Catalytic Pretreatments and Microwave Assisted Hydrolysis of Lignocellulosic Raw Materials

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Academic Dissertation

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Abstract

There is nowadays a strong concern about decreasing oil supplies and global warming leading to ever increasing interest in biobased fuels and chemical production. The utilization of lignocellulosic raw materials for liquid biofuels and chemicals is a challenging task due to raw materials’ rigid structure which is resistant towards any actions to break it. Thus the raw materials should be pretreated to reach an economically vital process.

Catalytic and alkaline oxidation presented here are novel, highly selective and effective methods to separate carbohydrates from lignin in different lignocellulosic materials. Both pretreatment methods are carried out at 120 – 140 °C for 4 – 20 hours under 10 bar oxygen pressure and in alkaline water solution. In catalytic oxidation also copper(II) phenanthroline catalyst is used. The obtained carbohydrate rich fraction is remarkably easy to hydrolyze with enzymes to corresponding sugars. The carbohydrate rich material from catalytic oxidation is nearly quantitatively converted into corresponding monohydrates in 24 hours by enzymatic hydrolysis. The enzyme loading can also be significantly decreased compared to standard methods, which helps to decrease the total costs of the process.

Catalytic and alkaline oxidation pretreatment methods can be applied also to chromated copper arsenite treated waste wood, which is considered as hazardous waste. When combined with very mild sulfuric acid extraction and enzymatic hydrolysis 90 % sugar yield is obtained from the carbohydrate material. The sugars can be further utilized in chemical industry, which makes this method an interesting way to recycle hazardous waste.

In a process of turning lignocellulosic materials into sugars hydrolysis plays the key role. The hydrolysis is usually carried out enzymatically or with acids. They both have their disadvantages, such as long reaction times, poor yields or corrosive effect. As an alternative to traditional acid hydrolysis a microwave assisted mild acid hydrolysis is presented here. It can be applied to xylan, which is converted to xylose quantitatively in just one minute with 2 wt-% hydrochloric acid. When applied to milled birch wood 70 % yield of xylose is obtained. Subjecting alkaline oxidation pretreated birch to a three-step reaction, all the xylan and 66 % of the cellulose is hydrolyzed to corresponding sugars.
**Preface**

This work has been carried out during years 2008-2013 at the Laboratory of Inorganic Chemistry, University of Helsinki.

I am truly grateful to my supervisors, Professors Markku Leskelä and Timo Repo for their professional guidance and continuous support. I am especially grateful for the opportunity to work in the interesting field of biorefineries, the chance to make my own choices and all the help I got when solving the problems followed by those choices.

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Helsinki, November 2013,

Maija Hakola
List of original publications

This thesis is based on the following publications:


The publications are referred to in the text by their roman numerals.
### Abbreviations

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AlkOx</td>
<td>Alkaline oxidation pretreatment</td>
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<tr>
<td>CatOx</td>
<td>Catalytic oxidation pretreatment</td>
</tr>
<tr>
<td>CuBipy</td>
<td>Copper(II) bipyridine</td>
</tr>
<tr>
<td>CuPhen</td>
<td>Copper(II) phenanthroline</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>5- HMF</td>
<td>5-hydroxymethylfurfural</td>
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<tr>
<td>NTA</td>
<td>Nitrilotriacetic acid</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<td>SSF</td>
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**1. Introduction**

Petroleum industry has become truly vital to the world during last decades. Current energy systems are mainly based on it. Fossil resources are also a highly important carbon source and raw material in chemical industry.\(^1\)\(^-\)\(^3\) The fact that the oil supplies are decreasing and the oil peak has already been accomplished and the growing concern about greenhouse gases have pushed the scientists all over the world to seek for alternatives.\(^3\)\(^-\)\(^4\) Biobased raw materials are a promising choice as they are abundant, renewable and widely available. Even today we are using a great variety of starch and sucrose based transport fuel components, chemicals and end-products. For example the amount of bioethanol in gasoline has been increasing and polyethylene is produced from sugarcane. However, these first generation starch and sucrose based chemicals are under constant criticism as starch and sucrose are edible and thus compete with the food production.\(^5\) The second generation biofuels and chemicals are produced from lignocellulosic materials, which are non-edible. These include for instance wood and various side streams of food industry and agriculture, like straw and sugarcane bagasse.

Even though the lignocellulosic materials are abundant and widely available in nature, using the lignocellulosic waste material as a raw material in chemical industry could help solving many problems.\(^1\) Since the 1990’s a lot of attention has been directed to waste prevention instead of waste remediation, which can be seen also in chemistry as the principles of green chemistry.\(^6\) Nevertheless, all the waste cannot be prevented, but some could be recycled as a raw material in fuel and chemical production. This would also increase the value of waste dramatically. As an example, wood treated with chromated copper arsenite (CCA) is considered as hazardous waste in many countries.\(^7\) Though the manufacture of the CCA-treated wood is prevented by EU and the US government the waste management problems will last for the next decades as the lifetime of CCA-treated wood is 30-50 years. Traditionally CCA-treated waste wood is disposed within landfills or burned to create energy.\(^7\)\(^-\)\(^8\) It has been estimated that disposing to landfills increases the costs of waste with approximately 400 $/t of biomass, while electricity generation produces 60-150 $/t of biomass. When average bulk chemicals are produced the value increases to 1000 $/t of biomass.\(^1\)\(^,\)\(^2\)
Whether from waste or straight from nature, the utilization of lignocellulosic materials needs rather complex and still expensive processes. Lignocellulosic materials consist of three main polymers: cellulose, hemicelluloses and lignin. Cellulose and hemicelluloses are polymers of monosaccharides while lignin is built up of different phenols. These three components are tightly packed and form a highly stable and resistant structure. To reach the monosaccharides, first the macrostructure of the material needs to be broken down. This step is called pretreatment. After the pretreatment the revealed polysaccharides are hydrolyzed usually either with enzymes or acids. Only then the monosaccharides can be used for further processing in fuel and chemical production. There is a need for efficient pretreatment methods, as the hydrolysis and the following steps are dependent on the effectiveness of this first step. A profitable and usable pretreatment method should (1) make easily hydrolysable cellulose, (2) avoid losses of hydrolysable carbohydrates, (3) avoid formation of toxic compounds and (4) be cost-effective. \(^9\)

The pretreatment step is followed by hydrolysis, where the sugar polymers are broken down into monosaccharides. Nowadays the hydrolysis is usually done enzymatically. Enzymes are extremely selective, but also very vulnerable to inhibiting compounds.\(^{10,11}\) Thus very careful pretreatment is needed prior enzymatic hydrolysis. As normal for biological processes, the hydrolysis times are quite long, up to days. The prices of hydrolysis enzymes have been decreasing, but they are still rather expensive. As a consequence, new enzyme recycling methods are studied. Besides enzymes acids have been used in hydrolysis for decades, but also not without problems. The strong acids cause serious corrosion problems, and, on the other hand, diluted acids require usually high reaction temperatures. Acid hydrolysis also lacks selectivity and often significant amount of side products are formed.
2. The Scope of the Study

When this doctoral theses project was started the main aim was to develop a novel pretreatment method for the second generation bioethanol production. The focus was only on lignocellulosic materials and the first generation raw materials were not studied at all. Researchers in Laboratory of Inorganic Chemistry in University of Helsinki had been studying catalytic oxidation of lignin model compounds and their suitability in pulp bleaching already for some time. However, the processes were unsuitable for the paper industry because of the decomposition of cellulose fibers, but offered a great starting point for development of the new catalytic pretreatment method, which was urgently needed.

