hectares [10]. It has numerous traditional applications in rural areas worldwide (including forage and bedding for livestock, use as structural material for dwellings and fences, and as weaving material for ropes, mats, and carrying nets), but it is rarely, if ever, really cultivated. Probably for the same reason and also because it grows profusely and uncontrolled wherever it occurs only few data are available on its production [10, 11]. Despite looking fairly similar everywhere, reed may vary a lot depending on the location and growth conditions [12–15] with quality and yield of the produced biomass varying accordingly [16–18].

Due to its fast-growing properties and high biomass yields (estimated to range between 15 to 35 tonnes on dry basis per hectare annually for the aboveground biomass), reed is increasingly recognized as a promising source of renewable energy [19–23]. The properties of common reed as an energy plant closely resemble those of reed canary grass (*Phalaris arundinacea*) grown increasingly as a dedicated energy crop [24–27]. Their carbon content (45–47% (*w*/*w*) on a dry basis) is fairly similar, as well as their minimum heating value (approximately 17 MJ/kg). In contrast to reed canary grass, common reed does not need to be fertilized and is more productive [12, 28]. It can be collected for bioenergy throughout the year; green reed is ideally suited for the production of biogas [29], whereas delayed harvest gives a dry and storable material that can be directly burned in boilers as shreds, pellets, briquettes, or whole bales, without artificial drying [30]. The level of undesired elements that may cause fouling and corrosion problems in the boilers when released during combustion (e.g., nitrogen, sulfur, chlorine, or potassium) are significantly reduced when the feedstock is harvested after it has overwintered in the field compared to collecting it in the end of the growing season [28, 31, 32]. Harvesting at various seasons and locations year by year helps to avoid thinning or other means of destruction. This flexibility in the availability and possible uses of reed may make it an attractive raw material in various multi-feedstock technologies to produce bioenergy.

The few investigations carried out so far with common reed as an energy crop have primarily been focusing on the exploitation of its lignin content (20–25%, *w*/*w*) to produce solid fuel [30, 33, 34], and despite its relatively high cellulose (33–36%, *w*/*w*)

and hemicellulose (20–22%, *w*/*w*) contents, its potential convertibility to fuel ethanol and related chemicals has not been deeply investigated so far. In fact, reed is among the least studied lignocellulose crops ever considered for liquid biofuel production and to the best of our knowledge the present paper is one of the first to report experimental research in this topic.

The overall aim of the present work was to use the common reed as feedstock for the production of bioethanol. This was done by (1) pretreating the reed by wet oxidation to produce a cellulose-enriched feedstock with improved accessibility to hydrolytic enzymes, (2) enzymatically converting the wet oxidized material into glucose, and (3) fermenting cellulose-enriched solids to ethanol using simultaneous saccharification and fermentation.

Materials and Methods

Chemicals

All chemicals were of analytical grade and obtained from Sigma–Aldrich (St. Louis, MO, USA). Enzymes used in the hydrolysis experiments were obtained from Novozymes A/S (Bagsværk, Denmark). Distilled water was used throughout, except for the chromatographic procedure wherein Millipore water was used.

The Feedstock

Reed (approximately 10 kg in total on dry basis) was collected from the shores of Lake Balaton in Western Hungary. It was cut in February 2005 when the lake was frozen. The stalk above the ice with a height of about 1.7 m was harvested by cutting manually. The approximately 0.4 m of stalk below the ice was not used. Collected stalks were air-dried to 94% dry matter (DM), chopped, and milled to a particle size of 2 mm using an IKA-MF 10 laboratory grinder (IKA, Stauffen, Germany). This milled reed was stored at room temperature and used as the starting material throughout the work.

Pretreatment of Reed by Wet Oxidation

Wet oxidation (WO) of reed was carried out in a specially designed 2-L loop reactor constructed at Risø National Laboratory [35–37] using similar experimental conditions that were previously found to be suitable to treat other hollow-structure stalks [38, 39]. Alkali (2 g/L Na_2CO_3) was added to 60 g DM reed in 1 L of water. This was followed by the addition of oxygen (12 bar at room temperature) to the remaining gas volume. The gas–liquid mass transfer was accomplished by mixing with an impeller wheel. Pretreatment reactions were carried out at different temperatures (185, 190, 195, and 200 °C) at a constant reaction time of 12 min. Due to the good heat-transfer conditions (the reactor was mounted on a rack facilitating the control of temperature by immersing the reactor in an appropriate heating or cooling bath), the heating and cooling times were relatively short (3 and 1 min, respectively). Each WO treatment was performed in duplicates and the order of the experiments was randomized. The slurries obtained after WO were separated into a liquid fraction (hereafter referred to as the hydrolysate) and a solid fraction (also referred to as the fibrous fraction) by filtration. Filter cakes were dried in a climate chamber at 20 °C and 65% relative humidity to a constant weight. These samples were used as feedstock for hydrolysis and fermentation studies.

