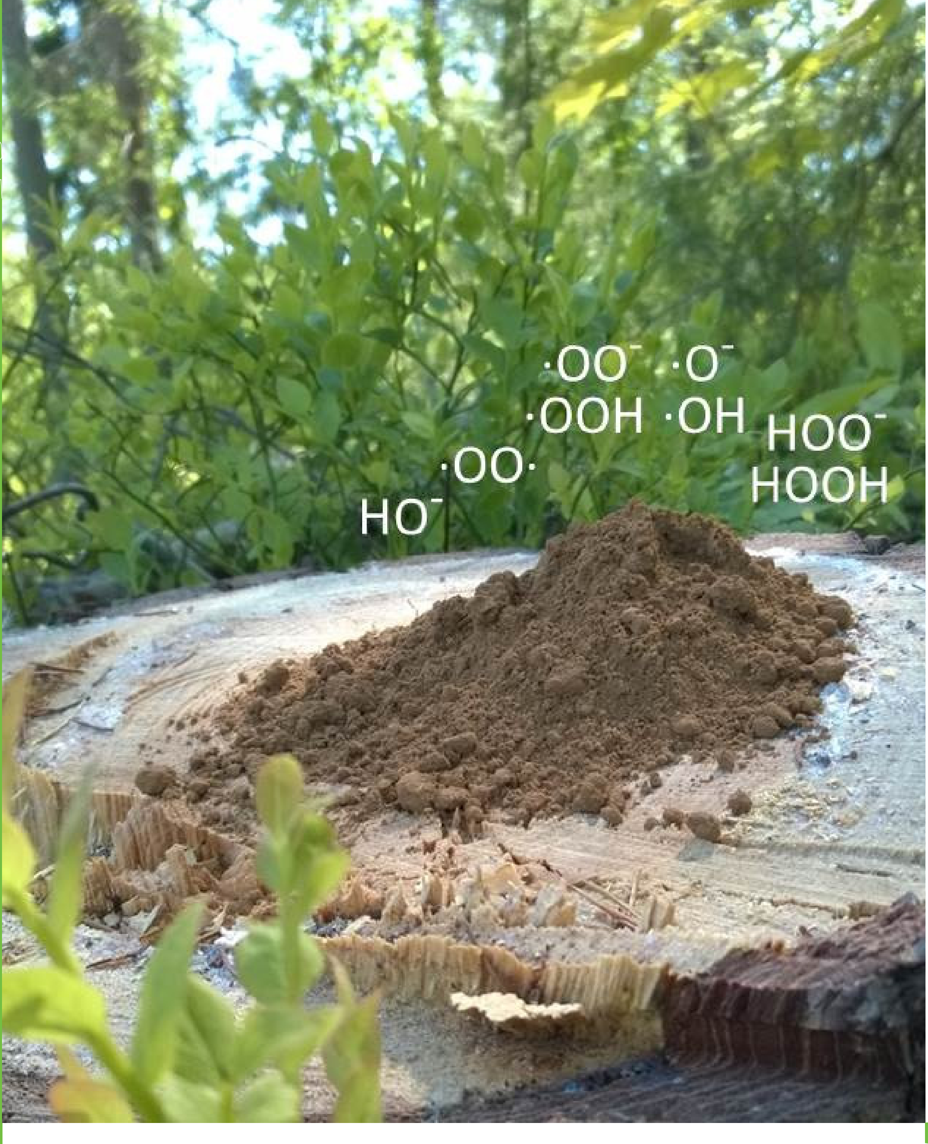

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Chemical and enzymatic oxidation using molecular oxygen as a means to valorize technical lignins for material applications

Anna Kalliola

VTT SCIENCE 99

Chemical and enzymatic oxidation using molecular oxygen as a means to valorize technical lignins for material applications

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Abstract

Oxidation by molecular oxygen (O_2) is one of the lignin modification methods. O_2 is active towards phenolic groups, which are particularly abundant in kraft and soda lignins. The main aim of this thesis was to apply oxidation by O_2 to modify technical lignins to enhance their utilization for polymeric chemicals and material applications. O_2 oxidation was aided by using either alkaline conditions or laccase enzyme as a catalyst. In addition, oxygen delignification of pulp was studied using kraft lignin as a model substrate to provide data for a mechanistic model for the process. Lignin oxidation mechanisms by O_2 under alkaline conditions and laccase catalysis are discussed.

A simple alkali-O_2 oxidation method under high lignin content was developed to increase the water solubility of soda lignin, desirable for dispersing applications. Lignin characterization was done directly from the reaction solution. Both the negative charge and the molecular mass of the lignin were controlled by the oxidation parameters, and especially by pH. Oxidation without controlling the pH decrease caused condensation and an increase in molecular mass. Oxidation under a constant pH of 11.5 clearly hindered the condensation and increased the negative charge. Oxidation at constant pH of 13 decreased molecular mass. The results indicate that the organic hydroperoxide formed via coupling of a phenoxyl radical with superoxide ($O_2\bullet^-$) is the key intermediate. The course of further reactions is dependent on the degree of protonation of this intermediate (pK_a 12–13) and is thus pH dependent. The hydroperoxide anion rearranges leading to degradation. Below pH 12, the protonated form decomposes back to the phenoxyl radical, which spontaneously undergoes coupling and thus induces condensation. Under laccase catalysis conditions, $O_2\bullet^-$ is not present and thus the reaction paths described above do not function. Therefore, the formed phenoxyl radicals couple with each other rather than degrade. O_2 has a significantly lower tendency to attach to the phenoxyl radical compared to $O_2\bullet^-$.

The oxidized soda lignin solutions were applied as ready-to-use products for concrete plasticizing. They were superior to commercial lignosulfonate and good in comparison to synthetic superplasticizers. The best performing lignin solution (oxidized at a constant pH of 11.5) also showed promising results in other concrete application tests.

To enhance the utilization of kraft lignin in composite applications, both laccase- and alkali-catalyzed O_2 oxidation were used to polymerize lignin-derived low-molecular phenolics for the reduction of VOCs. According to sensing and chemical analysis, the undesirable odor and the formation of VOCs under elevated temperatures were reduced to a greater extent by alkali- than by laccase-catalyzed oxidation. However, neither method led to adequate odor removal. In order to lower the glass transition temperature of lignin, functionalization with a hydrophilic phenolic compound was attempted. However, homogeneous polymerization of this compound was favored over coupling to lignin.

The operating conditions of alkali-O_2 oxidation could probably be optimized for targeted lignin characteristics, which would increase the further application potential of technical lignins. Laccase-catalyzed oxidation is best applied when lignin polymerization is desired.

Tiivistelmä

Hapetus molekyylisellä hapella (O_2) on yksi ligniinin muokkausmenetelmistä. O_2 reagoi fenolisten ryhmien kanssa, joita on runsaasti erityisesti sulfaatti- ja soodaligniineissä. Tässä työssä päätavoitteena oli soveltaa O_2-hapetusta teknisten ligniinien muokkaukseen, jotta niiden käyttö polymeerisenä kemikaalina ja materiaalisovelluksissa helpottuisi. O_2-hapetus toteutettiin joko alkalisissa olosuhteissa tai entsyymiavusteisesti lakkaasilla. Lisäksi työssä tutkittiin massan happidelignifiointia käyttäen malliaineena sulfaattiligniinia. Näin tuotettiin tietoa kyseisen prosessin mekanismimallinnusta varten. Työssä käsitellään ligniinin O_2-hapetusmekanismeja alkalisissa olosuhteissa ja lakkaasikatalysoidusti.

Soodaligniinille kehitettiin yksinkertainen korkeassa ligniinipitoisuudessa tapahtuva alkali-O_2 -hapetusmenetelmä, joka nostaa ligniinin vesiliukoisuutta dispergointisovelluksia varten. Ligniiniä luonnehdittiin suoraan reaktioliuoksesta. Sekä ligniinin negatiivista varausta että moolimassaa hallittiin hapetusparametreilla, erityisesti pH:n avulla. Hapetus ilman pH-laskun hallintaa aiheutti kondensaatiota ja kasvatti moolimassaa. Hapetus vakio-pH:ssa 11.5 esti selvästi kondensaatiota ja kasvatti negatiivista varausta. Hapetus vakio-pH:ssa 13 pienensi moolimassaa. Tulokset osoittavat, että orgaaninen hydroperoksidi, joka muodostuu fenoksiradikaalin ja superoksidin ($O_2^{\bullet-}$) yhdistyessä, on oleellinen välituote. Jatkoreaktioiden suunta on riippuvainen välituotteen protonoitumisasteesta (pK_a 12–13) ja siten pH:sta. Hydroperoksidianionin uudelleenjärjestäytyminen johtaa pilkkoutumiseen. pH:ssa alle 12 sen protonoitunut muoto hajoaa takaisin fenoksiradikaaliksi, joka yhdistyy spontaanisti toiseen vastaavaan ja siten aikaansaa kondensaatiota. Lakkaasikatalysoiduissa olosuhteissa $O_2^{\bullet-}$ ei esiinny, ja siksi yllä esitetyt reaktiopolut eivät toimi. Tämän takia muodostuneet fenoksiradikaalit yhdistyvät keskenään sen sijaan, että hajoaisivat. O_2 reagoi fenoksiradikaalin kanssa merkittävästi hitaammin kuin $O_2^{\bullet-}$.

Hapetettuja soodaligniiniliuoksia käytettiin sellaisenaan tuotteena betonin notkistuksessa. Ne toimivat selvästi paremmin kuin kaupallinen lignosulfonaatti ja olivat vertailukelpoisia suhteessa synteettisiin tehonotkistimiin. Parhaiten toiminut ligniiniliuos (hapetettu vakio-pH:ssa 11.5) oli lupaava myös muiden betonisovellustestien mukaan.

Sekä lakkaasi- että alkalikatalysoitua O_2-hapetusta käytettiin ligniiniperäisten, pienimolekyylisten fenoleiden polymerointiin sulfaattiligniinissä. Näin pyrittiin vähentämään haihtuvien yhdisteiden määrää ja edistämään ligniinin käyttöä komposiittisovelluksissa. Aistiin perustuvan ja kemiallisen analyysin mukaan alkalikatalysoitu

hapetus vähensi hajua ja haihtuvien yhdisteiden muodostumista korkeissa lämpötiloissa enemmän kuin lakkaasikatalysoitu. Kumpikaan menetelmistä ei kuitenkaan johtanut riittävään hajun poistoon. Ligniiniin yritettiin liittää vesiliukoinen fenolinen yhdiste, jotta sen lasinsiirtymälämpötila alenisi. Yhdiste kuitenkin homopolymeroitui sen sijaan, että olisi liittynyt ligniiniin.

Alkali-O_2 -hapetuksen toimintaolosuhteita optimoimalla saavutetaan hyvin todennäköisesti haluttuja ligniiniominaisuuksia, mikä edistäisi teknisten ligniinien sovelluspotentiaalia entisestään. Lakkaasikataloitu hapetus soveltuu parhaiten ligniinin polymerointiin.

Preface

This thesis work was carried out at VTT Technical Research Centre of Finland during the years 2009–2014 in four research projects: “Virtual pulp bleaching” in EffTech research program, “VOC reduction of lignin containing materials” in ERA-IB program, “Products from lignocellulose” in ERA-IB II program, and “ProLignin” in Wood Wisdom program. I acknowledge the financial support from the Finnish Funding Agency for Innovation (TEKES), the industrial partners of the projects, and VTT.

Foremost, I wish to express my warmest thanks to the thesis advisor, Dr. Tarja Tamminen for giving me the opportunity to work in these interesting projects, and for her help and encouragement during the years. Especially, her instant and valuable responses to all my questions as well as her positive feedback have been extremely important to me.

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June 2015, Espoo, Finland

Anna Kalliola

Academic dissertation

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List of publications

This thesis is based on the following original publications which are referred to in the text as I–IV (Appendices). The publications are reproduced with kind permission from the publishers.

I Kalliola, A., Kuitunen, S., Liitiä, T., Rovio, S., Ohra-aho, T., Vuorinen, T., Tamminen, T., 2011. Lignin oxidation mechanisms under oxygen delignification conditions. Part 1. Results from direct analyses. *Holzforschung* 65(4), 567–574.

II Kalliola, A., Savolainen, A., Faccio, G., Ohra-aho, T., Tamminen, T., 2012. Reducing the content of VOCs of softwood kraft lignins for material applications. *BioResources* 7(3), 2871–2882.

III Kalliola, A., Asikainen, M., Talja, R., Tamminen, T., 2014. Experiences of kraft lignin functionalization by enzymatic and chemical oxidation. *BioResources* 9(4), 7336–7351.

IV Kalliola, A., Vehmas, T., Liitiä, T., Tamminen, T., 2015. Alkali-O_2 oxidized lignin – A bio-based concrete plasticizer. *Industrial Crops and Products* 74, 150–157.

Author’s contributions

I The author planned the work together with the co-authors and supervised the experiments concerning lignin oxidation. She interpreted the results together with the co-authors and had the main responsibility for writing the publication.

II The author planned the work together with the co-authors and supervised the experiments, except for the odor panel and the thermal desorption test development. She interpreted the results together with the co-authors and wrote the publication.

III The author planned the work together with Tarja Tamminen and supervised the experiments, except for the synthesis of hydrophilic derivatives. She interpreted the results together with the co-authors and wrote the publication, except the part concerning synthesis.

IV The author planned the work together with the co-authors and supervised the experiments, except for the mortar and concrete tests. She interpreted the results together with the co-authors and wrote the publication.

Contents

Appendices

List of abbreviations

AoSOX1	*Aspergillus oryzae* sulfhydryl oxidase
BDt	Bone dry ton (dry solids 100%)
CEM II	Cement type Plussementti CEM II B-M (S-LL) 42,5 N
Ester V-PEG	Vanillic acid poly(ethylene glycol) methyl ester
Ether V-PEG	Vanillic acid poly(ethylene glycol) methyl ether
G	Guaiacyl unit
Glenium	Polycarboxylate-based superplasticizer
H	*p*-Hydroxyphenyl unit
HBT	1-hydroxybenzotriazole
$HO\bullet$	Hydroxyl radical
HOO^-	Hydroperoxyl anion
HW	Hardwood
Indulin AT	Commercial softwood kraft lignin
k	Rate constant (reaction dependent)
M_n	Molecular mass, number average
M_w	Molecular mass, weight average
MMD	Molecular mass distribution
O_2	Molecular oxygen
$O_2\bullet^-$	Superoxide anion radical
$O\bullet^-$	Oxyl anion radical
pH_{Init}	pH, initial
pK_a	Acid constant (10-base logarithm of dissociation coefficient K_a)

r-MaL	*Melanocarpus albomyces* laccase
S	Syringyl unit
SE	Softwood kraft lignin, provided by Stora-Enso
SL	Soda lignin from wheat straw
SW	Softwood
t	Time
$T_{init.}$	Temperature, initial
T_g	Glass transition temperature
TaLcc1	*Thielavia arenaria* laccase, Lcc1
TaLcc2	*Thielavia arenaria* laccase, Lcc2
TD-MS/GC	Thermal desorption-gas chromatography/mass spectrometer
ThL	*Trametes hirsuta* laccase
UV	Ultra-violet
VB-Parmix	Polycarboxylate-based superplasticizer
VOC	Volatile organic compound
w-%	Weight percentage
WRDA 90 D	Lignosulfonate-based concrete plasticizer

1. Introduction

1.1 Technical lignins in the lignocellulosic refinery context

Lignocellulosic biomass is primarily composed of polysaccharides, cellulose and hemicelluloses, and aromatic lignin. In soft- and hardwoods, the share of lignin is 25–32% and 18–25%, respectively (Sixta et al., 2006a).

Large quantities of lignin are available from the fractionation processes of the lignocellulosic refineries, meaning chemical pulping and the emerging biorefineries. The primary aim of these industries is the efficient conversion of the biomaterials into valuable products. However, lignin conversion to value-added products is a challenge.

