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# Development of Chemical and Biological Processes for Production of Bioethanol: Optimization of the Wet Oxidation Process and Characterization of Products

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# Abstract

The combination of the wet oxidation process (water, oxygen pressure, elevated temperature) and alkaline hydrolysis was proven to be efficient in pretreating agricultural crops for conversion to high-value products. The process was evaluated in order to efficiently solubilise the hemicellulose, degrade the lignin, and open the solid crystalline cellulose structure of wheat straw without generating fermentation inhibitors.

The effects of temperature, oxygen pressure, reaction time, and concentration of straw were investigated. The degree of delignification and hemicellulose solubilisation increased with reaction temperature and time. The optimum conditions were 15 minutes at 185°C, producing 9.8 g/L solubilised hemicellulose. For quantification of the solubilised hemicellulose the hydrolysis with 4 %w/v sulfuric acid for 10 minuteswas used. Even though the Aminex HPX-87H column was less sensitive towards impurities than the HPX-87P column. The former gave improved recovery and reproducibility, and was chosen for routine quantification of the hydrolysed hemicellulose sugars.

The purity of the solid cellulose fraction also improved with higher temperature. The optimum condition for obtaining enzymatic convertible cellulose (90%) was 10 minutes at 170°C using a high carbonate concentration. The hemicellulose yield and recovery were significantly reduced under these conditions indicating that a simultaneous optimal utilisation of the hemicellulose and cellulose was difficult.

Wet oxidation was compared with hydrothermal processing (without oxygen). The oxygen pressure and sodium carbonate concentration had little effect on the solubilisation of hemicellulose; however, by combining wet oxidation with alkaline hydrolysis the formation of 2-furfural, a known microbial inhibitor, was greatly reduced.

The wet oxidation was compared with steaming (steam, high temperature). Much more hemicellulose and lignin were solubilised by wet oxidation than by steaming. More cellulose was solubilised (and degraded) by steaming than by wet oxidation. Overall carbohydrate "losses" of 20.1 and 16.2% were found for the steaming and wet oxidation process, respectively. More 2-furfural was formed by steaming than by wet oxidation.

Preliminary calculations of the economics of the wet oxidation process showed that the raw material was the major contributor to the costs.

Preliminary studies of wet oxidation of flax resulted in high cellulose-rich fibres with high tensile strength for composite materials.

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# Contents

# Preface

This report presents the significant results of the work performed in the subproject "Optimisation of the Wet Oxidation Process and the Characterisation of Products" carried out in the Environmental Science and Technology Department at Risø National Laboratory from 01/07/94 to 31/12/96. This subproject was part of the project "Development of Chemical and Biological Processes for Production of Bioethanol" (J.No. EFP 1383/94-0003) carried out in collaboration with The Technical University of Denmark (DTU), Lyngby, funded by the Danish Ministry of Energy and Environment.

The project goal was to evaluate some biological and chemical processes for bioethanol production from fractionated lignocellulose, i.e. wheat straw. The wet oxidation process was studied as a pretreatment method to obtain 1) a solid cellulose-rich fraction accessible for enzyme treatment and 2) an aqueous hemicellulose-rich fraction without producing microbial inhibitors. The aqueous solutions achieved were sent to DTU for fermentation to ethanol. Analytical tools were developed to characterise the carbohydrate fractions.

**Acknowledgement**

The authors would like to thank Dr. Jürgen Puls, Institute of Wood Chemistry, Hamburg, Germany for analysis of some acid hydrolyzates for monosaccharide composition using borate-complex anion-exchange HPLC and for performing the steaming experiments. Thanks are also due to the Nordic Energy Research Programme for financing our study visit to Dr. Puls's laboratory in Hamburg. The assistance from Tomas Fernqvist, Trine Frederiksen, Birgit Jensen, Ingelis Larsen, Jette Bruun Nielsen, Annette Plöger, Tina Simonsen and Anders Woidemann at Risø National Laboratory is also gratefully acknowledged.

# 1 Introduction

Lignocellulosic biomass like wood and agricultural crop residues, e.g. straw and sugar-beet pulp, as well as new alternative industrial crops, *e.g.* flax, kenaf, hemp, miscanthus, willow, are potential raw materials for producing several high-value products like fuel ethanol, enzymes, xylitol, carboxylic acids, oligosaccharides, and biofibers, as up to 80% of the lignocellulose is polysaccharides (Bjerre *et al.*, 1996b; Biermann, 1983; Fan *et al.*, 1982; McGinnes *et al.*, 1983a). This use of raw materials is particularly important because of the large abundance of surplus plant material world-wide. These renewable raw materials look promising for replacing environmentally unfriendly fossil hydrocarbon raw materials, and hence, creating "green" products. In contrast to traditional fuels, fermentation ethanol does not contribute to the greenhouse effect, being a $CO_2$ neutral resource.

## 1.1 Lignocellulose

The production of ethanol from sugar and starch (fermentation by yeast) is a well-known technology, while making ethanol from lignocellulosic materials like straw and wood chips is more difficult and requires more process steps (Kosaric *et al.*, 1983). To optimise the processes the physical and chemical characteristics of lignocellulosic materials, located in the cell walls of all higher plants, must be understood. Lignocellulose is a complex raw material consisting of mainly three constituents, i.e. cellulose (35-45% of total dry weight), hemicellulose (20-40% in most annual plant residues mainly as xylan) and lignin (10-30%). The cellulose and hemicellulose are the most interesting for producing a variety of high-value products (Korte *et al.*, 1991; Schmidt & Bjerre, 1997a). Untreated lignocellulose is degraded only slowly by microorganisms due to the compact and stringent structure of cellulose and to the intimate association of the carbohydrates (cellulose and hemicellulose) with the lignin in plant cell walls (**Figure 1**) leaving very few reactive sites available for enzyme attachment (Büchert, 1990; Fengel & Wegener, 1989; Viikari *et al.*, 1991). Therefore, these potential sugar fractions are not directly accessible for enzyme treatment, and *e.g.* for ethanol production. Generally, if the carbohydrate content of lignocellulose is completely hydrolysed, cellulose is converted into glucose, while hemicellulose is converted into the pentoses xylose and arabinose and the hexoses glucose, galactose, and mannose.

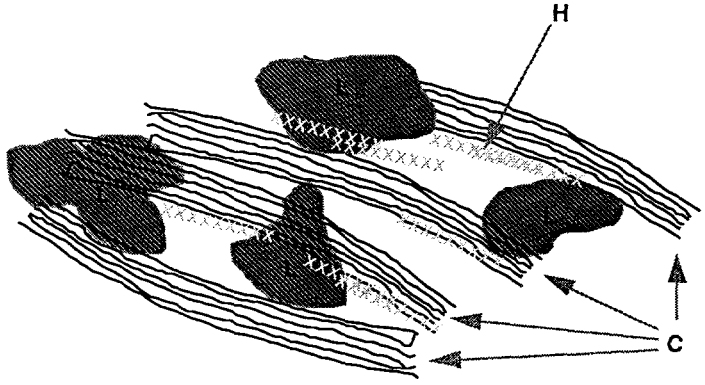


***Figure 1.*** *The hypothetical view of the fibre surface in lignocellulose: cellulose fibrils (C), lignin (L), and hemicellulose (H).*

### 1.1.1 Cellulose

Cellulose is a homogeneous linear high molecular weight polymer of D-glucose containing an average of 10,000 anhydroglucose units (**Figure 2**), and due to its β-1,4 linkages is a highly crystalline material resistant to enzymatic hydrolysis (Fan *et al.*, 1982). Extensive hydrogen bonds are formed between two parallel cellulose chains creating its highly rigid compact microfibril structure insoluble in water. Cellulose is unique as it is found only in microfibrils and never as individual molecules. The microfibrils are about 35 Å in diameter and contain about 40 cellulose chains in cross section. However, the microfibrils have both crystalline and amorphous regions (about two-thirds crystalline). Cellulose is the largest naturally occurring polymer and is up to 6 μm long with a molecular weight approaching two million. Considering its physical structure, cellulose is most suitable for use as fibre, *e.g.* in paper-making, fibreboards and reinforced composite materials or as a carbohydrate source for fermentation after hydrolysis to D-glucose (Büchert, 1990).

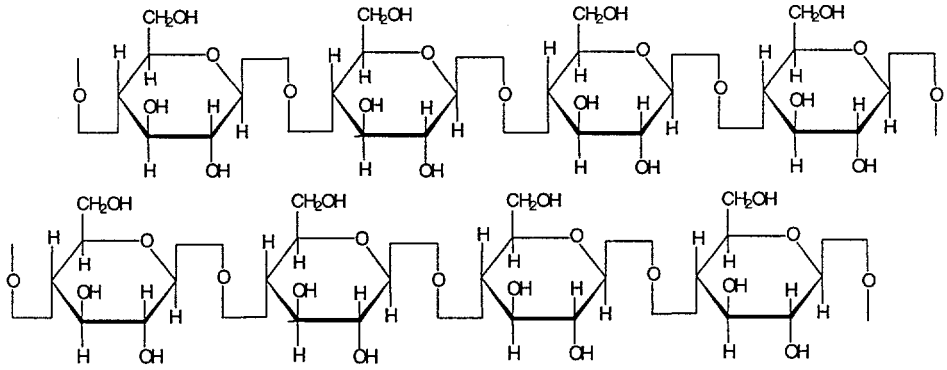

*Figure 2. The structure of the cellulose polymer.*

### 1.1.2 Lignin

Lignin is a strengthening material usually located between the cellulose microfibrils, where it serves to resist compressional forces. Besides its strengthening function, lignin also provides protection against attack by pathogens and consumption by herbivores, both insect and mammalian (Swain, 1979), due to its phenolic components.

Lignin is difficult to study as it is insoluble in most solvents primarily due to its high molecular weight (probably more than 10,000), complex structure, and chemical attachment to cellulose and other polysaccharides in the native state. This attachment is observed at various points mainly by ether linkages to the hydroxyl groups of polysaccharides. Lignins may be considered as three dimensional phenylpropane networks of phenolic compounds mainly based on the three aromatic alcohols, coniferyl, sinapyl, and p-coumaryl alcohol (**Figure 3**) (Fengel & Wegener, 1989) held together by ether and carbon-carbon bonds (Fan *et al.*, 1982). Lignin is formed by a free radical polymerisation reaction, which results in an absence of consistent repeating linkage between the monomeric units as proposed in **Figure 4**. This makes it even more difficult to completely characterise the chemical structure of the macromolecule. Therefore, lignins presumably always have variable structures. Most lignins also have varying amounts of related acids, specially ferulic, p-coumaric, and p-hydroxy benzoic acid, esterified to the core.