Analysis of Native and Pretreated Reed

The composition of the raw material as well as that of the dried solids obtained after WO was analyzed using a two-step acid hydrolysis procedure based on the method of Hägglund [40] as modified by Kaar et al. [41]. Briefly, about 0.15 g DM of sample was weighed and hydrolyzed in 1.5 mL of 72% H_2SO_4 for 1 h at 30 °C. After dilution with 42.5 mL of water the reaction mixtures were autoclaved at 121 °C for 1 h. Samples were filtered through a 0.2 μm cellulose ester filter (Schleicher and Schuell, Dassel, Germany) and monosaccharides were analyzed using high performance liquid chromatography (HPLC). Components of interest were separated on an organic acid Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, CA, USA) at 65 °C, equipped with a matching precolumn (Carbo-H). The mobile phase was 5 mM H_2SO_4 used at a flow rate of 0.5 mL/min. Monosaccharides were detected with a refractive index detector (Shimadzu Corp., Kyoto, Japan). Conversion factors for the dehydration on polymerization were 162/180 for glucose to cellulose, and 132/150 for the sum of xylose and arabinose to hemicellulose. Important to note, that although the employed column does not resolve xylose, mannose, and galactose, the composition of reed with xylose largely dominating over the other two compounds [42] allows the recognition of their common signal mainly as xylose.

The acid-insoluble residue of the solid fraction was separated from the acid hydrolysate by filtration and reported as Klason lignin. Ash content was determined by igniting samples at 550 °C for 3 h and then weighing.

Monosaccharides in the liquid fractions of WO-treated reed were quantified by HPLC after dilute sulfuric acid hydrolysis (4% (*w*/*w*) H_2SO_4, 121 °C, 10 min) of samples, using the procedure described above.

Enzymatic Hydrolysis of Pretreated Reed to Fermentable Sugars

To determine the enzymatic convertibility of pretreated fibers to glucose, solid fractions obtained after WO were hydrolyzed using cellulases. Solids (0.1 g DM) were diluted to 2% (*w*/*w*) in 0.2 M sodium acetate buffer (pH 4.8) in test tubes and after adding appropriate enzymes the resulting suspensions were incubated at 50 °C for 48 h with intensive magnetic stirring. Experiments were done in triplicates.

The enzyme preparation, Celluclast 1.5 L, which contains endoglucanase, cellobiohydrolase, and β-glucosidase, was added at 25 FPU/g solids DM. To improve the conversion of cellulose to glucose [43, 44], Novozym 188 was added at 25 IU/g solids DM as a complementary source of β-glucosidase. Enzyme activities were determined according to Ghose [45] for the overall cellulase activity and Berghem and Pettersson [46] for the β-glucosidase activity, and they were expressed as filter paper unit (FPU) and international unit (IU), respectively.

Supernatants of the reaction mixtures collected after 48 h of hydrolysis were analyzed by HPLC. The percentage (*w*/*w*) of total cellulose content in the pretreated material converted to glucose was calculated and expressed as enzymatically converted cellulose (ECC). The converted cellulose (g) is 90% of the glucose produced (g) because water binds during hydrolysis. Accordingly, the conversion factor introduced above (162/180) is to be used in these calculations too.

Simultaneous Saccharification and Fermentation of Pretreated Reed to Ethanol

To evaluate the efficiency of the pretreatments, solids obtained after WO were subjected to simultaneous saccharification and fermentation (SSF) as follows. Pretreated fibers were

suspended to 5% (*w*/*v*) DM in 150 mL of fermentation medium containing 1.0 g/L of KH_2PO_4, 0.3 g/L of $MgSO_4$, and 2 g/L of NH_4Cl in 200-mL flasks. After adjusting the pH to 4.8 and adding Celluclast 1.5 L (25 FPU/g cellulose) and Novozym 188 (25 IU/g cellulose), flasks were inoculated with *Saccharomyces cerevisiae* (2 g/L DM). The *S. cerevisiae* cells were harvested from a 1-day-old inoculum prepared under sterile conditions in the above salt medium with added glucose(50 g/L), peptone (5 g/L), and yeast extract (2.5 g/L) at 30 °C with shaking (250 rpm).