The potential for lignin production in the existing chemical pulping (kraft and the declining sulfite) processes is approximately 60 million tons annually, and the anticipated amount of lignin generated by future biorefineries is considerably higher. At the present, a vast amount of this lignin is incinerated on the production site, to provide energy in the manufacturing process. Only a very small part of it is isolated and sold as technical lignin (1–1.3 million tons per year), which can be further converted to value-added products. The main commercial source of technical lignins, the majority being macromolecular lignosulfonates, is the chemical pulping industry (Gosselink et al., 2004; Zhang et al., 2011). The lignin sales value varies from low grade to high grade with an estimate of €50–1200 per ton (Laurichesse and Avérous, 2014)

The potential of lignins to replace oil-based raw materials is being actively investigated for various specialty chemicals and material applications (Doherty et al., 2011; Laurichesse and Avérous, 2014; Schorr et al., 2014). Typically, before lignin can find targeted use in these applications, it needs to be modified to enhance the reactivity, solubility, or applicability in general.

Lignin can be modified in a variety of ways. One of these modification methods is oxidation. Several oxidizing chemicals exist, with molecular oxygen (O_2) being one of them. O_2 is an attractive oxidant, since it is inexpensive, at €0.06 per kg (Kangas et al., 2014), abundant, and nontoxic. O_2 oxidation of lignin can be aided using alkaline conditions or an enzyme as a catalyst. In valorization lignin for polymeric chemicals, or base material for material applications, it is essential that the oxidation treatment is performed under conditions that retain the polymeric structure of lignin.

1.2 Lignin

1.2.1 Structure

Lignin is a natural amorphous polymer found in plant biomass. In plant cell walls, it fills the spaces between cellulose and hemicellulose, and holds the lignocellulose matrix together. Native lignins are formed via enzymatic activation and the random oxidative polymerization of three monomeric building blocks: p-coumaryl, coniferyl, and sinapyl alcohols which give rise, respectively, to so-called p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) phenylpropanoid units. The units differ in the number of methoxyl groups: G contains a methoxyl in the C3 position, S in both C3 and C5, while H has no methoxyl groups (**Figure 1**). The chemical structure of softwood lignin (is mainly composed of G units, which have a greater tendency to crosslink than the more linear hardwood lignins composed of a mixture of predominantly G and S units with a small number of H units. Lignin in annual plants is rich in H units (Sjöström, 1993; Dimmel, 2010).

The dominant linkage in both native softwood and hardwood lignin is the β-O-4 (aryl alkyl ether) linkage (**Figure 1**). The other common linkages connecting the phenylpropane units include β-5 (phenylcoumaran), 4-O-5, β-1, dibenzodioxocin, 5-5 (mainly as part of dibenzodioxocin), and β-β linkages (Brunow et al., 1998; Boerjan et al., 2003; Dimmel, 2010) (**Figure 2**). Lignin is also linked to polysaccharides, forming lignin-carbohydrate complexes (Dimmel, 2010). Lignin is often described as a macromolecule with a wide polydispersity. Recently, Crestini et al. (2011) have reported, based on a combination of novel lignin characterization methods, that milled softwood lignin has a degree of polymerization of 11 or less, and hardwood lignin is only slightly larger. Thus, in this case oligomer would be a better description of lignin.

Lignin contains different functional groups, such as aliphatic hydroxyl, phenolic hydroxyl, methoxyl, carbonyl, and carboxylic acid, whose ratios vary, depending on origin (**Figure 1**) (Sjöström, 1993; Dimmel, 2010). However, carbonyl and carboxylic acid content in native lignin is relatively low. After processing, the prevalence of the linkages and functional groups is changed.

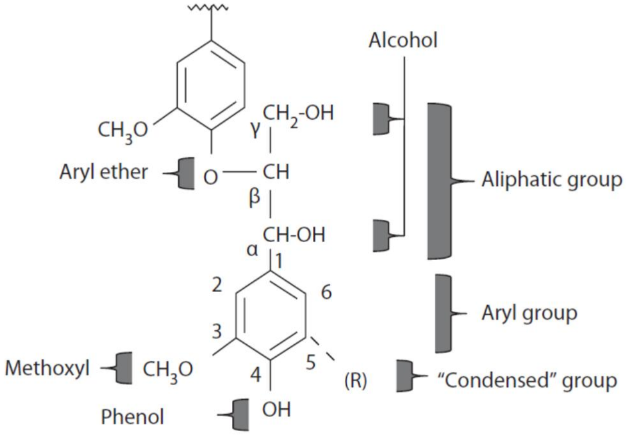


Figure 1. Lignin functional groups (Dimmel, 2010).

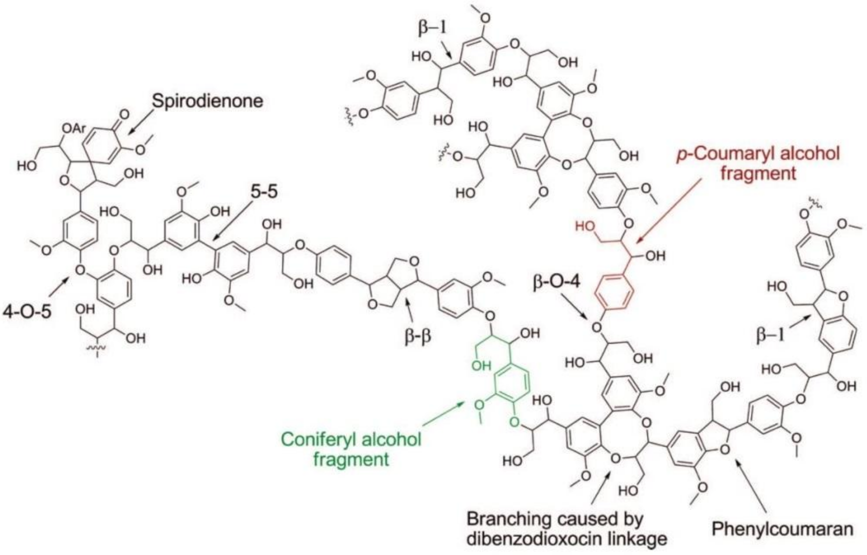


Figure 2. Schematic representation of a softwood lignin structure (Zakzeski et al., 2010, initially adopted from Brunow et al., 1998).

1.2.2 Extraction

The extraction of lignin from lignocellulosic material is done under conditions where lignin is gradually broken down into lower molecular mass fragments or solubilized by introduction of acidic functionality in lignin with concurrent breaking of the lignin-carbohydrate complexes.

The alkaline sulfate, meaning kraft process utilizing NaOH and Na_2S as pulping chemicals, is the main traditional method for pulping (95% of world production) (Gosselink et al., 2004) and hence produces the largest volume of lignin. The most abundant linkage in lignin, β-O-4, is the most susceptible to cleavage by kraft pulping, which induces a large number of phenolic hydroxyl groups within the structure of kraft lignin. The complex kraft delignification reactions also lead (among other things) to enol ether and stilbene linkages (Marton, 1971). A statistical scheme for softwood kraft lignin is shown in **Figure 3**, as proposed by Marton (1971).

The alkaline soda process does not utilize sulfur containing chemicals and is the predominant method for pulping annual plants (e.g. straw). The acid or neutral sulfite-pulping technology is still applied to some extent. In sulfite pulping, lignin is separated from cellulose and hemicellulose by the addition of sulfonic acid groups to the Cα position of lignin, thereby increasing the solubility of lignin in water (Sixta et al., 2006a). Related to the production of cellulosic ethanol and bio-diesel in the emerging biorefineries, additional sources of lignin will be available through various pretreatment technologies, of which the most promising can involve combinations of physical, chemical, biochemical, and thermal methods. Cooking methods in organic solvent (e.g. ethanol, formic acid, acetic acid, and methanol) produce so-called organosolv lignins, which, however, are not yet produced commercially (Doherty et al., 2011).

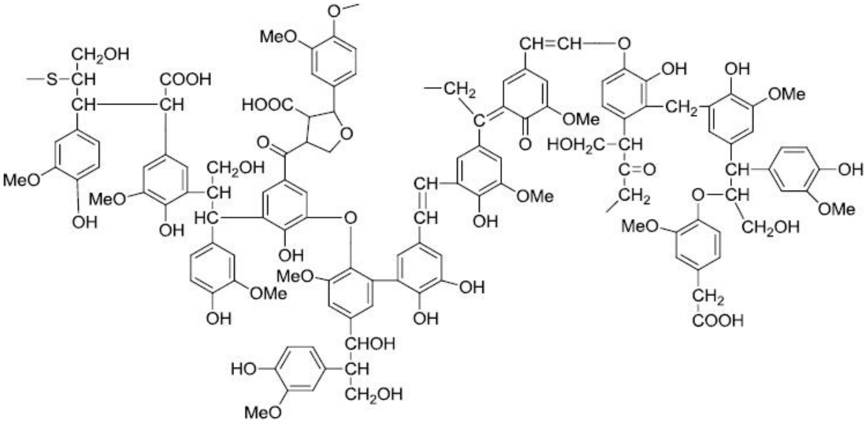


Figure 3. A scheme for softwood kraft lignin showing the large abundance of phenolic OH groups (Marton, 1971).

1.2.3 Isolation

The isolation of lignin from the processing liquor is based on acid precipitation or ultrafiltration followed by solid/liquid separation and drying. On an industrial scale, precipitation is used to obtain kraft lignin from black liquor, and filtration for the water-soluble, high molecular mass lignosulfonates from sulfite spent liquor (Vishtal and Kraslawski, 2011).

Two commercial processes for isolation of kraft lignin exist (Öhman, 2006; Tomani, 2010; Maki et al., 2012). In both processes, carbon dioxide is added to reduce the black liquor pH to 9–10, a point at which lignin precipitates from the solution. This is followed by filtration to obtain a lignin cake, which is either washed with sulfuric acid and water on the filter (FPInnovation-NORAM process) or reslurred in sulfuric acid, where it is then filtered and washed (LignoBoost process owned by Valmet). The FPInnovation-NORAM process also applies oxygen oxidation to black liquor prior to carbon dioxide, mainly for filtration-improving purposes.

In addition, a new, continuous SLRP (sequential liquid-lignin recovery and purification) process for producing lignin from black liquor is being developed by Liquid Lignin Company, LLC (Liquid Lignin Company, 2014). The process also applies carbon dioxide, but is reported to simplify integration with the host mill and to reduce capital and operating costs, when compared to the alternative processes.

1.2.4 Properties

The physicochemical properties of lignin depend on the source from which lignin is obtained and on the method of fractionation/extraction. Lignin properties including purity also determine how and where lignin can be further utilized.

Typically, lignins require alkali conditions to be dissolved. This is mainly because the dissociation of guaiacyl- and syringyl-derived phenols occurs at alkaline pH (Ragnar et al., 2000). In contrast to native lignins, the phenolic hydroxyl groups are among the most abundant functional groups in kraft lignins, determining its increased chemical reactivity and, consequently, its stability toward technical utility. Lignosulfonates are water soluble under most conditions because of the sulfonic acid/sulfonate group ($pK_a \leq 2$) in their backbone. The reactivity and physicochemical properties of lignins are also dependent, to a certain extent, on their molecular mass distribution (Doherty et al., 2011).

Lignin has poor thermal stability (Fenner and Lephardt, 1981) and melt-flow (softening) behavior under elevated temperatures. Amorphous polymers like lignin exhibit a glass transition, in which a hard, brittle, glass-like state is transformed into a rubbery or viscous state by heating. Relaxation phenomena are observed below and above the glass transition temperature (T_g), which indicates the temperature at which lignin undergoes softening. Lignin T_g depends on the amount of water and polysaccharides (Hatakeyama and Hatakeyama, 2010), and is increased as the molecular mass and degree of condensation increases (Baumberger et al., 2002), and is decreased as the solubility in organic solvents increases (Lora and Glasser, 2002). However, in general, T_g will be lower the greater the mobility of the lignin molecules, meaning less crosslinking between the macromolecules via intermolecular hydrogen bonding (Gargulak and Lebo, 2000; Hatakeyama and Hatakeyama, 2010).

Lignins are often categorized as sulfur and sulfur-free lignins according to pulping chemistry (Laurichesse and Avérous, 2014) (**Table 1**). Sulfur lignins, except the lignosulfonates, have the disadvantage of odorous, sulfur containing volatile organic compounds (VOCs) releasing from the material, especially at elevated temperatures (Lora, 2008).

Information on the physicochemical properties of two commercial lignins: kraft lignin Indulin AT (MeadWestvaco Corp., Appomattox, VA, USA) and soda lignin Protobind 2400 (GreenValue SA, Alpnach Dorf, Switzerland) is given in **Table 2**.

Table 1. Characteristics of technical lignins (Laurichesse and Avérous, 2014).

Lignin type	Sulfur-lignins		Sulfur-free lignins	
	Kraft	Lignosulfonate	Soda	Organosolv
Aspect				
Raw materials	Softwood Hardwood	Softwood Hardwood	Annual plants	Softwood Hardwood Annual plants
Solubility	Alkali Organic solvents	Water	Alkali	Wide range of organic solvents
Number-average molar mass (M_n – g mol^{-1})	1000–3000	15,000–50,000	800–3000	500–5000
Polydispersity	2.5–3.5	6–8	2.5–3.5	1.5–2.5
T_g (°C)	140–150	130	140	90–110

Table 2. Physicochemical properties of commercial kraft (Indulin AT) and soda (Protobind 2400) lignin (adapted from Schorr et al., 2014).

	Indulin AT	**Protobind 2400**
Insoluble Klason lignin (%)	92 ± 2	79 ± 1
Soluble Klason lignin (%)	4.4 ± 0.3	9 ± 1
Total sugars (%)	1.0 ± 0.2	1.3 ± 0.1
Ash (%)	3.59 ± 0.7	1.61 ± 0.06
C (%)	63.9	61.2
H (%)	5.6	6.3
N (%)	0.39	0.93
S (%)	2.1	2.0
O (%)	24.4	28.0
OCH_3(%)	11.2 ± 0.8	14.8 ± 0.2
Formula C9	$C_9H_{8.13}N_{0.05}S_{0.11}O_{2.1}(OCH_3)_{0.66}$	$C_9H_{9.42}N_{0.11}S_{0.12}O_{2.5}(OCH_3)_{0.93}$
M C9 (g mol^{-1})	174.6	182.8
OH_{Phen} (mmol g^{-1} per C9)	3.37	2.84
OH_{Phen} /OH_{Aliph} (by FT-IR)	0.93	0.94
H/G/S	2/96/2	6/37/57
M_n	1248	838
M_w	4549	2802
PD	3.65	3.34
Dp_n	7.1	4.4
Dp_w	26.1	14.6
T1 (°C)	156	185
T50% (°C)	632	415
Residues (%)	34	35
Coal for 1000 °C (%)	31	33
DTG max (°C)	378	366
T_g (°C)	132	59

The number average (Dp_n) and mass average (Dp_w) degree of polymerization were calculated by dividing M_n or M_w by the molecular mass of C9 calculated from the elemental analysis. T1 = first temperature of degradation; T50% = temperature for 50% of mass losses; Coal for 1000°C = residues - ash; DTG max = temperature for maximal degradation; T_g = glass transition temperature determined by thermomechanical analysis (TMA) measurements. N_2 was used as sample purge and balance purge gas in determination of the thermal properties.

1.2.5 Current and potential commercial applications

The products potentially derived from lignin can be separated into categories: (1) power, fuel, and syngas products; (2) macromolecules; and (3) low-molecular weight aromatic or phenolic compounds (Holladay et al., 2007). Since the focus of this work is to valorize technical lignins to polymeric chemicals and base material for material applications, the macromolecular category is reviewed.

In general, polymeric/macromolecular lignins can be used as surface active agents, in thermosetting resins/materials, and in thermoplastic blends (Glasser, 2001; Stewart, 2008). Technical lignins, other than water-soluble lignosulfonates, require increased hydrofilicity to be applied as surfactants, dispersants, or polyelectrolyte-based applications. For the base material applications, thermosets, and thermoplastics, technical lignins mainly require enhancement of their thermal properties (thermal stability and melt-flow).

There are many commercial applications in different industries where lignosulfonates, including the sulfite-pulping by-product lignin and the post-sulfonated alkali lignins, are used because of their surface-active properties, initiated by both the hydrophobic and hydrophilic moieties in the macromolecule. These applications are reviewed in several publications (Gargulak and Lebo, 2000; Lora, 2008; Doherty et al., 2011; Zhang et al., 2011). The predominant utilizations of lignosulfonates, with a weight of 700 000 t annually (Plank, 2004), are dispersants (plasticizers) for cement particles in concrete preparation. However, currently, lignosulfonates are more and more displaced by synthetic superplasticizers, due to their superior properties when used as additives to high-strength concrete (Stern and Schwarzbauer, 2008). The other applications of lignosulfonates include aids for animal-feed pelleting, oil-well drilling, or dust control. They are also used to a smaller extent for dispersants for carbon black, dyes or pigments, and products for specialty markets such as pesticides, emulsifiers, specialty chelates, and scale inhibitors.

