CATALYSED ALKALINE OXIDATION AS A WOOD FRACTIONATION TECHNIQUE

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Alkaline oxidation (AlkOx) is an effective fractionation technique for lignocellulosic raw materials. The efficiency of the AlkOx treatment can further be enhanced by using a catalyst (CatOx). Both CatOx and AlkOx provide a fiber fraction containing readily hydrolysable carbohydrates that can be utilized in biotechnical processes and a liquid fraction containing solubilized lignin and reaction products from various biomass components. The effects of different fractionation conditions on yields and chemical composition of solubilized and insoluble fractions were investigated. Two temperatures and two reaction times were studied with and without a catalyst. The composition and content of carbohydrates in the fiber and liquid fractions were examined. The generation of aliphatic carboxylic acids as oxidation products was also investigated. The catalytically assisted oxidation was more efficient than the alkaline counterpart in dissolution of wood components under a four-hour treatment period resulting in higher dissolution of hemicelluloses. A longer reaction time of 20 hours leveled out the differences between the oxidation processes. Comparison of different bases showed that similar solubilisation of dry matter was obtained with NaOH, KOH, and Na_2CO_3. Oxidation in Na_2CO_3 caused higher dissolution of glucomannan and greater acid production. The dissolution of hemicellulose and lignin, and their oxidation to acids was most efficient in the first 4 hours of oxidation.

INTRODUCTION

The use of lignocellulosic biomass as a source of chemicals and fuels has been a subject of interest recently due to concerns about climate change and the need to find an alternative to fossil resources. Currently, the main transport biofuel products on the market are bioethanol, biodiesel, and biogas. Bioethanol has the largest market by far, although biodiesels are also expected to increase in popularity (Mosier et al. 2002). Biomass valorization concentrates mainly on cellulose, but an economically viable production necessitates the valorization of all components of lignocellulosic biomass. Approximately only 2% of the lignins available from the pulp and paper industry are used commercially, and the remainder is burned as a low value fuel: however, lignin has significant potential as an aromatic resource for bulk chemical and fuel production (Zakzeski et al. 2010; Hallac et al. 2009).

Pretreatment is one of the most critical steps in converting lignocellulosic materials into bioethanol or other chemicals produced by fermentation. It decomposes the cell wall and separates the material into its components, partly degrading the biopolymers into smaller fragments. This kind of fractionation is needed to produce carbohydrate material readily hydrolysable to sugars by enzymes (Chen et al. 2006). Efficient pretreatment also reduces the cellulose crystallinity and increases the porosity of the material; however, fractionation should not cause remarkable carbohydrate yield losses (Sun and Cheng 2002). In addition, production of toxic or inhibiting compounds should be minimal, and the pretreatment should be technically and economically feasible (Kumar et al. 2009).

Several pretreatments have been studied relative to degradation purposes (Zheng et al. 2009). Among these are steam explosion, organosolv pulping, acidic pretreatments, and wet oxidation

Uncatalyzed steam-explosion, also named as autohydrolysis, is a technology in which only steam water is used to destroy the physical structure of lignocellulosic material. During the pretreatment, the hemicellulose is often hydrolyzed by organic acids such as acetic acids and other acids formed from acetyl or other functional groups, released from biomass. In addition, water itself also possesses certain acidic properties at high temperature, which further catalyze hemicellulose hydrolysis (Avellar and Glasser 1998; Taherzadeh and Karimi 2008). Steam-explosion is typically conducted at a temperature of 160 to 270℃ for several seconds to a few minutes before pretreated contents are discharged into a vessel for cooling. The action of steam explosion can be further enhanced by addition of catalyst such as SO_2, H_2SO_4, and CO_2. Catalyzation improves hemicellulose removal and generates less inhibiting compounds (Zheng et al. 2009).

In the organosolv pretreatment process, organic or aqueous organic solvent with inorganic acid catalysts are used to dissolve the lignin and separate the fibres (Sun and Cheng 2002). Organosolv pulping can remove lignin from fibres selectively, resulting in a pulp of higher yield and viscosity and less condensed residual lignin (Lohrasebi and Paszner 2001). The benefit of the organosolv process is the possibility of recycling the cooking liquid, which decreases the process costs. In addition, it is also obligatory, since the solvent may be may be inhibitory to the growth of organisms, enzymatic hydrolysis, and fermentation (Sun and Cheng 2002).

Dilute acid pretreatment is also a widely used pretreatment method. It is conducted typically using sulfuric acid at high temperature (160 to 200 °C) (Shuai et al 2010). Dilute acid treatment dissolves hemicellulose and partially hydrolyzes cellulose. Serious equipment corrosion problems and extensive condensation of lignin limit the usefulness of this pretreatment. In addition, dilute acid pretreatment can achieve satisfactory levels of cellulose saccharification for agricultural residues and some hardwood species, but it is not effective for softwoods. Sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) has been recently developed for robust and efficient bioconversion of softwoods (Zhu et al. 2009). The process involves treatment of wood chips under acidic conditions using 8 to 10% bisulfite and 1.8 to 3.7% sulfuric acid at 180 °C for 30 min. By this pretreatment almost complete hemicellulose separation, partial delignification, and lignin sulfonation were achieved, offering improved recovery of fermentable sugar, when compared with dilute acid pretreatment. Moreover, a benefit of

SPORL pretreatment is its applicability of variety lignocellulosic raw material (Tian et al. 2011).

The above mentioned pretreatment methods are usually carried out under acidic conditions. Wet oxidation is a pretreatment that is performed under neutral environment. It involves the treatment of the biomass with water and air, or oxygen, at temperatures between 120 and 350°C and at elevated pressures (> 1 MPa) (McGinnis et al. 1983; Bjerre et al. 1996; Palonen et al. 2004).

In more recent developments, wet oxidation has been combined with an alkaline-aided hydrolysis, utilizing either sodium carbonate or lime as the base (Bjerre et al. 1996; Chang et al. 2001). The alkaline wet oxidation is typically applied to biomasses having a low lignin content, such as wheat straw, clover, and ryegrass (Galbe and Zacchi 2002; Bjerre et al. 1996; Martin et al. 2008). This process has also been used as a pretreatment of softwood and hardwood in order to produce bioethanol and biogas (Palonen et al. 2004). The alkaline wet oxidation process has been found to convert many organic polymers to oxidized compounds, such as low molecular weight carboxylic acids, or even to CO_2 and H_2O (Taylor and Weygandt 1974; Klinke et al. 2002); even 65% of wheat straw lignin could be removed by wet oxidation.

Enhancement of the alkaline oxidative fractionation by the addition of a catalyst is a new attractive approach (Korpi et al. 2004), especially in the case when pH of the reaction is strongly alkaline (Hakola et al. 2010). Alkaline oxidation can be catalyzed by a copper-phenanthroline complex, while copper(II) ions form oxygen-activating copper–diimine complexes e.g with 1,10-phenanthroline (phen) and its substituted derivatives (Korpi et al. 2006). According to Korpi et al. (2004) the in situ complexed Cu–phen is an active catalyst for the oxidation of lignin compounds and is thus a potential catalyst for the biomass pretreatment process.