SSF was performed under semi-anaerobic conditions in capped flasks allowing off-gas outlet but restricting air inlet. The evolution of carbon dioxide known to correlate well with the metabolic activity of the yeast was monitored by following the volume of the gas outlet. This was done using an apparatus constructed at the Budapest University of Technology and Economics which was based on the rationale of the HaloteC Alcohol Fermentation Monitor (HaloteC Instruments BV, Zoetremeer, The Netherlands). The metabolic activity of eight fermentations was monitored simultaneously via the off-gas signal. For the registration of mass-flow as well as for data handling compatible software was used. SSF experiments (duplicates of each) were carried out with magnetic stirring at 32 °C under low agitation (50 rpm) for 3 days.

Flasks were sampled at the end of the fermentation, which corresponded with the off-gas signal reaching a plateau. The concentration of ethanol in the samples was determined by HPLC, as described earlier. The ethanol yield is given as a percentage of the theoretical, which is 0.51 g of ethanol per gram of glucose.

Results and Discussion

Analysis of the Pretreated Material

Slurries obtained after WO were subjected to phase separation and the resulting solid and liquid fractions were analyzed for their compositions (Table 1). To obtain a better understanding on the performance of WO under various temperatures, the distribution of recovered cellulose and hemicellulose between the two phases was evaluated with a special view on the non-recovered portions (losses) of the two components (Fig. 1).

After WO at different temperatures the recovery of insoluble solids (calculated with reference to the DM content of samples before treatment) ranged between 54.1% and 63.7% with the highest value obtained using the lowest temperature. As a function of increasing temperature, a clear decrease in the recovery of reed DM in the solids was observed. Accordingly, the solubilization of reed followed an opposite order and was more evident at higher temperatures.

Each WO treatment produced a cellulose-enriched solid fraction with glucan contents ranging between 42.4% and 49.2%, which were by 29.3% to 50.0% higher than that of untreated reed (32.8%). As expected, WO at the lowest temperature (185 °C) gave the best recovery of cellulose in the fibrous fraction (88.3%), while the highest glucan content in the pretreated solids (49.2%) was achieved by WO at 195 °C. Important to note, that the recovery of cellulose in this sample was only slightly lower than the maximum recovery obtained (87.1% vs. 88.3%). In contrast, a further increase in the processing temperature by only 5 °C (i.e., up to 200 °C) resulted in a dramatic decrease in cellulose recovery (70.0%). This was, however, not accompanied by a proportionally higher recovery of cellulose-derived sugars in the liquid fraction of the corresponding treatment. Although the portion of glucan recovered as soluble sugars in the hydrolysates clearly followed the order of

Table 1 Composition of solid and liquid fractions obtained after WO treatment of reed (60 g/L reed DM, 2 g/L Na_2CO_3, 12 bar O_2, 12 min) at different reaction temperatures (185, 190, 195, and 200 °C) with reference to the untreated material.

	Untreated reed	Reed pretreated by WO at various temperatures			
		185 °C	190 °C	195 °C	200 °C
Composition of pretreated solids with reference to the untreated material (g/100 g raw material DM)					
DM	100	63.73 (100)	61.66 (100)	58.07 (100)	54.12 (100)
Glucan	32.82	28.97 (45.46)	28.38 (46.03)	28.59 (49.23)	22.97 (42.44)
As percentage of the original	100%	88.27%	86.47%	87.10%	69.99%
Xylan	17.38	10.46 (16.42)	8.37 (13.57)	7.35 (12.66)	3.31 (6.12)
Arabinan	2.53	0.75 (1.17)	0.49 (0.80)	0.00 (0.00)	0.00 (0.00)
Xylan+Arabinan	19.91	11.21 (17.59)	8.86 (14.37)	7.35 (12.66)	3.31 (6.12)
As percentage of the original	100%	56.30%	44.48%	36.92%	16.64%
Lignin	24.85	12.98 (20.37)	12.19 (19.78)	10.35 (17.82)	11.16 (20.62)
As percentage of the original	100%	52.23%	49.05%	41.65%	44.91%
Ash	2.08	2.16 (3.39)	1.94 (3.15)	1.93 (3.33)	1.97 (3.64)
Composition of liquid side fractions with reference to the untreated material (g/100 g raw material DM)					
Glucan	–	1.44 (0.96)	1.58 (1.05)	1.71 (1.14)	1.82 (1.21)
As percentage of the original	–	4.39%	4.80%	5.21%	5.54%
Xylan	–	6.05 (4.13)	8.01 (5.46)	9.26 (6.32)	8.65 (5.90)
Arabinan	–	0.89 (0.60)	1.05 (0.72)	1.03 (0.68)	0.82 (0.56)
Xylan+Arabinan	–	6.94 (4.73)	9.06 (6.18)	10.29 (7.00)	9.47 (6.46)
As percentage of the original	–	34.86%	45.50%	51.68%	47.56%