In the first phase of this project the target was on finding a suitable copper(II) diimine catalyst for pretreatment of lignocellulosic materials and optimizing the reaction conditions for maximum ethanol yield. After obtaining encouraging results the ideas of a new research topic followed each other. While optimizing the catalytic pretreatment another alkaline pretreatment method was invented. A great variety of novel research topics have been developed around these two pretreatment methods in our laboratory as well as in other laboratories, e.g. VTT – Technical Research Centre of Finland and Department of Pharmacy in University of Tartu.

The idea of applying the novel oxidative pretreatment methods to preservative treated waste wood followed by the experiments with fresh lignocellulosic materials. After a while we had a method of turning hazardous waste into a valuable raw material for chemical industry.

The pretreatment studies were naturally continued by hydrolysis studies. For a catalyst chemist it was hard to swallow the incomparable efficiency of enzymes in hydrolysis. The answer was found in the combination of old knowledge and new technology, and a microwave assisted mild acid hydrolysis was found and optimized. During this study a lot of questions have been answered, but as it is common in science, many more questions have arisen.
3. Lignocellulosic raw materials for chemical production

Biorefinery is a similar concept as oil refinery, only the raw material is renewable and biobased instead of oil. The goal is to produce a great variety of different intermediate and/or end products from one or several starting materials. In the optimal case all the raw material is utilized without any unusable side products.\textsuperscript{4,11} Chemical pulp mills are an example of one kind of biorefineries.\textsuperscript{11} There the main product is pulp made of mainly cellulose and the whole energy need of the mill is covered with the energy from burning the lignin and hemicelluloses.\textsuperscript{4} They also have other smaller side products which help in getting a bigger profit from the raw material. Norwegian Borregaard is a good example of a highly developed biorefinery.\textsuperscript{12} They produce widely different products, like specialty cellulose, bioethanol, different lignin based products, vanillin and fine chemicals. Both bulk chemicals and fine chemicals are produced from one raw material.

3.1 Variety of lignocellulosic raw materials

Often when lignocellulosic materials are considered wood is the primary material. However, wood is a rather valuable material and it has important roles in paper industry and as construction material. It is also growing quite slowly especially in northern countries. Recently different lignocellulosic waste streams have gained attention because the production of those materials does not necessarily increase the costs of the total process. Different waste streams to be considered are, for example, municipal waste, forestry waste and agricultural waste. Naturally there are also challenges with waste materials that need to be considered. For example, wastes are usually fairly heterogeneous which can make the utilization more complex.\textsuperscript{1,4}

3.2 Cellulose, hemicelluloses and lignin

The second generation raw material for biorefineries, lignocellulose, is mainly cellulose (40-50 % of dry weight), hemicelluloses (20-30 % of dry weight) and lignin (20-30 % of dry weight). There is also a small amount of different kind of extractives. Together these components allow the growth of large plants and protect them against attack by microorganism and insects. That is also the reason why the utilization of lignocellulosic
material is not easy and none of the components should be abandoned to get a biorefinery economically viable.

The three main components of lignocellulose differ from each other significantly, which gives the grand opportunity to produce very different products from one material. Cellulose is a linear polymer consisting of β-D-glucopyranosyl monomers linked together with β(1→4)-glycosidic bond. The length of a cellulose polymer depends on the plant material and for example in wood the degree of polymerization is around 10 000. The single polymer chains are linked together with strong hydrogen bonds and form microfibrils of 30-200 single chains. The microfibrils have both, crystal and amorphous regions, which together ensure the great strength and flexibility needed in plant cell walls.\textsuperscript{13}

Hemicelluloses, on the other hand, are heteropolysaccharides with varying structure. The polymer chains are held together by hydrogen bonds and van der Waals forces. In softwoods galactoglucomannan consisting of galactose, glucose and mannose, is the main hemicellulose. Also arabinogluronoxylan is present, but in smaller amounts. In hardwoods the main hemicellulose is glucuronoxylan and also some glucomannan is present.\textsuperscript{13,14}

Contrary to cellulose and hemicelluloses, lignin does not consist of monosaccharides but is a phenolic polymer. It is irregular and amorphous and formed primarily by free-radical polymerization of \textit{p}-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol (Figure 1).\textsuperscript{13,15} These three precursors are present in different lignocellulosic materials in different ratios. For example in softwood, almost only coniferyl alcohol is present, whereas hardwood lignin consists of coniferyl alcohol and sinapyl alcohol, and in grasses and herbaceous crops all three components can be found. The precursors react with each other and form a phenolic macromolecule. Since softwood lignin has a lower methoxyl content than hardwood lignin, it is more condensed and resistant to chemical conversion (Figure 2).\textsuperscript{13,15}
Figure 1. The structures of lignin precursors a) \( p \)-coumaryl alcohol, b) coniferyl alcohol, and c) sinapyl alcohol.

Figure 2. An example of the structure of lignin showing different bonds between lignin precursors.\textsuperscript{13}
3.3 The top value added chemicals from biomass

The current idea of a biorefinery based on the second generation raw materials has its history among bioethanol production. Even though the bioethanol production has been studied widely, the technology, chemistry and biochemistry still need improvement to get the process profitable. The profitability can be improved with production of various high-value products at the same time.\textsuperscript{4}

The amount and variety of chemicals that can be produced from biobased materials is almost indefinite.\textsuperscript{2} The US Department of Energy, DOE, has listed 12 top value added chemicals derived from sugars. Those molecules can act as building blocks for a number of high-value biobased chemicals and materials.\textsuperscript{16} The building block chemicals are

1. 1,4-succinic, fumaric and malic acids
2. 2,5-furandicarboxylic acid
3. 3-hydroxypropionic acid
4. aspartic acid
5. glucaric acid
6. glutamic acid
7. itaconic acid
8. levulinic acid
9. 3-hydroxybutyrolactone
10. glycerol
11. sorbitol
12. xylitol/arabinitol

Also the top value added chemicals from lignin have been listed. Contrary to the sugar derivatives, lignin derivatives could not be listed at molecular level but only as product types. Lignin is potentially used in power, fuel and syngas production, or as a starting material for macromolecules, aromatics and miscellaneous monomers. The utilization in power, fuel and syngas production, e.g. purely as a carbon source, is considered as a near-term opportunity. The utilization as macromolecules aims to high molecular weight products where the structure imparted by nature is retained and is considered as a mid-term opportunity. The production of aromatics requires the breaking up lignin’s macromolecular structure but maintaining the aromatic nature of the obtained molecules.\textsuperscript{17}
4. Pretreatment of lignocellulosic materials for further processing

The structure of lignocellulosic materials is very rigid and resistant towards any actions to break it. Nevertheless, generally the macrostructure needs to be broken down to separate and utilize the different building blocks, cellulose, hemicelluloses and lignin, efficiently. This separation step is called pretreatment.4

The ideal pretreatment should18,19

1. loosen the plant cell walls,
2. minimize the use of energy, chemicals, and capital equipment,
3. use inexpensive chemicals,
4. require simple equipment,
5. decrease the crystallinity of cellulose,
6. maximize the enzymatic susceptibility,
7. minimize the carbohydrate loss,
8. not impregnate the wood with toxic chemicals which would inhibit the saccharifying enzymes or microbes which utilize the sugars,
9. maximize the recovery of lignin as a valuable by-product,
10. be effective with a wide range of biomass raw material, and
11. be capable of being scaled up to industrial size.