The blending of two or more polymers provides the ability to tailor material properties to achieve specific goals with a higher value. Typically in blends, ionic linkage or hydrogen-bonding interactions connect lignin functional groups to the other polymer. A recent review by Doherty et al. (2011) discusses protein–lignin blends, starch–lignin blends, epoxy–lignin composites, phenol-formaldehyde resins where all or part of the phenol is derived from lignin, polyolefin–lignin blends, lignin blends with vinyl polymers, lignin–polyester blends, lignin as a component of polyurethanes, synthetic rubber–lignin blends, graft copolymers of lignin, and the prospects of lignin incorporation into further polymer systems. However, most of these copolymers and polymer blends are still searching for commercial applications.

Arboform® biocomposite is a commercial application of lignin in fiber-reinforced thermoplastics. The composite consists of isolated lignin, natural fibers (from wood or annual plants), and natural additives (plasticizers), which are processed in conventional thermoplastic processes such as press or injection molding (Naegele et al., 2002).

Lignin modification by derivatizing substituents (via covalent bonds) offers opportunities for tailoring lignin properties. Because of the hydroxyl groups in lignin, esterification and etherification are the most prominent among the derivatization alternatives. The chemical modification of lignin plasticization enhances the glass transition and reduces the brittleness of lignin-derived polymers, both of which are characteristics desirable, for example, in polymer coating and composite applications (Hult et al., 2013; Laurichesse and Avérous, 2014). To obtain “green” surfactants and dispersants with improved performance, lignins have been chemically modified to increase their solubility using various methods of alkylation, sulfonation, carboxymethylation, ozonation, and so on (Månsson and Öster, 1988; Kosikova et al., 2000; Ouyang et al., 2009; Homma et al., 2010; Cerrutti et al., 2012).

Future utilization of polymeric lignin has been anticipated by several authors (Brosse et al., 2011; Doherty et al., 2011; Nandanwar et al., 2012; Schorr et al., 2014). The new emerging applications of lignin are principally projected to be sustainable alternatives to non-renewable products such as polyurethanes, epoxy and phenolic resins (thermosets), and thermoplastic polymers, as well as corrosion inhibitors (surface treatment). However, it has been pointed out that lignin must be reacted with suitable petrochemical compounds to reach acceptable properties for the resulting thermosetting materials, due to its lack of thermal stability or functions available to participate with the curing processes (Raquez et al., 2010). In summary, several potential applications exist for technical lignins, often after being essentially modified, but their commercial utilization always involves a balance between price and performance.

1.3 Oxidation of lignin using O_2 under alkaline conditions

The roots of lignin oxidation are in chemical pulp bleaching, wherein several oxidizing chemicals are sequentially used to remove and modify residual lignin (1.5–4.5 w-% of unbleached fiber (Gellerstedt, 2010)) to increase pulp brightness. The bleaching sequence typically starts with oxygen in alkali media, with the pH in the range between 10 and 13. Under the conditions used in oxygen delignification, there are a large number of both oxygen derivatives and lignin structures present, thus giving complexity to their reactions.

1.3.1 Oxygen delignification

Oxygen delignification is normally carried out at medium pulp consistency (10–12 w-%) with all the chemicals, meaning gaseous oxygen, aqueous sodium hydroxide, and magnesium salt being introduced in a mixer followed by further reaction in a tower for 30–60 minutes at around 90–100°C. Shorter oxygen stages, as well as a more powerful dual stage, are also used. The oxygen gas charge used in commercial softwood oxygen delignification systems often ranges from 15 to 25 kg per ton of pulp (1.5–2.5 w-% of pulp) (McDonough, 1996; Sixta et al., 2006b).

Pulp oxygen delignification reduces the number of free phenolic groups in the residual lignin, and increases the number of condensed structures and carboxyl groups (Gierer, 1987). Oxidation of etherified lignin structures also takes place in the oxygen stage, but the delignification reactions are, in this case, much slower than the oxidation of phenolic structures. The reactions of the etherified lignin structures are mediated by hydroxyl radicals, as these structures are resistant to direct oxygen attack (Gierer, 1987, 1997).

Catalytic alkaline oxygen oxidation for pulp delignification (Suchy and Argyropoulos, 2001; Gaspar et al., 2007), as well as for obtaining value-added products from lignin (Crestini et al., 2010; Zakzeski et al., 2010; Azarpira et al., 2014) have been a subject of many studies. However, they are not reviewed in this work.

1.3.2 Molecular oxygen

In its ground state, oxygen exists as a diatomic molecule, O_2 (dioxygen). The two unpaired electrons in the outermost orbital of O_2 have parallel spins. Both electrons are seeking electrons with the opposite spin direction. Because of this property, O_2 has a tendency to react with substrates that have sites of high electron density. O_2 takes up one electron at a time to the molecular orbital – in a process called one-electron reduction – and accordingly, the reduction products of O_2 are formed (Sixta et al., 2006b).

In stepwise reduction (**Figure 4**), O_2 is reduced to water, while the substrate is oxidized. In the first stage, a superoxide anion ($O_2\bullet^-$) is formed. It can protonate to form a hydroperoxyl radical (HOO•). Protonization does not, however, take place under alkaline conditions, as the superoxide anion has a pK_a value of 4.8. In the next stage, the hydroperoxyl radical receives an electron and forms a hydroperoxyl anion (HOO^-), the anion of hydrogen peroxide (HOOH). Both peroxide and its anion occur under oxygen delignification conditions. In the third stage, hydrogen peroxide receives an electron and forms a hydroxyl radical (HO•) that is in equilibrium with an oxyl anion radical ($O\bullet^-$). Water is formed when the hydroxyl radical receives an electron and a proton (Gratzl, 1992; Sixta et al., 2006b).

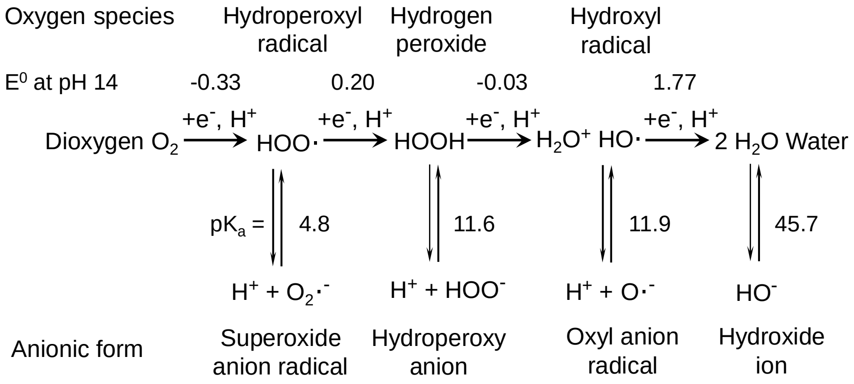


Figure 4. Dioxygen reduction proceeding in four consecutive one-electron steps (E^0 standard reduction potential) (Sixta et al., 2006b initially adopted from Gratzl, 1992).

In order to initiate a reaction (reach the activation energy) under the conditions of alkaline O_2 treatment, an increase in the temperature and the ionization of the functional group (phenolic hydroxyl in lignin) are necessary to facilitate the electron transfer to O_2 and its derivatives (Sixta et al., 2006b).

In O_2 delignification, purely inorganic reactions also take place, as the oxygen derivatives react with each other or with transition metals, such as Fe, to form further radicals and ions (Bielski et al., 1985; Buxton et al., 1988; De Laat and Le, 2005). The hydroxyl radical (HO•) produced, for example by Fenton chemistry, is extremely reactive, and the most unselective of the oxygen species.

Dioxygen and its derivatives react with different lignin structures: O_2, $O_2•^-$, HO•, and $O•^-$ function as electrophiles attacking the high electron density sites in lignin, while only HOO^- functions as a nucleophile attacking the low electron density sites (e.g. carbonyl carbons) (Gierer, 1987, 1990, 1997).

1.3.2.1 Solubility

The solubility of oxygen gas and the diffusion of dissolved O_2 in the aqueous phase are essential for the oxidation of residual lignin in pulp, or lignin in solution as such.

Medium-consistency oxygen delignification systems use pressure to improve mass transfer, by reducing gas volume and bubble size, hence improving the solubility of oxygen gas. In addition to the partial pressure of O_2 in the gas phase, other important factors affecting O_2 solubility are temperature and the presence of inorganic solutes such as NaOH.

Correlation (Broden and Simonson, 1979) and thermodynamic-based (Tromans, 1998a, 1998b, 2001) models have been used to estimate the solubility of O_2 in solutes as a function of O_2 pressure and temperature in equilibrium conditions. The elevated temperature that is beneficial to oxidation reaction kinetics is detrimental to gas volume and solubility. In general, the solubility of O_2 has its minimum at 100 °C. The solubility of O_2 in NaOH solution is less than that in water. According to Tromans (1998a, 1998b), at 100°C and under O_2 pressure of 700 kPa, the solubility of O_2 in NaOH solutions of 1.50, 1.00, and 0.50 M is 0.09, 0.12, and 0.16 g l^{-1}, respectively, and in water 0.19 g l^{-1}.

By assuming that the oxygen gas charge is about 2 w-% of pulp, or 2 g l^{-1} at 10% pulp consistency, only about 10% of this charge is dissolved in the alkali solution at saturation level. However, the initial O_2 charge of 2 w-% of pulp equals roughly a 100 w-% charge of residual lignin, assuming that the pulp contains 2 w-% of residual lignin. Accordingly, at saturation level the dissolved O_2 charge equals about 10 w-% of residual lignin.

1.3.2.2 Consumption

Typically, when investigating the degree and selectivity of pulp oxygen delignification, more attention has been paid to process variables other than the oxygen charge (ensured to be enough), including alkali charge, temperature, pressure, and reaction time (Li et al., 1996; McDonough, 1996).

However, the purpose of the study by Salmela et al. (2004) was to estimate the amount of oxygen that is actually consumed or needed in different reactions during an industrial oxygen delignification process (two-stage O_2 delignification for softwood kraft pulp). Based on a detailed chemical analysis of filtrate compounds and computation of material balances, it was observed that oxidation of lignin, as well as inorganic sulfur-containing ions, in the process liquor consumed a considerable amount of molecular oxygen. The main oxygen-consuming reaction products were dissolved lignin, carbonate, and sulfate. Alkali in the form of hydroxyl ions (HO^-) takes part in the reactions, especially with carbohydrates producing carboxylic acids. Thus, part of the oxygen bound to reaction products also originated from alkali. Due to the complexity of the reactions taking place in the process, it was impossible to state the exact amounts of oxygen or alkali involved in the reactions and bound to dissolved products. However, a rough estimation indicated that only 4–10 kg per BDt (0.4–1.0 w-% of pulp), being about half of the O_2 charged, was actually consumed in the reactions. Thus, a considerable amount of O_2 was lost in vent gases.

1.3.3 Reactivity of lignin structures

Traditionally, model compounds have been used in reaction mechanistic and kinetic studies. The reactions of lignin model compound under oxygen delignification conditions have been reviewed by Northey (2001). The relative reactivity of model compounds with different functionality with oxygen is shown in **Figure 5**. The structures with free phenolic hydroxyl groups have been reported to be clearly more reactive than non-phenolic. The reactivity of lignin units containing different numbers of methoxyl groups is as follows: S > G > H. Catechol with two hydroxyl groups in the aromatic ring, is clearly more reactive then the aforementioned.

Since the oxidation rate in the reactions is highly affected by the system parameters such as pH, temperature, oxygen charge, and reactant charge, the comparison of the reactivity of different dimeric model compounds is difficult, due to the variable conditions used in the experiments. However, the approximate order of the reactivity of bonds/dimeric compounds can be stated as follows: The phenolic stilbenes and vinyl ethers degrade rapidly across the Cα-Cβ double bond, while stilbenes oxidize over a hundred times faster than vinyl ethers. The compounds with a free phenolic group and β-1, β-O-4 (with α-carbonyl or α-hydroxyl), 5-5, or β-5 bonding react an order of magnitude more slowly than vinyl ethers. The dimeric compounds, not including free phenolic groups, were found to be unreactive.

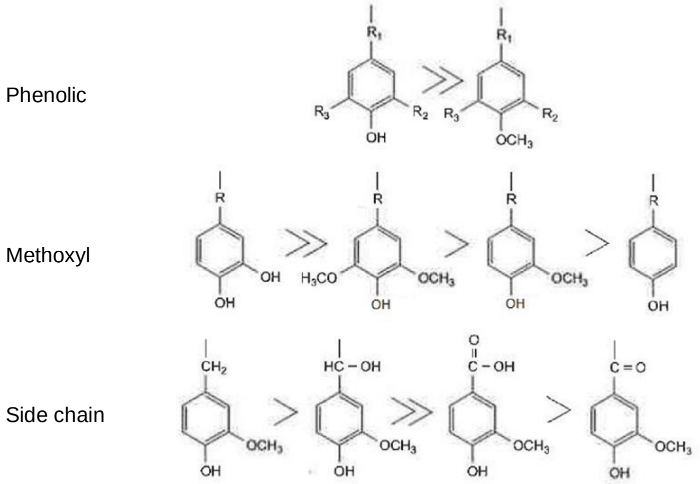


Figure 5. Relative reactivity of lignin model compounds with oxygen (adopted and modified from Northey, 2001; Sixta et al., 2006b).

1.3.4 Mechanism

Several reviews (Chang and Gratzl, 1980; Gierer, 1982, 1986, 1997; Gratzl, 1990, 1992) have been written on the mechanism involved in the lignin degradation by oxygen-derived species under alkali conditions. The review by Chang and Gratzl (1980) summarizes the early studies (e.g. Eckert et al., 1973; Kratzl et al., 1974; Gierer and Imsgård, 1977a; San Clemente et al., 1981) in which phenolic lignin model compounds were reacted with dioxygen, and presents the fragmentation patterns of the phenolic lignin structures under oxygen-alkali conditions. The review by Gierer (1997) summarizes the results of the model compound studies (Gierer et al., 1992, 1994, 1996a, 1996b) on the $HO\bullet/O\bullet^-$ and $HO_2\bullet/O_2\bullet^-$ radicals involved in lignin oxidation, and presents the reaction schemes of the non-phenolic lignin structures with the oxygen-derived radicals.

1.3.4.1 Initiation

In alkaline media, the free phenolic hydroxyl groups in lignin are ionized to produce phenolates. O_2 reacts with phenolate, forming phenoxyl radical, the first oxidation product, and a superoxide radical anion ($O_2\bullet^-$), the one-electron reduction product of oxygen (**Figure 6** A). Optionally, the hydroxyl radical ($HO\bullet$), formed during oxygen treatment, can react with phenolate generating a phenoxyl radical and being eliminated as a hydroxide anion (**Figure 6** B) (Gierer, 1982; Sixta et al., 2006b).