Contents of compounds as g/100 g pretreated material DM are given in parentheses

increasing WO temperatures, no significant difference in their absolute concentration (ranging between 4.4% at 185 °C to 5.5% at 200 °C) was observed. This means, that WO at 200 °C resulting in the lowest recovery of cellulose in the solids (70%) performed the poorest also in terms of overall cellulose recovery (75.5%). The other treatments characterized by a significantly lower loss of glucan (7.3% to 8.7% vs. 14.5% at 200 °C) were fairly comparable from this perspective (Fig. 1A).

The removal of hemicellulose from the fibrous fraction with a maximized recovery of derivative carbohydrates in the hydrolysate and a minimized formation of undesired degradation products [47–49] is a key that a viable pretreatment process has to answer. The latter issue was however not addressed in the present study. The hemicellulose content decreased gradually in the solids as a function of increasing pretreatment temperature, dropping to as low as 6.1% from the original 19.9% at the highest temperature applied (200 °C). The recovery of hemicellulose-derived sugars in the hydrolysate followed an opposite order and improved gradually from 34.9% to 51.7% with increasing WO temperature from 185 to 195 °C. However, a further increase in the processing temperature by 5 °C (up to 200 °C) resulted in a significant decrease in hemicellulose recovery in the liquid fraction (47.6%), supposedly because the higher severity under these conditions facilitated the formation of low molecular weight degradation products from hemicellulose. For the overall recovery of hemicellulose a similar pattern was observed than for cellulose. Namely, the WO treatments at 180, 190, and 195 °C performed similarly to each other with a low net loss of material (8.9–11.4%), while the treatment at 200 °C resulted in a much poorer overall recovery with 35.8% of the initial hemicellulose content non-recovered in either fraction (Fig. 1B).

The lignin content, which was 24.9% in the untreated material, dropped to 17.8% to 20.6% in the solid residues after WO, corresponding to a relative decrease by 17.0% as the lowest