Softwoods are generally considered to be a difficult lignocellulosic raw material to hydrolyze to sugars for fermentation, primarily owing to the nature and amount of lignin. When compared to hardwoods, softwoods contain more hemicellulose, and they have lower xylose content but higher mannose content (Mabee et al. 2006).

The goal of the study was to evaluate an alkaline pretreatment method for the production of bioethanol from softwood. The focus of the present paper is on the chemistry that takes place in alkaline oxidation and the effects of different process parameters, in particular the role of the catalyst in a laboratory scale batch reactor. Other process parameters studied were temperature, treatment time, reaction volume, liquid/gas ratio, and the alkali source.

The decomposition of spruce raw material was monitored by analyzing the carbohydrate composition and yield of the fiber fraction and the compounds formed during the fractionation and dissolved in alkaline liquors. Soluble carbohydrates were analysed with anion exchange chromatography, dissolved lignin with UV measurements, and the formation of small aliphatic carboxylic acids with capillary electrophoresis (CE). The evaluation of the effects of pretreatments on enzymatic hydrolysis yield will be reported separately.

EXPERIMENTAL

Raw Materials

Three kind of particle size were used, depending on the reactor volume. Industrial wood chips consisting mainly of Norway spruce (*Picea abies*) were obtained from a Finnish pulp mill. Part of the chips was ground into wood meal using a Wiley-mill equipped with a 5 mm sieve. Softwood saw dust was collected from a Finnish saw mill and had an average particle size of 2 mm x 2 mm x 10 mm.

Oxidation Treatments

Four different decomposition treatments were carried out. In the first and second series, catalyzed alkaline oxidation (CatOx) was compared with alkaline oxidation (AlkOx). Reaction volume, temperature, and time were variables in both series, as shown in Table 1. In addition, an excess of oxygen was fed into the reactor to kept pressure at a constant level in series I. Sodium carbonate was the alkali used in these experiments. In the third series, oxidations using different alkali sources (calcium hydroxide, sodium hydroxide, potassium hydroxide, and sodium carbonate) without a catalyst were compared. Detailed reaction conditions are listed in Table 1. The fourth series was also catalytically assisted, but the reaction volume was one liter and the experiment was performed in four parallel reactions with different reaction times (1, 4, 8, and 20 h).

In the case of catalyzed oxidation, 0.84 mmol L^{-1} of $CuSO_4*5H_2O$ and 1.66 mmol of L^{-1} phenanthroline were added to the reaction solution. The reagents were dissolved and added to the preheated autoclave (volume of 1 or 2 liters) equipped with a stirrer or added to a stirred tank reactor (volume of 40 liters). The substrate was added to the reactor, stirring was started, and the autoclave was closed and pressurized immediately with 10 bar of oxygen and then heated to the reaction temperature of either 120 °C or 140 °C. The liquid to wood ratio and oxygen loading as well as consistency are compiled in Table 1.

In all experimental series, the reactors were cooled down after the reaction completion and the pressure was released. At temperatures below 50 °C, the reactor was opened and the solid material was washed and separated with vacuum filtration to remove any soluble material. The sugar compositions of the filtrates were then analysed. Part of the filtrate was acidified with 1 M HCl to a pH of 2.5. The precipitate (lignin and some carboxylic acids) was recovered by centrifugation (4000 rpm, 15 min), washed with water (adjusted to a pH of 2.5), centrifuged, and finally freeze-dried.

Analytical Methods

Carbohydrates

The carbohydrate content and composition were determined as monosaccharides using acid hydrolysis and high performance anion exchange chromatography with pulse amperometric detection (HPAEC/PAD) (Willför et al. 2009). A CarboPac PA-1 column 4×250mm (Dionex, Sunnyvale, CA, USA) was coupled with a Dionex ICS-3000 series chromatograph equipped with a pulsed amperometric detector (Dionex ICS-3000). For monosaccharide analysis the system was equilibrated with 15 mM sodium hydroxide.

Table 1. Conditions Used in the Decomposition Tests

Series	Sample code used in Figures and Tables	Sample form	Liquid–to–wood ratio (v/w)	Alkali (conc. 0.25 M)	Catalyst	T (°C)	Oxygen loading (g O_2/g wood)	Liquid volume / reactor volume (Liter) (% fill)	Reaction time (hours)
I	AlkOx 120 °C 4 h 75 %	Chips	20	Na_2CO_3	No	120	0.1[*]	30 / 40 (75 %)	4
	CatOx 120 °C 4 h 75 %	Chips	20	Na_2CO_3	Yes	120	0.1[*]	30 / 40 (75 %)	4
	AlkOx 140 °C 4 h 25 %	Chips	20	Na_2CO_3	No	140	0.7[*]	10 / 40 (25 %)	4
	CatOx 140 °C 4 h 25 %	Chips	20	Na_2CO_3	Yes	140	0.7[*]	10 / 40 (25 %)	4
II	CatOx 120 °C 20 h 50 %	Wood meal	20	Na_2CO_3	Yes	120	0.2	1 / 2 (50 %)	20
	CatOx 120 °C 20 h 10 %	Wood meal	20	Na_2CO_3	Yes	120	2.1	0.2 / 2 (10 %)	20
	AlkOx 120 °C 20 h 50 %	Wood meal	20	Na_2CO_3	No	120	0.2	1 / 2 (50 %)	20
	AlkOx 120 °C 20 h 10 %	Wood meal	19	Na_2CO_3	No	120	2.1	0.2 / 2 (10 %)	20
III	AlkOx $Ca(OH)_2$ 120 °C 20 h 10 %	Saw dust	20	$Ca(OH)_2$	No	120	2.1	0.1 / 1 (10 %)	20
	AlkOx NaOH 120 °C 20 h 10 %	Saw dust	20	NaOH	No	120	2.1	0.1 / 1 (10 %)	20
	AlkOx KOH 120 °C 20 h 10 %	Saw dust	20	KOH	No	120	2.1	0.1 / 1 (10 %)	20
	AlkOx Na_2CO_3 120 °C 20 h 10 %	Saw dust	20	Na_2CO_3	No	120	2.1	0.1 / 1 (10 %)	20
IV 1-4		Saw dust	20	Na_2CO_3	Yes	120	2.1	1 / 2 (50 %)	1, 4, 8 or 20

* oxygen loading at start; pressure was kept constant by feeding excess O_2.

After sample injection, 15 mM sodium hydroxide flowed through the column for 2 min, and from 2 to 36 min 100% of ultra-pure water was run isocratically. A solution of 300 mM NaOH was added to the column effluent before the PAD cell at a flow rate of 0.4 mL min^{-1}. The column was washed with a solution of 100 mM NaOH and 300 mM sodium acetate for 3 min and then washed with 300 mM sodium hydroxide for 4 min. The flow rate was set to 1 mL min^{-1}, column temperature to 30°C, and sample temperature to 15°C. Injection volume was 25 µL. The resulting data were processed using Chromeleon software.