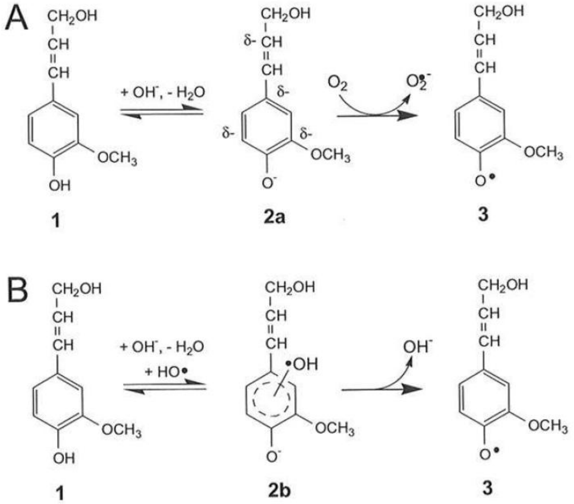
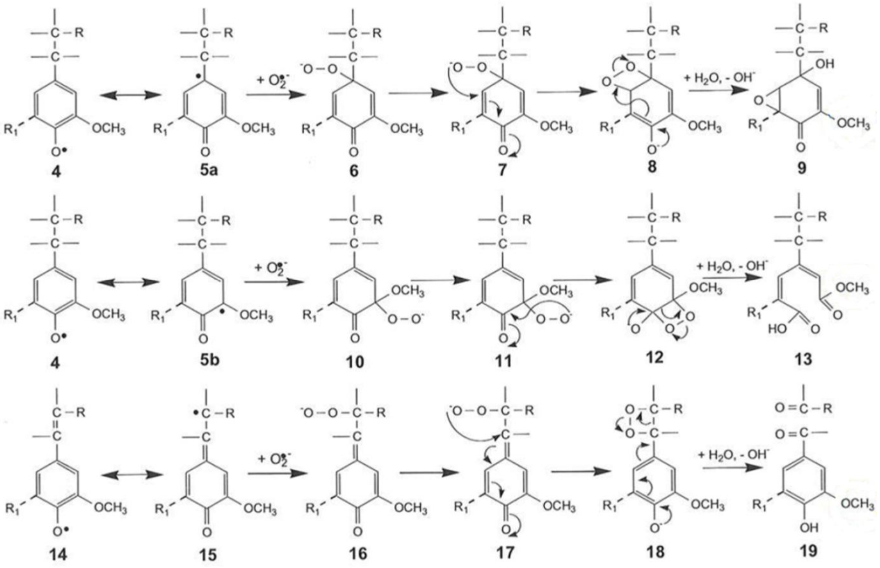


Figure 6. Formation of the phenoxyl radical by oxygen (A) and the hydroxyl radical (B) (Sixta et al., 2006b).

1.3.4.2 Reactions of phenolic lignin

Phenoxyl radicals exist in different mesomeric forms. Delignification proceeds when a superoxide radical anion ($O_2\bullet^-$) attaches to any of the mesomeric forms of the resonance-stabilized phenoxyl radical (5a, 5b and 15) (**Figure 7**). The new intermediates are cyclohexadienone hydroperoxides (6 and 10) with a mesomeric β-structure (16). The **hydroperoxide anion** reacts further by intramolecular nucleophilic attack by the peroxide anion within the molecule (8, 12, and 18) and rearranges to form the primary oxidation products: **oxirane** (9), **muconic acid ester** (13), or **carbonyl** (19) structures. The formation of muconic acid (13) ester is a result of ring opening by cleavage of the C-C bond, while the formation of the carbonyl (19) structure is caused by cleavage of the Cα-Cβ bond (Gierer and Imsgård, 1977a; Gierer, 1982, 1986).

Chang and Gratzl (1980) have presented that the *p*-hydroperoxide anion intermediate (6) can give an enol in addition to the oxirane structure. In addition, if the side chain in the *p*-hydroperoxide anion intermediate is a carbinol-, carbonyl-, or carboxylic function, *p*-quinone formation with the side chain elimination is predominant. The *o*-hydroperoxide anion intermediate (10, also presented in **Figure 8**) forms a **muconic acid** structure and methanol by the elimination of the methoxyl group (hydrolysis reaction following the ring opening) or alternatively ***o*-quinone** and methanol (**Figure 8** A) (Chang and Gratzl 1980, Gratzl 1990).



R = H, OAr, Ar or Alk

Figure 7. Formation of hydroperoxide intermediates in alkaline media followed by an intramolecular nucleophilic attack of the hydroperoxide anions and formation of primary oxidation products (Sixta et al., 2006b, initially adopted from Gierer 1982).

1.3.4.3 Rate-determining step and delignification kinetics

The intermediate hydroperoxide anion structure (a pK_a of 12–13) exists in equilibrium with its protonated form under alkaline conditions. The protonated hydroperoxide intermediate decomposes homolytically back to a phenoxyl radical and a hydroperoxyl radical (HOO•). The phenoxyl radicals may spontaneously couple with each other to form dimeric products (Gierer and Imsgård, 1977a; Chang and Gratzl, 1980) (**Figure 8** B). The importance of pH on the course of coupling was revealed when, at pH 9 or below, 4-ethylguiaicol gave biethylguiacol exclusively, whereas at pH 13.7 or above, no dimerization took place (San Clemente et al., 1981). The formation of biphenyl structures is an undesired reaction during pulp oxygen delignification, since the 5-5 bond is very stable and can hardly be cleaved. It has been suggested that C-C coupling of the phenoxyl radicals is the dominating coupling mode, rather than C-O for derivatives with alkyl in *p*-position (Kratzl et al., 1974).

Recently, Ji et al. (2009a, 2009b) studied the kinetics of oxygen delignification on softwood kraft pulps of different kappa numbers in a continuous stirred tank reactor. Because the dissolved oxygen and alkali concentrations in the reactor were constant, the rate of lignin removal could be determined from the dissolved lignin content in the outflow stream, measured by UV absorption. The delignification kinetics were determined at different temperatures, oxygen pressures, and caustic concentrations. The kinetics were first order in residual lignin content. The reaction order in terms of NaOH suggested that the rate-determining step involves an acidic lignin active site with a pK_a almost 2 units higher than that of phenol in lignin (10.5 for Indulin AT at room temperature (Norgren and Lindström, 2000)). The mechanism proposed by Chang and Gratzl (1980) was adopted to explain the kinetics. The kinetics of phenolic delignification could be described by assuming that the decomposition of the hydroperoxide anion at C5 of the aromatic ring is the rate-determining step. The pH dependence of the kinetics suggested that the protonated hydroperoxide does not fragment into muconic acid or quinone structures. In addition, since the dissolved lignin mostly retained its aromatic nature, and methanol was released essentially quantitatively during delignification, it was supposed that quinone formation is the dominant pathway.

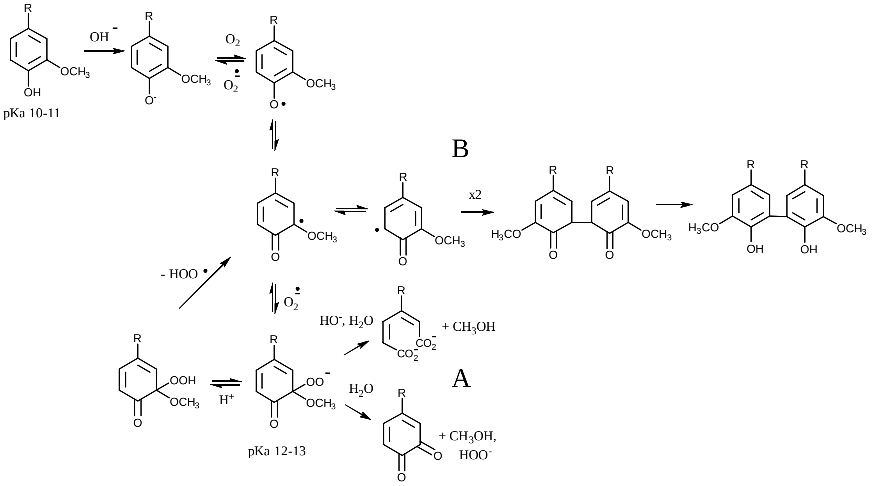


Figure 8. Reaction paths of lignin with oxygen (adopted from Chang and Gratzl, 1980; Ji et al., 2009a, 2009b). Route A: degradation (simplification). Route B: coupling.

1.3.4.4 Secondary reactions

Model compound studies have also shown that the primary oxidation products arising from the hydroperoxide anion intermediates may undergo further alkaline promoted (non-oxidative) and oxidative degradation, as well as ring closure reactions (forming lactone or furoic acid structures). The degradation schemes have been outlined in several publications. Kuitunen et al. (2011) have adopted the schemes in developing a model for lignin oxidation under oxygen delignification conditions (**Figure 9**).

During the secondary oxidation, oxirane undergoes a series of reactions forming smaller molecular mass structures with carboxylic functionality (e.g. S23, **Fig-**

ure 9 a) (Gierer and Imsgård, 1977a, 1977b). Muconic acid structures react easily further, forming a lactone structure via a ring closure reaction (nucleophilic addition), especially under neutral or acidic conditions (Gierer and Imsgård, 1977a; Chang and Gratzl, 1980; Pieken and Kozarich, 1990), or they may oxidize to smaller structures with simultaneous formation of formic and oxalic acids (**Figure 9** d). *o*-Quinone reacts further with hydrogen peroxide and results in an epoxide structure (Chang and Gratzl 1980, Gratzl 1990), or alternatively with a hydroperoxyl anion, resulting a muconic acid structure (**Figure 9** c) (Gierer and Imsgard 1977a, Gierer 1986). According to Gellerstedt et al. (1980), the reaction between p-quinone (S16, **Figure 9** b) and the hydroperoxide anion finally leads to the formation of maleic and acetic acid.

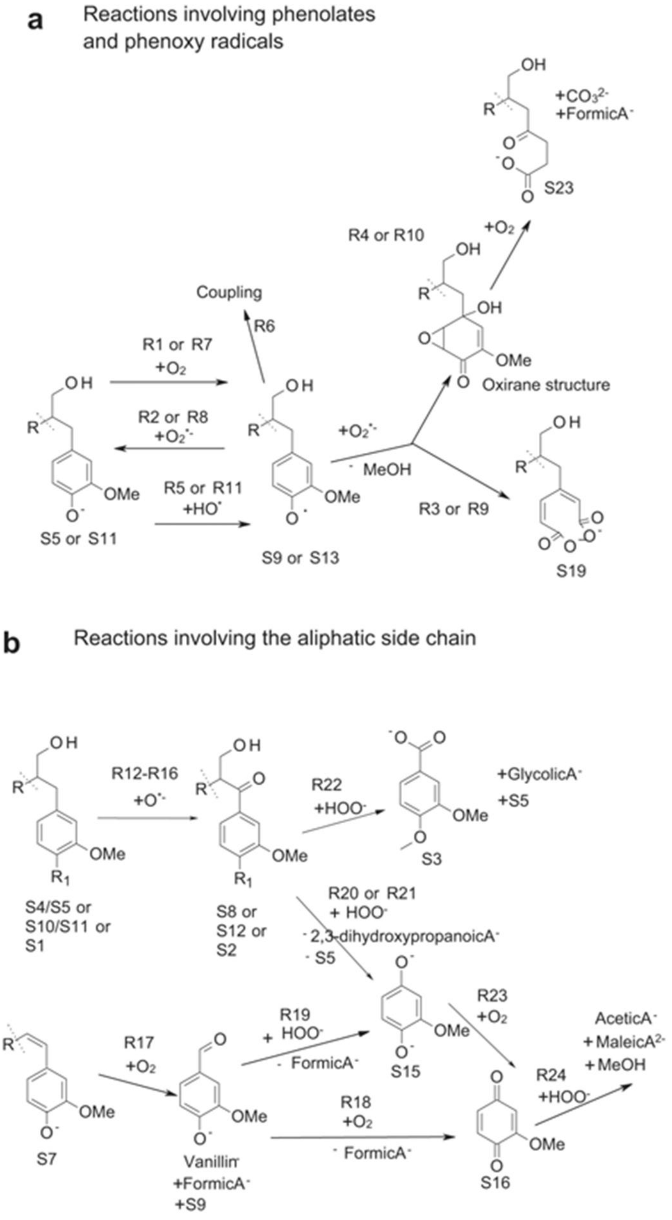
The several different secondary reactions taking place during lignin oxidation are obviously condition dependent. If the oxygen oxidation is continued under suitable conditions, most of the lignin structures will finally end up as carbon dioxide.

1.3.4.5 Model of oxidation chemistry

A comprehensive phenomenological model of lignin oxidation chemistry was recently presented by Kuitunen et al. (2011). It is based both on the reaction mechanisms proposed in the literature (reviewed in the previous chapter) and on experimental work (Kalliola et al., 2011 (publication I); Rovio et al., 2011) on oxidization of kraft lignin under oxygen delignification conditions. Commercial softwood kraft lignin (Indulin AT) was selected as a model compound. Lignin oxidation was followed by analyzing samples taken from the reactor and recording the pressure drop. The results of several analysis methods showed the changes in lignin structure during oxidation. In addition small molecular mass carboxylic acids and CO_2 were determined. The model development aimed to identify the reaction routes that would reproduce these experimental observations.

The reactions in the chemistry model fall into four main categories, involving: (a) phenolates and phenoxyl radicals, (b) the aliphatic side chain, (c) chromophoric structures, and (d) degradation of aliphatic organic compounds (**Figure 9**). The traditional classification of reactions is based on the mechanism of formation of hydroperoxides, fragmentation of hydroperoxides, and involvement of radicals, whereas the classification by Kuitunen et al. (2011) was based on the reactive sites or organic structures. The stoichiometry of the reactions was defined by conserving mass and electroneutrality. The reaction kinetic parameters were adapted from the literature or determined in the study, if not available. In addition to the reactions of the organic structures, reactions involving only inorganic species were incorporated. The key intermediate, hydroperoxide structure, and its pH dependency (Ji et al. 2009a, 2009b) were not considered in the model.

As conclusions of the study, it was elucidated that the degradation reactions starting from the phenolates consume a major part of the oxygen and produce protons. The dominating oxidation paths lead to the formation of muconic acid and an oxirane structure, both which are very vulnerable to secondary/further degradation, which also consumes a lot of oxygen and hydroxyl ions, and forms smaller lignin units containing carboxylic groups, oxalic acid, formic acid, and carbon dioxide. Carbon dioxide acts as a buffer affecting the evolvement of the pH.



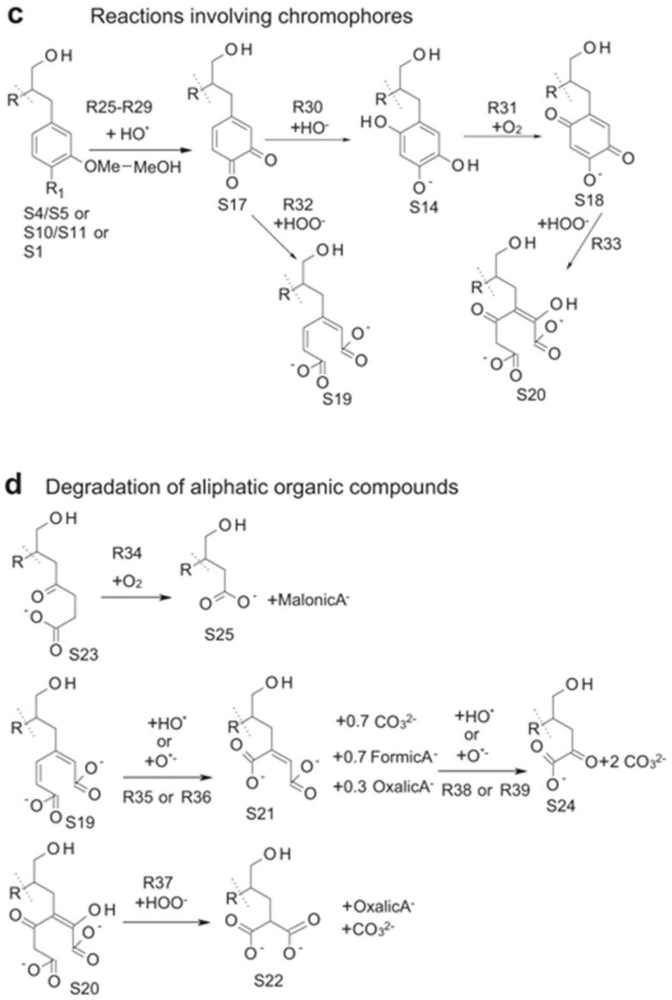


Figure 9. Reaction routes and structures of lignin oxidation by oxygen (adopted from Kuitunen et al. 2011).

1.3.4.6 Reactions of non-phenolic lignin

The very reactive hydroxyl radicals (HO•) are generated via the superoxide radicals and hydrogen peroxide. Hydroxyl radicals add rapidly to the π-electron system of the aromatic ring. This results in a short-lived charge-transfer adduct (e.g. 33) (**Figure 10**) that decays under alkaline conditions to give isomeric hydrocyclohexadienyl radicals (34, 37, and 41). The hydrocyclohexadienyl radicals may then undergo two different types of reactions: 1) oxidation by molecular oxygen (**Figure 10**), or 2) elimination of the added hydroxyl radical as a hydroxide anion or water.