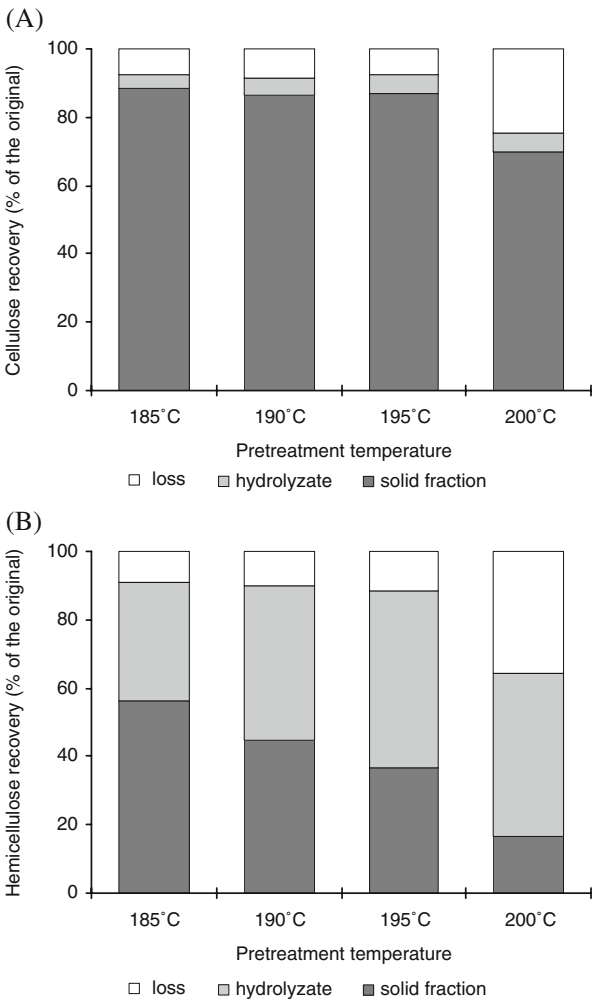

Fig. 1 Recovery of cellulose (**A**) and hemicellulose (**B**) in the solids fraction (*dark fill*) and in the hydrolysate (*light fill*) obtained after WO treatment of reed (60 g/L reed DM, 2 g/L Na_2CO_3, 12 bar O_2, 12 min) performed at different processing temperatures (185, 190, 195, and 200 °C). The loss of material (*no fill*) is defined as the nonrecovered portion of cellulose and hemicellulose, respectively, originally present in untreated reed

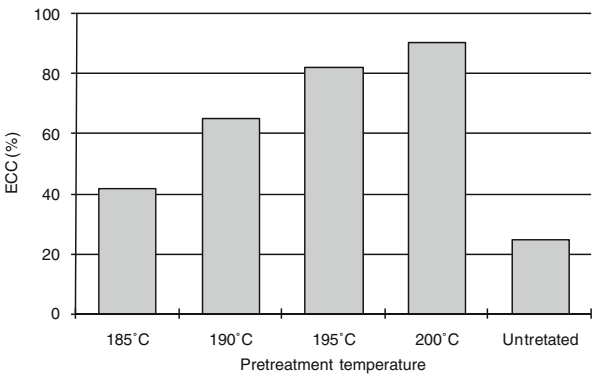

Fig. 2 Enzymatic convertibility of WO-treated reed fibers (60 g/L reed DM, 2 g/L Na_2CO_3, 12 bar O_2, 12 min) as a function of pretreatment temperature (185, 190, 195, and 200 °C), compared to untreated reed used as the control. Conversion of cellulose to glucose obtained after 48 h of hydrolysis (2% solids DM, pH 4.8, 50 °C) using a mixture of Celluclast 1.5 L (25 FPU/g biomass DM) and Novozym 188 (25 IU/g biomass DM) is reported as ECC

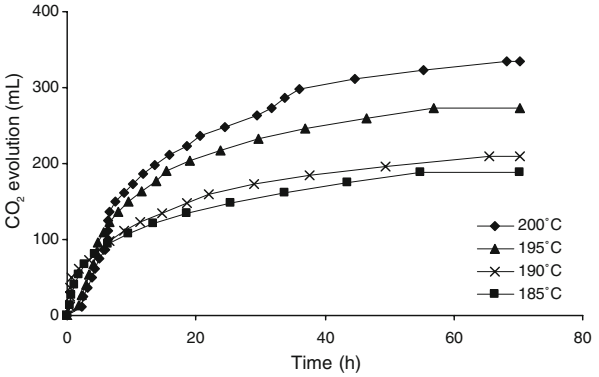

Fig. 3 Simultaneous saccharification and fermentation of pretreated reed fibers originating from WO (60 g/L reed DM, 2 g/L Na_2CO_3, 12 bar O_2, 12 min) performed at four different reaction temperatures (185, 190, 195, and 200 °C) as monitored via off-gas release. Conditions of the SSF were: 5% (*w*/*v*) of WO reed DM, salt medium (1.0 g/L KH_2PO_4, 0.3 g/L $MgSO_4$, 2 g/L NH_4Cl), 25 FPU/g cellulose of Celluclast 1.5 L, 25 IU/g cellulose of Novozym 188, *S. cerevisiae* corresponding to 2 g/L DM, pH 4.8, 32°C, 3 days

(200 °C) and 28.3% as the highest (195 °C). As a more informative measure of lignin removal, the portions of solubilized lignin (ranging between 47.8% to 58.3% of the original) were considered. The highest delignification (58.3%) was obtained after WO at 195 °C.

Conversion of Pretreated Reed to Ethanol

Analysis of pretreated material in comparison to the untreated reference provides a good basis to predict the efficiency of a given treatment. This information on its own, however, does not guarantee a reliable choice of optimal pretreatment methodology. In the present case, it was essential to collect information also on the enzymatic digestibility of cellulose-enriched solids of WO-treated reed to fermentable sugars first and, thereafter, check its convertibility to ethanol.