There is an urgent need for an efficient and cost effective pretreatment and recently a great variety of different methods have been developed. In the next chapters some of the state-of-art pretreatment methods are described in more detail as well as catalytic and alkaline pretreatment methods developed in this thesis.

4.1 Steam explosion

Steam explosion is one of the most widely studied pretreatment methods. The process was developed already in 1925 and has been originally used in hardboard manufacture. In steam explosion a cylinder is first filled with wood chips or other preferred raw material. The filled cylinder is sealed and pressurized. The chips are permeated by saturated steam and develop high internal pressures. Usually the reaction temperature varies from 160 °C to 238
After a certain time (1-10 min) the cylinder is opened and due to the sudden decompression the chips are defibrated. The process can be catalyzed with H₂SO₄ or SO₂. Also the chemical structure of wood is affected, the lignin barrier is disrupted, and hemicelluloses are partially broken down and solubilized in water. The accessibility of cellulose to enzymatic hydrolysis is largely increased due to larger pores and a higher surface area. In fact, steam explosion can enhance the rate of enzymatic hydrolysis up to ten folds compared to non-treated materials.

Different raw materials have been studied widely in steam explosion, including various hardwood and softwood species, rice straw and hulls, corn stalks, stover and corncobs, sugarcane bagasse, wheat straw, forage sorghum, mandarin and lemon peel waste, and cotton gin waste. Usually steam explosion has been applied to ethanol production and has thus been followed by enzymatic hydrolysis and fermentation. However, also the production of D-lactic acid, acetone-butanol and methane has been studied.

One of the drawbacks of steam explosion is the degradation of sugars and formation of enzyme inhibiting compounds. For example acetic, formic and levulinic acids, 5-HMF, syringaldehyde, 4-hydroxybenzaldehyde and vanillin are often formed during steam explosion. All of those are not necessarily really inhibiting, but the synergism and cumulative effects may cause inactivation of enzymes.

### 4.2 Organosolv pretreatment

Organosolv pretreatment offers potential for meeting many of the criteria of a successful pretreatment method. The method was developed already in the 1930’s, primarily for pulping. In the organosolv method lignocellulosic material is treated with water-solvent-catalyst mixture where the catalyst and water hydrolyze the lignin-lignin and lignin-carbohydrate bonds and the solvent dissolves lignin. The organic solvent used can be distilled and recycled and the obtained lignin fraction can be utilized in other applications. Some of the hemicelluloses are decomposed during the treatment, but that can be prevented with dilute acid treatment prior the organosolv treatment. Organosolv treatment can be applied to various lignocellulosic materials including different softwood and hardwood species, wheat straw, barley straw, sugarcane bagasse, perennial plants and bamboo.
Usually methanol\textsuperscript{53,54}, ethanol\textsuperscript{47,51,55-57,60-62} and butanol\textsuperscript{19} are used in different rations with water as a solvent in organosolv pretreatment, but also acetone\textsuperscript{52} can be used. These solvents have, however, low boiling points and there are some restrictions due to the risks from high operation pressure and highly volatile and flammable solvent. Thus recently other solvents have been introduced. For example glycerol, a solvent with a high boiling point and a biodiesel production by-product, have given comparable results to ethanol cooking.\textsuperscript{63} What comes to catalysts, acids and bases are used, sulfuric acid\textsuperscript{19,48,50,51,55,57,61,62}, phosphoric acid\textsuperscript{53}, hydrochloric acid\textsuperscript{57} and sodium hydroxide\textsuperscript{19,47,49,51} being the most common. Also FeCl$_3$\textsuperscript{60} and MgCl$_2$\textsuperscript{51,57} work as catalysts. The pretreatment time can vary from minutes to hours, and the reaction temperature from room temperature close to 240 °C.

There are some drawbacks also in organosolv pretreatment, for example rather heavy washing steps with organic solvents and water, the increased energy consumption due to the distillation of the solvents and the extremely tight and efficient control of the process because of the organic solvents. These keep the costs of the process high.\textsuperscript{64}

4.3 Wet oxidation

A reaction involving oxygen and water at elevated temperature and pressure, wet oxidation, has been applied for treatment of polluted soil and waste water already since 1986. More recently the method has been found to be effective also in pretreatment of certain lignocellulosic materials. During the wet oxidation the crystalline structure of cellulose is opened and cellulose can be collected as solid fraction. At the same time, some of the lignin is decomposed and hemicelluloses are dissolved. The process can be catalyzed by alkaline addition.\textsuperscript{65} The pretreatment is carried out at temperatures from 130 °C to 200 °C for 5 to 15 min and under 3 to 12 bar oxygen pressure.\textsuperscript{65,66} Wheat straw\textsuperscript{65-67} has been studied most as a raw material, but also other materials like corn stover\textsuperscript{68}, softwood\textsuperscript{69} and sugarcane bagasse\textsuperscript{70} have been successfully studied. Due to the low solubilization of lignin the pretreatment has not been as promising with wood as with other materials.\textsuperscript{67} Certain compromises have to be done in wet oxidation. At high reaction temperatures part of cellulose and hemicelluloses is degraded leading to lower sugar yields and formation of fermentation inhibitors. At lower temperatures lignin is left in the carbohydrate fraction and the hydrolysis and fermentation levels are decreased.\textsuperscript{71,72} But as an advantage compared to
steam explosion and organosolv pretreatment no enzymes inhibiting by-products, like furfural and 5-HMF, are formed. 65

4.4 Catalytic oxidation – results and discussion on publication I

In situ-prepared copper(II) diimine catalysts have been designed for catalytic oxidation of benzylic alcohols, which also work as lignin model compounds. 73 These catalysts are water based, stable and can use dioxygen as oxidant. Previous pulp bleaching studies have shown that copper(II) phenanthroline in alkaline water solution is in fact active in delignification. However, during the reaction also severe degradation of cellulose fibers occurs, making the method unsuitable for pulp industry. As in sugar and/or ethanol production the quality of cellulose fibers is not relevant, the use of copper(II) diimine catalysts in alkaline conditions was studied as a pretreatment process for enzymatic hydrolysis of carbohydrates.

The catalytic oxidation pretreatment experiment (CatOx) was typically carried out in a one liter autoclave equipped with a magnetic stirrer and oil bath heating. Usually 5 g of dry raw material was used with 100 ml of alkaline water solution. After the reaction the solution was filtered and the solid carbohydrate rich fraction was collected. The solution contained the dissolved lignin and hemicelluloses.

While searching the most efficient catalyst for pretreatment, a large amount of different diimine ligands were studied. In all the reactions the raw material was decomposed to some extent and at least part of the lignin was solubilized (Figure 3). The effectiveness of the reaction was followed as a mass loss, e.g., how much of the raw material was solubilized. The lost mass was mainly lignin, which could be seen with bare eyes as pulp-like light colored product. The composition of the solid fraction from CatOx was quantitatively determined and compared to the pretreated material from steam explosion (Figure 4). During CatOx a significant amount of lignin was dissolved and the amount increased with increased reaction time. Also, approximately half of hemicelluloses were dissolved, whereas cellulose remained in the solid fraction. On the other hand, during steam explosion lignin and cellulose remained in the solid fraction and all hemicelluloses were dissolved.