The oxidation leads to hydroxylation (**Figure 10** A) or, in combination with the elimination of methanol (demethoxylation) and hydrolytic cleavage of an alkyl aryl ether bond, to dealkoxylation with the formation of o-quinone structures (**Figure 10** B, also presented in **Figure 9** c). Analogously, the hydrocyclohexadienyl radicals from conjugated structures yield quinone methide intermediates, which further react and result in the cleavage of the conjugated double bond (**Figure 10** C). The same oxidation products, together with the corresponding reduction products, may also arise from the hydroxyl adducts by disproportionation (Gierer, 1997).

The second reaction mode, the elimination of the added hydroxyl radicals, leads to side chain oxidation or cleavage of the Cα-Cβ bond. The elimination reaction may also lead to phenolic coupling (Gierer, 1997).

Hydroxyl radicals do not open the aromatic ring (neither phenolic nor non-phenolic) without the co-operation of molecular oxygen.

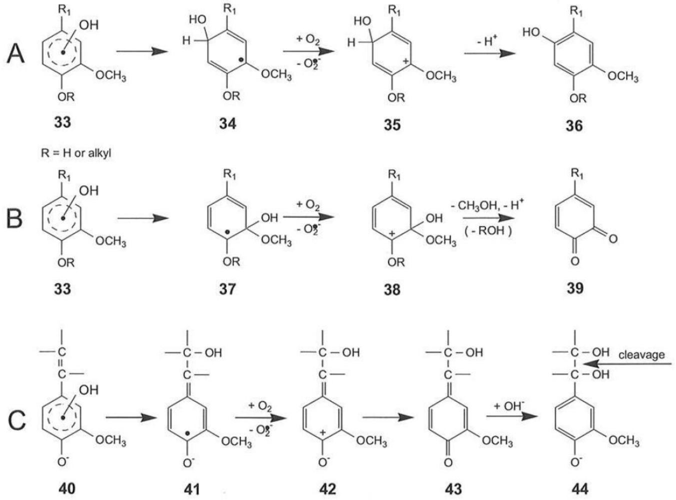


Figure 10. Reactions of the hydroxyl radical adduct of aromatic and ring-conjugated structures (Sixta et al., 2006b, initially adapted from Gierer, 1997)

1.3.5 Applications to technical lignins

Alkaline oxidation of residual lignin (in pulp fibers or isolated from the fibers) by O_2 has been studied extensively in order to improve and optimize bleaching techniques. Oxidation of kraft lignins by O_2 (Imai et al., 2007; Kalliola et al., 2011 (publication I); Yada and Meshitsuka, 2006) has been studied mainly for the same purposes. In these studies, oxidation has been conducted under the conditions prevailing in the oxygen stage, with a low lignin content (<1 w-%) in the reaction solution, a rather high oxidant dose on lignin, and a temperature at or above 90°C, accompanied by fairly a long residence time. These conditions have degraded lignin strongly, partly turning it into low molecular mass components.

Another application of alkaline O_2 oxidation that is intended to degrade lignin is the production of artificial vanillin, a flavor constituent. Vanillin has been produced commercially since 1930 from lignosulfonates, by air oxidation under highly alkaline conditions (Van Ness, 1983). Alkaline O_2 oxidation for the conversion of softwood (Mathias et al., 1995) and hardwood (Rodrigues Pinto et al., 2013) black liquor, and softwood (Fargues et al., 1996a; Araújo et al., 2010; Rodrigues Pinto et al., 2011) and hardwood (Villar et al., 2001; Rodrigues Pinto et al., 2011) kraft lignins to phenolic aldehydes, especially to vanillin, has also been carried out. In most of these batch oxidation studies, the highest yield on vanillin, up to 10% of the initial lignin, has been obtained using lignin content of around 6–8 w-%, very aggressive pH conditions (initial pH close to 14), temperatures of almost 150°C, and O_2 partial pressure of 3 to 5 bars (constant). It has been observed that independent of the lignin source, pH is the most important variable in vanillin production. High alkaline pH is required both for the production of vanillin and to retain its yield (studies on Indulin AT (Fargues et al., 1996a; Araújo et al., 2010)).

O_2 oxidation under alkaline conditions, retaining lignin polymeric structure or favoring lignin condensation, has not been widely studied as a means to valorize lignin. However, three old patents in which lignin O_2 oxidation was done at high lignin content (up to 20 w-%), beneficial for down-stream processing, exist. One of these reports production of polycarboxylic lignin rich in carboxylic functionality in lignin backbone (Lin, 1975). The other two report polymerization of lignin using O_2 and formaldehyde (Benko and Daneault, 1975; Lin, 1980). The modified lignins were found to have good dye-dispersing properties.

According to Lin (1975), the water-soluble polycarboxylic lignin could be obtained by an oxygen-alkali process from kraft or soda lignin. The effect of reaction temperature on the yield and precipitation point of carboxylic lignin was mainly tested. The preferable lignin content was reported to be 15 w-%. The operation conditions of initial pH around 13, temperature most preferably 140°C, reaction delay 2 hours, and the use of 1 to 1.5 parts O_2 per 1 parts lignin (w/w), induced an O_2 uptake of about 0.25 parts per 1 part lignin. Thereby, degradation of lignin to non-recoverable non-lignin products is minimized, condensation of lignin to less water-soluble material is minimized, and the formation of carboxylic groups in lignin is maximized. The carboxylic lignin could be recovered by spray drying. It was reported that gelling of lignin always occurred when the concentration of lignin solution in the oxidation process was about 18 w-%.

1.4 Oxidation of lignin using O_2 and laccase as a catalyst

Reactions catalyzed by enzymes are regarded as an environmentally friendly alternative to chemical methods, which typically use harsh conditions. In addition, due to their high selectivity, bio-catalytic procedures can be very effective in the conversion of specific substrates. In general, enzymes are exploited in degradation, as well as non-destructive modification of synthetic and natural polymers, and also in polymer synthesis.

The most extensively studied lignolytic enzymes include laccases and manganese-dependent peroxidases (Grönqvist et al., 2003), which have been used for crosslinking and grafting of lignaceous materials and for the production of polymers from phenolics. In addition, laccases have both commercial and potential applications in a variety of industrial fields, including the pulp and paper, textile, and food industries (Rodríguez Couto and Toca Herrera, 2006; Ba et al., 2012; Asgher et al., 2014). The advantage of using laccases over peroxidases in an industrial process is that laccases are robust and stable, and they only require molecular oxygen as an oxidant. Not only is peroxide significantly more expensive than oxygen (Kangas et al., 2014), but it can easily be over-dosed, which may inactivate peroxidases.

1.4.1 Laccases

Laccases (EC 1.10.3.2) are multi-copper containing oxidoreductive enzymes. Fungal laccases are the most widely encountered in the literature. Well-known laccase-producing fungi (yeast and molds) include *Trametes*, *Pleurotus*, *Coprinus*, *Myceliophtora*, *Phlebia*, *Pycnoporus*, *Rhizoctonia*, and *Schizophyllum*. Laccase or laccase-like activity has also been demonstrated by plants, some insects, and a few bacteria. Fungal laccases have an acidic isoelectric point (pI) of around pH 4.0 (varying from 2.6 to 6.9). These enzymes were found to exhibit their highest activity at pH optima in the acidic range (depending on test conditions). The pH optima are very much substrate-dependent. While the oxidation of ABTS occurs at an optimal pH of 4 or lower, the optimal pH is between 4 and 7 for 2,6-dimetoxyphenol (DMP) and guaiacol with a bell-shaped curve expressing activity versus pH. The mean optimum reaction temperature is 55°C (ranging from 25 to 80°C), although the thermostability of fungal laccase varies considerably. In general, the half-life at 50°C ranges from minutes to several hours (Baldrian, 2006; Strong and Claus, 2011).

1.4.2 Mechanism

Laccase contains four copper atom centers, which perform the four electron reduction of molecular oxygen to water. No other reduction species of O_2 are known to form. As the next step, oxidation of various aromatic substrates, especially phenols, takes place, generating a free radical in the substrate (more specifically 4 times one electron oxidation per reduction of O_2 to water) (Solomon et al., 1996; Giardina et al., 2010). In lignin, only phenolic subunits are attacked by laccase, with the reaction product of the catalysis being phenoxyl radical (**Figure 11** A) (Crestini et al., 2003, 2010). It has been presented (Kratzl et al., 1974) that O_2 can react with phenoxyl radicals. However, the main reaction pathway of the phenoxyl radicals generated by laccase is considered to be spontaneous polymerization. In addition, disproportionation reactions of phenoxyl radicals yield side chain oxidation products (Cα oxidation) (Crestini et al., 2003, 2010).The other reaction pathways are not that likely to occur, due to the slow kinetics of O_2 addition to the phenoxyl radical species. For example, the rate constant (k) for the reaction of a tyrosine-derived phenoxyl radical (TyrO•) with O_2 has been determined to be $< 10^3$ M^{-1} s^{-1} (Jin et al., 1993). In oxidation under alkaline conditions, a superoxide anion (O_2•$^-$) is present (**Figure 6** A). Phenoxyl radical species react with O_2•$^-$ at a high rate: for example, k(TyrO• + O_2·$^-$) is 1.5×10^9 (Jin et al., 1993), k(G• + O_2•$^-$) is 1.4 × 10^9, and k(H• + O_2•$^-$) is 1.7×10^9 M^{-1} s^{-1} (Jonsson et al., 1993).

1.4.2.1 Laccase-mediator system

Mediators, acting as intermediate substrates for laccases, enable laccase to indirectly oxidize large molecules, and even non-phenolic substrates (Reid et al., 2010). Laccase in combination with a mediator has been applied, for example, for delignification of kraft pulps (Bourbonnais and Paice, 1997; Call and Mücke, 1997).

A possible reaction mechanism mediated by one of the mediator molecules, 1-hydroxybenzotriazol (HBT) appears to proceed as follows (Crestini et al., 2003). O_2 activates laccase, which oxidizes the mediator to form an oxybenzotriazolyl radical (HBT•). HBT• oxidizes lignin to form alternatively a phenoxyl or benzylic radical (**Figure 11** B). The benzylic radical has a high degree of reactivity towards O_2 ($k < 10^{10}$ M^{-1} s^{-1}) (Von Sonntag and Schuchmann, 1991; Gierer et al., 1992). Such an intermediate reacts by O_2 addition, yielding a side chain oxidation product with the simultaneous generation of HOO•. Its anion, O_2•$^-$, reacts with the phenoxyl radical, and thus the formation of side chain oxidation and aromatic ring cleavage products or substructures are favored, while 5-5 and/or 4-O-5 coupling reactions are depressed. In the study by Crestini et al. (2003) HBT, did not act as a mediator in the oxidation of non-phenolic lignin subunits (residual kraft lignin). The utilization of synthetic mediators in industrial processes, however, is hindered by their high cost and the possible generation of toxic species (Reid et al., 2010).

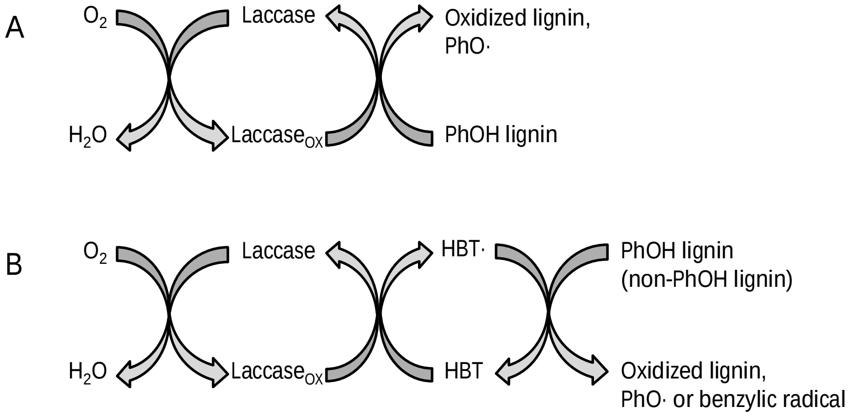


Figure 11. Schematic presentation of oxidation of lignin using laccase (A) and a laccase-HBT mediator system (B) as a catalyst (adopted from Crestini et al. 2003).

1.4.3 Applications to technical lignins

Mattinen et al. (2008) have shown that different technical lignins – soda lignin from flax, enzymatic mild acidolysis lignin from spruce, and dioxane lignin from eucalyptus species – can be activated and polymerized to different degrees by laccase (*Trametes hirsuta*). The solubility of lignin at the treatment pH (4.5) appeared to be an important criterion for the reactivity. Therefore, the suspensions for the laccase treatments were prepared by dissolving lignin first in alkaline water, after which the pH was decreased slowly using 1 M HCl, and finally set to the target pH using a proper buffer. The dissolution-pH adjustment procedure was intended to reduce the agglomerates in lignin to maximize its reactivity.

Molecular and structural heterogeneity of technical lignins can be reduced by sequential extraction fractionation using organic solvents (Ropponen et al., 2011; Boeriu et al., 2014). Recently, it was shown by Fiţigău et al. (2013) that low molecular mass fractions of technical lignins (M_w < 4000 g mol^{-1}), containing a large number of free phenolic hydroxyl groups with adjacent free *o*-positions, non-sterically hindered, are the best substrates for laccase (*Trametes versicolor*). Lignin polymers with a molecular mass of up to 17500 g mol^{-1} were obtained. Lignin fractionations and laccase treatments were done using 50 v-% acetone in water. The use of water-miscible organic solvents increases the solubility of lignin, which was also pointed out to be an important factor of the reactivity in the laccase-catalyzed process (Fiţigău et al., 2013). Recently, in comparing different types of technical and emerging lignins, higher lignin-laccase (*Trametes hirsuta*) reactivity in slightly acidic water was found to correlate with lower lignin molecular mass and higher amounts of monomeric phenolics (West et al., 2014).

Polymerization of commercial lignosulfonates by laccases, with and without mediators in varying reaction conditions, has also been confirmed recently

(Areskogh et al., 2010a, 2010b; Prasetyo et al., 2010). Interestingly, the study by Areskogh et al. (2010b) was intended to improve the plasticizing properties of lignosulfonates for cement/concrete applications. The modification method was a combination of commercial fungal laccase treatment followed by ozonolysis (at pH 7) aiming to generate high molecular mass lignosulfonate structures enriched with carboxylic groups.

Laccases have also been applied to provide a route for the addition of desired functionalities to lignin, as the phenoxyl radicals are potential sites for coupling reactions with the functionalizing reactant (phenolic) radicals. Lund and Ragauskas (2001) investigated the incorporation of water-soluble phenols with carboxylic or sulfonic acid groups onto kraft lignin by laccase (*Trametes villosa*) catalysis in 60 v-% dioxane in water. The oxidative coupling between lignin and a phenol was most strongly illustrated by the incorporation of guaiacol sulfonate onto lignin, which made the lignin water-soluble at a pH of 2.4.

A chemo-enzymatic polymerization pathway has been reported as a means to graft acrylamide and acrylic acid onto lignosulfonates. In this process, the role of laccase (*Trametes versicolor*) appeared to catalyze the formation of phenoxyl radicals in lignin, which further induced the formation of organic peroxide-derived radicals required as initiators in co-polymerization (Mai et al., 2000a, 2000b, 2001, 2002). In the investigations by Hüttermann et al. (2000), laccase activated lignosulfonate with relative high radical density was shown to react as a crosslinking agent after being mixed into a kraft lignin solution. The activated lignosulfonate was also postulated to react with nucleophiles such as cellulose and starch, via covalent bonds.

2. Aims

The main aim of this thesis was to apply oxidation by O_2 to modify technical lignins to enhance their utilization for polymeric chemicals and material applications. O_2 oxidation was aided using alternatively alkaline conditions or a laccase enzyme as a catalyst. In addition, oxygen delignification of pulp was studied using kraft lignin as a model substrate, to provide data for a mechanistic model for the process. More specifically, the aims were:

I To provide detailed information on alkaline O_2 oxidation of softwood and hardwood kraft lignins for the development of a mechanistic model for the pulp oxygen delignification process (Publication I).

II To evaluate different approaches aiming to reduce VOCs of softwood kraft lignins. Laccase and sulfhydryl oxidase catalyzed oxidation was tested as a potential means. In addition, O_2 oxidation at alkaline pH and room temperature was investigated (Publication II).

III To find ways to soften softwood kraft lignin to enhance its utilization for composite applications. The primary route studied was via the introduction of hydrophilic functionality into the lignin structure by laccase-catalyzed oxidation. O_2 oxidation at alkaline pH was studied as an alternative (Publication III).