Pretreatment by WO improved the enzymatic convertibility of reed cellulose to glucose under all process conditions applied (Fig. 2). There was a gradual increase in obtained EEC

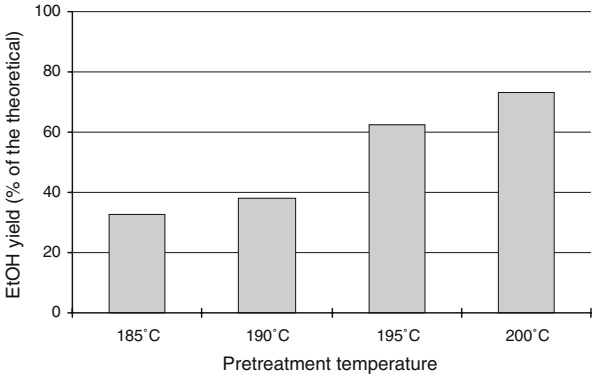

Fig. 4 Ethanol yield after SSF of WO-treated reed fibers (60 g/L reed DM, 2 g/L Na_2CO_3, 12 bar O_2, 12 min) as a function of pretreatment temperature (185, 190, 195, and 200 °C). Conditions of the SSF were: 5% (*w*/*v*) of WO reed DM, salt medium (1.0 g/L KH_2PO_4, 0.3 g/L $MgSO_4$, 2 g/L NH_4Cl), 25 FPU/g cellulose of Celluclast 1.5 L, 25 IU/g cellulose of Novozym 188, *S. cerevisiae* corresponding to 2 g/L DM, pH 4.8, 32 °C, 3 days. Yields are given in the percentage of the theoretical based on the cellulose contents of WO-treated reed fibers

from 41.7% to 90.5% as a function of increasing WO temperature from 185 to 200 °C, with the best conversion being 3.7 times higher than that obtained with the untreated control (24.7%). As visualized from the off-gas signal (Fig. 3) proportional to CO_2 release, yeast cells were metabolically active at least for 48 h under the applied conditions. The evolution of CO_2 obtained with different WO-treated samples followed the order of the increasing reaction temperatures that the samples were subjected to during the WO treatment. The obtained ethanol yield—ranging from 32.5% to 73.2% of the theoretical—followed a similar order, with the highest ethanol production obtained when WO of reed was carried out at 200 °C as the pretreatment temperature (Fig. 4).

Conclusion

The present paper reports data on the performance of wet oxidation at various temperatures (185, 190, 195, and 200 °C) in terms of its ability to improve the enzymatic digestibility of reed for bioethanol production.

All applied WO treatments resulted in a cellulose-enriched solid fraction and a liquid fraction rich in hemicellulose derived sugars, with a considerable rate of delignification of fibers obtained in all cases. In general, the increase in pretreatment temperature improved the performance of WO as far as 195 °C, while a further increase in reaction temperature to 200 °C resulted in a drop in some of the quality measures. As the best treatment, WO at 195 °C resulted in a cellulose recovery in the solids close to the highest obtained (87.1% vs. 88.3% achieved at 200 °C), and the highest recovery of hemicellulose derived carbohydrates in the hydrolysate (51%). The delignification of solids was also the highest (58.4%) in this case. Enzymatic digestibility and SSF of WO-treated reed fibers improved gradually with increasing the temperature of the treatment. Under the applied conditions, the highest conversion of cellulose to glucose (90.5%) and the highest ethanol yield after SSF (73.2% of the theoretical) were obtained with reed pretreated at 200 °C. Nevertheless, because high recovery was considered a priority in this preliminary study, WO at 195 °C was considered as the best pretreatment method studied, which resulted in a conversion of cellulose to glucose and a theoretical ethanol yield only by approximately 9% lower than those obtained at 200 °C.

Based on its availability and the obtained results reed can be considered as a candidate raw material in a multi-feedstock second-generation ethanol technology, probably not only in the close proximity of its natural habitat but also where it can efficiently be cultivated as a dedicated energy crop.

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References

1. den Hartog, C., Květ, J., & Sukopp, H. (1989). *Aquatic botany*, *35*, 1–4. doi:10.1016/0304-3770(89)90062-4.
2. Ditlhogo, M. K. M., James, R., Laurence, B. R., & Sutherland, W. J. (1992). *Journal of Applied Ecology*, *29*, 265–276. doi:10.2307/2404495.
3. Cowie, N., Sutherland, W. J., Ditlhogo, M. K. M., & James, R. (1992). *Journal of Applied Ecology*, *29*, 277–284. doi:10.2307/2404496.