The reaction conditions for catalyst screening were selected based on experiments with benzylic alcohols and preliminary experiments with spruce. With 20 hours reaction time at 100 °C and under 10 bars oxygen pressure, phenanthroline and bipyridine together with copper were found to be the most effective catalysts in lignin removal.
Figure 3. Ligands used in catalytic pretreatment of spruce wood and their effect on mass loss. Reactions were carried out with 21.0 mg (84.0 µmol) CuSO₄·5 H₂O, ligand:metal molar ratio 2:1, 5 g spruce (dry weight), 2.6 g of Na₂CO₃, 100 ml H₂O, at temperature 100 °C, for 20 h, and with oxygen pressure (10 bar).

Based on the catalyst screening, copper(II) phenanthroline (CuPhen) and copper(II) bipyridine (CuBipy) were chosen for reaction condition optimization experiments. Both were used in reaction time and temperature optimization experiments (Figure 5) and CuPhen in base concentration (Figure 6) and oxygen pressure experiments (Figure 7). Also the amount of catalyst was screened as well as the ratio of metal and ligand. The results showed that the optimal reaction conditions were:

- 10 bars oxygen pressure,
- 0.168 mmol of phenanthroline and 0.084 of copper(II) in 100 ml of aqueous solution
- 0.25 M Na₂CO₃ concentration and
- 100 °C for 20 h or 120 °C for 5 h.
Figure 4. Carbohydrate and lignin contents of untreated spruce wood before any treatment, after catalytic pretreatment (Cat.Ox.) as a function of the reaction time and after steam explosion (SE Spruce). Lignin was analysed as Klason lignin. Carbohydrates were analysed after acid hydrolysis by HPLC. The CatOx reactions were carried out at 100 °C under 10 bar oxygen pressure with copper(II) phenanthroline as a catalyst.

![Chemical components](image)

**Chemical Components (% of dry weight)**
- Glucan
- Other neutral polysaccharides
- Others (pectin, ash, extractives, unknown oxidation products)
- Lignin

Figure 5. The effect of temperature and reaction time on mass loss with CuPhen (a) and temperature in mass loss in 20 h in CatOx with CuPhen and CuBipy (b). The reactions were carried out under 10 bar oxygen pressure.
Figure 6. The effect of dosage of sodium carbonate on mass loss in CatOx. The reaction was carried out with CuPhen catalyst at 100 °C for 20 h under 10 bar oxygen pressure.

Figure 7. The effect of applied gas and its pressure on mass loss in CatOx. The reaction was carried out with CuPhen catalysts at 120 °C for 5 h.

In scanning electron microscopy (SEM) images it is clearly seen that after CatOx the isolated carbohydrate rich material is very similar to chemical pulp: the macrostructure of the raw material is destroyed and cellulose fibers are revealed (Figure 8).
Figure 8. SEM images of a) nontreated spruce wood, b) chemical spruce pulp, and c) the filtered solid fraction from CatOx pretreatment. The images were taken with a Hitachi S-4800 field-emission scanning electron microscopy instrument. The samples were sputter-coated with a 5 nm layer of Pt/Pd prior to imaging.

The efficiency of CatOx as a pretreatment method was tested with enzymatic hydrolysis of the carbohydrate rich fraction and compared to the hydrolysis of steam exploded material. The solid fractions from selected experiments with CuPhen were hydrolyzed to sugars (Figure 9). While the one hour pretreatment was not enough for a good sugar yield, already four hours
pretreatment gave optimal sugar yield and the extended pretreatment time did not improve the hydrolyzability. In similar hydrolysis conditions, only less than 60% of the carbohydrates in steam exploded spruce wood were hydrolyzed.

Figure 9. Enzymatic hydrolysis of catalytically pretreated spruce wood and steam exploded spruce wood (SE spruce). The hydrolysis products were analyzed as reducing sugars and total carbohydrates by acid hydrolysis and HPLC. The CatOx reactions were carried out at 120 °C under 10 bar oxygen pressure.

The carbohydrate rich fraction from the 4 h catalytic oxidation pretreatment was also exposed to simultaneous saccharification and fermentation (SSF) (Figure 10). A prehydrolysis of 18 h was applied prior SSF. The cellulosic material was very fast converted into ethanol and already in 10 h almost maximum ethanol concentration was achieved. In 20 h the value reached close to 50 g L⁻¹ with 15% dry substrate content. Interestingly, the carbohydrate fraction can be converted into ethanol with SSF directly in the pretreatment reaction solution without additional filtration steps. This shows that no inhibiting by-products are formed.

When the adsorption of monocomponent enzymes during hydrolysis of CatOx pretreated spruce wood was studied it was found that a high amount of the enzymes were desorbed after the hydrolysis. This indicates that the enzymes could possibly be recycled without additional desorption treatment. On the contrary from steam exploded spruce wood the enzymes were not desorbed.⁷⁴
Figure 10. SSF of the catalytically oxidized spruce fibre fraction in anaerobic conditions in a bench-top bioreactor at dry matter content of 15 % at 35 °C, pH 5 with initially 3 g d.w. S. cerevisiae cells/L. Substrate was prehydrolyzed before fermentation with 10 FPU Celluclast/g d.w. and 1000 nkat Novozym 188/g d.w. for 18 h at 45 °C, pH 5. Catalytic pretreatment was carried out for 4 h at 120 °C and with 1,10-phenanthroline and CuSO₄·5 H₂O as catalyst at 0.25 mol/l Na₂CO₃ concentration, with 10 bar oxygen pressure.

4.5 Alkaline oxidation – results and discussion on publication II

Alkaline oxidation pretreatment (AlkOx) is a similar method as CatOx, but without the copper(II) phenanthroline catalyst. The reaction was carried out for 20 h at 120 °C or for 5 h at 140 °C, under 10 bar oxygen pressure in alkaline water solution. Also the obtained solid material was very similar in AlkOx and CatOx: the carbohydrate content was over 93 % in both and the material was extremely easily hydrolyzed, lignin was dissolved, as well as part of the hemicelluloses. Thus the only significant difference is in the reaction conditions. The catalyst in CatOx allows shorter reaction times and/or lower reaction temperatures.

In the CatOx studies Na₂CO₃ was selected as alkali agent. With AlkOx also other alkaline solutions, NaOH, KOH and Ca(OH)_2, were studied. It was found out that AlkOx is not very dependent on the alkali used, but the carbohydrate content of the obtained solid fractions were similar with Na₂CO₃, NaOH and KOH. Only Ca(OH)_2 was an exception and the pretreatment did not work very well, most probably because of the low solubility of Ca(OH)_2 in water.

An interesting feature of the AlkOx treated material is its very high enzymatic hydrolyzability. By decreasing the enzyme loading the total costs of sugar and ethanol
production can be decreased as the enzymes and enzyme costs are an important part of the overall production costs.\textsuperscript{75,76} With AlkOx the standard hydrolysis experiments were carried out with 10 FPU/g enzyme dosage and very good sugar yields were achieved with all studied materials, bagasse, birch and spruce (Figure 11). Decreasing the enzyme dosage to 4 FPU/g still produced acceptable sugar yields and only 2 FPU/g was found to be a too low dosage for good hydrolysis. Thus, with AlkOx treated materials the enzyme dosage can be reduced up to 60\% compared to steam exploded material without a significant effect on the sugar yields.

When the hydrolysis results from AlkOx and steam exploded spruce, birch and bagasse were compared, the advantage of AlkOx was especially seen with spruce. While after AlkOx 84\% of the carbohydrate rich material was hydrolyzed, with steam exploded material the yield was only 52\%. Also with bagasse the sugar yields were a little better after AlkOx treatment than after steam explosion.