Carboxylic acids

Separations of the carboxylic acid standards and liquid fractions of the oxidation samples were performed using a P/ACE MDQ capillary electrophoresis instrument (Beckman-Coulter, Fullerton, CA, USA) equipped with a photodiode array UV detector. The background electrolyte (BGE) was fine tuned to enhance the resolution between closely migrating acids and for quantifying the C(5)-C(6) hydroxy acids (Rovio et al. 2010). Briefly, anionic compounds were analyzed using the BGE solution containing 20 mM 2,3-pyrazine dicarboxylic acid, 30 mM tricine, 2 mM $BaCl_2$, 0.5 mM cetyltrimethyl-ammonium bromide, and 2 M urea at a pH of 8.06 (± 0.02, adjusted with triethanol-amine). Uncoated fused silica capillaries with a 50 µm I.D. and a length of 100/110 cm (effective length/total length) were employed in the analyses. The samples were injected at a pressure of 0.5 psi (34.5 mbar), and the injection time was set to 20 s. The capillary and samples were thermostatted to +15 °C. The detection wavelength was set to 281 nm using an indirect detection mode. Before the measurements, the new capillaries (from Teknolab Trollåsen, Norway) were conditioned by sequentially rinsing with 0.1 M sodium hydroxide, 0.1 M HCl, and ultra-pure water. The capillaries were rinsed with each solution for 20 min and then with an electrolyte solution for 5 min. Between analyses, the capillaries were rinsed with 0.1 M HCl for 3 minutes, 0.1 M NaOH for 1 min, and the electrolyte solution for 5 min, all under a pressure of 20 psi. Prior to CE analyses, the oxidation samples were diluted to a ratio of 1:10 (v/v) with ultra-pure water.

Lignin content

The content of the dissolved, aromatic lignin was measured from the soluble fraction UV spectroscopy at 280 nm using an absorptivity of 20 L g^{-1} cm^{-1} (Tamminen and Hortling 2001).

Total organic carbon

Total organic carbon (TOC, SFSEN 1484:1997) was determined at Kymen Laboratorio Oy (Finland).

Size exclusion chromatography (SEC)

Size exclusion chromatography (SEC) was performed in a Waters HPLC system. Two Ultrahydrogel (pore sizes of 250 Å and 120 Å, Waters Assoc. USA) columns were linked in series. The detection was carried out with an UV at 280 nm and refractive index (RI) detectors in series. Isocratic chromatography was performed using 0.1 M $NaNO_3$ as the eluent, which was pumped through the columns at a rate of 0.5 mL/min. Each sample

was diluted with a ratio of 1:10 (v/v) with eluent and filtered with 0.45 um disposable filters prior to the injection of 50 µL into the SEC system. The analysis temperature was 30 °C. The molecular weight distributions (MWD) and the average number and weight average molecular weights (Mn, Mw) of the lignin and polysaccharides were calculated using pullulan standards (5900-212000 Da). The system was controlled and data were analysed using Empower 2 software.

RESULTS AND DISCUSSION

General Composition of the Fractions

Catalyzed alkaline oxidation produces fiber fractions with high enzymatic hydrolysability (Hakola et al. 2010). The outcome of both the alkaline and catalyzed reactions can be compared by examining the mass loss, i.e. the proportion of the dissolved lignin and hemicellulose from the original dry weight of the wood.

During the decomposition of wood under oxygen-alkaline conditions, with or without a catalyst, the wood material was divided into two fractions: the solid cellulose rich fraction and the water soluble fraction. Figures 1a, 1c, and 1e present the percentage portions of both the solid and soluble fractions obtained using different reaction conditions. A more detailed distribution of the monosaccharides, small aliphatic carboxylic acids, and lignin in the soluble fraction is depicted in Figs. 1b, 1d, and 1f.

Catalytically assisted decomposition of wood under oxygen-alkali conditions increased the degradation of spruce compared to its counterparts treated in alkali without catalyst (Figs. 1a and 1c). The resulting material from batch reactor was, however, inhomogeneous, containing also unreacted particles, and further scale-up of the technique is needed in order to evaluate its feasibility in full scale industrial process.

A clear difference between non-catalyzed and catalyzed treatment can be observed in the 120°C experiment, where the portions of soluble fractions were 14% and 28%, respectively. By increasing the reaction temperature to 140°C and lowering the filling ratio of the reactor, the difference became smaller, but still the catalyzed reaction was more efficient. Increasing the reaction time to 20 h leveled out the dissolution efficiency between the AlkOx and CatOx treatments, while the proportions of fiber fraction and dissolved fraction became similar (Fig. 1c). The loading of the reactor had a small effect on fractionation. The lower filling ratio, 10% versus 50%, enhanced the dissolution, however.

Over 35% of the dry matter was solubilized in the alkaline or catalytic oxidation when the loading was 10% or 25% of the reactor volume (Figs. 1a and 1c). The higher oxygen volume as a result of lower liquid volume likely enhanced the oxidation reaction and solubilisation. Lower liquid volumes might improve the mixing of the suspension, decrease the concentration gradients, and increase the solubilisation of oxygen throughout the reaction. These observations need to be taken into account when scaling up the method. The proportions of monosaccharide, carboxylic acid, and lignin in the soluble fractions followed the trend of the total soluble fraction (Fig. 1b). In the four-hour experiments, the proportions of chemical components in the liquid fraction were 6 to 12%, 10 to 20%, and 1 to 4% of the raw material dry weight for lignin, carboxylic acid,

and monosaccharides, respectively. The increase in the reaction time from 4 h to 20 h had greater affect on the proportion of dissolved lignin and monosaccharides, while the proportions nearly doubled when compared to the shorter treatment (Fig. 1d). Catalytically assisted treatment produced only more dissolved monosaccharides than the alkaline treatment, but its effect on lignin solubility was not as clear. Most likely, the dissolution of lignin was more affected by the increased reaction time coupled with the action of alkali.

Quite similar solubilisation was obtained with the three bases with the exception of $Ca(OH)_2$ (Fig. 1e). Among these three experimental series the highest yield of dissolved substances was 43%, which was achieved with 0.25 M Na_2CO_3. The cumulitive amount of the recognized dissolved compounds was about 30% (Fig. 1f), which means that 3 to 11% of the dissolved matter remained unidentified. The unexplained part may include other degradation products that cannot be determined with the applied methods, and unidentified carboxylic acids. In addition, among the uncalculated compounds is carbon dioxide, which is the final degradation product of carboxylic acids (Suzuki et al. 2006).

The fractionation with calcium hydroxide was more complicated to study, since calcium hydroxide was not soluble in the applied concentration and conditions. This also explains the high yields of fiber fraction in the experiments (Fig. 1e). The unrealistic high result can be explained with undissolved $Ca(OH)_2$, oxalic acid which precipitates as calcium oxalate, and undissolved lignin, which was not monitored among the identified compounds in the dissolved fraction (Fig. 1f). The differences in chemical compositions of the dissolved fractions of NaOH, KOH, and Na_2CO_3 in 20 hours of treatment were minimal.