4. Ostendorp, W. (1989). *Aquatic Botany*, *35*, 5–26. doi:10.1016/0304-3770(89)90063-6.
5. Tscharntke, T. (1992). *Conservation Biology*, *6*, 530–536. doi:10.1046/j.1523-1739.1992.06040530.x.
6. Rolletschek, H. (1999). *Limnologica*, *29*, 86–92. doi:10.1016/S0075-9511(99)80043-7.
7. Chambers, R. M., Meyerson, L. A., & Saltonstall, K. (1999). *Aquatic Botany*, *64*, 261–273. doi:10.1016/S0304-3770(99)00055-8.
8. Marks, M., Lapin, B., & Randall, J. (1994). *Natural Areas Journal*, *14*, 285–294.
9. Warren, R. S., Fell, P. E., Grimsby, J. L., Buck, E. L., Rilling, G. C., & Fertik, R. A. (2001). *Estuaries*, *24*, 90–107. doi:10.2307/1352816.
10. Allirand, J. -M., & Gosse, G. (1995). *Biomass and Bioenergy*, *9*, 441–448. doi:10.1016/0961-9534(95)00042-9.
11. Kauppi, P., Selkäinaho, J., & Puttonen, P. (1983). *Annales Botanici Fennici*, *20*, 51–55.
12. Daniels, R. E. (1991). *Aquatic Botany*, *42*, 41–48. doi:10.1016/0304-3770(91)90104-D.
13. Lissner, J., & Schierup, H. -H. (1997). *Aquatic Botany*, *55*, 247–260. doi:10.1016/S0304-3770(96)01085-6.
14. Kühl, H., Koppitz, H., Rolletschek, H., & Kohl, J. G. (1999). *Aquatic Botany*, *64*, 235–246. doi:10.1016/S0304-3770(99)00053-4.
15. Hansen, D. L., Lambertini, C., Jampeetong, A., & Brix, H. (2007). *Aquatic Botany*, *86*, 269–279. doi:10.1016/j.aquabot.2006.11.005.
16. Granéli, W. (1989). *Aquatic Botany*, *35*, 99–109. doi:10.1016/0304-3770(89)90070-3.
17. Weisner, S. E. B., & Granéli, W. (1989). *Aquatic Botany*, *35*, 71–80. doi:10.1016/0304-3770(89)90068-5.
18. Lenssen, J. P. M., Menting, F. B. J., van der Putten, W. H., & Blom, C. W. P. M. (1999). *Aquatic Botany*, *64*, 151–165. doi:10.1016/S0304-3770(99)00012-1.
19. Kresovich, S., Wagner, C. K., Scantland, D. A., Groet, S. S., & Lawhon, W. T.(1981). The utilization of emergent aquatic plants for biomass energy systems development. Report to Solar Energy Research Institute, Golden, Colorado Aquatic Species Program.
20. Kresovich, S., Wagner, C. K., Scantland, D. A., & Lawhon, W. T. (1981). *Biomass*, *1*, 127–144. doi:10.1016/0144-4565(81)90021-4.
21. Duke, J. A. (1983). *Handbook of Energy Crops: Phragmites australis (Cav.) Trin. ex Steud.* Center for New Crops and Plants Products, Purdue University, West Lafayette, IN. Available from: http://www.hort.purdue.edu/newcrop/duke_energy. Accessed: May 15, 2008.
22. Fren, K. (1997). *Plants for A Future: Edible and Useful Plants for A Healthier World*. Hampshire: Permanent.
23. Hagelberg, E., & Lyytinen, S. (2007). in: *Read Up on Reed* (Ikonen, I., and Hagelberg, E., eds.), Southwest Finland Regional Environment Centre, Turku, Finland, pp. 94-101.
24. Börjesson, P. (1999). *Biomass and Bioenergy*, *16*, 137–154. doi:10.1016/S0961-9534(98)00080-4.
25. Bridgeman, T. G., Jones, J. M., Shield, I., & Williams, P. T. (2008). *Fuel*, *87*, 844–856. doi:10.1016/j.fuel.2007.05.041.
26. Jasinskas, A., Zaltauskas, A., & Kryzeviciene, A. (2008). *Biomass and Bioenergy*, *32*, 981–987. doi:10.1016/j.biombioe.2008.01.025.
27. Pahkala, K., Aalto, M., Isolahti, M., Poikola, J., & Jauhiainen, L. (2008). *Biomass and Bioenergy*, *32*, 1009–1015. doi:10.1016/j.biombioe.2008.02.004.
28. Landström, S., Lomakka, L., & Andersson, S. (1996). *Biomass and Bioenergy*, *11*, 333–341. doi:10.1016/0961-9534(96)00041-4.
29. Hansson, P. A., & Fredriksson, H. (2004). *Agriculture Ecosystems & Environment*, *102*, 365–375. doi:10.1016/j.agee.2003.08.005.
30. Granéli, W. (1984). *Biomass*, *4*, 183–208. doi:10.1016/0144-4565(84)90056-8.
31. Burvall, J. (1996). *Biomass and Bioenergy*, *12*, 149–154. doi:10.1016/S0961-9534(96)00064-5.
32. Hadders, G., & Olsson, R. (1996). *Biomass and Bioenergy*, *12*, 171–175. doi:10.1016/S0961-9534(96)00047-5.
33. Björk, S., & Granéli, W. (1978). *Ambio*, *7*, 150–156.
34. Monti, A., Di Virgilio, N., & Venturi, G. (2008). *Biomass and Bioenergy*, *32*, 216–223. doi:10.1016/j.biombioe.2007.09.012.
35. Ahring, B. K., Jensen, K., Nielsen, P., Bjerre, A. B., & Schmidt, A. S. (1996). *Bioresource Technology*, *58*, 107–113. doi:10.1016/S0960-8524(96)00090-9.
36. Bjerre, A. B., Olesen, A. B., Fernqvist, T., Ploger, A., & Schmidt, A. S. (1996). *Biotechnology and Bioengineering*, *49*, 568–577. doi:10.1002/(SICI)1097-0290(19960305)49:5<568::AID-BIT10>3.0.CO;2-6.
37. Schmidt, A. S., & Thomsen, A. B. (1998). *Bioresource Technology*, *64*, 139–151. doi:10.1016/S0960-8524(97)00164-8.
38. Klinke, H. B., Ahring, B. K., Schmidt, A. S., & Thomsen, A. B. (2002). *Bioresource Technology*, *82*, 15–26. doi:10.1016/S0960-8524(01)00152-3.