The superiority of AlkOx treatment compared to steam explosion was observed in fermentation as well. Only 20\% ethanol yield was obtained with steam exploded spruce and 30\% yield with bagasse when fermented in 12\% consistency with commercial yeast able to utilize only C6 sugars. On the other hand, AlkOx produced easily fermentable materials. Fermentation of AlkOx treated spruce gave a maximum ethanol yield of 49 g/l, which corresponds to 80\% yield. With AlkOx treated bagasse, which has lower C6 sugar content than spruce, the obtained ethanol yield was a little lower, 38 g/l.

More value for the total process could be added with efficient valorization of the lignin. After CatOx and AlkOx the dissolved lignin can be precipitated with acid and further utilized. The obtained lignin is highly interesting as it is totally sulfur free. The utilization has been studied for example in pharmaceutical purposes.\textsuperscript{77}
Figure 11. Effect of enzyme dosage and pretreatment type on enzymatic hydrolyzability of steam exploded and alkaline oxidized bagasse (A), birch (B), and spruce (C). Enzymatic hydrolysis at 1 % dry matter content with Celluclast 1.5 L (2-10 FPU/g) and Novozyme 188 (100 nkat/g).
4.6 Summary of pretreatments

A lot of research has been done around the world to develop a new and cost effective pretreatment method for lignocellulosic materials for second generation biofuel and chemical production. All current pretreatment methods still have some serious drawbacks. CatOx and AlkOx are highly selective methods that produce easily enzymatically hydrolysable carbohydrate material. The enzyme loading in hydrolysis can be decreased up to 60 % compared to the standard loading which helps to decrease the costs of the whole ethanol or chemical production. In addition, the obtained lignin from CatOx and AlkOx is totally sulfur free and has a great potential in value added applications. To make CatOx more suitable for large scale applications, the catalyst should be recycled. The recycling of the current catalyst is challenging because it is homogeneous and difficult to isolate from the reaction mixture. Also even shorter reaction time would be desirable.

5. Chromated copper arsenite treated waste wood as a raw material for sugar production

Wood is an excellent construction material, but it is easily damaged by fungi and bacteria. To prevent the damage wood preservatives have been used for decades in exterior use such as decks, fences and utility poles. One of the most widely used preservatives contains copper, chromium and arsenite salts and is called chromated copper arsenite (CCA). Copper and arsenic are used to protect the wood against fungi, bacteria, insects and marine borers and chromium acts as a “fixing” agent for copper and arsenic. There is a substantial amount of the metal ions left in the wood when it is disposed, regardless of the leaching to the environment that occurs during the 30-50 years lifetime of CCA-treated wood. The significant amount of these metal ions makes the waste management of CCA-treated wood challenging and CCA-treated waste wood is actually considered as hazardous waste for example in the US and EU.

The production of CCA-treated wood has been dramatically decreased due to EU and different national regulations. However, the waste management issue stays relevant due to the long lifetime of CCA-treated wood. It has been estimated that in the UK the amount of
disposed CCA-treated wood would be 870 000 m$^3$ by 2061. This also means that the amount of CCA-treated wood in post-consumer wood stream would actually increase until 2061.$^{79}$

5.1 The waste management

The traditional way of treating CCA-treated waste wood is landfilling, which is relatively inexpensive, though it requires large disposal areas. Also, numerous studies have shown that copper, chromium and arsenic are leaching from CCA-treated waste wood which can cause significant pollution in soil water and have potential impacts on the environment and human health. Especially the leaching of arsenic is a problem, as the amount exceeds 5 mg/l which is the limit for hazardous waste for example in the US.$^{7,81-90}$

Besides landfill pyrolysis and combustion are traditionally common ways of treating wood waste and they have been applied also in waste treatment of CCA-treated wood. The thermal treatments allow efficient energy recovery and, when compared to landfill disposal, enable more controlled separation of the metal ions.$^{91}$ However, there are some serious drawbacks in burning CCA-treated wood. Though almost all copper and chromium can be retained in the ash, arsenic is more easily evaporated and is thus evolved into the gas phase. The amount of arsenic released into the air can be decreased by lowering the burning temperature and reducing the air supply.$^{89,92-95}$ However, the municipal waste incinerators and most industrial waste incinerators are not designed to retain this type of toxic elements in such high concentrations. Conventional pyrolysis systems are also operated at too high temperatures to prevent the vaporization of arsenic. And above all, even a low temperature pyrolysis ($< 350 \, ^\circ C$) does not solve the problem totally, since 10-15 % of arsenic is still evaporated.$^{79,96}$

When landfilling of CCA-treated waste wood is compared to combustion in waste-to-energy facilities it can be pointed out that there are pros and cons in both of them. Combustion is nearly twice more expensive, but at the same time energy is produced. Also, less landfill area is required in combustion, but as a disadvantage more of the metals are leached from the ash than from the waste material in landfills.$^{97}$
5.2 The recycling studies

If CCA-treated waste wood is recycled, wood fibers and the metal ions should both preferably be reused. Various biological and chemical methods for removing the metal ions have been developed, but unfortunately they all still have their limitations.98

Different fungi have been studied in biological treatment of CCA-treated wood. The fungi produce organic acids that decompose the wood and simultaneously the leached metals can be recovered. Often the fungi decompose only a part of the wood and thus also only part of the metals is released.79,99,100 Recently good results have been obtained with a biological two-step process, where two lactic acid bacteria were used. Over 86 % of the metals were removed in four days extraction and wood remained otherwise nearly intact.80 A combination of oxalic acid extraction and bacteria remediation has also given promising results where 70-100 % of the metals were removed.101,102 Though the use of fungi is considered as an environmentally friendly process as no chemicals are used, the loss of potentially valuable wood material and treatment times up to several weeks are clear disadvantages.99-102 Also ensuring the efficient bacterium growth is challenging.103

As mentioned, besides biological treatment also chemical treatment of CCA-treated waste wood has been studied. Although the metal ions rarely can be totally removed, the extraction of most of the metals makes the waste at least non-hazardous.104 However, if the wood fibers would be reused they need to be certified to be free of all chemical additives to ensure healthy working environment for the mill workers and acceptance of end-users.98

Sulfuric acid extraction is one of the most studied methods of chemical treatment. A recently discovered three step extraction process is able to remove up to 99 % of arsenic and copper and 95 % of chromium. The experiments have been carried out in pilot scale. Also the precipitation of the removed copper, chromium and arsenic has been studied with the extraction process with very good results: 99 % of the preservatives could be collected. Also citric acid and acetic acid extractions have been studied, but the results are not as promising as with sulfuric acid.105-107

Among chelating agents, ethylenediaminetetraacetic acid (EDTA), nitrilotriacetic acid (NTA) and oxalic acid have gained attention. The ability to form stable metal complexes makes them interesting extractive agents. EDTA is one of the most common chelators used to bind metal ions. Especially copper was very efficiently removed from CCA-treated waste
wood, whereas most of the chromium and arsenic remained in the wood. On the other hand, oxalic acid is not effective in copper removal, but fairly good in chromium and arsenic extractions. When the pH of an oxalic acid solution is adjusted to 3.2, hydrogen oxalate, which removes all the metal ions effectively, is formed. EDTA or NTA extraction followed by oxalic acid extraction is also an effective method, where 90-100% of the metal ions are removed. As there are some environmental concerns related to EDTA, an easily biodegradable alternative, SS-ethylenediamine-N,N′-disuccinic acid, has also been used in CCA-treated waste wood extraction studies. The results are similar to EDTA as copper is the easiest to remove, but the extraction yields of chromium and arsenic are even better than with EDTA. A great variety of other possibilities for the extraction has been studied, for example wood vinegar, chitin and chitosan and supercritical carbon dioxide, but not with any great success.