IV To apply alkali-O_2 oxidation to increase the water solubility of wheat straw soda lignin by the introduction of acidic groups in the polymer. The oxidized lignin solution was used as such for concrete plasticizing, in comparison to commercial lignosulfonate and synthetic superplasticizer (Publication IV).

3. Materials and methods

A summary of the materials and methods used in the thesis is presented in this section. The most important and most often used measurements and analysis as well as the application tests are briefly reviewed. Detailed descriptions can be found in the original publications I–IV.

3.1 Technical lignins, enzymes, and derivatives

Three commercial technical lignins and one non-commercial technical lignin were used as raw materials in the studies reported in publications I–IV (**Table 3**).

Four laccases and a sulfhydryl oxidase were tested in the studies reported in publications II and III, using different dosages with respect to the substrate, which was lignin or a hydrofilic derivative (**Table 4**). A mediator, 1-hydroxybenzotriazole (HBT), was used with a dose of 5 % with respect to lignin, in addition to laccases in two experiments (II).

In the studies concerning lignin functionalization (III), hydrophilic derivatives, vanillic acid poly(ethylene glycol) methyl ester and ether were synthesized and applied (**Figure 12**).

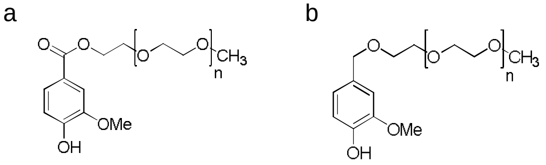


Figure 12. a) Vanillic acid poly(ethylene glycol) methyl ester (Ester V-PEG), and b) ether (Ether V-PEG) (III).

Table 3. Technical lignins.

Species	Extraction process	Lignin trade name	Producer	Abbreviation	Publication
Softwood	Kraft	Indulin AT	MeadWestvaco Corp.	SW / Indulin AT	I, II, III
Hardwood	Kraft	PC1369	MeadWestvaco Corp.	HW	I
Softwood	Kraft	-	Stora-Enso	SE	II
Wheat straw	Soda	Protobind 1000	GreenValue SA	SL	IV

Table 4. Laccases and sulfhydryl oxidase.

Enzyme	Produced in	pH optimum	Producer	Abbrev.	Dose[b] (nkat g^{-1} or %)	Publication
Trametes hirsuta laccase	Native host	5	VTT	ThL	100	II
Thielavia arenaria laccase Lcc1[a]	*Trichoderma reesei*	6	Roal Oy	TaLcc1	60-600	II, III
Thielavia arenaria laccase Lcc2[a]	*Trichoderma reesei*	6	Roal Oy	TaLcc2	60-600	II, III
Melanocarpus albomyces laccase[a]	*Trichoderma reesei*	8	VTT	r-MaL	30; 0.1, 1	II, III
Aspergillus oryzae sulfhydryl oxidase	*Trichoderma reesei*	7.5	VTT	AoSOX1	0.1, 1	II

[a] produced as recombinant enzyme. r-MaL, i.e. recombinant MaL.

[b] ThL, TaLcc1, and TaLcc2 activity measurements with ABTS; doses nkat g^{-1}. r-MaL activity measurements with guaiacol; doses nkat g^{-1} or on protein bases in respect to lignin. AoSOX1 doses on protein bases in respect to sulfur in lignin.

3.2 O_2 oxidation of lignin

3.2.1 Under alkaline conditions

Prior to the oxidation, lignin was dissolved in 0.1–2.5 M NaOH, depending on the lignin content in the reaction solution. In most of the experiments, the initial pH in the reaction solution was targeted to be 13 or slightly above.

The experiments were performed in a 1-L (I, III, and IV) or 2-L (II) stainless steel pressure batch reactor (Parr Instrumental Co., Moline, IL, USA) equipped with baffles and a turbine-type impeller stirrer (operated ≥ 600 rpm), an electric heating jacket, a gas inlet, and a gas release valve. The equipment did not include cooling.

For the constant pH oxidations a 1.8-L batch reactor, iControl RC1e™ HP100 Hastelloy (Mettler-Toledo GmbH, Switzerland) equipped with baffles, gas stirrer, a pH control loop and a pump to introduce 5 M NaOH to the reaction solution under the O_2 excess was used (IV). The equipment included temperature control. The constant pH oxidations were performed using isothermal operating mode.

The quantity of the lignin solution in the reactor (300–800 g in the 1-L, 1000 g in the 2-L, and 720 g in the 1.8 L reactor) and the initial O_2 pressure (5–40 bar) were varied in order to set the desired initial O_2 load in respect to lignin. In general, the higher the lignin content, the higher the O_2 pressure.

The operating conditions in terms of lignin content in the reaction solution, initial pH and temperature, and reaction period applied in the studies are summarized in **Table 5**.

Table 5. Operating conditions of alkali-O_2 oxidations applied to technical lignins.

Lignin (w-%)	**$pH_{Init.}$ (-)**	**$T_{Init.}$ (°C)**	**O_2 load on lignin (w-%)**	**t (min)**	**Publication**
0.75	12.7	90, 110	28, 41	240*	I
10.0	10.7**	22	8	60	II
16.7, 25.0	12.2, 13.3	40	36, 24	30	III
0.75–25.0	12.7–13.4	40–80	12–35	30	IV

*samples were taken as a function of the reaction period.

**after lignin dissolution, the pH of the reaction solution was lowered to 10.7 using 1 M HCl.

3.2.1.1 O_2 consumption

During the alkali-O_2 oxidations, the reactor pressure and temperature were recorded. In the onset of the reaction (Init), part of the O_2 introduced in the reactor is dissolved in the liquid phase (L), the main part being in the gas phase (G) (1). Both temperature and pressure affect the solubility of O_2 (2). The dissolved O_2 starts to consume in lignin reactions. Simultaneously new O_2 is dissolved in the solution causing a pressure decrease. The oxygen consumed in the experiments

(O_2 uptake; mol mol^{-1} lignin or w-% on lignin) (4) was estimated from the reactor pressure data by applying the ideal gas law equation and the values of water vapor pressure (3).

$$n(O_2)_{\mathrm{Init}} = n(O_2)_{\mathrm{Init,\,L}} + n(O_2)_{\mathrm{Init,\,G}} \tag{1}$$

$$n(O_2)_{\mathrm{L}} = (p - p_{\mathrm{H2O}}) \times k_{\mathrm{eq\_O2}} \times V_{\mathrm{L}} \tag{2}$$

$$n(O_2)_{\mathrm{G}} = \frac{(p-p_{\mathrm{H2O}})\times V_{\mathrm{G}}}{RT} \tag{3}$$

$$\frac{n(O_2)}{n(lignin)} = \frac{(n(O_2)_{\mathrm{Init}}-n(O_2)_{\mathrm{Final}})\times M(lignin)}{m(lignin)} \tag{4}$$

Where

$n(O_2)$ = molar amount of O_2 [mol]
p = reactor pressure [Pa]
p_{H2O} = water vapor pressure (T dependent) [Pa]
$k_{\mathrm{eq\_O2}}$ = equilibrium constant for dissolved O_2 (T dependent) [M Pa^{-1}]
V = volume [m^3]
R = universal gas constant, 8.3144 [m^3 Pa K^{-1} mol^{-1}]
T = temperature [K]
$n(lignin)$ = molar amount of lignin [mol]
$m(lignin)$ = mass of initial lignin in liquid phase [g]
$M(lignin)$ = molecular mass of phenyl propane unit (C9), 180 [g mol^{-1}]

3.2.2 Using laccase as a catalyst

Prior to the laccase-catalyzed oxidation, lignin was dissolved in 0.1–0.5 M NaOH, depending on the lignin content in the reaction solution. Thereafter, the pH adjustment to a desired level was done slowly stepwise, using 1 M HCl. The dissolution-pH adjustment procedure (Mattinen et al., 2008) was intended to reduce the agglomerates and increase the reactivity of the lignin.

At analytical scale, the reactivity of enzymes towards the lignin was evaluated by monitoring dissolved oxygen consumption in the reaction solution. Monitoring was performed with an Oxy-10 mini-sensor oxygen meter (PreSens, Germany) in a closed 1.9 ml vessel, based on dynamic luminescence quenching (II, III).

Selected treatments with promising enzyme dosages were repeated at a larger laboratory scale (2-L Parr reactor) with higher lignin content (5 and 10 w-%) and O_2 boosting (0.5 MPa) (II). The operating conditions in terms of lignin content in the reaction solution, initial pH and temperature, and reaction period applied in the studies are summarized in **Table 6**.

In the lignin functionalization experiments, the dosing of the lignin and the derivative was based on the content of phenolic hydroxyl groups in the substrates, since they are the reactive sites in laccase-catalyzed oxidation. The content of phenolics in Indulin AT and in the ester/ether derivative was 4.3 (Hult et al., 2013) and 1.4 mmol g^{-1}, respectively. The molar ratio of lignin: derivative was set to 3:1, which equals a mass ratio of 1:1. In the experiments, laccases were dosed as nkat g^{-1} substrate, meaning lignin or the derivative. Laccase treatments were also performed with one-component systems containing only lignin or the derivative as references.

Table 6. Operating conditions of laccase-catalyzed O_2 oxidations applied to technical lignins.

Lignin (w-%)	pH (-)	T (°C)	O_2 load on lignin (w-%)	t (min)	Publication
1.0, 2.5, 5.0	5, 6, 8	22	-*	120	II
5.0, 10.0	5, 6, 8	22	16, 8	60, 120	II
2.5**	6, 8	22	-*	120	III

*only O_2 dissolved in the solution under atmospheric pressure at room temperature.

**also in the case of a two-component system containing lignin and the derivative (III).

3.3 Characterization methods

Molecular mass (I–IV)

Molecular mass distributions and average molecular masses (M_n, M_w) of the lignin material in the solutions were measured by size exclusion chromatography (SEC), using PSS MCX 1000 and 100,000 columns in 0.1 M NaOH eluent (25 °C) with UV detection (280 nm). M_n and M_w were calculated in relation to polystyrene sulfonate standards.

UV-lignin (I, IV)

The change in the aromatic lignin content during oxidation was followed as UV-lignin at 280 nm based on absorptivity values of 22.4 l g^{-1} cm^{-1} for SW (Indulin AT) and 22.2 l g^{-1} cm^{-1} for HW kraft lignin (absorptivities determined in the initial concentration 7.5 g l^{-1}). An absorptivity value of 25.0 l g^{-1} cm^{-1}, measured for soda lignin from wheat straw (SL i.e. Protobind 1000), was used in determining the aromatic lignin content of SL and oxidized SL solutions.

Charge (I, IV)

In study I, the total charge, giving the sum of all acids in SW and HW kraft lignin solutions, was measured by conductometric titration. The conductivity of the sample solution was registered as a function of titrate addition (both 0.1 M HCl and 0.1 M NaOH; pH range 2.5–11.5). The total negative charge of the sample (mmol g^{-1} initial lignin) was calculated using the time difference between the equivalent points of acid and base titration curve and the initial lignin content in solution. The method is a slight modification of the method described by Zakis (1994).

In study IV, a modification of a potentiometric acid-base titration method developed for dry lignin samples (Rahikainen et al., 2013) was applied to determine the charge directly from the oxidized SL solutions. In the modified method, a given quantity of the oxidized lignin solution containing initially 75 mg of lignin (e.g. 0.5 g in the case of a solution containing initially 15 w-% lignin) was diluted to 5 g using 0.2 M NaOH. Thereafter, the CO_2 species were removed from the sample by acidification, and the procedure was continued as described by Rahikainen et al. (2013). After re-dissolution of the lignin solution in CO_2 species-free NaOH, the solution was titrated with 0.1 M HCl under N_2 atmosphere. The blank sample included both the alkali quantity initially existing in the lignin solution and the additional quantity of the 0.2 M NaOH. The blank was prepared and titrated similarly to the oxidized lignin solution. Linear estimation was used to obtain a titrant volume, V(HCl) for a given pH level, pH_i (i = 11.7, 11.6, 11.5, . . ., 2.0). The titrant volume consumed during the blank titration at a given pH level, V(HCl_{Blank} at pH_i), was subtracted from that consumed during the sample solution titration, V(HCl_{Sample} at pH_i). The corresponding negative charge of lignin (mmol g^{-1}) at pH_i was computed using the information on the volume difference, V(HCl at pH_i), titrant concentration, and the initial lignin content in the sample solution. A negative charge at pH 6 is reported as an indication of anionic character.

Solubility (IV)

A simple acidification-centrifugation procedure was used to evaluate the water solubility of the oxidized SL lignins as a function of pH from 2.5 to 7. Acidification of a given volume of sample solution to a target pH level was done using 1 M HCl. Thereafter, the precipitate was separated by centrifugation at 10 000 g for 30 minutes, the supernatant was decanted off, and the precipice was dried overnight at 105°C. The lignin precipitation yield (%) was computed based on the initial lignin content in solution.

Glass transition temperature (III)

T_g of polymers is usually observed by differential scanning calorimetry (DSC) as a stepped increase in the heat capacity (Cp) of the sample during heating due to enhancement of molecular motion in the polymer. Other measurement techniques also exist.

Here, 5 to 8 mg of sample was weighed into a standard aluminum crucible (oxidized at 500°C prior to use). The crucible was closed and the lid was pricked to allow evaporation of volatile substances during the measurement. The DSC was used at a heating rate of 10°C min^{-1}, and a flow of dry N_2 was used to purge the measurement cell. The drying of the lignin sample was carried out, followed by isothermal conditioning at 105°C for 20 minutes. The thermogram of the lignin sample was recorded across a temperature range from 25 to 250°C. The thermogram of the Ester-PEG was recorded twice over the temperature range from -80 to

50°C. The T_g was determined as a midpoint temperature of the baseline shift in the glass transition region.

Odorimetry (II)

In this sensing procedure, a wet sample volume containing 5 g of lignin dry solids was placed in a small crucible and stabilized inside closed glass containers (500 ml) for 48 hours at 40 ºC. Thereafter, the odor panel members (ten qualified experts) evaluated the odor intensity of each sample using a scale of 1 to 6, which was selected according to VDA-270 (1992) recommendations. The stabilized samples were also graded using the numbers 0 to 10 by individual perception. The least odor-intense sample got the number 0 and the most odor-intense was assigned the number 10. The panel was able to evaluate a maximum of 6 of the samples at a time. Three separate sessions were conducted.

Thermal desorption (II)

The thermal desorption method (TD-MS/GC) was developed to simulate the formation of volatile degradation products at the elevated temperatures prevailing in injection molding. TD measurements were carried out with a Pyrolab pyrolyzer unit connected to a gas chromatography (GC)/mass spectrometer (MS). About 2.5 mg of the freeze-dried sample was weighed accurately and heated at 150 and 190ºC for 5 minutes in a pyrolysis chamber. Thereafter, volatiles were led to the gas chromatography column for separation and mass spectral identification and quantification. Sulfur and phenol compounds were tentatively identified using commercial mass spectra library Nist05. Quantification of guaiacol was performed with an external standard calibration (guaiacol, 98 percent, Aldrich).

3.4 Application testing

Plasticizing performance (IV)

The plasticizing performance of the oxidized lignin solutions was evaluated stepwise, proceeding from the easiest medium to the most demanding. The initial testing was done in cement pastes, thereafter in mortar, and lastly the performance of selected samples was verified in concrete. In preparing cement pastes, mortar, or concrete, the oxidized lignin solution (or the references) was added last in the mixture.

In the mortar and concrete tests, cement type Plussementti CEM II B-M (S-LL) 42,5 N (Finnsementti) (CEM II for short) was applied. Mortar was prepared using a standard sand mixture, CEM II, and water, with the following mass ratio: 48.6: 36.0: 15.4. The fluidity of the mortar was studied using the Haegermann flow table (Ø 300 mm) method (DIN 1060) and a mold with a diameter of 100 mm. Haegermann flow value represents the spread (diameter) of mortar (**Figure 13** b), which is formed after pulling up the mold filled with the material (a). Concrete was prepared according to EN1766 type C(0,40), with fine and coarse stones aggregates, CEM II, and water. A slump test was applied to evaluate the workability of the concrete (SFS-EN 12350-2). Slump value represents the slump of concrete (**Figure 13** d), which is formed after pulling up the cone (height 300 mm) filled with the material (c).