Carbohydrates

The carbohydrate content of the spruce raw material was 66 and 65% for the chips and saw dust, respectively. A minor part (1 to 6% of the raw material dry weight) of the carbohydrates in the raw material was found in the soluble fractions, whereas most of the carbohydrates remained in the solid fraction after the fractionation (Fig. 2). The carbohydrate content of the solid fraction after fractionation was between 40 and 60% of the raw material dry weight, and the respective carbohydrate yield was between 66 and 92% of the raw material carbohydrates. The solubilized fraction contained only oligosaccharides and no monosaccharides. Most likely, the reducing ends of the soluble carbohydrates were oxidized under experimental conditions, as the soluble fraction gave no response to reducing sugars assay (data not shown). In general, the absence of monosaccharides and the low concentration of solubilized oligosaccharides may be explained by their further oxidation into carboxylic acids. As the total carbohydrate yield varied between 72% and 99% of the raw material carbohydrate content, it is obvious that carbohydrate yield losses occurred.

The composition of the dissolved carbohydrate material is basically similar to that reported earlier for wet oxidized spruce (Palonen et al. 2004), i.e. xylose, mannose, arabinose, galactose, and glucose, illustrating that mainly the hemicelluloses (arabinoxylan and galactoglucomannan) dissolve while the cellulose remains in the fiber fraction (Fig. 2).
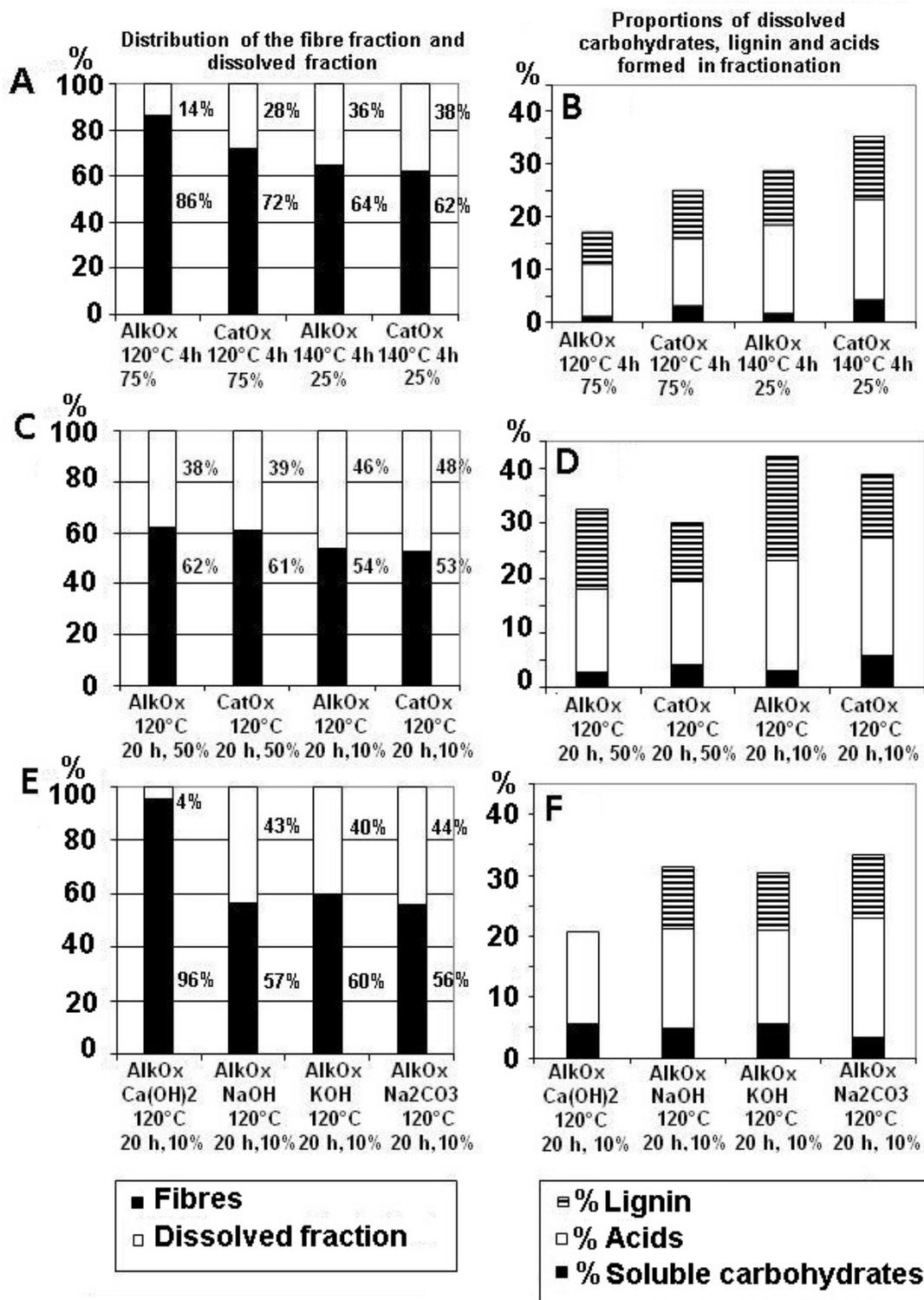
Fig. 1. Percent distribution of fibre fraction and soluble fraction after oxidation experiments in series I (A), II (C), and III (E). Portions of soluble carbohydrates, lignin, and small carboxylic acids found in dissolved fraction in series I (B), II (D), and III (F). The details of fractionation conditions of the samples are described in Table 1.

In addition, the figures show that under more severe conditions, i.e. with a catalyst, 20 hours of reaction time, and a higher temperature of 140 °C, there was a higher carbohydrate content in the liquid fraction. The shorter reaction time (4 h), lower temperature (120 °C), and Na_2CO_3 as an alkali all resulted in lower carbohydrate dissolution.

The oxidations were carried out with either 0.25 M Na_2CO_2, NaOH, KOH, or $Ca(OH)_2$, producing highly alkaline conditions (pH≥12) early in the reaction. During oxidation part of the alkali was consumed and after 20 hours the pH was usually between 7.5 and 8.5.

The dissolution of arabinoxylan was higher than that of galactoglucomannan in all experiments, but an especially clear difference was seen between the CatOx and AlkOx treatments (Figs. 2a and 2c). In addition, the comparison of alkali sources showed that the dissolution of galactoglucomannan was enhanced when $Ca(OH)_2$, KOH, or NaOH was used. The lower mannose content after oxidation with Na_2CO_3, however, can be explained by the degradation of galactoglucomannan, derived monosaccharides, and more pronounced acid production (Figs. 1f and 2e). The monosaccharide content and composition of the fiber fraction were also determined (Figs 2b, d, f). As expected, the majority of this fraction consisted of cellulose, but portions of 17-22%, 14-19%, and 12-18% of hemicelluloses, galactoglucomannan and arabinoxylan, were also observed in series I-III, respectively.