Rather strong oxidative agents, hydrogen peroxide and sodium hypochlorite, are quite efficient in metal ion removal from CCA-treated waste wood, but the wood itself is destroyed to some degree. Thus the reuse must be carefully considered. Also steam explosion and pyrolysis have been studied for opening the chemical structure of CCA-treated wood to provide better removal of metal ions with different acid extractions or bacterial fermentation. However, the pretreatment did not improve the extraction yield but in fact reduced it. Electrodialysis would allow the recycling of the CCA-treated wood and the metals collected. The amount of removed metal ions with electrodialysis is rather high, over 85%, but as a severe drawback two weeks reaction time is needed.

5.3 Oxidative pretreatment and enzymatic hydrolysis – results and discussion on publication III

Instead of considering CCA-treated waste wood simply as waste that needs rather complex disposal methods, it can be considered as a highly available raw material for sugar and chemical production. This way the originally hazardous material would be turned into something valuable. When CCA-treated waste wood is processed with a suitable pretreatment method the carbohydrates it contains can be isolated and further hydrolyzed to monomers or other preferred products. In an optimal process also the copper, chromium and arsenic ions are recovered and recycled, and the lignin is utilized.
CatOx and AlkOx pretreatments were applied to CCA-treated waste wood. The treatments were carried out at 120 °C for 20 hours under 10 bar oxygen pressure. During CatOx and AlkOx 56-58 % of the raw material was dissolved, including most of the lignin, part of the hemicelluloses and some of the metal ions. While the raw material contained 3.8 g/kg of copper, 5.3 g/kg chromium and 0.9 g/kg arsenic, after CatOx the contents in carbohydrate rich fraction were 0.9 g/kg, 2.6 g/kg and 0.5 g/kg and after AlkOx 0.7 g/kg, 3.4 g/kg and 0.5 g/kg, respectively (Figure 12).

![Figure 12](image.png)

**Figure 12.** The amount of copper, chromium and arsenic in CCA-treated waste wood before pretreatment (raw material), after CatOx pretreatment (CatOx), and after AlkOx pretreatment (AlkOx).

The solid carbohydrate rich fraction was exposed to enzymatic hydrolysis. After 24 hours hydrolysis already 74 % sugar yield was obtained from the material treated with CatOx and 58 % sugar yield from the material treated with AlkOx (Figure 13). The results show that even though the pretreated materials still contained some of the metal ions, the ions are not very significantly inhibiting enzymes responsible of the hydrolysis.

To still improve the hydrolysis yields the materials from CatOx and AlkOx treatments were further exposed to additional extraction either with EDTA, hydrogen oxalate or mild sulfuric acid and the enzymatic hydrolysis was carried out after the extractions. A significant improvement in hydrolysis was seen with CatOx treatment combined with EDTA extraction and AlkOx treatment combined with hydrogen oxalate extraction, the sugar yields being 97 % and 93 %, respectively. It is worth of noting that when the pretreatments were combined with extraction with 0.07 N sulfuric acid, close to 90 % sugar yields were obtained. This is a very
interesting result when also the economy is considered as sulfuric acid is a cheap and easily available chemical.

Figure 13. Enzymatic hydrolysis of catalytic and alkaline pretreated materials without extraction (CatOx and AlkOx), after sulfuric acid (CatOx H$_2$SO$_4$ and AlkOx H$_2$SO$_4$), EDTA (CatOx EDTA and AlkOx EDTA) and sodium hydrogenoxalate extractions (CatOx hydrogenoxalate and AlkOx hydrogenoxalate).

5.4 Summary of CCA-treated waste wood and its waste management

The waste management of CCA-treated wood is a task that will follow us for the next decades. The task is very challenging due to the toxic chemicals present in the treated wood. Even though the possible disposal methods have been widely studied we are still lacking an excellent method.

CatOx and AlkOx together with very mild sulfuric acid extraction would offer a promising opportunity to transform hazardous waste into various valuable chemicals. The obtained carbohydrate rich fraction is notably easily hydrolyzed to sugars by enzymes and the sugars can act as a possible raw material for further processing to chemicals. The separation of copper, chromium and arsenic from the reaction mixture still needs to be studied to obtain information of the whole economy of the process.
6. Hydrolysis of cellulose and hemicelluloses

Cellulose and hemicelluloses are abundant polymers of different monosaccharides. They offer a great opportunity for chemical industry all over the world as they are widely available and can be utilized in a wide spectrum of chemical processes. To exploit the monosaccharides in chemical industry the cellulose and hemicellulose polymers need to be depolymerized, e.g. hydrolyzed. Hemicelluloses have irregular, amorphous structure and a lower degree of polymerization compared to cellulose. Thus hemicelluloses are easier to hydrolyze than the partly crystalline cellulose.

Generally the hydrolysis of cellulose and hemicelluloses is done either with enzymes or acids. The basic chemistry of converting carbohydrates into corresponding monosaccharides with mineral acids has been known for over 100 years. Already during World War II acid hydrolysis was used in ethanol production from wood. However, the applied methods were rather expensive and after the war the raise of petroleum industry almost ended the studies of biomass utilization for chemical production. Recently the concern of sustainability and the reduced oil supplies have raised the biomass conversion again high on the scientific agenda. This has led also to the development of novel hydrolysis methods among all, including enzymatic hydrolysis.

6.1 Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose and hemicelluloses has been studied since the late 1970’s and it has gained a lot of attention. It is a highly selective hydrolysis method and with a suitable combination of enzymes very pure monosaccharides or their mixtures can be obtained. In cellulose hydrolysis usually endoglucanases, exoglucanases and β-glucosidases are used. Endoglucanases cleave randomly internal bonds, exoglucanases cleave two to four units from the ends of the cellulose chains, and β-glucosidase hydrolyses the oligosaccharides into monosaccharides. Exoglucanases can be found in two different forms, one that acts at the non-reducing end of cellulose and one that acts at the reducing end. Similarly, xylanases are used for hydrolysis of xylan and so on.

Enzymatic hydrolysis is usually carried out at room temperature and in close to neutral conditions. Thus the operation costs are relatively low, and the requirements for the reactors
are acceptable. On the other hand, despite of extensive research, enzymes are still rather expensive and very vulnerable to inhibiting side products.\textsuperscript{11} Also the hydrolysis times are quite long, often a day or more. Nature has designed the structure of lignocellulosic materials to be very resistant towards biological attack. Thus a pretreatment step, which softens the lignocellulosic material and breaks down cell structures to a large extent, is needed prior to the enzymatic hydrolysis.\textsuperscript{120}

### 6.2 Acid hydrolysis

A wide variety of acid solutions have been used in cellulose hydrolysis, including hydrochloric acid and sulfuric acid, hydrofluoric acid and different organic acids. Often the problem with acid hydrolysis is the requirement of strong acids or, on the other hand, poor sugar yields. In industrial applications concentrated acids cause many problems related to corrosion and poor recyclability of the acid. However, for hydrolysis in analytical studies concentrated sulfuric acid is widely used and high sugar yields are obtained.\textsuperscript{121} Often diluted sulfuric acid has been favored in industrial applications. The hydrolysis is traditionally carried out in a two-stage process, where in the first step hemicelluloses are hydrolyzed (190 °C) and in the second stage the cellulose is subjected to harsher hydrolysis conditions (215 °C). The hemicelluloses sugar yields are around 80-90 \%, but the glucose yield is only about 50 \%.\textsuperscript{120}