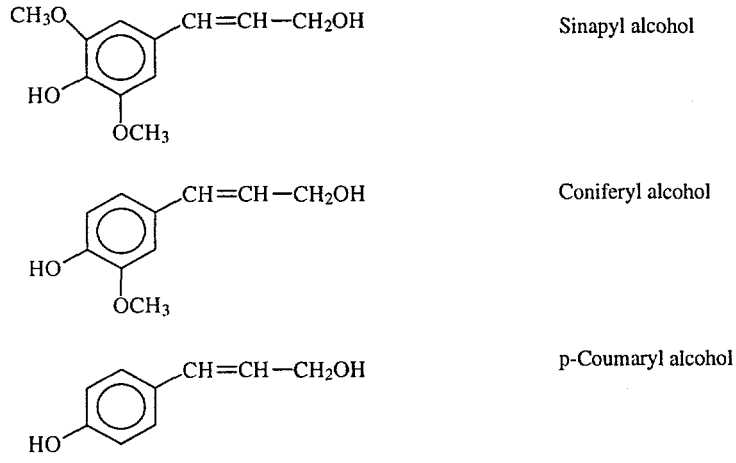


*Figure 3. The structure of lignin building blocks.*

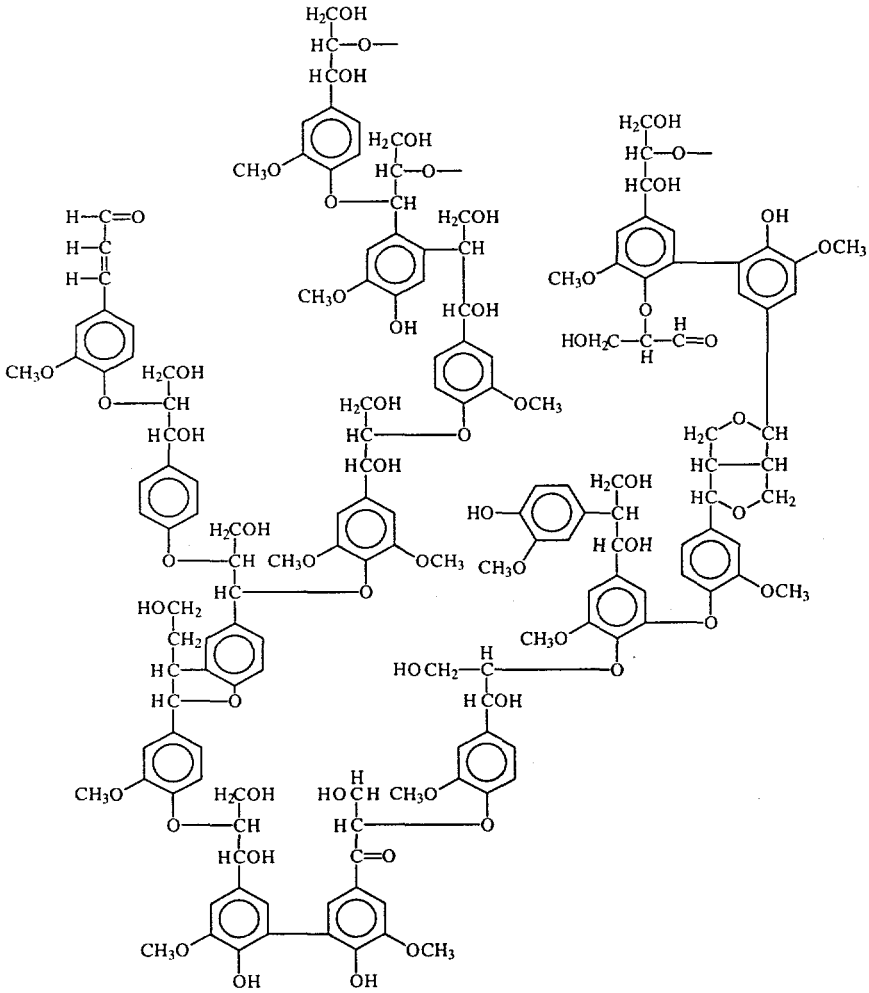

*Figure 4. The proposed structure of lignin in hardwood (Fengel & Wegener, 1989).*

Lignins from hardwood and grasses contain less coniferyl alcohol than softwood and considerably more sinapyl-derived units (**Figure 3**), and are also referred to as syringyl lignins. By increasing the sinapyl-derived content, major changes occur in the macromolecular structure. Because both methoxy groups are neighbouring the phenolic hydroxyl, they interfere spatially with reactions of this group and result in fewer ether bonds (**Figure 4**). The presence of the second methoxy group also considerably reduces carbon-carbon bonding. Consequently, this lignin has a lower degree of polymerisation, less condensation, and more free phenolic hydroxyls than softwood lignin. The lignin from grasses also has more related acids, particularly 3-12% p-coumaric acid and 1-3% ferulic acid, than have woods. This explains its greater fraction of soluble lignin. Hence, hardwoods are easier to delignify than softwood, and grasses easier than hardwood.

### 1.1.3 Hemicellulose

Hemicellulose is referred to mainly as a "cellulose"-associated plant cell wall polysaccharide, which can be extracted in alkaline solution. The role of the amorphous hemicellulose is to provide linkage between lignin and cellulose (Fan *et al.*, 1982). Unlike cellulose it has a heterogeneous structure consisting of various different sugars. Besides, the molecules are much shorter (DP < 200), more highly branched, and usually substituted. The most abundant of the hemicelluloses are the xylans (Coughlan & Hazlewood, 1993). Like most other polysaccharides, the monosaccharides are linked together with glucosidic bonds. Wheat straw hemicellulose consists mainly of arabino-4-O-methylglucurono xylan (**Figure 5**) with a degree of polymerisation of approximately 70 (Coughlan & Hazlewood, 1993).

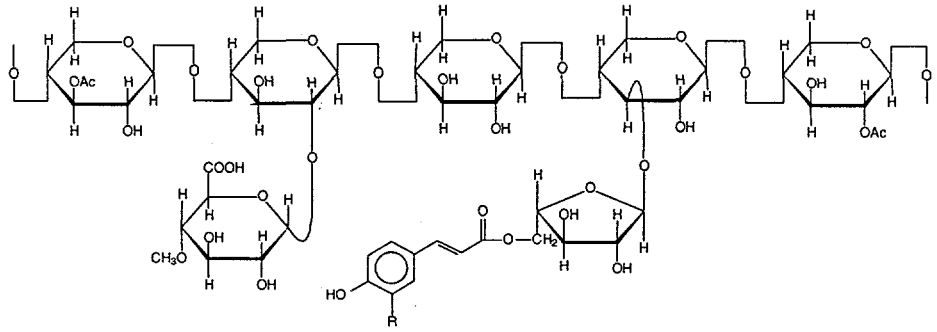


***Figure 5.*** *The theoretical structure of wheat straw (grasses) xylan (Puls & Schuseil, 1993). (Ac = acetyl groups, ferulic acid (R = OMe), p-coumaric acid (R = H), Me = methyl group)*

The polymer backbone is a xylan chain of D-xylose units with β-1,4 linkages. The xylan backbone is acetylated in the C-2 and C-3 position, up to 70% of the cell wall xylan, but only to about 2% in grasses. These acetyl-groups are an important barrier for the enzymatic hydrolysis (Kong *et al.*, 1992). The xylan is substituted with several different side-groups, such as L-arabinofuranose (by α-1,3 bonds) and 4-O-methyl-D-glucuronic acid (by α-1,2 bonds) (**Figure 5**), with about 1.5 arabinose and one 4-O-methyl-D-glucuronic acid for every 10 xylose backbone units. Grasses may have both 4-O-methyl and non-methylated glucuronic acid side groups (only about 1%). The deacetylated hemicellulose consists of about 85% D-xylose and 15% L-arabinose (Fidalgo *et al.*, 1993). Some of the L-arabinosyl residues are esterified with ferulic and p-coumaric

acids, which can form a dimer that is used in the cross-linking of the xylan molecules. Additionally, the ferulic and p-coumaric residues may be involved in covalent linking the xylan to lignin, since these residues are also present in the lignin (**Figure 4**).

## 1.2 Pretreatment

As the two polysaccharides, cellulose and hemicellulose, are not directly available for bioconversion, a pretreatment is needed to overcome the physical barrier of lignin and make the sugars available for the microorganisms (Büchert, 1990; Fengel & Wegener, 1989; Viikari *et al.*, 1991). Several pretreatment processes have been developed for lignocellulose, which function by an enlargement of the inner surface area. This is accomplished partly by solubilisation of the hemicellulose and partly by degradation of the lignin, which resutls in a fractionating of the components and opening of the cellulose structure. The processes are: milling and grinding, pyrolysis, high-energy radiation, high-pressure steaming, alkaline or acid hydrolysis, gas treatment (chlorine dioxide, nitrogen dioxide, sulfur dioxide, ozone), hydrogen peroxide treatment, organo-solvent treatment, hydrothermal treatment (water, elevated temperature), steam explosion, wet oxidation, and biological treatment (Fan *et al.*, 1982; Hörmeyer *et al.*, 1988; McGinnis *et al.*, 1983a).

When biomass is treated with water or steam alone, or with a small amount of acid, the process is classified as prehydrolysis, autohydrolysis, steaming, or steam explosion; when high-pressure oxygen or air is present, the reaction is called wet oxidation (Biermann, 1983). As autohydrolysis and steam explosion use only water and heat, these processes are relatively inexpensive. Additionally, if reaction temperatures and times are kept moderate, there is minimal carbohydrate degradation, but minimal fractionation as well (Biermann, 1983). However, in order to achieve a high yield and recovery of the carbohydrates high temperatures (> 200°C) are needed (Biermann, 1983) inducing carbohydrates degradation. The pretreatment method most often used is a steaming/steam explosion at a temperature of 190-230°C (Saddler *et al.*, 1993), an autohydrolysis process due to the hydrolytic action of acetic acid liberated from the acetyl xylan (Korte *et al.*, 1991; Saddler *et al.*, 1993).

Another method which has been applied in this project is wet oxidation (water, oxygen pressure, elevated temperature) (McGinnis *et al.*, 1983a; Bjerre *et al.*, 1996a; 1996b). Although, the additional requirement of oxygen and alkaline makes the wet oxidation process more expensive, the combination of the wet oxidation process and alkaline hydrolysis very efficiently fractionates wheat straw lignocellulose to a cellulose-rich and a hemicellulose-rich fraction (Biermann, 1983; Bjerre *et al.*, 1996b; Schmidt & Bjerre, 1997b) at relatively low temperatures (150-200°C).

The utilisation of hemicellulose hydrolyzates from steaming and steam explosion is complicated by the presence of degradation products of both sugar and lignin, *e.g.* 2-furfural and 5-hydroxymethyl-2-furfural (Büchert *et al.*, 1988; Büchert, 1990; McGinnis *et al.*, 1983). The production of these compounds is undesirable because of their known inhibitory effect against micro-organisms (Büchert, 1990; Yu *et al.*, 1987) requiring a costly detoxification step *prior* to fermentation (Büchert, 1990; von Sivers *et al.*, 1994). Von Sivers *et al.* (1994) estimated that such a detoxification step would account for about 22% of the total cost of producing ethanol from lignocellulosic materials. However, since the aliphatic aldehydes and saturated carbon bonds are very reactive under wet oxidation conditions (Bjerre & Sørensen, 1994) these inhibitors were not expected to be produced in great amounts or even to be stable in such a system.

Hence, the costly detoxification step might be bypassed making wet oxidation a more promising pretreatment process. Basically, the wet oxidation process had been found to convert a large number of organic molecules (*e.g.* lignin) to $CO_2$, $H_2O$ and simpler and more oxidised organic compounds, which are mainly low molecular weight carboxylic acids (Taylor & Weygandt, 1974; McGinnis *et al.*, 1983b). Furthermore, wet oxidation has been shown to produce fewer toxic compounds from the carbohydrate part of the lignocellulosic material (*e.g.* 2-furfural and 5-hydroxymethyl-2-furfural) than steaming (Schmidt *et al.*, 1996; this report).