Table 2 compiles the results of the carbohydrate yields in the solid and liquid fractions. The total monosaccharides yield reveals clearly how much carbohydrate was lost, i.e. it gives information about the magnitude of the degradation of carbohydrates in the acids. This range varied between 3 and 28 percent, with the least degradation occurring with AlkOx, at 120°C, for 20 h, and a filling ratio of 50%, and the most degradation occurring with AlkOx, Na_2CO_3, at 120°C, for 20 h, and a filling ratio of 10%, indicating that the high filling ratio of the reactor hindered the oxidation reactions.

Lignin

Alkaline SEC is a common relative method for determining the molecular weight of lignins, as reviewed by Baumberger et al. (2007). The separation was calibrated here with pullulan standards in accordance with quoted literature, which can be considered as adequate for comparative purposes. SEC analyses of the dissolved fractions of series I and II reveal that the dissolved fraction contains both lignin and carbohydrate-based oligomeric substances having rather low molecular weights monitored with UV and RI detectors (Table 3). The four-hour reaction time with AlkOx treatment and a filling ratio of 75 % was not sufficient to dissolve lignin or hemicelluloses completely, and it was supposed that only small molecular weight lignin or oligosaccharides were dissolved; therefore, the molecular weight results obtained from this experiment are not comparable with its CatOx counterpart. The CatOx fractionation produced smaller lignin and polysaccharide oligomers than the AlkOx treatment during the 20 hour oxidation. This difference is not as clear as in the four-hour fractionations, except in the case of 140 °C. In addition, the polydispersities were lower in the CatOx samples than in the AlkOx samples in the 20 hour treatments. This indicates relatively narrow molecular weight distributions of dissolved substances.

Table 2. Carbohydrate Fraction Yield and Total Yield

SAMPLE	Fraction	GLU	MAN	XYL	GAL	ARA	Total mono-saccharides
AlkOx 120°C 4 h 75%	solid	103	59	86	47	77	92
	liquid	0.1	1	6	11	14	1
	total	103	60	92	58	90	93
CatOx 120°C 4 h 75%	solid	89	56	61	30	49	78
	liquid	0.7	6	20	21	30	4
	total	89	62	81	50	79	82
AlkOx 140°C 4 h 25%	solid	99	41	71	29	50	83
	liquid	0.2	1	12	14	26	2
	total	99	42	83	43	76	86
CatOx 140°C 4 h 25%	solid	100	48	54	20	38	83
	liquid	1.1	7	28	21	37	6
	total	101	55	82	42	74	89
CatOx 120°C 20 h 50%	solid	105	61	46	11	24	88
	liquid	0.9	11	40	37	52	8
	total	106	72	86	49	75	96
CatOx 120°C 20 h 10%	solid	106	55	41	6	19	87
	liquid	1.1	14	42	37	52	9
	total	107	69	83	43	71	96
AlkOx 120°C 20 h 50%	solid	109	61	64	14	37	93
	liquid	0.3	3	24	21	41	4
	total	110	64	88	36	78	97
AlkOx 120°C 20 h 10%	solid	102	49	57	8	30	85
	liquid	0.3	3	25	25	47	5
	total	103	52	82	33	78	90
AlkOx Ca(OH)$_2$ 120°C 20 h 10%	solid	93	26	31	5	20	70
	liquid	2.0	15	36	18	51	9
	total	95	40	66	23	71	78
AlkOx NaOH 120°C 20 h 10%	solid	84	35	36	12	24	67
	liquid	1.0	11	31	38	59	8
	total	85	46	67	50	83	74
AlkOx KOH 120°C 20 h 10%	solid	88	41	36	40	26	70
	liquid	1.4	15	31	40	53	15
	total	89	56	68	79	78	85
AlkOx Na$_2$CO$_3$ 120°C 20 h 10%	solid	83	36	40	11	27	66
	liquid	0.5	5	25	26	56	5
	total	84	41	66	37	83	72

* Yield is expressed as % of the raw material carbohydrate content
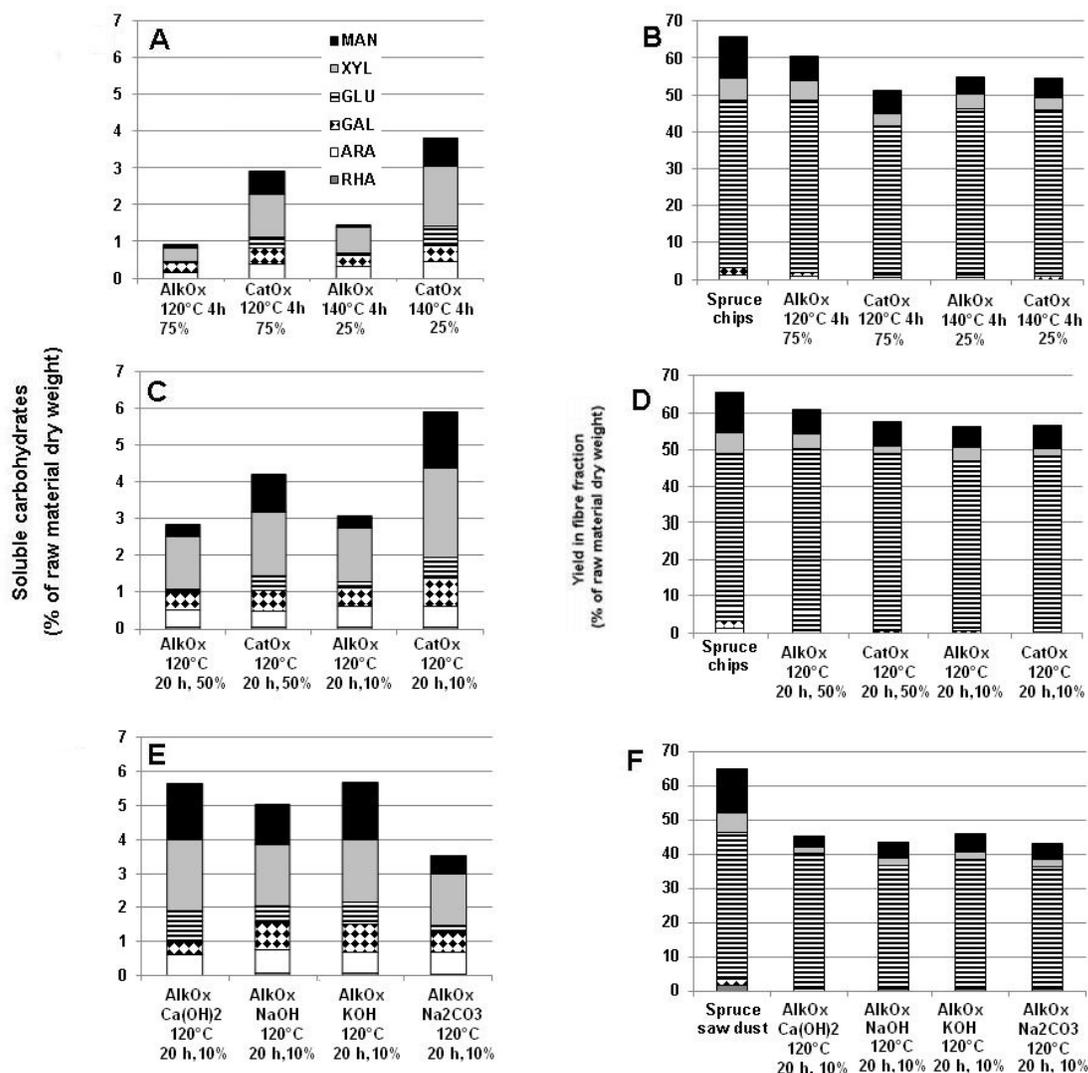
Fig. 2. Monosaccharide composition in the soluble fraction (A, C, and E) and the fibre (B, D, and F) fraction after acid hydrolysis in series I - III. The carbohydrate composition of spruce chips, wood meal, and saw dust are also presented in Figs. B, D, and F, respectively. The composition was determined by HPAEC-PAD after acid hydrolysis and presented as % (w/w) of monosaccharides in the original raw material. The details of the fractionation conditions are shown in Table 1.