### 6.3 Microwaves in chemical reactions

Microwaves were applied to chemical research already in the early 1970’s with standard household microwave ovens. The observation of significant rate accelerations increased the interest notably in 1980’s and resulted in the design of more advanced microwave reactors.\textsuperscript{122} The present microwave reactors ensure accurate temperature control, and rapid heating and cooling rates. The possibility of direct heating of the reaction mixture reduces energy consumption and saves time.\textsuperscript{123,124}

Microwaves have been applied to hydrolysis of carbohydrates to enhance the sugar yields. Promising results have been obtained with phosphoric acid in the hydrolysis of carbohydrates in grass clippings.\textsuperscript{125,126} Some solid acids have also been successfully studied.\textsuperscript{127-129} Regardless of the microwave reactors the reaction times are still rather long or the required temperatures relatively high.
6.4 Microwave assisted mild acid hydrolysis – results and discussion on publication IV

As promising hydrolysis results with phosphoric acid had been obtained in previous studies, herein sulfuric acid, phosphoric acid and hydrochloric acid were studied in hydrolysis of xylan, AlkOx birch and milled birch chips. In preliminary studies with AlkOx birch temperatures from 150 °C to 200 °C, reaction times from 1 min to 10 min and acid concentrations from 0.1 wt% to 3 wt-% were screened. The screening showed clearly that with all the selected acids 200 °C was too high a temperature as hardly any sugars were detected after the reactions. An acid concentration of 2 wt-% was selected for further experiments as the best sugar yields were obtained with that. Depending on the temperature and acid, the best sugar yields were obtained either in a short reaction time or a longer time, therefore, the further reactions were carried out in reaction times of 1, 2, 3, 5 and 7 min.

In xylan hydrolysis the reactions were carried out also at 140 °C as xylan is easier to hydrolyze than cellulose. The results with xylan were very good. With phosphoric acid the maximum xylose yield was 84 % and with sulfuric acid 98 %. Especially with phosphoric acid the xylose yield significantly increased with increasing temperature (Figure 14) and the optimal reaction conditions were 175 °C and 2 min. At lower temperatures the xylose yield could be improved to some extent by prolonging the reaction time. The trend between xylose yield and temperature was quite similar with sulfuric acid though not so obvious, and already at 175 °C the yield started to decrease. Thus the optimal reaction conditions were 160 °C and 1 min. Contrary to phosphoric acid and sulfuric acid the xylose yield was decreasing with increasing temperature when hydrochloric acid was used. The optimal reaction conditions were 140 °C and 1 min and almost a quantitative yield of xylose was obtained. This is a significantly fast hydrolysis at a relatively low temperature. It also seems that xylose is only little decomposed at 140 °C as the yields are high also with 2 and 3 min reaction times.
Figure 14. Xylose yields in pure xylan hydrolysis. The reactions were carried out with 2 wt-% acid concentration at temperatures 140-160 °C for 1-3 min.
As the results with xylan were nearly quantitative, the hydrolysis experiments were also carried out with milled birch wood as raw material. The target was to hydrolyze the birch xylan selectively. It was known that sulfuric acid is efficient in cellulose hydrolysis, so only phosphoric acid and hydrochloric acid were selected for these experiments. Phosphoric acid was shown to be highly selective towards xylose as no glucose was detected after the reactions. The trend between reaction temperature and xylose yield was similar as with pure xylan, i.e., increasing temperature increased the sugar yield (Figure 15). In the optimal reaction conditions, at 175 °C in 5 min, 64 % xylose yield was obtained. With hydrochloric acid a little better xylose yield, 70 %, could be obtained at 150 °C in 3 min. Unfortunately the selectivity was lost as also a significant amount of glucose was obtained, especially at higher temperatures. However, the hydrolysis of xylan in milled birch wood was fast and it could be carried out at relatively low temperatures.

The tight and resistant structure of wood usually restricts the hydrolysis rate, therefore, AlkOx pretreatment was applied to birch chips prior the microwave assisted hydrolysis to enhance especially the hydrolysis of cellulose. During the pretreatment 47 wt% of the wood material was solubilized and the solid fractions, which were further hydrolyzed, contained 22 % xylan and 67 % cellulose. Also with this material, phosphoric acid was selective towards xylose and only at 175 °C some glucose was detected. Nevertheless, sulfuric acid was the most effective in xylan hydrolysis (Figure 16). The best xylose yield was almost 90 %, which corresponds to ca. 50 % of the original amount of xylose in birch as some of the hemicelluloses were lost in the pretreatment step.
Figure 15. Xylose yields in birch hydrolysis. The yields are calculated as a percentage of the total amount of xylose in birch chips. The reactions were carried out with 2 wt% acid concentration at temperatures of 150-175 °C for 2-5 min.
Figure 16. Xylose and glucose yields in AlkOx birch wood hydrolysis. The yields are calculated as a percentage of the total amount of xylose or glucose in AlkOx birch. The reactions were carried out with 2 wt% acid concentration at temperatures of 150-175 °C for 2-5 min.
The glucose yields in these hydrolysis experiments were not as good as it was expected. The unreacted solid material left after the hydrolysis reactions indicated that some of the starting material did not react. Thus further multistep reactions were carried out where after the first hydrolysis the unreacted material was separated with filtration from the sugar solution and exposed to a second and possibly a third additional hydrolysis steps. With filtration and separation between the hydrolysis steps the further decomposition of obtained sugars was avoided. A three-step hydrolysis with sulfuric acid was the best option among various combinations. The first step was carried out at 160 °C for 2 minutes and the second and third at 175 °C for 7 minutes each. With this kind of multistep hydrolysis all the xylose was hydrolyzed and the glucose yield was 66 %. In a similar reaction but without the third step, the glucose yield was only 55 %. This strongly indicates that the formation of glucose still proceeds after the second hydrolysis step and when the formed glucose is removed its decomposition is prevented.

### 6.5 Summary of the hydrolysis

Although the hydrolysis of cellulose and hemicelluloses has been studied for a long time it is still a challenging task. A microwave reactor together with a simply mild acid hydrolysis is an extremely fast method to produce xylose from xylan or milled birch chips. The method can be used also in multi-step hydrolysis, which gives a rather satisfying glucose yield in a total reaction time of 16 min. The difference in sugar yields and optimal reaction conditions between different acids can be partly explained with the different pH of the solutions.\textsuperscript{130}

When these results from microwave assisted mild acid hydrolysis are compared to enzymatic hydrolysis it is obvious that the reaction times are significantly shorter. The xylose yields are comparable to the yields in enzymatic hydrolysis. The glucose yields are relatively good, though not reaching the quantitative yields of enzymatic hydrolysis.
The concern of sustainability and the reducing oil supplies are the driving forces behind the need for biobased fuels and chemicals. The lignocellulosic materials are interesting raw materials as they are abundant, widely available and non-edible. However, there are challenges in their utilization in the chemical industry. The macrostructure of lignocellulosic materials is very tight and resistant. A lot of studies have been done to find a proper pretreatment method to loosen the structure and to separate the different fractions, cellulose, hemicelluloses and lignin, for further processing.