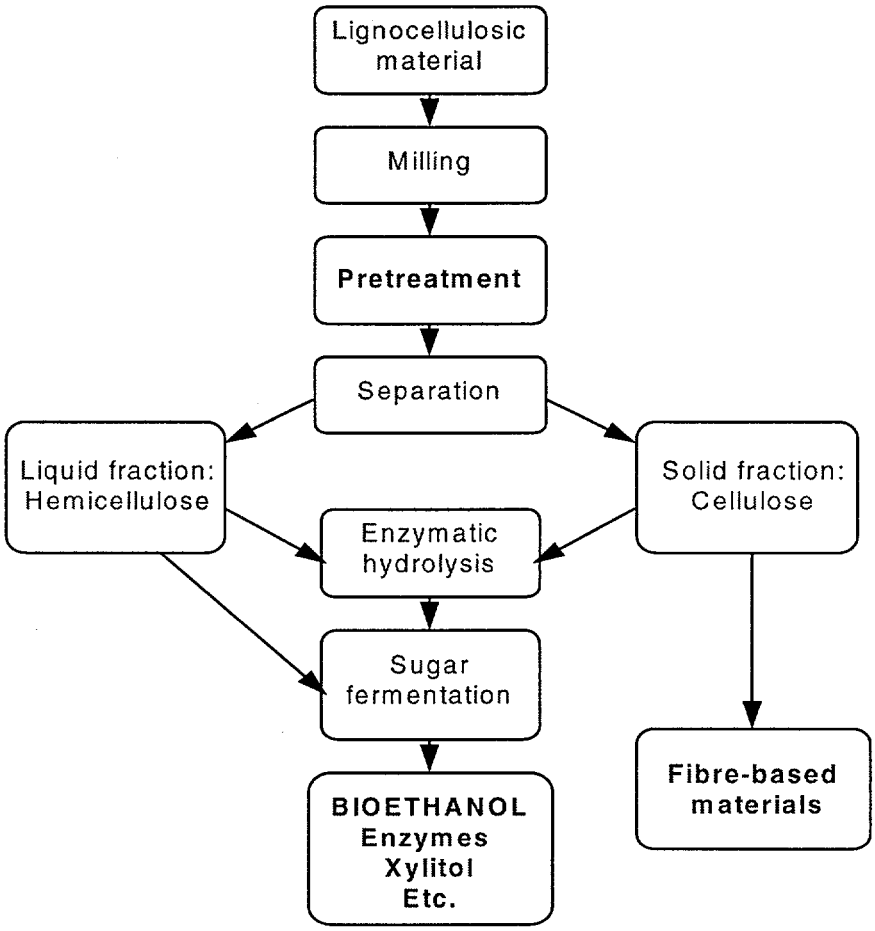


***Figure 6.*** *The flow scheme of possible utilisation of the carbohydrates of lignocellulosic materials in an integrated physical/chemical and biological treatment.*

Annual plants and hardwood excel from softwood by their uniform hemicellulose composition (mainly xylan) and their comparatively low lignin content (Fan *et al.*, 1982). Such facts may favour an utilisation of the solubilized hemicelluloses and enable comparatively mild conditions to be present for hemicellulose solubilisation. By pretreatment the polysaccharides become accessible for bioconversion or the cellulose fibre fraction is purified for production of high quality fibre-based products as illustrated in **Figure 6**. The cellulose-rich fibers derived from wet oxidation were bleached during the process due to the present oxygen (Bjerre & Pallesen, 1994).

For the treatment of polluted soil and wastewater, Risø National Laboratory has since 1986 applied wet oxidation (Bjerre & Sørensen, 1992; 1994; Sørensen *et al.*, 1990) and consequently has developed an extensive expertise in handling this

process. Previous to this project, wet oxidation has not been combined with alkaline hydrolysis of lignocellulosic materials. Nevertheless, good results have been obtained by this treatment of other polymeric materials, generating products available for biological degradation (Sørensen & Bjerre, 1992; Bjerre & Sørensen, 1994).

## 1.3 Summary

Previously, the pretreatments have been optimised mainly with respect to the hydrolysis yield of the cellulose fraction (Fan *et al.*, 1982), although up to 40% of the lignocellulose may be hemicellulose, particular in annual plant residues. Only cellulose was applied in the production of ethanol, and the hemicellulose and lignin fractions were burned mainly to provide energy. To create a more efficient and overall economically feasible process the utilisation of the lignin and especially the hemicellulose fraction for production of either ethanol or co-products (*e.g.* other chemicals) has recently attracted increasing interest (Büchert, 1990). Hence, this project investigates the potential solubilisation of the hemicellulose fraction in wheat straw by wet oxidation and to some extent also by steaming pretreatment processes.

In Denmark following the banning of burning straw in the fields the main agricultural waste product is wheat straw rather than wood. Generally, straw has a lower cellulose and lignin content than wood, but a higher hemicellulose content (Fan *et al.*, 1982). Previously, the wet oxidation of lignocellulosic material had been investigated in reactors with relatively long heating and cooling times (McGinnis *et al.*, 1983a), making the total processing time both lengthy and increasingly inaccurate. A reactor which requires shorter processing times has been used in this investigation (Bjerre & Sørensen, 1992; Sørensen *et al.*, 1990), hence providing milder conditions.

The importance of the different wet oxidation process parameters was evaluated by a statistical factorial design. The process parameters (temperature, reaction time, oxygen pressure and alkaline addition) were optimised in order to maximise the concentration of solubilised hemicellulose and to some extent also a highly convertible cellulose. The formation of furfural inhibitors was investigated in order to find process conditions (chemical additions) that will minimise the formation without significantly decreasing the concentration of solubilised hemicellulose. A few steaming experiments were performed for comparison purposes.

Preliminary fermentation of the aqueous fraction with the fungus *Aspergillus niger*, a known producer of cellulases and hemicellulases, was examined in order to use the solubilised hemicellulose as the sole carbon source. This microorganism was used as a kind of test-organism for determining the fermentability of the hemicellulose-rich fraction following the pretreatment process.

# 2 Materials and Methods

## 2.1 Raw Materials

The wheat straw from two different harvest years was grown at Risø National Laboratory. *Prior* to pretreatment the wheat straw was chipped to increase its total surface area. The flax was kindly supplied by B. Pallesen, Department of Plant Production, Denmark.

***Table 1.*** *The composition of the raw materials: wheat straws and flax.*

| Raw material | NCWM (%w/w) | Hemicellulose (%w/w) | Cellulose (%w/w) | Total lignin (%w/w) | Ash (%w/w) |
|---|---|---|---|---|---|
| Wheat straw (1990) | 12.0 | 35.5 | 40.8 | 10.5 | 1.4 |
| Wheat straw (1993) | 18.8 | 32.8 | 38.0 | 8.9 | 1.4 |
| Flax | 14.1 | 12.9 | 68.3 | 4.8 | ≈0 |

NCWM = Non-Cell Wall Material (pectin, protein etc.)
Concentration given in % of dry weight.

## 2.2 Pretreatment

The wet oxidation was carried out in a specially designed loop-reactor (2 L) constructed at Risø National Laboratory (**Figure 7**) with a working volume of 1 L (Bjerre & Sørensen, 1992; Bjerre *et al.*, 1996b). The reactor was made of Sandvik Sanicro 28 (27% Cr, 31% Ni, 3.5% Mo, 1% Cu) and mounted on a rack facilitating the control of temperature by immersing the reactor in an appropriate heating and cooling bath. Due to the excellent heat-transfer conditions, the relaxation time was short (**Figure 8**), which made it suitable also for studies of the reaction kinetics. The lignocellulosic material was mixed with $Na_2CO_3$ and water before adding the oxygen pressure and heating the suspension. Sodium carbonate was chosen as base because it was cheaper than most other bases since lime was not suitable to use in the process. The steaming was carried out in Hamburg by J. Puls who treated the raw material with saturated steam for 10 minutes (Korte *et al.*, 1991). After the pretreatments, the biomass suspension was filtered to separate the solid cellulose-rich fraction (the filter cake) from the liquid hemicellulose-rich fraction (the filtrate). The pH of the filtrate was measured and the filter cake dried and weighed and the composition of the two fractions was then analysed.

## 2.3 Drying

Previously, the solid fractions were dried at 80°C *prior* to analysis; however, this might cause a significantly change in the chemical structure of the solids. Hence, presently the solid fractions are dried at 65% relative humidity at 20°C until equilibrium is achieved (after about one week). Stored at these conditions the chemical structure of the solid fraction was stable for up to 10 years (J. Puls, personal communication).

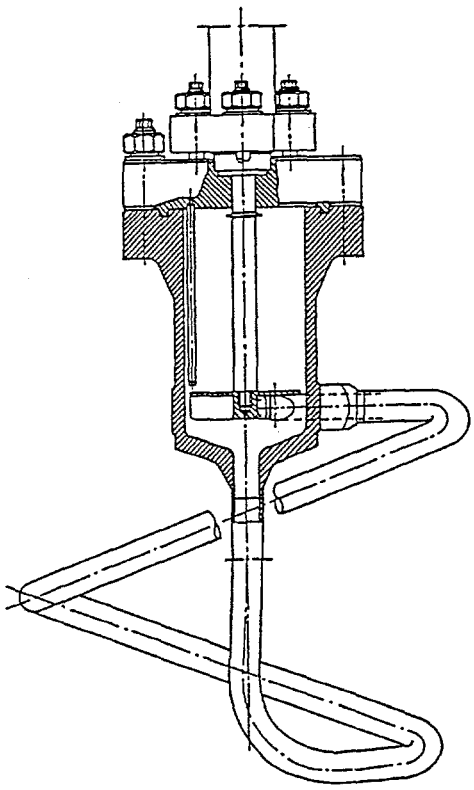

*Figure 7. The reactor with tubular loop and impeller designed and constructed at Risø National Laboratory.*

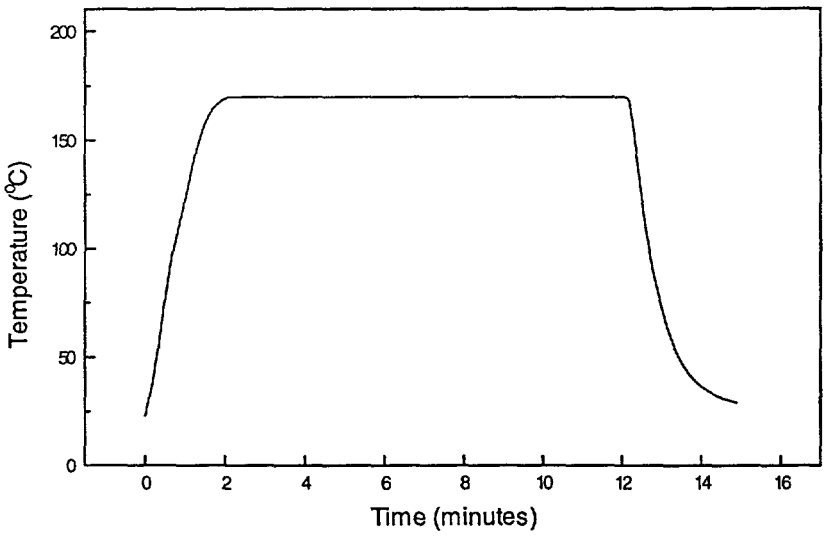


*Figure 8. The heating and cooling profile of the loop-reactor with a holding time of 10 minutes.*

## 2.4 Precipitation of Hemicellulose

A method was developed to precipitate the soluble hemicellulose from wet oxidised wheat straw by adding acetone (1:1 volume) while mixing for 30 minutes. After centrifugation (1500 rpm, 5 minutes) the concentrated hemicellulose suspension was dialysed with water for 24 hours and then freeze-dried.