As a reference for the oxidized lignins, the following commercial plasticizer products were used: WRDA 90D (Grace Construction Products), a conventional lignosulfonate plasticizer; Glenium C151 or Glenium 51 (BASF) (Glenium for short) and VB-Parmix (Finnsementti), all three being polycarboxylate-based superplasticizers. The dosing of oxidized lignin solutions or the reference products was based on their active matter content with respect to cement. In the case of mortar and concrete, dosages of 0.4 and 0.2–1.5 w-% were used, respectively. A defoamer agent, tributylfosfate (TBF) with a dosage of 2.5 w-% in respect to oxidized lignin, was used in one of the concrete experiments (SL constant pH 2ox (2.5% TBF)).

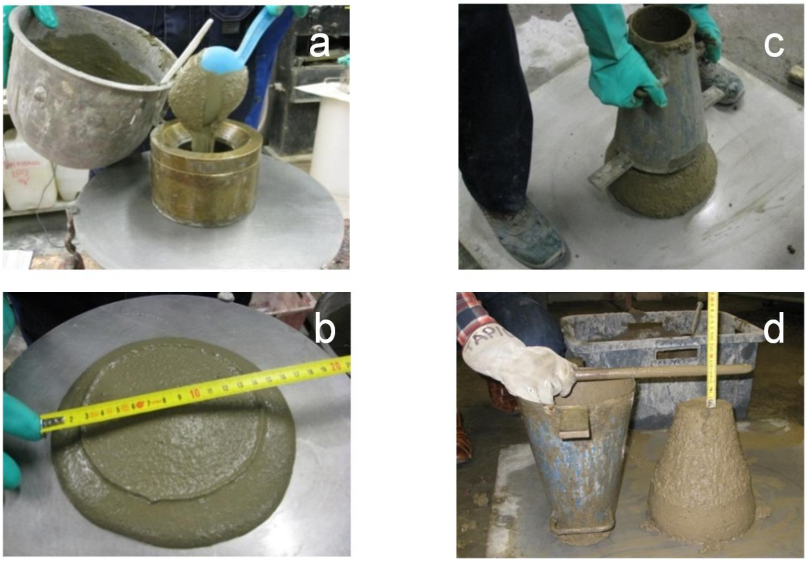


Figure 13. Illustrations of Haegermann flow (a, b) and slump (c, d) tests.

Air content, hydration, and compression strength (IV)

The air content of fresh concrete was determined according to SFS-EN 12350-7. Cement hydration kinetics were followed by measuring the heat flow of the plasticized cement paste using conduction calorimetry. The compression strength and density of the matured concrete was measured after 28 days, according to standard EN 196-1.

4. Results and discussion

4.1 Oxidation of lignin using O_2 under alkaline conditions

Depending on the aim of each study, the oxidation by O_2 was intended to degrade lignin (publication I), retain its polymeric structure or cause polymerization (II, III, IV), or introduce hydrophilic functionality into the lignin structure (III).

4.1.1 Delignification conditions

Publication I reports the study wherein oxidation of softwood and hardwood kraft lignins was observed under the conditions of oxygen delignification. The aim of the study was to provide detailed information on alkaline oxygen oxidation of softwood and hardwood lignins for the development of a mechanistic model for the pulp oxygen delignification process.

Lignin oxidation was followed by analyzing samples taken from the reactor and recording the reactor pressure. Publication I presents the results from the direct analyses of the oxidation experiments. In order to follow the reaction kinetics and determine the relative importance of different reaction routes for the kinetic model, it was necessary to quantify the degradation products, in addition to the detection of structural changes in the polymeric lignin. Therefore, the oxidized lignin samples obtained were further characterized by advanced analysis methods, to distinguish those carboxylic acids bound to lignin macromolecule and those bound to aliphatic acids. These results are presented in a following paper by Rovio et al. (2011). The model development was published by Kuitunen et al. (2011).

Kraft lignins were selected as starting materials, because of the large amounts of lignins needed for the experiments, which could not be isolated from pulp residual lignins. The structures of the dissolved and residual lignin are supposed to be rather similar (Akim et al., 2001; Fu et al., 2005), even though the structural changes induced by cooking are more pronounced in the dissolved lignin. For example, the content of phenolic hydroxyls in dissolved lignin has been measured to be approximately twofold greater than that in residual lignin (Gellerstedt and Lindfors, 1984; Robert et al., 1984). Residual lignin composition in the modeling was based on that reported in the literature, although its reactions were based on the data obtained from the kraft lignin experiments.

In the experiments, the initial concentration of kraft lignin, 7.5 g l^{-1} (0.75 w-%), corresponded to the content of fiber-bound lignin in the oxygen delignification of high-kappa number pulp (approx. 50) at 10% consistency. Two temperatures (90 and 110°C) and two initial oxygen pressures (0.6 and 0.9 MPa) were used (**Table 7**).

Table 7. Operating conditions for oxidation of kraft lignins from softwood (SW) and hardwood (HW). Initial O_2 loads corresponded to 28 and 41 w-% on lignin.

Exp. #	Kraft lignin	Initial pressure (MPa)	T (°C)	Code
1	SW	0.94	90	0.9 MPa, 90°C, SW
2	SW	0.94	110	0.9 MPa, 110°C, SW
3	SW	0.64	90	0.6 MPa, 90°C, SW
4	SW	0.64	110	0.6 MPa, 110°C, SW
5	HW	0.64	90	0.6 MPa, 90°C, HW

Figure 14 a–i illustrates the changes occurring in kraft lignin oxidation as a function of reaction time. In all experiments, during the first 60 minutes the lignin reactivity was high, and a very intense stage was observed during the first 20 minutes. This is readily visible from high oxygen consumption (a), pH decrease (b), and fast increase in total charge (c). The evolvement in charge (total negative charge) was due to the increase in low molecular mass carboxylic acids, and acids bound to lignin macromolecule (Rovio et al., 2011). The intense beginning was also clearly seen as degradation of aromatic lignin (d) and decreasing content of free phenols (e), with a simultaneously increasing proportion of conjugated phenols (f). The decrease in conjugated phenols (e.g. vanillin) under the harsher conditions was due to their oxidation. The fast increasing methanol concentration (g) correlated with the degradation of aromatic lignin and decreasing content of free phenols. The greatest change in lignin color occurred during the first 30 minutes, however, only in the case of hardwood kraft lignin (h). Monitoring the average molecular mass (M_w) of the lignin macromolecule (i) clearly demonstrated that depolymerization is intense at the beginning of the reaction, as ether bonds in lignin were also degraded. According to all observed data, the reactions decelerated after the first 60-minute period. After 120 minutes, the reactions did not significantly advance.

CO_2 is formed in the further oxidation reactions of lignin degradation products. According to total inorganic carbon (TIC) analyses, 7.0–8.4% of the carbon from the initial lignin was in the form of CO_2 species in the reaction solution in the end of the 4-hour reaction period (I). In addition, small part of the formed CO_2 had been released to gas phase (Kuitunen et al., 2011).

Elevating the reaction temperature from 90°C to 110°C increased the oxidation rate of the process, which is in accordance with the general kinetic theory. The effect of pressure was not as significant as that of temperature. The results correspond well to general theories regarding the lignin oxidation mechanism, and are also in agreement with the knowledge of hardwood kraft lignin being more reactive than softwood kraft lignin.

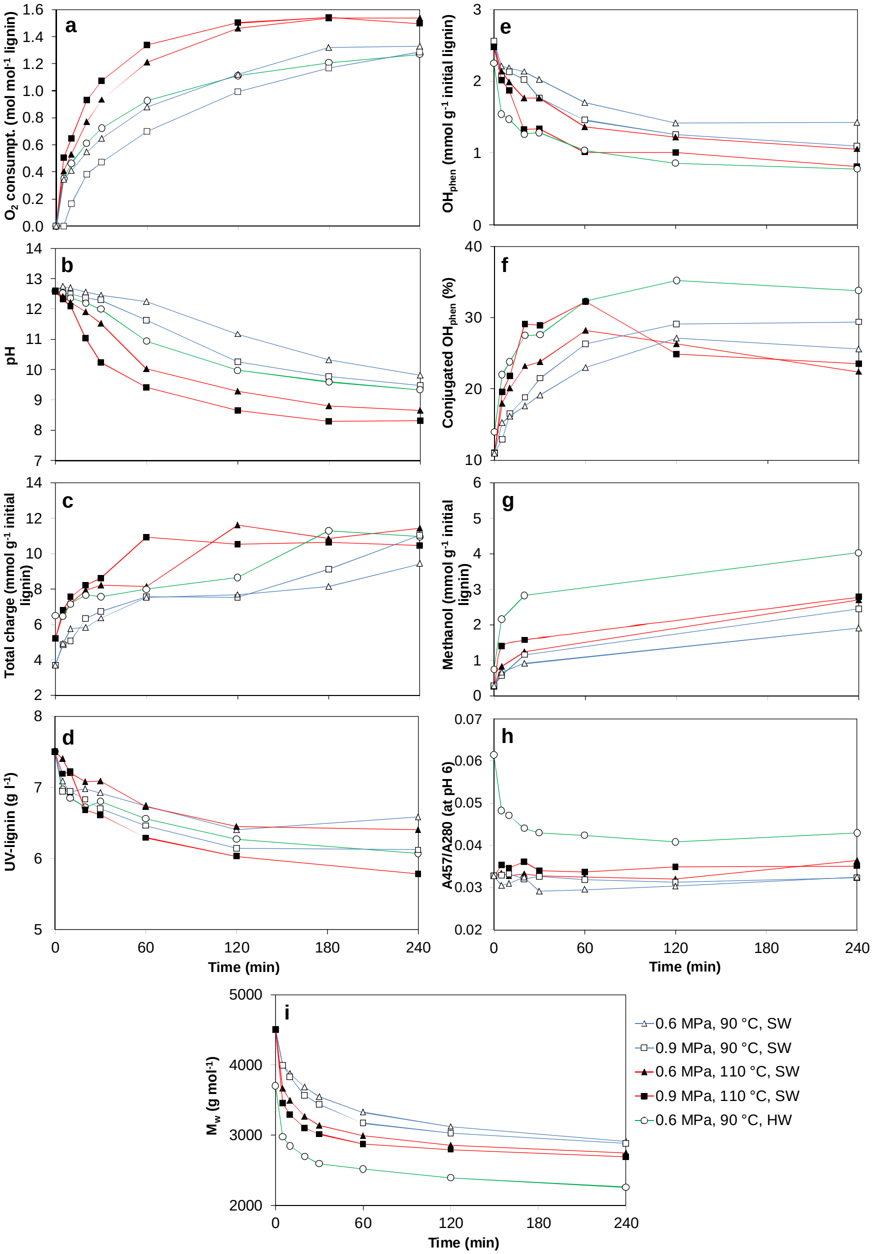


Figure 14. a–i) Changes in the data indicated on the y-axis as a function of reaction time of oxygen delignification. Initial concentration of kraft lignin 0.75 w-%. Oxidation conditions and codes according to Table 7.

The model development aimed to identify the reaction routes that would reproduce the experimental observations presented in publication I and by Rovio et al. (2011). Based on the results, the reaction schema of lignin oxidation by oxygen was elucidated by Kuitunen et al. (2011). It is presented in **Figure 9** (Chapter 1.3.4.5 Model of oxidation chemistry). The model development was part of a larger entity, creating phenomena-based models for kraft pulp bleaching (Tarvo, 2010) and more recently for pulping (Kuitunen, 2014). The phenomena-based modeling approach has been used, for example in simulation of industrial A/D stage bleaching chemistry (Kalliola et al., 2012).

4.1.1.1 O_2 consumption

Salmela et al. (2004) elucidated that the reactions taking place during industrial two-stage oxygen delignification of softwood kraft pulp consume oxygen roughly 4–10 kg per BDt. Taking into consideration the kappa number reduction reported (run 1: 6.9 and run 2: 10.6), the O_2 consumption per unit kappa number decrease was 0.6–0.9 kg per BDt. The specific O_2 consumption of industrial oxygen delignification has also been evaluated elsewhere (McDonough, 1996), with an average value of 1.4 kg per BDt for softwoods. According to laboratory studies, the O_2 consumption per unit kappa number decrease has been 0.5–0.6 kg per BDt (Berry et al., 2002).

The O_2 consumption in the industrial process (Salmela et al., 2004) is equal to 0.39–0.63 g O_2 by g residual lignin removed, assuming that a unit kappa number corresponds to 1.5 g residual lignin per kg fiber. In the course of modeling industrial oxygen delignification, the O_2 consumption was estimated to be 1 g O_2 by 1 g residual lignin (van Heiningen et al., 2003; Sixta et al., 2006b). The O_2 consumption of organic carry-over is also considered in the model. The inorganic reactions that consume oxygen, especially the oxidation of sulfur-containing ions, are likely to be included in these estimates, as their contribution could not be considered separately.

During oxidation of softwood kraft lignin (I), the O_2 consumption after a 1-hour reaction delay corresponded to 0.18–0.22 g O_2 by 1 g lignin (1–1.2 mol O_2 mol^{-1} lignin), and at the end of the 4-hour reaction to 0.23–0.27 g O_2 (1.2–1.5 mol O_2 mol^{-1} lignin). Imai et al. (2008), also measured O_2 consumption of softwood kraft lignin under similar oxygen alkali conditions and reported slightly higher O_2 consumption over 4 hours. Thus, the results indicate that there is a big difference in O_2 consumption between kraft lignin oxidation and residual lignin oxidation in industrial delignification. Residual lignin oxidation consumes at least approximately twice as much O_2 as kraft lignin oxidation.

Untreated white liquor, an alkali source in the oxygen stage, is known to reduce the degree of delignification when compared to the usage of oxidized white liquor or sodium hydroxide. This is reported to be due to the presence of the sulfide (Sixta et al., 2006b). Analogously, the residual sulfide that enters the oxygen stage within kraft brownstock reduces the degree of delignification, because its oxidation potential is partly wasted on oxidation of the sulfide ion (HS^-) to sulfate (SO_4^{2-})

instead of residual lignin. Therefore, the difference in O_2 consumption between kraft lignin oxidation and industrial pulp oxygen delignification can be partly due to the O_2 consumption of sulfide carry-over, caused by insufficient brownstock washing and/or incomplete oxidation of white liquor in industrial applications.

4.1.2 Alkali-O_2 oxidation for producing a soda lignin-based concrete plasticizer

Concrete is one of the most used materials in the world. It is a mixture of cement, water, and fine and coarse stone aggregate. By using less water, it is possible to make stronger concrete. Plasticizers, such as water soluble lignosulfonates, or synthetic superplasticizers (organic polyelectrolytes), are often added to the concrete mixture to enhance flow-properties when using a low water content. Especially, the use of superplasticizers is growing in all types of concrete applications (Ramachandran et al., 1998). Annual demand for the plasticizing admixtures can be estimated to be 1.5–15 million tons, assuming that half of the annual concrete production of 18 000 million tons (GEreports, 2014) is prepared using a plasticizer dosage of 0.06–0.6 w-% on cement (Ramachandran et al., 1998). In the last decades, polycarboxylate-based superplasticizers specially developed for concrete plasticizing purposes have been displacing lignosulfonates, especially in Western Europe and other developed regions (Stern and Schwarzbauer, 2008). The polycarboxylates offer superior flow-properties without affecting much of the other qualities of concrete (Ramachandran et al., 1998; Plank, 2004).

In a highly alkaline concrete mixture, the working mechanism of the superplasticizers, having negatively charged functional groups (sulfonic or/and carboxylic) and a branched structure, is based on both electrostatic and steric repulsion between individual cement particles to achieve a full dispersion (Ramachandran et al., 1998; Yamada et al., 2000). In the case of lignosulfonates, it is postulated that the dispersing effect is mainly due to the electrostatic repulsion between the cement particles (Taylor, 1997).

For the substitution of lignosulfonates by applying alkali lignin other than post-sulfonated lignin, only a few studies concerning cement dispersing exist. Ozonation of kraft lignin (Detroit, 1973) has been stated to result in a water-soluble product that has shown better dispersing performance than lignosulfonate products in a wide variety of organic and inorganic suspensions, including cement paste. Unmodified alkali lignins (Nadif et al., 2002) or fractions thereof (Takahashi et al., 2014) have shown dispersing performance of cement particles in mortar accompanied by satisfying strength properties of the cured material. Alkali lignins have also been reacted with epoxylated polyethylene glycol derivatives, and tested in cement to yield slightly better dispersing performance and bending strength than when using lignosulfonates (Uraki et al., 2012; Aso et al., 2013). However, the commercial utilization of a new plasticizer always depends on a combination of price and performance.