All of the current pretreatment methods still have some disadvantages and thus new pretreatment methods were studied in this thesis. Two novel pretreatment and fractionation methods for lignocellulosic materials, CatOx and AlkOx, were developed. These methods are highly selective: cellulose remains nearly intact and only a small amount of hemicelluloses is decomposed. The obtained carbohydrate material is easily hydrolyzed by enzymes. For example, the carbohydrate material obtained from four hours CatOx reaction can be almost quantitatively hydrolyzed to corresponding sugars in only 24 hours. Similar sugar yields can be achieved with AlkOx treated material, though the pretreatment time or temperature needs to be increased compared to the CatOx treatment. Low enzyme dosages can be used, which helps in reducing the overall costs of the processes. In addition, the obtained lignin is an interesting raw material, for example, for chemical industry as it is totally sulfur free. Both pretreatment methods can be applied to a great variety of different lignocellulosic raw materials like spruce, birch and eucalyptus wood, sugarcane bagasse and even to CCA-treated waste wood.

The waste treatment of CCA-treated wood is challenging due to the metal ions it contains. It is usually used either in landfills or thermally treated to produce energy. Both methods have their disadvantages. With landfills the main concern is the leaching of metals, especially arsenic. Arsenic is also the major problem in thermal treatment as it is easily evaporated in the process. However, CatOx and AlkOx enable the utilization of this hazardous waste as a raw material in the chemical industry. After CatOx or AlkOx pretreatment the obtained carbohydrate rich fraction can be enzymatically hydrolyzed to sugars. The pretreatments can be enhanced with a very mild sulfuric acid extraction, which leads to an increase in the sugar yield.
A lot of work has been done for improving the hydrolysis of cellulose and hemicellulose. Enzymatic hydrolysis is highly selective as even quantitative sugar yields can be obtained. Unfortunately, enzymatic hydrolysis is usually quite slow, with the reaction times starting from 24 h. In addition, enzymes are very sensitive to inhibiting compounds and require a selective pretreatment method.

The traditional way for hydrolysis is acid hydrolysis. It also suffers from some disadvantages like quite poor glucose yields from cellulose with mild acids and a corrosive effect of strong acids. Microwave assisted mild acid hydrolysis can offer a faster method for obtaining sugars from hemicelluloses and cellulose fast and with relatively good yields. With 2 wt-% hydrochloric acid in microwave reactor xylan can be quantitatively hydrolyzed to xylose in only one minute at 140 °C. With a three-step reaction using 2 wt-% sulfuric acid 66 % of the cellulose in AlkOx treated birch wood is hydrolyzed to glucose in only 16 min.

The pretreatment and hydrolysis methods introduced in this thesis form an ensemble, which offers a possibility to convert carbohydrates from different lignocellulosic materials, even from hazardous waste, to corresponding sugars. The obtained sugars can be further utilized in chemical industry. This ensemble offers an interesting option for current pretreatment and hydrolysis methods.
Experimental methods

General procedure for CatOx and AlkOx pretreatments (I, II)

In CatOx the in situ made catalyst solution is prepared by dissolving 30 mg (0.168 mmol) of 1,10-phenanthroline, 21 mg (0.084 mmol) of CuSO$_4$ · 5 H$_2$O, and 2.6 g (24.5 mmol) of Na$_2$CO$_3$ in 100 ml of deionized water. The catalyst solution together with 5 g of lignocellulosic raw material (dry weight) is added to a preheated autoclave equipped with magnetic stirrer and oil bath heating. The reaction is carried out for 1-20 h at elevated temperatures (100-120 ºC) and with oxygen pressure (10 bar).

AlkOx is carried out similarly, but without adding 1,10-phenanthroline and CuSO$_4$ · 5 H$_2$O. At optimal reaction conditions the reaction time is 20 h in 120 ºC or 5 h in 140 ºC.

Pretreatment and extraction experiments for CCA-treated waste wood (III)

The reaction scheme is shown in Figure 17. The CatOx and AlkOx pretreatments were carried out according to the general procedure with 20 h reaction time and 120 ºC reaction temperature. In CatOx no CuSO$_4$ · 5 H$_2$O was added, but 1,10-phenanthroline was assumed to be able to form the catalyst with the copper ions already present in CCA-treated wood. Both pretreatments, CatOx and AlkOx, were carried out for 10 times and the solid fractions were filtered and washed with water. The filtered solid fractions were combined. For further extractions and hydrolysis experiments the solid fractions were divided in four parts, one to be hydrolyzed as such and three to the additional extractions.

For the extraction experiments part of the solid fraction obtained in the pretreatments was placed in a flask together with 100 ml of extractant. The solution was stirred with magnetic stirrer. Due to the heating of the reaction mixture a condenser was used with hydrogenoxalate. The reaction parameters can be seen in Table 1.
CCA-treated waste wood

\[ \rightarrow \]

Catalytic oxidation

or

Alkaline oxidation

\[ \rightarrow \]

Optional extraction with EDTA, hydrogen oxalate or $\text{H}_2\text{SO}_4$

\[ \rightarrow \]

Enzymatic hydrolysis of carbohydrates to sugars

**Figure 17.** A process flow chart of pretreatment and hydrolysis of CCA-treated wood.

<table>
<thead>
<tr>
<th>Extractant</th>
<th>Reaction temperature</th>
<th>Reaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>$0.07 \text{ N H}_2\text{SO}_4$</td>
<td>room temperature</td>
<td>22 h</td>
</tr>
<tr>
<td>1 % EDTA</td>
<td>room temperature</td>
<td>24 h</td>
</tr>
<tr>
<td>0.125 M hydrogen oxalate*</td>
<td>72 °C</td>
<td>6 h</td>
</tr>
</tbody>
</table>

**Table 1.** Reaction parameters of extractions.*) pH of the solution was adjusted to 3.2 with Na$_2$CO$_3$.

**General procedure for enzymatic hydrolysis (I, III)**

The hydrolysis of washed solid fractions was carried out using a commercial cellulose mixture, Celluclast 1.5 L FG 10 FPU/g dry matter and $\beta$-glucosidase Novozym 188 100 nkat/g dry matter. The hydrolysis experiments were performed in 50 mM sodium acetate buffer (pH 5) in magnetically stirred test tubes at 1 % (w/v) dry matter concentration. The temperature was controlled at 45 °C. The hydrolysis experiments were done in triplicates. The reducing sugars released were monitored by using the dinitrosalisylic acid (DNS) assay.
Microwave assisted mild acid hydrolysis (IV)

Before AlkOx pretreatment the birch chips were pre-extracted two days with boiling hexane and one day with boiling acetone to remove extractives. The pretreatment was carried out according to the standard procedure for 20 h at 120 °C. After the pretreatment the solid fraction was separated with filtration, washed with 100 ml of deionized water and dried in room temperature. The dry material was ball milled for 3 minutes with 400 rpm (Planetary Mono Mill pulverisette 6, Fritsch GmbH, Germany) to obtain more easily processed material.

For hydrolysis xylan or birch samples, 100 mg of material was weighted into a microwave vial and 2 ml of 2w-% acid solution was added together with a magnetic stirrer bar. The sample was placed in a microwave reactor (Biotage Initiator) and heated at the desired temperature for a specific time. The reaction conditions are listed in Table 1. After the hydrolysis the birch wood samples were filtered. All reaction solutions were diluted to 50 ml with deionized water and neutralized with aqueous NH$_3$ solution. The HPLC sample was syringed through a 0.45 µm filter and analyzed. All hydrolysis experiments were done in duplicates and the yields were calculated as mean values.

For sugar analysis by HPLC a light scattering detector (Agilent Technologies 1200 Series) was used with a Carbohydrate-NH2 (150 mm) column. Samples were run at 30 °C and eluted at 1.0 ml/min with ACN:H$_2$O (80:20).
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