## 2.5 Acid Hydrolysis

The acid hydrolysis was optimised in order to determined the solubilised hemicellulose in the wet oxidation filtrate as monomeric sugars (Bjerre *et al.*, 1996c). The 10 mL of filtrate was mixed with 10 mL $H_2SO_4$. The best overall hydrolysis was treatment with 4 %w/v $H_2SO_4$ at 121°C for 10 minutes at 121°C. After acid hydrolysis, the solutions were filtered (0.45 µm) in order to remove the water-insoluble residue (Karr & Brink, 1991; Puls, 1993).

## 2.6 Analyses

The developed and existing analytical methods were evaluated and upgraded continually throughout the project.

### 2.6.1 Fibres

The raw materials and the treated solid fractions were analysed for their concentration of the different fibers: hemicellulose, cellulose and lignin, and the non-cell wall material (NCWM) (water-soluble substances or extractives) by using the gravimetric method of Goering & van Soest (1970). This method is based on the determination of the neutral (NDF) and acid detergent fibre (ADF) followed by a permanganate procedure and incineration. For lignin determination this method avoided interference from protein material and residual waxes present in the wheat straw, which would have given a higher lignin content if the Klassons method had been applied.

In the NDF analysis, the sample material was boiled in a neutral detergent solution for 1 hour, after which the suspension was filtered quantitatively, washed, dried, and weighed. The solid fraction was defined as the NDF. In the ADF analysis, the sample material was boiled for 1 hour in an acid detergent solution (0.5 M sulfuric acid). The solid fraction after filtration and drying was defined as the ADF. The lignin content was determined by treating the ADF residual with potassium permanganate and acetate-buffer for 90 minutes (Goering & van Soest, 1970). The solid residual fraction was then incinerated. The hemicellulose content was calculated to be the solid removed by the ADF analysis, the lignin content the solid removed by the permanganate step, and the cellulose content the solid removed by the incineration step. Additionally, the content of NCWM was the solid removed in the NDF analysis. All samples were analysed in duplicate and results were given as a dry matter percentage.

### 2.6.2 Enzymatic convertibility of cellulose

To evaluate the efficiency of the pretreatment, a method to determine the convertibility of the solid cellulose fraction to fermentable glucose was developed using a mixture of two enzymes: the cellulase Celluclast and the $\beta$-glucosidase Novozym 188 (kindly donated by Novo Nordisk A/S, Bagsværd, Denmark)

(Bjerre *et al.*, 1996b). Celluclast: 1.2 g enzyme/mL, corresponding to an activity of 1500 NCU/mL (1 NCU is the amount of enzyme necessary for producing 1 µmol glucose/min from CMC). Novozym 188: 250 CBU/g (1 CBU is the amount of enzyme necessary for producing 1 µmol glucose/min from cellobiose). The enzymatic hydrolysis was optimised with respect to enzyme concentration, hydrolysis temperature, and time. The developed method was as follows: approximately 10 mg dried filter cake were suspended in 1 mL 0.2 M acetate buffer (pH = 4.8). The enzymes were added and the volume adjusted to 5 mL with deionized water. The activities of the enzymes in the final suspension were: Celluclast: 13.9 NCU/mL and Novozym 188: 0.46 CBU/mL. The mixture was hydrolysed for 24 hr at 50°C. After filtration the concentration of D-glucose in the filtrate was determined by HPLC. Samples were analysed in duplicate and results given as percentage dry matter of cellulose converted to glucose.

### 2.6.3 Quantification of sugars

The acid hydrolysates were purified by combined precipitation and an ion exchange treatment developed in this project (Bjerre *et al.*, 1996c). The sulfate ions were precipitated by $Ba(OH)_2$. After centrifugation (removal of $BaSO_4$) any remaining ions were eliminated by treatment with Amberlite GC-120 (100-200 mesh, $H^+$) and Dowex 1x4 (50-100 mesh, $OH^-$) (both Fluka). Samples were purified in duplicate. The recovery of glucose, xylose, and arabinose in the purification procedure was between 82 and 93%. The monosaccharides were then quantified by an HPLC cation exchange (Aminex HPX-87H column (Biorad)) with a matching precolumn at 63°C. The sugars was eluted with 0.004 M $H_2SO_4$ at a flow-rate of 0.6 mL/min and detected by their differential refractometer index (Knauer).

### 2.6.4 Quantification of furfurals

To investigate the presence of 2-furfural and 5-hydroxymethyl-2-furfural in the pretreated filtrate an HPLC-method (Nucleosil 5C-18 column) was developed. A linear gradient of methanol (10-90%) in 0.02 M $NaH_2PO_4$ (pH 3.0) at a flow-rate of 0.7 mL/min was used. The furfurals were detected by a diode array detector at 280 nm using authentic compounds as standard (Bjerre *et al.*, 1996b). Samples were analysed in duplicate.

### 2.6.5 Quantification of carboxylic acids

The carboxylic acids was determined by ion chromatography (HPICE-AS1 Dionex 4000 i) with 1 mM HCl as eluent at 0.8 mL/min with a combined conductivity and UV (204 nm) detection (Bjerre *et al.*, 1996b). Samples were analysed in duplicates.

### 2.6.6 COD-determination

The chemical oxygen demand (COD) was analysed using the potassium dichromate method at the Municipal Food and Environmental Control Unit, Køge, Denmark.

## 2.7 Fermentation

*Aspergillus niger* IBT 13099 was kindly supplied by DTU, Denmark. Stock cultures were made on PDA (Potato Dextrose Agar) at 30°C. *A. niger* was grown at 30°C for 114 hours in submerged culture of wet oxidised wheat straw supplemented with several salts (pH 5.5, 100 rpm) (Bjerre *et al.*, 1996b). β-Xylosidase activity was measured by incubating the intact mycelia with p-nitrophenyl-β-D-xylopyranoside (Bjerre *et al.*, 1996b; Stålbrand *et al.*, 1992). The released p-nitrophenol was measured spectrophotometrically at 410 nm, and activity was defined as the release of one μmol of p-nitrophenol/min.

# 3 Optimisation of Wet Oxidation

## 3.1 Preliminary Studies

Some biological and chemical processes for bioethanol production from fractionated lignocellulose were evaluated. The wet oxidation process was studied as a pretreatment method to obtain 1) a solid cellulose-rich fraction assessible for enzyme treatment and 2) an aqueous hemicellulose-rich fraction without producing microbial inhibitors.

### 3.1.1 Evaluation of process parameters

The wet oxidation process (water, oxygen, and elevated temperature) was investigated under alkaline conditions as pretreatment of wheat straw to facilitate conversion of the cellulose to glucose and solubilisation of the hemicellulose for fermentation purposes without producing microbial inhibitors. By using the specially constructed reactor, the wet oxidation process was optimised with respect to both reaction time and temperature, and to a lesser extent the alkaline and oxygen addition using a low concentration of wheat straw (20 g/L) to avoid restraining important process variables. These experiments were carried out on wheat straw harvested in 1990. Wet oxidation combined with alkaline hydrolysis efficiently degraded lignin by oxidation to carboxylic acids (Bjerre *et al.*, 1996b). A higher reaction temperature and a shorter reaction time gave the maximum fractionation. The solid cellulose-rich fraction was purified, where the cellulose content increased from 41 %w/w to nearly 80 %w/w under these conditions. Generally, a higher temperature gave a better fractionation within the first 10 minutes (**Figure 9**).

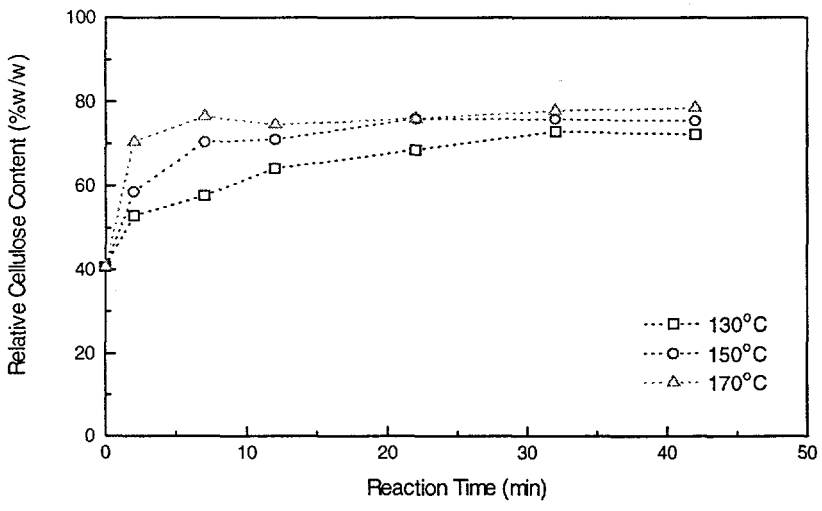


***Figure 9.*** *The effect of wet oxidation temperature and reaction time on the relative cellulose content in the solid fraction (%w/w dry weight) after pretreatment (20 g/L wheat straw (1990), 10 g/L $Na_2CO_3$, 10 bar $O_2$).*

The best wet oxidation conditions for obtaining enzymatic convertible cellulose were 170°C and 5-10 minutes giving about 85 %w/w conversion of the cellulose

to glucose (**Figure 10**). Most of the hemicellulose was dissolved, and approximately 45% of the dissolved hemicellulose was identified as saccharides in the wet oxidation filtrate. Although some saccharides were oxidized to carboxylic acids and $CO_2$, neither 2-furfural nor 5-hydroxymethyl-2-furfural (HMF) were observed following the wet oxidation treatment under the given wet oxidation conditions. On the contrary, the mass balance based on the chemical oxygen demand (COD) showed that all major components in the filtrate were low molecular weight carboxylic acids and saccharides (Bjerre *et al.*, 1996b).

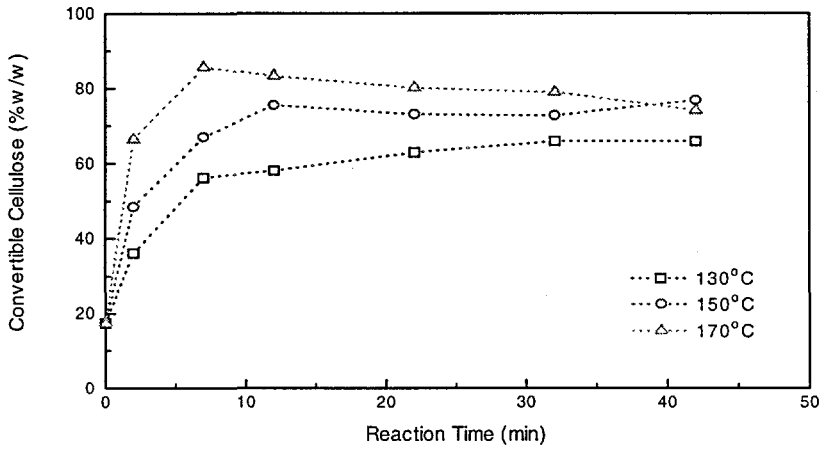


***Figure 10.*** *The effect of wet oxidation temperature and reaction time on the enzymatic convertibility of the cellulose in the solid fraction to glucose after pretreatment (20 g/L wheat straw (1990), 10 g/L $Na_2CO_3$, 10 bar $O_2$). Conditions for the enzymatic conversion were 50°C, pH = 4.8, 24 hours incubation.*

This preliminary study provided an insight of some important process variables. First of all, a large amount of sodium carbonate (alkaline) was needed in order to have an optimal utilisation of the solid cellulose-rich fraction (Bjerre *et al.*, 1996b). Secondly, the hemicellulose yield was significantly reduced when the process was run optimally for the cellulose utilisation. These process conditions gave only 1.8 g hemicellulose/L (measured as monosaccharides after acid hydrolysis) in solution corresponding to 40 % of the theoretical yield. This indicated that a simultaneous optimal utilisation of the hemicellulose and cellulose was difficult to achieve.