39. Varga, E., Schmidt, A. S., Réczey, K., & Thomsen, A. B. (2003). *Applied Biochemistry and Biotechnology*, *104*, 37–50. doi:10.1385/ABAB:104:1:37.
40. Hägglund, E. (1951). *Chemistry of wood* pp. 420–428. New York: Academic.
41. Kaar, W. E., Cool, L. G., Merriman, M. M., & Brink, D. L. (1991). *Journal of Wood Chemistry and Technology*, *11*, 447–463. doi:10.1080/02773819108051086.
42. Boschker, H. T. S., & Cappenberg, T. E. (1998). *FEMS Microbiology Ecology*, *25*, 79–86. doi:10.1111/j.1574-6941.1998.tb00461.x.
43. Szczodrak, J. (1988). *Biotechnology and Bioengineering*, *32*, 771–776. doi:10.1002/bit.260320608.
44. Spindler, D. D., Wyman, C. E., Grohmann, K., & Mohagheghi, A. (1989). *Applied Biochemistry and Biotechnology*, *20/21*, 529–540. doi:10.1007/BF02936507.
45. Ghose, T. K. (1987). *Pure and Applied Chemistry*, *59*, 257–268. doi:10.1351/pac198759020257.
46. Berghem, L. E. R., & Pettersson, L. G. (1974). *European Journal of Biochemistry*, *46*, 295–305. doi:10.1111/j.1432-1033.1974.tb03621.x.
47. Palmqvist, E., & Hahn-Hägerdal, B. (2000). *Bioresource Technology*, *74*, 25–33. doi:10.1016/S0960-8524(99)00161-3.
48. Klinke, H. B., Olsson, L., Thomsen, A. B., & Ahring, B. K. (2003). *Biotechnology and Bioengineering*, *81*, 738–747. doi:10.1002/bit.10523.
49. Klinke, H. B., Thomsen, A. B., & Ahring, B. K. (2004). *Applied Microbiology and Biotechnology*, *66*, 10–26. doi:10.1007/s00253-004-1642-2.