Comparison of the SEC chromatograms obtained with Ultrahydrogel columns using UV and RI detectors reveals that polysaccharides are dissolved as small fragments of oligosaccharides with a Mw range of 600 to 1600. The AlkOx treatment favored the dissolution of larger fragments (Fig. 3). On the other hand, CatOx fractionation produced even larger oligosaccharide fragments with a Mw of 1600 to 1900, monitored with the RI detector. Moreover, for the 20 h experiments, the CatOx treatment dissolved more lignin with a Mw between 3900 and 4000 than the corresponding AlkOx treatments, as seen in the chromatograms monitored with the UV detector.

Comparison of the precipitation yields of lignin in series I and II indicates that the yields were larger in the AlkOx samples than in the CatOx samples for both the 4 h and 20 h fractionations. At longer fractionations these correlated well with the amount of dissolved lignin as presented in Fig. 1d. The lowest yields, 17.6 % and 13 %, were obtained in the CatOx samples in which the filling ratios were 25 % and 10 %, respecttively. This indicates that the co-operation of catalyst and oxygen promoted lignin degradation. Moreover, the lower yield correlates with the higher amount of oxalic acid, which is the oxidation product of lignin (Fig. 4).
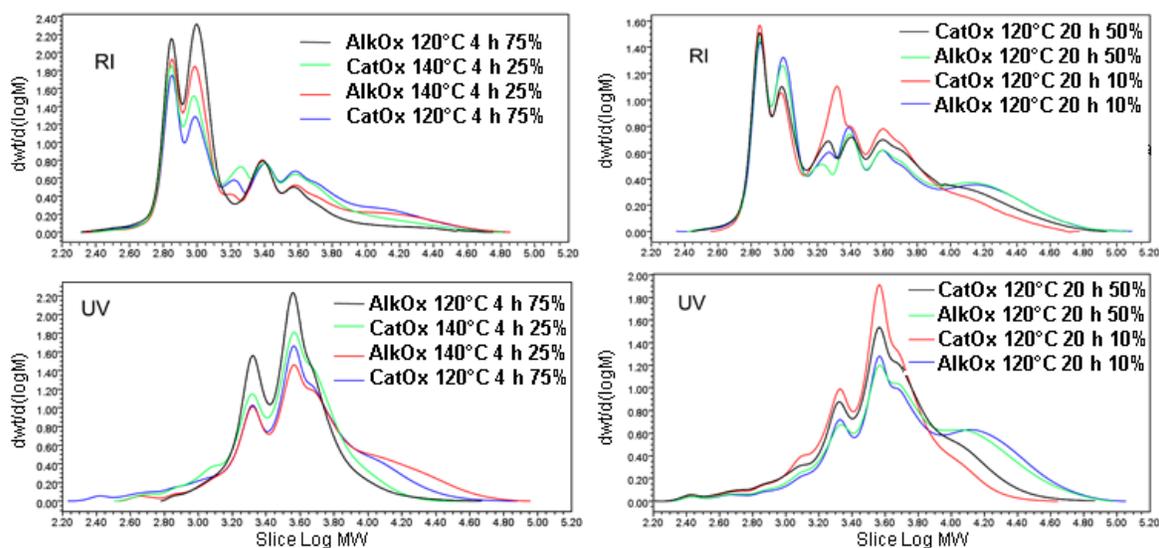
Fig. 3. SEC chromatograms of series I (left) and II (right) obtained from both the RI and UV detectors. The details of the fractionation conditions are described in Table 1.

Acids

The acidic pretreatment of lignocellulosic materials forms a variety of degradation products, which have an inhibiting effect on both the enzymatic action and the fermentation by yeast or bacteria. Among those compounds are phenols, aromatic aldehydes, soluble lignin compounds, and organic acids, such as formic and acetic acids (Sassner et al. 2006; Mosier et al. 2005; Chen et al. 2006; Hendriks and Zeeman 2009). The AlkOx and CatOx processes produce considerable amounts of the mentioned aliphatic carboxylic acids; therefore it is justified to study the nature and concentrations of the acidic compounds formed during the fractionations.

Both the AlkOx and CatOx treatments of spruce chips showed a release of lignin along with hemicelluloses. The acids were formed via the degradation of the phenolic structures of lignin and the degradation of hemicelluloses. The reactions are similar to those reactions proposed to occur in conventional oxygen delignification (Kuitunen et al. 2011; Kadla et al. 1999; Gierer 1986). The hemicelluloses are degraded through peeling reactions and chain cleavage.

Table 3. Average Molecular Weights (Mn, Mw), Polydispersities (Mw/Mn), and Precipitation Yields. *

Sample	Mw (g mol^{-1})	Mn (g mol^{-1})	PD (Mw/Mn) (g mol^{-1})	Mw (g mol^{-1})	Mn (g mol-1)	PD (Mw/Mn) (g mol^{-1})	Yield of precipitation (%)
	NaNO$_3$ /UV pullulan std			NaNO$_3$/ RI pullulan std			
AlkOx 120°C 4 h 75%	4 000	3 000	1.4	2 300	1 100	2.1	20.3
CatOx 120°C 4 h 75%	10 600	2 600	2.1	4 200	1 400	3	20.4
AlkOx 140°C 4 h 25%	7 100	3 300	2.1	3 900	1 300	3	33.9
CatOx 140°C 4 h 25%	4 100	2 600	1.6	3 300	1 300	2.5	17.6
CatOx 120°C 20 h 50%	5 700	2 600	2.2	5 200	1 600	3.3	26.7
CatOx 120°C 20 h 10%	4 500	2 500	1.8	4 100	1 600	2.6	13.0
AlkOx 120°C 20 h 50%	9 000	3 300	2.7	6 300	1 600	3.9	38.7
AlkOx 120°C 20 h 10%	9 700	3 500	2.8	6 100	1 600	3.8	40.2

* Precipitation yield was calculated using an absorptivity value of 20 L g^{-1} cm^{-1}.