The solubilised hemicellulose together with the carboxylic acids were sufficient to function as the sole carbohydrate source for *Aspergillus niger* producing exo-β-xylosidase. Moreover, growth experiments using *A. niger* indicated that no inhibitors were produced during the wet oxidation treatment at the applied conditions (Bjerre *et al.*, 1996b). By using wet oxidation as a pretreatment of lignocellulose, inhibitor problems in the following fermentation process (*e.g.* to ethanol production) might be avoided.

### 3.1.2 Concentration of biomass

Wet oxidation at low wheat straw level (20 g/L) solubilised a low amount of hemicellulose. A higher starting concentration of wheat straw (60 g/L) was applied in order to be able to use the hemicellulose as the sole carbohydrate source for ethanol-producing microorganisms. These experiments were carried out on wheat straw harvested in 1993. The 60 g/L wheat straw level was the

highest that could be employed in the loop-reactor without causing problems during the wet oxidation: poor mixing properties leading to a blockage of the reactor-loop. Approximately the same amount of carbonate was applied to a 3 times larger amount of wheat straw which resulted in milder process conditions and a more economically feasible process. The fractionation still took place within the first 10-15 minutes; however, in this case the fractionation was less complete (**Figure 11**) (Bjerre *et al.*, 1996a). The convertibility of the cellulose fraction was reduced to about 70% compared to the nearly 90% conversion obtained for the lower wheat straw level of 20 g/L (**Figure 10**). Using the higher straw concentration and a lower carbonate addition, the concentration of solubilised hemicellulose increased to more than 9.8 g/L (measured as monosaccharides after acid hydrolysis). This was sufficient to act as the sole carbohydrate source for ethanol-producing microorganisms.

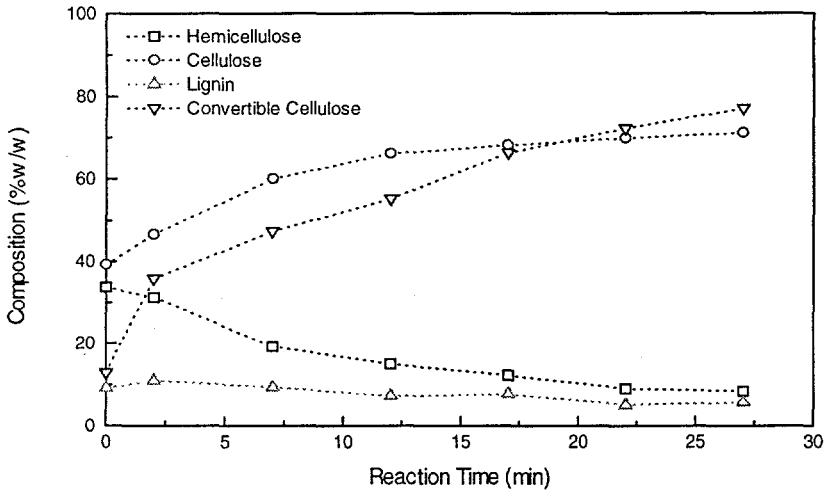


*Figure 11. The effect of wet oxidation reaction time on the relative content of lignin, hemicellulose and cellulose in the solid fraction (%w/w dry weight) and the enzymatic conversion of cellulose into glucose after pretreatment (60 g/L wheat straw (1993), 6.5 g/L $Na_2CO_3$, 185°C, 12 bar $O_2$).*

### 3.1.3 Precipitation of hemicellulose

The hemicellulose content determined as monosaccharides after acid hydrolysis accounted for only 62% of the theoretical value. Even though some hemicellulose was degraded to $CO_2$, $H_2O$, and to simpler and more oxidised organic compounds (mainly low molecular weight carboxylic acids formed during wet oxidation and acid hydrolysis), this could not explain the apparent hemicellulose loss of 38%. Therefore, the quantification of hemicellulose by acid hydrolysis and HPLC was accompanied by precipitation of the solubilised hemicellulose by addition of acetone, quantifying 20-30% more hemicellulose (**Table 2**) (Bjerre *et al.*, 1996a). However, this accounted for only about 75% of the theoretical hemicellulose in solution. By acetone precipitation, the lower molecular weight hemicellulose units were not precipitated, but remained in solution since the average molecular weight of the precipitate was higher than the soluble hemicellulose. Furthermore, some larger molecular weight lignin degradation products might coprecipitate with the hemicellulose. The recovery of the hemicellulose from wet oxidized filtrates needs further investigations.

*Table 2 Comparison of precipitation (with acetone) and hydrolysis (acid hydrolysis and HPLC of sugars) for quantification of the solubilised hemicellulose from wet oxidation of wheat straw (1993).*

| Wet Oxidation Conditions[a] | | | Hemicellulose | | | |
|---|---|---|---|---|---|---|
| Tempe-rature (°C) | $O_2$-pressure (bar) | Time (min) | Hemicellulose precipitated (g/L) | Total sugars (g/L) | Hemicellulose as sugars[b] (g/L) | Diffe-rence (%) |
| 150 | 6 | 5 | 3.3 | 2.8 | 2.5 | 36 |
| 150 | 6 | 15 | 4.6 | 4.0 | 3.5 | 30 |
| 185 | 6 | 5 | 7.7 | 7.5 | 6.6 | 16 |
| 185 | 12 | 5 | 8.0 | 7.5 | 6.6 | 21 |

a: Using 60 g/L wheat straw (1993) and 6.5 g/L $Na_2CO_3$.
b: Calculated as monomeric hemicellulose units and not as monosaccharides.

### 3.1.4 Flax to fibres

The solid cellulose-rich fraction may either be converted to a fermentation substrate for ethanol production after enzymatic hydrolysis to glucose or used in fibre composites (**Figure 6**) generating other high-value co-products and making the ethanol production from the hemicellulose fraction more economical. Due to their excellent mechanical properties lignocellulosic fibres have received a lot of interest as a replacement for industrial traditional fibres such as asbestos and glass in composite applications, *e.g.* thermoplastics or cement. A minor study of the suitability of the wet oxidation process for production of such fibres has been performed (Bjerre & Pallesen, 1994). Due to the high content of cellulose in flax (**Table 1**) this crop looks promising for fibre utilisation. These preliminary experiments look promising as the content of both lignin and hemicellulose was considerably reduced and the tensile strength of the fibre was very high (Schmidt & Bjerre, 1997a).

## 3.2 Wet Oxidation Pretreatment of Wheat Straw

### 3.2.1 The factorial design

The effect of the oxygen pressure, reaction temperature, and time was evaluated by a statistical $2^3$-factorial design in order to determine the importance of the different wet oxidation process variables (**Table 3**). In the design, the concentration of wheat straw and $Na_2CO_3$ were kept constant. As only one replicate of each different experiment was carried out, the interaction between all three factors was assumed to be negligible (ABC = 0) and, hence, used as $S_0$ in the 2-way analysis of variance (ANOVA).

*Table 3. The $2^3$-factorial design.*

| Factor | High level | Low level |
|---|---|---|
| Oxygen pressure (A) | 12 bar | 6 bar |
| Reaction temperature (B) | 185°C | 150°C |
| Reaction time (C) | 15 min | 5 min |

Other variables were held constant: 60 g/L wheat straw (1993) and 6.5 g/L $Na_2CO_3$

The experiment showed that reaction time and temperature had a significant effect on the degree of fractionation (Schmidt & Bjerre, 1997b). The reaction temperature was the most important variable of the three factors and had a significant effect on the formation of nearly every product. The reaction time had an effect on the formation of some of the products, *e.g.* xylose and arabinose (measured by HPLC after acid hydrolysis of the hemicellulose-rich fraction). On the other hand, the $O_2$ pressure had a significant effect only on the concentration of isobutyric acid (Schmidt & Bjerre, 1997b).

### 3.2.2 Effect of reaction temperature and time

The reaction temperature as an important variable in the wet oxidation process was investigated in more detail. The optimal temperature was 185°C under the given conditions (60 g/L wheat straw (1993); 6.5 g/L $Na_2CO_3$; 12 bar $O_2$; 15 minutes) (**Figure 12**). This temperature gave the highest concentration of solubilised hemicellulose (measured as monosaccharides after acid hydrolysis) (**Figure 12**) as well as the highest convertibility of cellulose to glucose (Schmidt & Bjerre, 1997b). The total amount of carboxylic acids increased with temperature, and the composition changed. The acids measured were acetic, formic, glycolic, oxalic, malic, and isobutyric acid. At high temperatures (> 180°C) acetic, formi, and glycolic acid were formed in relatively high amounts, whereas at low temperatures (150°C) all acids were present in similar amounts (Schmidt & Bjerre, 1997b).

The reaction time affected the solubilisation of the hemicellulose under the given conditions (60 g/L wheat straw (1993); 6.5 g/L $Na_2CO_3$; 12 bar $O_2$) at 185°C (**Figure 13**) and 200°C (Schmidt & Bjerre, 1997b) in a way that at the higher temperature a shorter reaction time was necessary to obtain the highest concentration, 15 and 10 minutes, for the two temperatures respectively. This illustrated that the wet oxidation process was a balance between solubilisation of the hemicellulose and a degradation of the solubilized hemicellulose to other products, *e.g.* $CO_2$ and $H_2O$. The longer the reaction time the more hemicellulose was solubilised, but also the more hemicellulose was degraded (Schaleger & Brink, 1978). The optimum reaction temperature and time were found to be 185°C for 15 minutes, giving a yield of 9.8 g/L solubilised hemicellulose (measured as monosaccharides after acid hydrolysis).

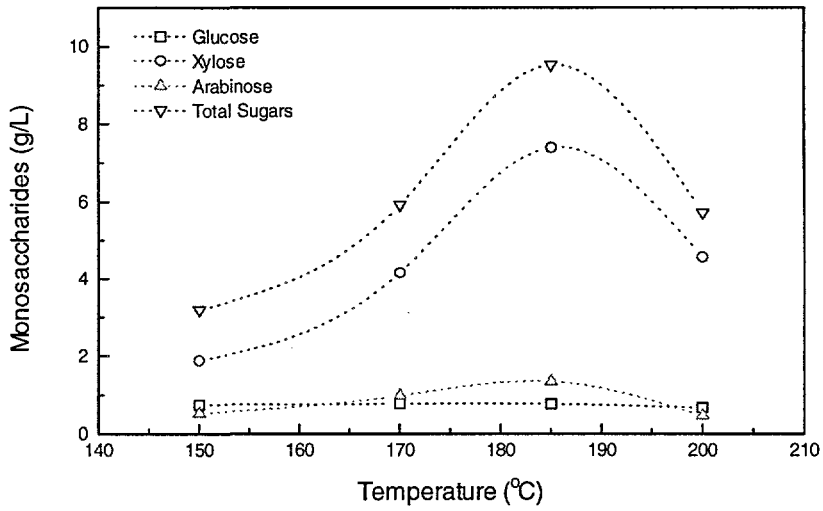


***Figure 12.*** *The hemicellulose concentration (measured as monosaccharides after acid hydrolysis) in the filtrate obtained by the wet oxidation process (60 g/L wheat straw (1993), 6.5 g/L $Na_2CO_3$, 12 bar $O_2$, 15 minutes) as a function of the reaction temperature.*

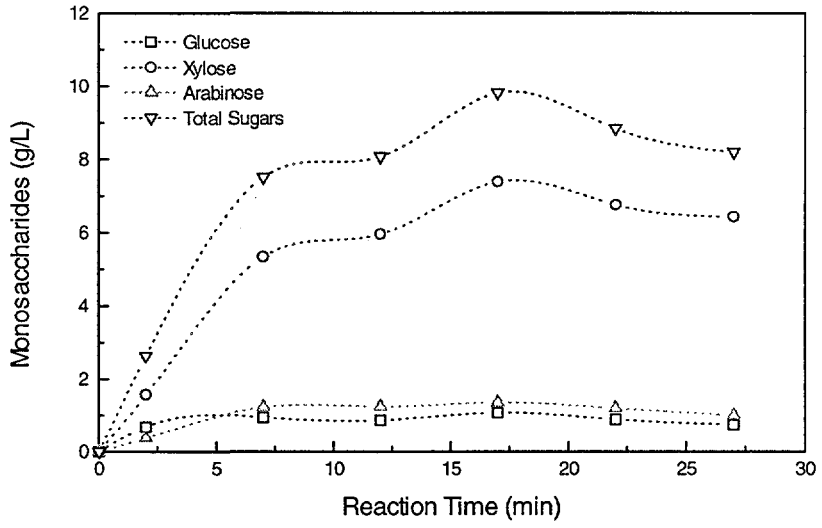


***Figure 13.*** *The hemicellulose concentration (measured as monosaccharides after acid hydrolysis) in the filtrate obtained by the wet oxidation process (60 g/L wheat straw (1993), 6.5 g/L $Na_2CO_3$, 12 bar $O_2$, 185°C) as a function of the reaction time.*

### 3.2.3 Hydrothermal reference experiments

By combining wet oxidation with alkaline hydrolysis the formation of 2-furfural, a known microbial inhibitor, was minimised (Bjerre *et al.*, 1996b). At higher concentrations of wheat straw (60 g/L) 2-furfural was detected (3 ppm) in the filtrate (Bjerre *et al.*, 1996a), but was much lower than commonly found by steam explosion. When autohydrolysis reactions are run under conditions of high

temperature and/or long time to solubilise hemicellulose efficiently, the carbohydrates undergo a series of secondary reactions, including rearrangements and dehydration, to form 2-furfural (from the pentoses) and 5-hydroxymethyl-2-furfural (from the hexoses) (Biermann, 1983), which may be further oxidized to levulinic acid (Fengel & Wegener, 1989). These furfurals may undergo self-condensation reactions to form polymers or may react with lignin to form copolymers (Biermann, 1983), which also can act as microbial inhibitors. The main difference between wet oxidation and most other pretreatment processes, *e.g.* steaming and autohydrolysis, is the addition of the oxygen under alkaline conditions. The effect of these two parameters on the 2-furfural formation was investigated in order to find process conditions (with respect to chemical additions) that minimised their formation.

***Table 4.*** *The formation of 2-furfural from hydrothermal pretreatment of wheat straw (wet oxidation: 60 g/L straw (1993); 6.5 g/L $Na_2CO_3$; 12 bar $O_2$; 185°C; 15 minutes).*

| Pretreatment | pH | Pentoses (g/L) | 2-Furfural (ppm) | 2-Furfural/Pentose (mg/g) |
|---|---|---|---|---|
| Wet Oxidation | 5.9 | 8.74 | 3 | 0.3 |
| No Oxygen | 7.0 | 8.36 | 2 | 0.2 |
| No Base | 3.0 | 9.68 | 76 | 7.8 |
| No Oxygen/Base | 4.9 | 6.71 | 55 | 8.2 |

The experiments were based upon the best wet oxidation process for the solubilisation of hemicellulose (60 g/L wheat straw (1993), 185°C, 6.5 g/L $Na_2CO_3$, 12 bar $O_2$, 15 minutes). The experiments were carried out without oxygen and/or without carbonate addition. When carbonate was absent (low pH) a higher concentration of 2-furfural (about 30 times higher) was formed than when carbonate was present (**Table 4**) (Ahring *et al.*, 1996a). This indicated that the reaction of pentoses to 2-furfural to some extent was catalysed by hydrogen ions. The experiments with the lowest pH-value formed the most 2-furfural. On the other hand, oxygen did not have a great effect on the formation of 2-furfural at the studied conditions. 5-Hydroxymethyl-2-furfural was not found in any of the treated samples. Additionally, after treatment oxygen significantly affected the filterability of the suspension, which is nearly impossible to filter when oxygen was absent in the process.

When carbonate was absent but oxygen present, a relatively low content of hemicellulose remained in the solid fraction (**Figure 14**). When either carbonate or oxygen were absent a high quality solid fraction was obtained with a high cellulose content with a high conversion to glucose. On the other hand, when both carbonate and oxygen were absent a low quality solid fraction was obtained with a relatively higher content of hemicellulose, a lower relative content of cellulose and a low convertibility of the present cellulose. Hence, either the base or the oxygen has to be present in order to achieve a high solubilisation of the hemicellulose from the straw.

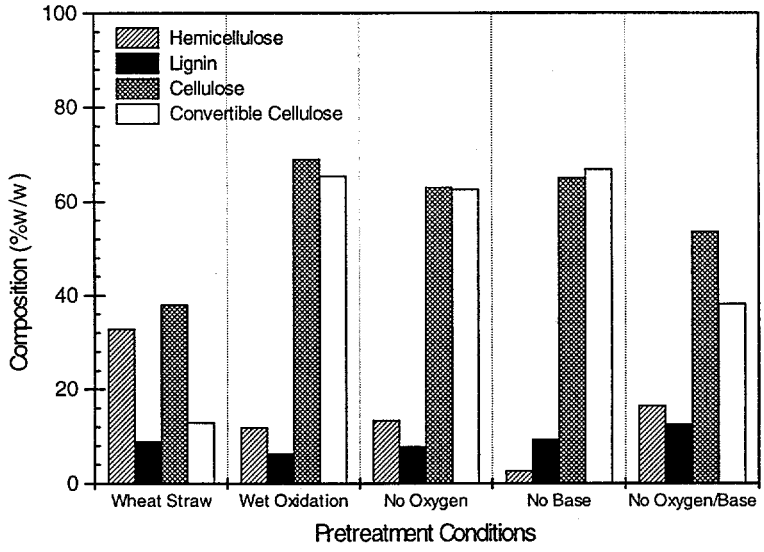


***Figure 14.*** *The characteristics of the solid fraction - the relative content of lignin, hemicellulose, and cellulose (%w/w dry weight) and the enzymatic convertibility of the cellulose to glucose - after hydrothermal pretreatment (wet oxidation: 60 g/L wheat straw (1993); 6.5 g/L $Na_2CO_3$; 12 bar $O_2$; 185°C; 15 minutes).*

The oxygen level significantly affected the amount of carboxylic acids formed (**Table 5**) as a result of the reaction with other compounds, in particular lignin-related compounds (phenol derivatives), which are very reactive during the process (Bjerre & Sørensen, 1994; Taylor & Weygandt, 1974). A similar amount of carboxylic acids was found when either base or oxygen were absent, which was also similar to the result of the wet oxidation experiment. Hence, the formation of acids was not the only reason for the observed difference in the final pH-values (**Tables 4** and **5**).

***Table 5.*** *The formation of low molecular weight carboxylic acids from hydrothermal pretreatment of wheat straw (wet oxidation: 60 g/L straw (1993); 6.5 g/L $Na_2CO_3$; 12 bar $O_2$; 185°C; 15 minutes).*

| Carboxylic acids (g/L) | Wet Oxidation | Without Oxygen | Without Base | Without Oxygen/Base |
|---|---|---|---|---|
| Acetic acid | 2.1 | 1.7 | 1.2 | 0.7 |
| Formic acid | 1.4 | 0.4 | 1.7 | 0.1 |
| Glycolic acid | 1.1 | 0.4 | 0.7 | 0.1 |
| Oxalic acid | 0.7 | 0.3 | 0.6 | 0.3 |
| Malic acid | 0.2 | 0 | 0.2 | 0 |
| Isobutyric acid | 0 | 1.4 | 0 | 0 |
| Total acids | 5.5 | 4.2 | 4.5 | 1.2 |

### 3.2.4 Effect of oxygen and alkaline addition

The addition of both sodium carbonate and oxygen makes wet oxidation an expensive pretreatment process. In order to lower the cost, the effect of these two parameters on the hemicellulose solubilisation was investigated. The effect was not as important as other process parameters, *e.g.* the temperature. However, a tendency for a high concentration of oxygen to give the most solubilised hemicellulose was observed (Ahring *et al.*, 1996b). On the other hand, the sodium carbonate and oxygen addition had a distinguished effect on the formation of 2-furfural in the process (**Table 6**). The lower the level of carbonate the higher the concentration of 2-furfural. The addition of carbonate could be decreased by 38% (from 6.5 g/L to 4 g/L) with minimal formation of 2-furfural.

***Table 6.*** *The effect of adding sodium carbonate and oxygen on the formation of 2-furfural (ppm) in the hemicellulose-rich filtrate from wet oxidation of wheat straw (60 g/L straw (1993), 185°C, 15 min.).*

| Oxygen (bar) | $Na_2CO_3$ (g/L) | | | |
|---|---|---|---|---|
| | 0 | 2 | 4 | 6.5 |
| 0 | 55 | 2 | 1 | 2 |
| 3 | 101 | 5 | 1 | 1 |
| 6 | 67 | 13 | 2 | 1 |
| 12 | 76 | 27 | 8 | 3 |

## 3.3 Comparing Wet Oxidation and Steaming

### 3.3.1 Efficiency of the fractionation process

The wet oxidation was compared with steaming (steam, high temperature, no chemicals) in order to evaluate the efficiency of the wet oxidation process. During our visit to Dr. Puls's laboratory in Hamburg, steaming experiments with the same wheat straw material as used in the wet oxidation experiments (from 1993) were performed. Much more hemicellulose and lignin were solubilised from the straw by wet oxidation (81% and 55%, respectively) than by steaming (46% and 18%, respectively) (**Table 7**). On the other hand, more cellulose was solubilised (and degraded) by steaming (16%) than by wet oxidation (6%). Furthermore, a considerably higher amount of solubilised hemicellulose (measured as monosaccharides after acid hydrolysis) was obtained after wet oxidation (16.6 g sugar/100 g straw) than after steaming (6.7 g sugar/100 g straw) (**Table 8**).