The reactions of both lignin and hemicellulose with molecular oxygen, perhydroxyl/superoxide radicals (HO_2^*/O_2^{*-}), hydroperoxide anions (HOO^-), and hydroxyl radical/oxide anion radicals (HO^*/O^{*-}) cause degradation of those macromolecules and the formation of small aliphatic carboxylic acids (Gellerstedt et al. 1980; Young and Gierer 1976; Bailey and Dence 1969; Kuitunen et al. 2011).

Capillary zone electrophoresis (CZE) with indirect UV detection was used for the simultaneous determination of inorganic anions and C(1)-C(6) aliphatic carboxylic acids liberated in the lignocellulosic processed samples (Rovio et al. 2010). From the analytical point of view, the separation of the aliphatic carboxylic acids is a challenging task, since they have similar mass-to-charge ratios and similar molecular structures. The optimized method was applied for determining the carboxylic acids in the soluble fractions of the oxidation treatments.

Both volatile and non-volatile carboxylic acids were detected. The volatile acids include formic and acetic acids, while the non-volatile acids include dicarboxylic acids and hydroxy acids. The dominating acids in the experiments were formic, acetic, oxalic, and glycolic acid both in alkali-oxidized and catalytically-oxidized samples. Moreover, malonic, fumaric, succinic, malic, lactic, 3-hydroxypropionic, 2-OH-butyric, and 2.5-di-OH-pentanoic acids were found in smaller quantities (Fig. 4). In general, the acid profiles were similar in all experiments with only small variations in the concentrations of

individual acids. Most likely, the reason for this is the alkaline oxidative environment which promotes similar reactions despite the nature of the alkali.

The most crucial reaction parameters with respect to the formation of the acids were the presence of a catalyst, reactor filling, and reaction temperature. The catalytically assisted oxidation produced more acid regardless of the reaction temperature. Catalyzation had the greatest affect in the formation of formic acid and oxalic acid. Oxalic acid has been generally recognized as a degradation product of lignin (Kuitunen et al. 2011; Bailey and Dence 1969).

The total acid formation was enhanced both in the AlkOx and CatOx experiments at a temperature of 140 °C. The acid production in both treatments was balanced with a reaction time of 20 hours; however, the lower reactor filling (10% instead of 50%) had a positive effect on wood degradation and oxidation of dissolved lignin and hemicelluloses, leading to the increase in acid formation from *ca*. 15% to 20% (Fig. 4). Comparison of bases showed similar acid productions, but slightly more oxalic and minor acids were formed in samples treated with Na_2CO_3, NaOH, and KOH than with $Ca(OH)_2$, which in turn favors the generation of formic and acetic acids.

The composition of the major acids was similar to what was observed in the wet oxidation of wheat straw (Bjerre et al.1996) and the mixture of clover and ryegrass (Martín et al. 2008). In addition, Samuelson and Sjöberg (1976) studied spent liquor obtained from oxygen bicarbonate cooking of birch and found the same major acids: formic, acetic, oxalic and glycolic acids. Shuai et al. (2010) observed that dilute acid and SPORL pretreatments produced formic acid levels of 7.4 and 1.9 g L^{-1}, respectively, whereas the amount of formed acetic acid were 5.3 and 2.7 g L^{-1}, respectively. Moreover, Larsson et al. (1999) reported, that steam explosion of acid treated spruce chips produced 1.6 g L^{-1} of formic acid and 2.4 g L^{-1} of acetic acid. It should be noted that acidic pretreatments produce levulinic acid, which is a potent inhibiting compound too (Palmqvist and Hahn-Hägerdal, 2000). In our study the concentrations of formic acid and acetic acid varied within the ranges 0.8 to 2.5 g L^{-1} and 0.9 to 3.3 g L^{-1}, respectively.
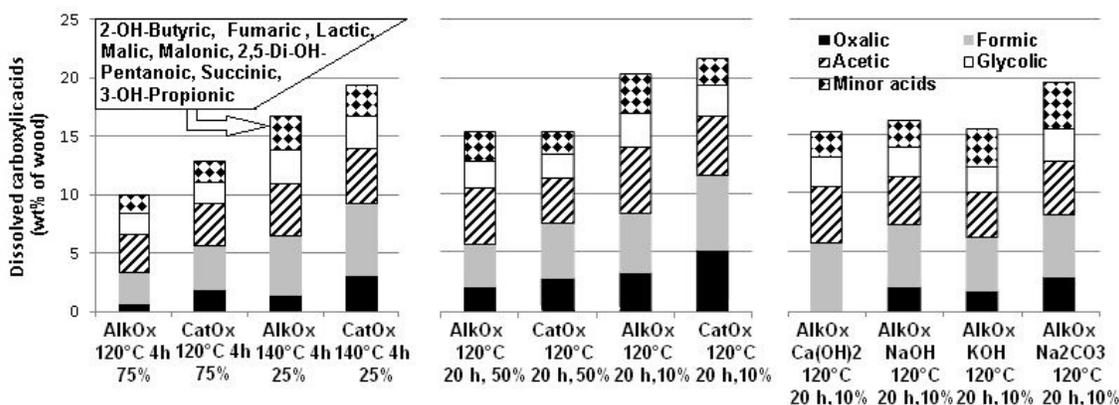
Fig. 4. Distribution of four main aliphatic acids and sum of minor acids found in the soluble fraction in series I - III after oxidation. Acid composition was determined by capillary electrophoresis as described in Chapter Methods and presented as % acid from the original raw material. The details of fractionation conditions are described in Table 1.

Kinetics of Catalyzed Oxygen Delignification

The progress of the reaction was monitored by analyzing the dissolved material as a function of time.

Analysis of the samples of the CatOx reaction mixture at predetermined intervals showed increased dissolution of hemicelluloses after one hour of oxidation (Fig. 5, center). After four hours of treatment the carbohydrates were solubilized more slowly. The content of carbohydrates in the fiber fraction originating from hemicellulose decreased by half of its original concentration during 20 hours of the reaction. The cellulose was not dissolved significantly, as the concentration of glucose decreased by only five per cent (Fig. 5, left).

Almost half of the acetic acid and formic acid was generated during the first hour, and such generation continued over the course of the oxidation. The formation of glycolic, oxalic, and 2,5-di-OH-pentanoic acids was more vigorous during the first four hours and became slower thereafter (Fig. 5, right).