***Table 7.*** *The percentage of cellulose, hemicellulose, and lignin solubilised from the solid fraction by pretreating the wheat straw by wet oxidation (60 g/L straw (1993), 6.5 g/L $Na_2CO_3$, 12 bar $O_2$, 185°C, 15 minutes) and steaming (200 g straw (1993), 205°C, 10 min).*

| Pretreatment | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|---|---|---|---|
| Wet Oxidation | 5.7 | 80.5 | 54.5 |
| Steaming[a] | 16.3 | 45.8 | 18.0 |

a: Carried out by Dr. J. Puls, Hamburg, Germany.

***Table 8.*** *The concentration of solubilised hemicellulose (measured as monosaccharides after acid hydrolysis) by wet oxidation and steaming pretreatment (conditions given in* ***Table 7****).*

| Sugars | Wet Oxidation (g/100 g straw) | Steaming[a] (g/100 g straw) |
|---|---|---|
| Rhamnose | 0[b] | 0.09[b] |
| Mannose | 0[b] | 0.15[b] |
| Arabinose | 2.25 | 0.77[b] |
| Galactose | 0.75[b] | 0.42[b] |
| Xylose | 12.32 | 4.18[b] |
| Glucose | 1.28 | 1.08[b] |
| Total sugars | 16.60 | 6.71[b] |

a: Carried out by Dr. J. Puls, Hamburg, Germany.
b: Quantified by Dr. J. Puls, Hamburg, Germany.

### 3.3.2 Furfural formation

The formation of 2-furfural in the steaming of wheat straw was compared to that in the wet oxidation process. In all steaming experiments, 2-furfural was formed, although mostly at the higher temperatures (**Table 9**). The solubilisation of hemicellulose was very low in the steaming experiments compared to wet oxidation (**Table 8**), but the ratio of furfural/pentoses was a factor of 5 to 26 higher (**Table 9**). In general, more 2-furfural was formed by steaming than by wet oxidation.

*Table 9. The comparison of 2-furfural formation by wet oxidation and steaming (conditions given in **Table 7**). The steaming experiments were performed at 3 different temperatures.*

| Pretreatment | Pentoses (g/L) | 2-Furfural (mg/L) | 2-Furfural/Pentoses Ratio (mg/g) |
|---|---|---|---|
| Wet Oxidation | 8.74 | 3.0 | 0.34 |
| Steaming (180°C)[a] | 1.52 | 2.5 | 1.66 |
| Steaming (190°C)[a] | 3.10 | 6.7 | 2.15 |
| Steaming (205°C)[a] | 2.37 | 20.8 | 8.78 |

a: Carried out by Dr. J. Puls, Hamburg, Germany.

### 3.3.3 Mass balances

Mass balances were calculated to estimate the losses of the valuable carbohydrates, cellulose and hemicellulose, when using the two different treatments.

Wet oxidation:
(60 g/L straw (1993), 6.5 g/L $Na_2CO_3$, 12 bar $O_2$, 185°C, 15 minutes)

*Cellulose* (based on 100 g straw):

In (solid) + Accumulated = Out (solid) + Produced (glucose)
36.9 g + Accumulated = 34.8 g + 1.28 g ⇒ Accumulated = - 0.82 g

*Hemicellulose* (based on 100 g straw):

In (solid) + Accumulated = Out (solid) + Produced (other sugars)
31.8 g + Accumulated = 6.2 g + 15.3 g ⇒ Accumulated = -10.3 g

Steaming:
(200 g straw (1993), 205°C, 10 min).

*Cellulose* (based on 100 g straw):

In (solid) + Accumulated = Out (solid) + Produced (glucose)
36.9 g + Accumulated = 30.9 g + 1.1 g ⇒ Accumulated = - 4.9 g

*Hemicellulose* (based on 100 g straw):

In (solid) + Accumulated = Out (solid) + Produced (other sugars)
31.8 g + Accumulated = 17.3 g + 5.6 g ⇒ Accumulated = - 8.9 g

By wet oxidation 2.2% of the original cellulose and 32.4% of the original hemicellulose were converted to other products (*e.g.* carboxylic acids) and/or degraded (*e.g.* to $CO_2$ and $H_2O$). This gave an overall "loss" of carbohydrates of 16.2%. Whereas, by steaming, 13.3% of the original cellulose and 28.0% of the original hemicellulose were converted to other products and/or degraded, which

gave an overall "loss" of carbohydrates of 20.1%. In wet oxidation, hemicellulose was mainly converted and/or degraded; however, considerable more hemicellulose was found in solution, *e.g.* available for fermentation. In steaming, both carbohydrates were converted and/or degraded, which was in accordance with the known formation of both 2-furfural and 5-HMF. In general, the overall recovery of carbohydrates was greater for wet oxidation than for steaming.

## 3.4 Preliminary Economic Evaluation

A preliminary calculation of the economics of a continuous wet oxidation production plant was performed in order to estimate the production costs of the wet oxidation process and make a comparison between possible wet oxidation treatments. The following assumptions were made: (a) a capacity of the plant of 100 000 tons wheat straw per year with a dry content of 90%, (b) there was neither filtration nor detoxification *prior* to fermentation. Since wet oxidation is an exothermic process, the wheat straw suspension was needed to be heated to only about 150°C, after which the reaction temperature increased spontaneously to the required level. The water was assumed to be delivered free of charge. It was not possible to estimate the cost of the milling of the wheat straw *prior* to the wet oxidation process. The cost of the expenditures were assumed to be as followed: 0.50 Dkr/kg dry straw; 0.30 Dkr/kWh; 1 Dkr/$m^3$ oxygen (Hede Nielsen, Denmark) and 2 Dkr/kg $Na_2CO_3$ (Superfos, Denmark). In this estimate the cost of labour has not been included. As the implementation of the wet oxidation process to lignocellulose treatment had not been fully developed, three levels of expenditures (utilisation) were included in this calculation (**Table 10**). Furthermore, the regeneration of heat was assumed to be at least 80% by heat exchanging the incoming with the outgoing stream.

***Table 10.*** *The three levels of expenditures applied in the preliminary calculation of a continuous wet oxidation process of wheat straw.*

| Utilisation Level | $O_2$-Pressure[a] (bar) | $Na_2CO_3$[a] (g) | Heat Regeneration[b] (%) |
|---|---|---|---|
| High | 12 | 6.5 | 0 |
| Low | 3 | 4 | >80 |
| Lowest | 0.5 | 2 | >90 |

a: Based on 60 g wheat straw.
b: Heat regeneration by heat exchanging the incoming with the outgoing stream.

By using the estimated prices, the cost of treating the wheat straw by wet oxidation can be calculated taken when all process streams are taken into account (**Figure 15** and **Table 10**). In general, the raw material, wheat straw, appeared to be the major contribution to the costs (**Table 11**). At a high chemical utilisation level this was 35% of the overall cost. Whereas, when the process had the lowest utilisation level it contributed to nearly 80% of the overall cost. By the decrease in carbonate and oxygen requirement and the increase in heat regeneration the overall process cost decreased by 56%.

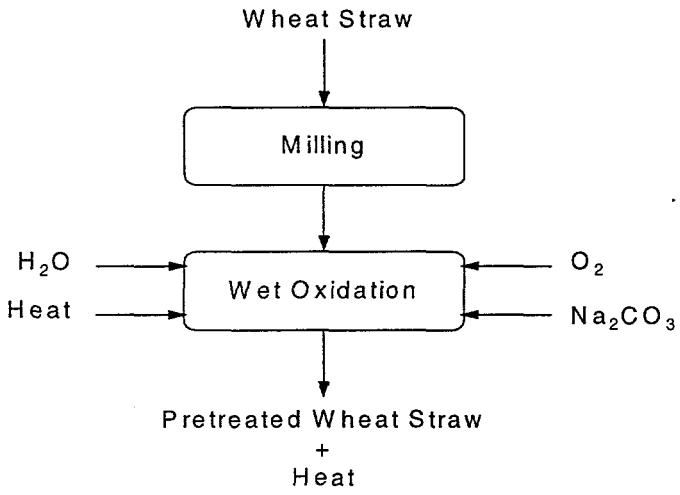


***Figure 15.*** *The different process streams in a continuous wet oxidation treatment of wheat straw.*

***Table 11.*** *Estimates of the cost of the individual process streams using the wet oxidation process (based on 1 kg wheat straw).*

| Process Stream[a] | Price (Dkr) | Utilisation (per kg straw) | | | Cost (Dkr per kg straw) | | |
|---|---|---|---|---|---|---|---|
| | | High | Low | Lowest | High | Low | Lowest |
| 1. Straw (kg) | 0.45 | 1 | 1 | 1 | 0.45 | 0.45 | 0.45 |
| 2. Milling | ? | ? | ? | ? | ? | ? | ? |
| 3. Oxygen[b] (kg) | 0.76 | 0.26 | 0.06 | 0.01 | 0.20 | 0.05 | 0.01 |
| 4. $Na_2CO_3$ (kg) | 2.00 | 0.11 | 0.07 | 0.03 | 0.22 | 0.13 | 0.07 |
| 5. Heat (kWh) | 0.30 | 1.43 | 0.29 | 0.14 | 0.43 | 0.09 | 0.04 |
| 6. Water (kg) | 0 | 16.67 | 16.67 | 16.67 | 0 | 0 | 0 |
| Overall | | | | | 1.29 | 0.72 | 0.57 |

a: Process streams are shown in **Figure 15.**
b: The applied pressure was calculated to mass assuming oxygen to be an ideal gas.

In this project, the final product was ethanol produced from fermentable sugars, therefore, the price of these fermentable sugars was calculated (**Table 12**). To simplify the calculation, the same amount of carbohydrates were assumed to become available for fermentation purposes at the 3 different utilisation levels. By using wet oxidation (60 g/L straw, 6.5 g/L $Na_2CO_3$, 12 bar $O_2$, 185°C, 15 minutes) the following available carbohydrates were found: 0.167 kg solubilised sugars per kg straw, 0.062 kg available solid hemicellulose per kg straw, and 0.231 kg solid convertible cellulose per kg straw. The cost of sugar significantly decreased when both fractions of available carbohydrates (solubilised as well as solid) was utilised. The utilisation of all available sugars was more important for the cost than a lower level of chemicals applied in the process.

*Table 12. The estimated prices for the three levels of wet oxidation process conditions (**Table 10**).*

| Utilisation Level | Price of treated straw (Dkr/kg straw) | Price of sugar (Dkr/kg sugars in solution) | Price of sugar (Dkr/kg total available sugar) |
|---|---|---|---|
| High | 1.29 | 7.75 | 2.82 |
| Low | 0.72 | 4.31 | 1.57 |
| Lowest | 0.57 | 3.41 | 1.24 |

# 4 Characterisation of Products

## 4.1 Quantification of Solubilised Hemicellulose

An investigation of the acid hydrolysis and HPLC analysis have been carried out in order to optimise the quantification of the solubilised hemicellulose fraction from wheat straw after pretreatment.

### 4.1.1 Acid hydrolysis

In connection with our visit to Institute of Wood Chemistry, Hamburg, Germany, we became aware that acid hydrolysis has to be optimised individually for a given lignocellulosic substrate. The optimal acid hydrolysis conditions for steam-exploded material was not necessarily the same as for wet oxidised material. At Puls's laboratory, 4 %w/v $H_2SO_4$ and 121°C for 40 minutes are used for hydrolysing freeze-dried steam-exploded material.

Different acid hydrolyses were performed in order to identify the optimal hydrolysis conditions (concentrations of acid and hydrolysis time) for quantification of the hemicellulose (Bjerre *et al.*, 1996c). Four different sugars were identified: xylose, arabinose, glucose and galactose. Hydrolyses were carried out using aqueous and freeze-dried samples. Hydrolysis with 4 %w/v $H_2SO_4$ gave a higher monosaccharide concentration than hydrolysis using 7 %w/v $H_2SO_4$ (**Figure 16**).

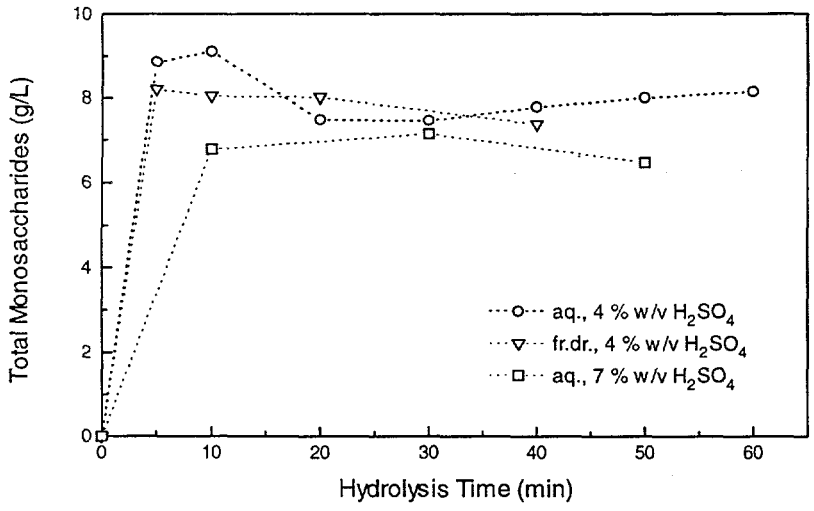


*Figure 16. The total monosaccharide concentration (measured by HPLC) as a function of the acid hydrolysis time under 3 different hydrolysis conditions (aq. = aqueous sample; fr.dr. = freeze-dried sample) at 121°C.*

Furthermore, a shorter hydrolysis time of 10 minutes was better than that of 40 or 60 minutes (previously used in our laboratory). The pentoses xylose and arabinose were especially sensitive to the acid hydrolysis. The best overall hydrolysis was obtained by treating an aqueous sample with 4 %w/v sulfuric acid for 10 minutes. However, these conditions were optimal only for the pentoses and not for the measurement of glucose, which was estimated by using a correction factor (factor = 1.19) (Bjerre *et al.*, 1996c).

During our visit to Dr. Puls's laboratory, the possibility of enzymatic hydrolysis of the hemicellulose from wet oxidized wheat straw was examined due to the absence of sugar degradation products like furfurals. Besides, the time-consuming sample purification procedure of the acid hydrolyzates (Bjerre *et al.*, 1996c) could be side-stepped by applying enzymatic hydrolysis. On the other hand, achieving complete degradation of the solubilised hemicellulose to monosaccharides might be complicated if this milder hydrolysis was used. A collaboration with Dr. Maija Tenkanen, VTT, Finland, has been initiated in order to further investigate suitable enzyme mixtures for hydrolysing hemicellulose.

### 4.1.2 Monosaccharide analysis

Within this project a method for quantification of sugars in acid hydrolyzates has been developed as a purification of the hydrolyzate followed by an HPLC-determination of the monosaccharides. The purification step was needed for removing interfering ions. This included precipitation of sulfate ions by barium hydroxide and an elimination of remaining ions by mixed-bed ion exchange: Amberlite GC-120 (100-200 mesh, $H^+$) and Dowex 1x4 (50-100 mesh, $OH^-$). The recovery in this purification procedure for glucose, xylose, and arabinose was 82-92 % (Bjerre *et al.*, 1996c).

Monosaccharide analysis was carried out using Aminex HPX-87P and HPX-87H HPLC columns with different resin ionic forms, respectively lead ($Pb^{2+}$) and hydrogen ($H^+$). The lead column (HPX-87P) separated all four sugars in the acid hydrolyzates with a better resolution, but sample purification was laborious and required the removal of all interfering impurities, which resulted in a poor reproducibility and a sugar recovery below 50%. The hydrogen column (HPX-87H) separated only glucose, xylose, and arabinose, whereas galactose was not separated from xylose. However, this column was less sensitive towards impurities and gave a better recovery and reproducibility. Therefore, the hydrogen column (HPX-87H) was chosen for routine quantification of the hydrolysed hemicellulose sugars with 0.004 M $H_2SO_4$ as eluent (Bjerre *et al.*, 1996c). The present column/detector system was able to determine monosaccharides; however, in order to obtain a greater understanding of the acid hydrolysis and to get a higher recovery a pulsed amperometric detector may be used, which also detects possible oligosaccharides after the acid hydrolysis.

## 4.2 Structure of Hemicellulose

The size of the hemicellulose molecule after pretreatment was investigated during our visit to Dr. Puls's laboratory and showed that the solubilised hemicellulose had a minimum of 15 xylose units ($X_{15}$), and the precipitated hemicellulose a minimum of 50 units ($X_{50}$). The hemicelluloses may be very heterogeneous and branched. These results were obtained by an advanced TLC unit with a scanner and computer analysis. When the solubilized hemicellulose was applied as fermentation substrate it is important that the microorganisms are able to form all enzymes necessary for the degradation of the solubilised hemicellulose to monomers. A complete characterisation of the solubilised hemicellulose structure may enable us to verify which enzymes are necessary.

# 5 Conclusions

The wet oxidation process of wheat straw has been studied as a pretreatment method to evaluate some biological and chemical processes for bioethanol production. Several pretreatment processes (wet oxidation, steaming, and hydrothermal treatments) have been investigated to provide a suitable feedstock for enzymatic hydrolysis and fermentation. From the present studies it could be concluded that:

- The combination of the wet oxidation process (water, oxygen pressure, elevated temperature) and alkaline hydrolysis was efficient in pretreating wheat straw and was optimised to efficiently solubilise the hemicellulose, degrade the lignin, and open the solid crystalline cellulose structure of wheat straw without generating microbial inhibitors 2-furfural and 5-hydroxymethyl-2-furfural.
- The degree of delignification and hemicellulose solubilization increased with the reaction temperature and time. The optimum conditions for wet oxidation were 185°C and 15 minutes, giving 9.8 g/L solubilised hemicellulose (measured as monosaccharides after acid hydrolysis).
- Analytical tools were developed and implemented to chemically characterise the two carbohydrate fractions from wet oxidation of wheat straw. An investigation of the acid hydrolysis and HPLC analysis has been carried out in order to optimise the quantification of the solubilised hemicellulose fraction. The best overall hydrolysis was obtained by a treatment with 4 %w/v sulfuric acid for 10 minutes. A hydrogen HPLC column (Aminex HPX-87H) was suitable for analysis of the monosaccharides, although galactose was not separated from xylose. Because this column was less sensitive towards impurities than a lead HPLC column (Aminex HPX-87P) and gave better recovery and reproducibility, it was chosen for routine quantification of the hydrolysed hemicellulose sugars.
- An improved purity of the solid cellulose-rich fraction was obtained at a higher wet oxidation temperature. The optimum wet oxidation conditions for obtaining enzymatic convertible cellulose (90%) was 10 minutes at 170°C using high carbonate concentration.
- The hemicellulose yield and recovery were significantly reduced when the process was run under optimal conditions for cellulose conversion. This indicated that a simultaneous optimal utilisation of the hemicellulose and cellulose was difficult to achieve.
- Wet oxidation was compared to hydrothermal processing (without oxygen) under neutral and alkaline conditions. The oxygen pressure and sodium carbonate concentration had little effect on the solubilisation of hemicellulose; however, by combining wet oxidation with alkaline hydrolysis the formation of 2-furfural, a known microbial inhibitor, was low.
- Comparison of the wet oxidation process with steaming (steam, high temperature, no chemicals) showed that more hemicellulose and lignin were removed from the straw by wet oxidation than by steaming. On the other hand, more cellulose was solubilised (and degraded) by steaming than by wet oxidation. An overall "loss" of carbohydrates of 20.1% and 16.2% was found for the steaming and wet oxidation process, respectively. More 2-furfural was formed by steaming than by wet oxidation. As the solubilisation of hemicellulose was considerably lower in the steaming experiments compared to wet oxidation the ratio of furfural/pentoses was substantially higher. Therefore, the wet oxidation appears to be more promising than steaming for pretreatment of wheat straw.

– Preliminary studies of wet oxidation of flax indicated that this process may also be used to efficiently produce high cellulose-rich fibres with high tensile strength for composite materials.
– Preliminary calculations of the economics of a continuous wet oxidation production plant showed that the biomass raw material was the major contributor to the cost of producing fermentable sugars. In order to make the wet oxidation process more economical both carbohydrate fractions should be utilised and the possibility of using the by-product should be further investigated, *e.g.* the use of cellulose in fibre-based materials and solid lignin for fuel in biogas generation or as raw material (monomers of the liquid lignin) for production of chemicals.

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Abstract (max. 2000 characters)

The combination of the wet oxidation pretreatment process and alkaline hydrolysis was investigated to efficiently solubilise the hemicellulose, degrade the lignin, and open the solid crystalline cellulose structure of wheat straw lignocellulose without generating fermentation inhibitors. The effects of temperature, oxygen pressure, reaction time, and concentration of straw were evaluated. The degree of lignin degradation and hemicellulose solubilisation increased with the reaction temperature and time. The optimum conditions were 15 minutes at 185°C, producing 9.8 g/L hemicellulose. For quantification of the solubilised hemicellulose the best overall acid hydrolysis was obtained by treatment with 4 %w/v sulfuric acid for 10 minutes. The Aminex HPX-87H column was less sensitive towards impurities than the Aminex HPX-87P column. The former gave improved recovery and reproducibility, and was chosen for routine quantification of hydrolysed hemicellulose sugars. The purity of the solid cellulose fraction also improved with higher temperature. The optimum condition for obtaining enzymatic convertible cellulose (90%) was 10 minutes at 170°C using a high carbonate concentration. The hemicellulose yield and recovery were significantly reduced under these conditions indicating that a simultaneous optimal utilisation of the hemicellulose and cellulose was difficult.The oxygen pressure and sodium carbonate concentration had little effect on the solubilization of hemicellulose; however, by combining wet oxidation with alkaline hydrolysis the formation of 2-furfural, a known microbial inhibitor, was minimal. The wet oxidation was compared with an alternative process, steaming. Much more hemicellulose and lignin were solubilised from the straw by wet oxidation than by steaming. More cellulose was solubilised (and degraded) by steaming than by wet oxidation. Overall carbohydrates "losses" of 20.1 and 16.2% were found for steaming and wet oxidation, respectively. More 2-furfural was formed by steaming than by wet oxidation. Preliminary calculations of the economics of the wet oxidation process showed that in general, the raw material was the major contributor to the costs. Preliminary studies of wet oxidation of flax resulted in high cellulose-rich fibres with high tensile strength.

Descriptors
BIOMASS, WHEAT, STRAW, HEMICELLULOSE, CELLULOSE, LIGNIN, ACID HYDROLYSIS, SACCHARIDES, FURFURAL, ENZYMATIC HYDROLYSIS, FERMENTATION, ASPERGILLUS

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