The dissolved lignin content was estimated based on UV analysis at 280 nm. For aromatic lignin, an absorptivity of 20 L g^{-1} cm^{-1} was used. In addition, the absorptivities of the isolated (unpurified) lignins were determined. Table 4 shows the yields of dissolved lignin based on the UV results. The content of dissolved aromatic lignin followed a similar dissolution pattern as the hemicelluloses, while the majority of the dissolution of lignin occurred during the first four hours of the reaction, and only minor changes were seen between 4 and 20 hours. About half of the dissolved lignin was precipitated by the acidification, and this proportion was not affected by the stage of the reaction. The precipitation yields in the kinetic series were larger than those of the AlkOx and CatOx samples (Table 3).

The formation of carboxylic acids was supposed to occur *via* both the oxidation of dissolved hemicelluloses and the degradation of aromatic lignin structures. It was presumed that the oxidation of lignin affected the absorptivity of the dissolved lignin. Surprisingly, a decrease in the absorptivity was not observed, but rather a slight increase.

Part of the fiber components may have dissolved in the form of degradation products and were not detected by the methods applied; therefore, the total content of dissolved organic material was reported as total organic carbon (TOC), and the contributions of lignin and carbohydrates to TOC were calculated using carbon contents of 0.60, 0.44, and 0.40 for lignin, carbohydrates, and carboxylic acids, respectively (Laine and Tamminen 2002).

Table 4. Precipitated Lignin, Dissolved Lignin, Precipitation Yield, and Absorptivity

Time (h)	Precipitated lignin, mg g^{-1}	Dissolved lignin, mg g^{-1}	Precipitation yield, %	Absorptivity of lignin precipitate, L g^{-1} cm^{-1}
1	44,5	100.4	44	17.5
4	87.3	185.6	47	20.1
8	78.0	195.2	40	22.6
20	90.1	194.0	46	22.1

*Weight is based on dry wood. Precipitation yield was calculated using an absorptivity value of 20. Absorptivity of the precipitate was at 280 nm.
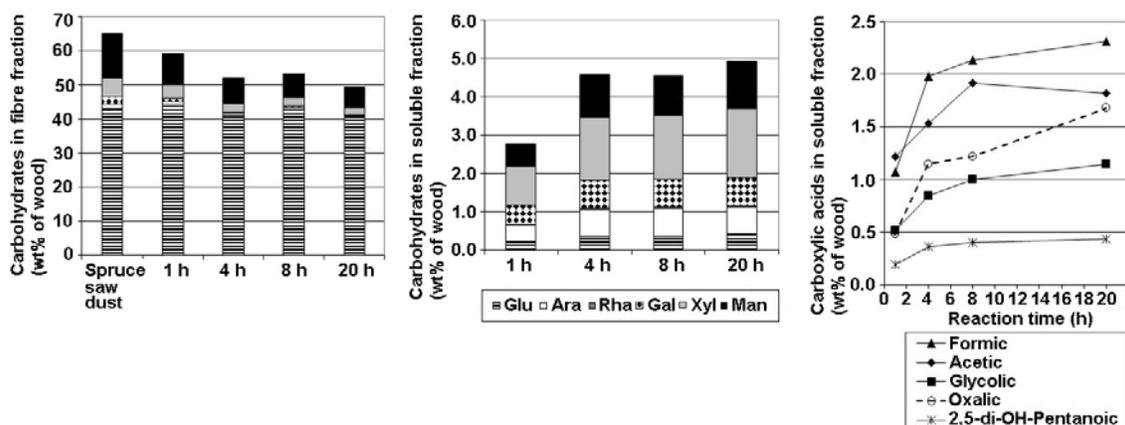
Fig. 5. The development of concentrations of different monosaccharides under 20 hours of CatOx oxidation in the fiber fraction (left) and in the soluble fraction (center). Formation of carboxylic acids under the same conditions is presented in the right figure. Details of fractionation conditions of the samples are described in Table 1, samples IV 1-4.

As seen in Fig. 6, lignin accounted for more than half the TOC, whereas the carbohydrates had only a small contribution. The contribution of carboxylic acids was approximately three times than that of the carbohydrates. About 20 to 25 percent of the TOC was composed of unidentified material.
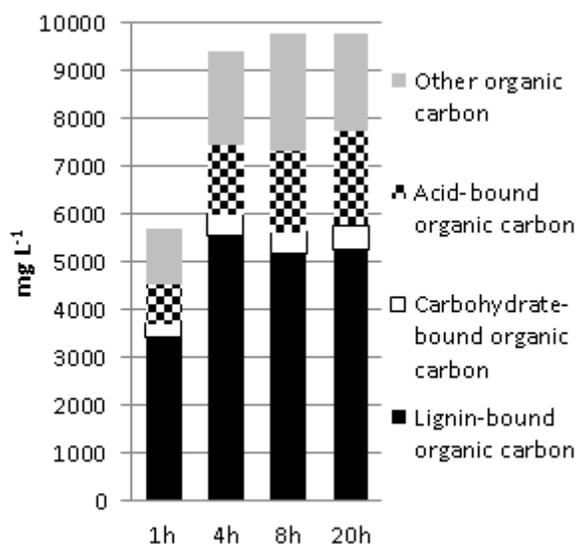
Fig. 6. Total dissolved organic carbon and its distribution between lignin, carbohydrates, and other organic material. Details of fractionation conditions of the samples are described in Table 1, samples IV 1-4.

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CONCLUSIONS

Alkaline oxidation can be catalyzed by a copper-phenanthroline complex to enhance the fractionation of lignocellulosic material and decrease the reaction time. This catalyzed oxidation (CatOx) process was studied in a laboratory scale reactor in order to understand the chemistry taking place, in particular the role of the catalyst, using Spruce wood as the raw material.

1. During the catalytic pretreatment the wood chips lost their macroscopic structure, and more than 28% of the dry wood material was solubilized during the 20 hours catalytic pretreatment. Both catalytic and alkaline oxidations resulted in a cellulose-rich fiber material. The solid material contained over 83% of the original total carbohydrates after four hours of catalytic oxidation at 140 °C, which was a similar value to that of a non-catalyzed counterpart. An extended pretreatment time did not alter the total yields of the fiber fraction, being 87 to 88% in CatOx and 85 to 93% in AlkOx samples. The applicability of the fiber fraction for bioethanol production was shown in earlier studies (Hakola et al. 2010).

2. The effectiveness of the catalyst in the dissolution of lignocellulosic material was also observed by analyzing the composition of the dissolution fraction. The closer examination of dissolved compounds revealed that the catalytically assisted oxidation was more efficient than its alkaline counterpart in the dissolution of carbohydrates, especially of those originating from glucomannan and arabino-xylan, which can be verified by analyzing the proportions of mannose and xylose in the dissolved fraction. In addition, the CatOx process was more efficient with a low reactor filling ratio.

3. The catalyst increased both the degradation of dissolved monosaccharides and the degradation of lignin to small aliphatic carboxylic acids. This observation was more pronounced with reactor filling ratios below 25%. The acid profile was quite similar in the presence and absence of the catalyst, although some variations in the concentration of individual acids were observed. Comparison of the four different bases showed that NaOH, KOH, and Na_2CO_3 were comparable in dissolution efficiencies.

ACKNOWLEDGMENTS

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