Publication IV reports the study in which an alkali-O_2 oxidation method to solubilize soda lignin from wheat straw was developed. The oxidation treatment was performed on high lignin dry solids, since the oxidized lignin solutions were applied as ready-to-use products for concrete plasticizing. The performance of the oxidized lignin solutions was evaluated using several testing methods, in comparison to commercial lignosulfonate and synthetic superplasticizers.

4.1.2.1 Controlling lignin characteristics

In contrast to the delignification of strongly degrading lignin (I), here (IV), the alkali-O_2 oxidation was intended to introduce acidic groups in the polymer while retaining its polymeric structure. Preliminary oxidation experiments were done to understand the impact of the oxidation parameters, especially of lignin content on the molecular mass and charge. Based on these results, the alkali-O_2 oxidation was further developed to optimize the properties of the oxidized lignin for concrete plasticizing performance.

As the aim was to produce a ready-to-use plasticizer with a substantially high active matter content, a high lignin content was intended. **Table 8** shows the data from the preliminary oxidations, in which the effect of the reaction parameters, lignin content (0.75–25 w-%), O_2 load (12–35 w-% on lignin), and temperature (50–80°C) was screened. A reaction period of 30 minutes was used in all oxidations, since previously (I) it was observed that lignin reactions are very intense during the first 20 minutes and decelerate after 60 minutes of oxidation. In **Figure 15** a–c, pH after oxidation (pH_{Final}), molecular mass (M_w), and O_2 uptake are plotted as a function of lignin content. **Figure 15** d shows the relation between pH_{Final} and M_w.

It is seen that the higher the lignin content, the lower the pH_{Final} until it is settled at a near neutral level (7.5–8) in the case of oxidation done at 15 and 25 w-% (a). The decrease in pH is caused by the degrading reactions starting from the phenolates and consuming the major part of the oxygen in the formation of the main products, muconic acid and oxirane structure, which are also very vulnerable to further oxidative degradation (Kuitunen et al. 2011). The decrease in pH is more pronounced the higher the concentration of the acidic products in a given volume of the lignin reaction solution. In addition to the degrading reactions indicated by the pH drop, it was noted that the higher the lignin content, the more condensed the lignin after oxidation (b). The increase in molecular mass was stronger when the decrease in pH was greater, with the increase being further accelerated by the 25 w-% lignin content (d). Only oxidation at very low lignin content (0.75 w-%), typical of pulp oxygen delignification, led to actual depolymerization of lignin measured, as reduced molecular mass (likewise in the case of SW and HW kraft lignins in I). Under the selected oxidation conditions, there was no clear relation between the O_2 uptake and lignin content (c) or accordingly between the O_2 uptake and pH_{Final} or molecular mass. O_2 consumption was fairly constant, 8–13 w-% on lignin, which corresponds to an uptake of 2.3–4.1 mmol g^{-1} lignin (0.42–0.74 mol O_2 mol^{-1} lignin).

To summarize the results of the preliminary experiments, it appears that the most important factors contributing to lignin condensation and increase in molecular mass are lignin density and the prevailing pH conditions in the oxidation. This is also supported by the fact that below pH 12, the hydroperoxide intermediates tend to protonate and decompose homolytically back to phenoxyl radicals, which can spontaneously combine by a 5-5 coupling reaction (**Figure 8**) (Chang and Gratzl, 1980; also noticed in II and III), or alternatively form 4-O-5 bonding. High lignin density favors the coupling reactions because the phenoxyl radicals are close to each other. In addition, the dissolution and diffusion of oxygen is hindered in viscous lignin-alkali solutions, which restricts the availability of oxygen species taking part in the degradation reactions. Condensation in a viscous lignin solution by O_2 under alkali conditions was already reported in an old patent (Lin 1975) and could be partly reduced by optimizing the O_2 load and reaction temperature (optimum at 140°C).

It has also been observed that pH is the most important variable when producing vanillin from technical lignins (Fargues et al., 1996a, 1996b; Araújo et al., 2010). High alkaline pH, close to 14, is required both for the formation of vanillin and to retain its yield, since at lower values of pH (<11.5), the vanillin oxidizes and degrades at a considerable rate. This observation also shows that it is crucial to maintain a highly alkaline pH to ensure the dissociation of the hydroperoxide intermediate. Only the hydroperoxide anion structure (pK_a 12–13) rearranges to primary oxidation products, with a phenolic aldehyde/carbonyl structure (e.g. vanillin) being one of those (**Figure 7**) (Chang and Gratzl, 1980; Sixta et al., 2006b).

Since the negatively charged functional groups of the plasticizers induce the electrostatic repulsion between the cement particles, the alkali-O_2 oxidation was further developed, in order to increase the negative charge, most preferably in a form of muconic acid structures in lignin polymer. Constant pH oxidation at a pH of 11.5 and sequential oxidation, with or without the constant pH control in the second stage were applied for this purpose. The aim in the sequential oxidations was to first induce lignin condensation at 25 w-% content during the initial stage (SL 25% 1ox), and thereafter at 15 w-% content during the following more aggressive stage (SL 15% 2ox or SL constant pH 2ox) to increase the negative charge. Condensation by 5-5 coupling does not consume the phenolic groups in lignin, which are thus able to react with O_2 in the next oxidation stage while being dissociated.

Data on the improved oxidations is shown in **Table 8**. In the case of SL 15% 2ox, the total consumption of NaOH increased up to 41 w-% on lignin when considering the NaOH requirements of both stages. The consumption of NaOH during the constant pH oxidations performed at 11.5 (SL constant pH ox) and at 13 after the initial oxidation (SL constant pH 2ox) was very high. The consumption of NaOH does not, however, necessarily reflect the real formation of carboxylic acids in the lignin polymer. Acidic, secondary oxidation products, including CO_2, are formed and they contribute to the pH (I) (Kuitunen et al., 2011; Rovio et al., 2011) and consume NaOH. The further oxidation of the primary oxidation products is probably impossible to avoid totally. The O_2 uptake on lignin was also increased by the constant pH oxidations (17–27 w-%), as a highly alkaline pH favored the oxidation of lignin rather than the secondary reactions.

Table 8. Operating conditions for alkali-O_2 oxidation of soda lignin (SL) from wheat straw and O_2 uptake of SL. Upper panel: preliminary oxidations. Lower panel: improved oxidations. A reaction period of 30 minutes was used in all oxidations. Samples marked in bold were selected for further characterization and evaluation.

Sample	**Lignin (w-%)**	**NaOH on lignin (w-%)**	**$pH_{Init.}$ (-)**	**$T_{Init.}$ (°C)**	**O_2 load on lignin (w-%)**	**O_2 uptake on lignin (w-%)**
	Preliminary oxidations					
SL 0.75%60C ox	0.75	53	13.1	60	20	8
SL 0.75%80C ox	0.75	53	13.1	80	23	11
SL 2.5%60C ox	2.5	31	12.7	60	14	8
SL 2.5%80C ox	2.5	31	12.7	80	14	9
SL 5% ox	5	38	13.3	80	12	10
SL 15% ox	15	23	13.1	55	17	8
SL 15% i.ox	15	23	13.1	60	35	10
SL 25% ox	25	30	13.4	50	30	13
SL 25% 1ox [a]	25	24	13.0	40	15	9
	Improved oxidations					
SL 15% 2ox	15	17 (41)*	13.0	75	34 (49)*	8 (17)*
SL constant pH ox [b]	15, 11.4_{Final}	65	13.1	70	35	23
SL constant pH 2ox [c]	15, 10.5_{Final}	75 (99)*	13.0	70	35 (50)*	18 (27)*

[a] initial oxidation stage prior to SL 15% 2ox and SL constant pH 2ox

[b] oxidation at pH 11.5

[c] oxidation at pH 13

* in parentheses: the total NaOH load, O_2 load and uptake in the case of sequentially oxidized SL.

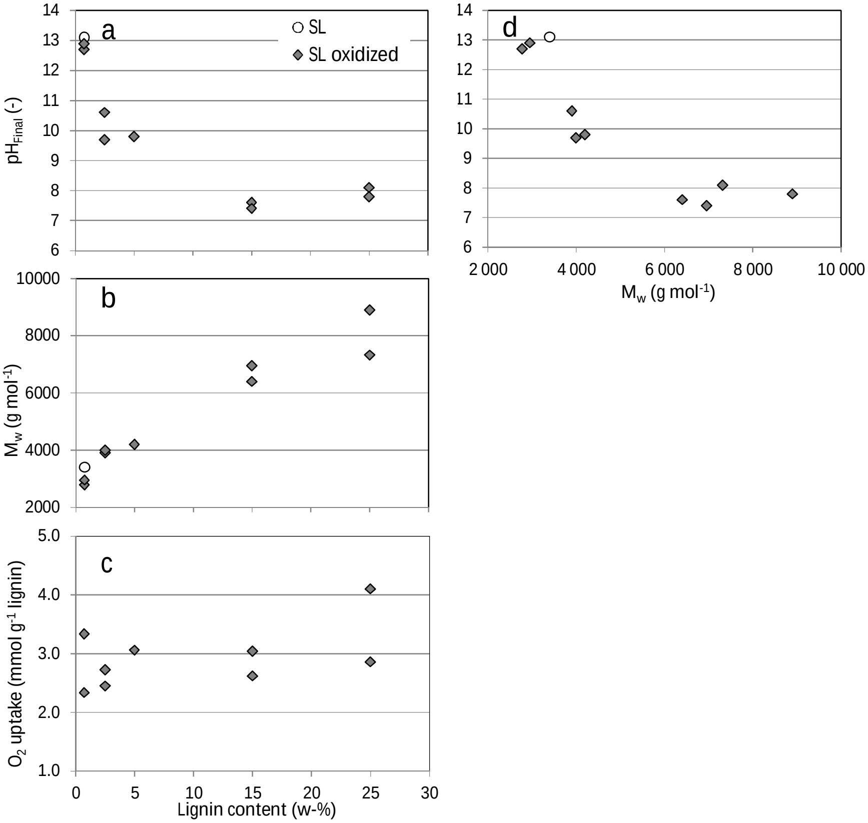


Figure 15. Data on the preliminary oxidation experiments of soda lignin (SL): a) pH after oxidation (pH$_{Final}$), b) molecular mass (M_w), and c) O_2 uptake as a function of lignin content in oxidation; d) relation between pH$_{Final}$ and M_w.

Selected samples from the preliminary experiments and those produced by the improved oxidation methods were characterized more carefully (Table 2 in IV).

The results indicate that the characteristics of soda lignin (SL) can be controlled by the oxidation parameters, especially by pH. Thus, different kinds of SL-based plasticizer solutions in terms of molecular mass (M_w 3200–7320 g mol^{-1}) and negative charge (3.2–6.9 mmol g^{-1}) were produced (**Figure 16**). All the other oxidation treatments except, the 2-stage oxidation in which the second oxidation was performed at a constant pH of 13, increased the molecular mass (M_w 3880 g mol^{-1}) and polydispersity (1.9) of SL. Oxidation at a constant pH of 11.5 (SL constant pH ox) hindered the condensation reactions, compared to those oxidations performed without pH control. The increase in polydispersity indicates that although the condensation reactions were prevailing, some of the lignin fragments were also degraded. Oxidation at a pH of 13 degraded lignin efficiently, since its M_w decreased from 7300 g mol^{-1} (SL 25% 1ox) to 3200 g mol^{-1} (SL constant pH 2ox).

Molecular masses of the commercial plasticizers used as references in the application testing differed greatly from each other, lignosulfonate (WRDA 90D) being small (M_w 3000 g mol^{-1}) and polycarboxylate (Glenium C151) being fairly large (M_w 15900 g mol^{-1}) with a wide molecular mass distribution (Table 2 in IV).

The total negative charge at pH 6 in the oxidized lignin solutions, determined by potentiometric titration, was more than doubled (in some cases more than tripled) when compared to that of un-oxidized SL solution (1.5 mmol g^{-1}). As described above, the negative charge can arise from the carboxylic functionality in lignin polymer or from the acidic, small molecular reaction products of lignin. Part of the charge may originate from the reduction products of O_2, especially hydroperoxyl radical (HOO•) (pK_a 4.8) (IV). Another plausible explanation is that the charge is due to the resonance stabilized quinone enol structures (hydroxyl-*p*-quinone, S18, **Figure 9** c), which have a pK_a value in acidic pH range (Zakis, 1994). The acidic quinone enol structures can also arise from the non-phenolic lignin. In this case, the mechanism proceeds via the addition of OH• to the non-phenolic lignin unit followed by O_2 oxidation forming an o-quinone structure (**Figure 10**), which further reacts to quinone enol structure.

According to UV280 measurements, 82–71% of the aromatic structures in SL remained after the improved oxidations (Table 2 in IV), meaning 18–29% were degraded. The content of phenolics in SL was recently quantified by ^{31}P NMR to be 3.4 mmol g^{-1} (Liitiä et al., 2014). Thus, assuming that the decrease in UV absorbance is due to degradation of phenolic structures, the quantity of the degraded phenolics in SL would correspond to 0.6–1.0 mmol g^{-1}. If the degradation only leads to the formation of muconic acid structures in lignin polymer, it would increase the negative charge by 1.2–2.0 mmol g^{-1}. However, the increase in negative charge measured directly from the treatment solutions of the improved oxidations was at least two-fold more. This indicates that some of the primary oxidation products were further oxidized to secondary products, thus increasing the overall amount of carboxylic acid functionalities. Since a clear correlation is seen between the O_2 uptake and negative charge at pH 6 (Table 2 in IV), a substantial part of the charge may originate from other acidic moieties than carboxylic acids bound to lignin.

Figure 17 illustrates the recovery yield of SL and oxidized SL as a function of pH, measured by an acidification-centrifugation procedure. The oxidation increased lignin solubility, which is desirable for dispersing and plasticizing applications. SL is soluble only above a pH of 7, while the oxidized SL with increased molecular mass was already above a pH of 4 to 5. Part of the lignin material in sample SL constant pH 2ox could not be recovered at pH 2.5. It seems that the increase in charge in lignin polymer is enough to enhance its solubility considerably, even though the molecular mass of the polymer is high. Recovery of lignin material by acidification may alter its structure. For example, muconic acids are reported to form lactones via a ring closure reaction, especially under neutral or acidic conditions (Gierer and Imsgård, 1977a; Chang and Gratzl, 1980; Pieken and Kozarich, 1990; Evtuguin and Robert, 1997). More specified characterization of the functionalities in oxidized lignin material was not considered to be necessary, since the solutions were to be used as such for the plasticizing applications.

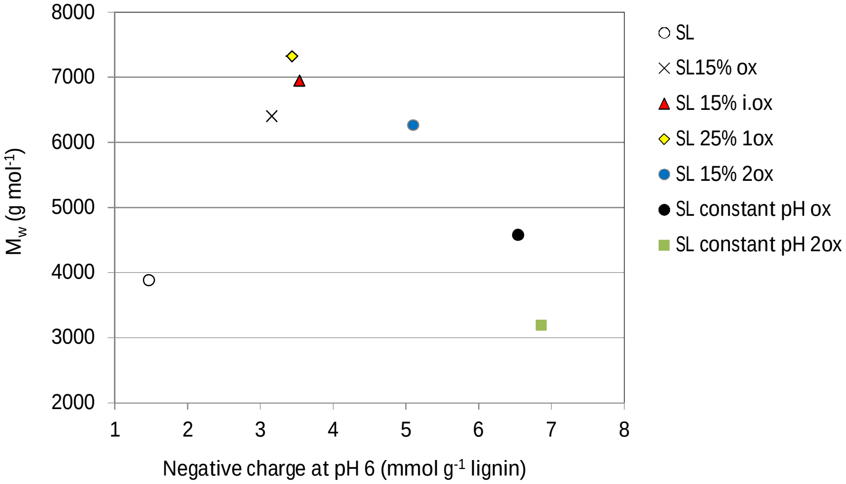


Figure 16. Impact of oxidation on soda lignin (SL) characteristics: Molecular mass (M_w) as a function of negative charge measured directly from the solution by potentiometric titration.

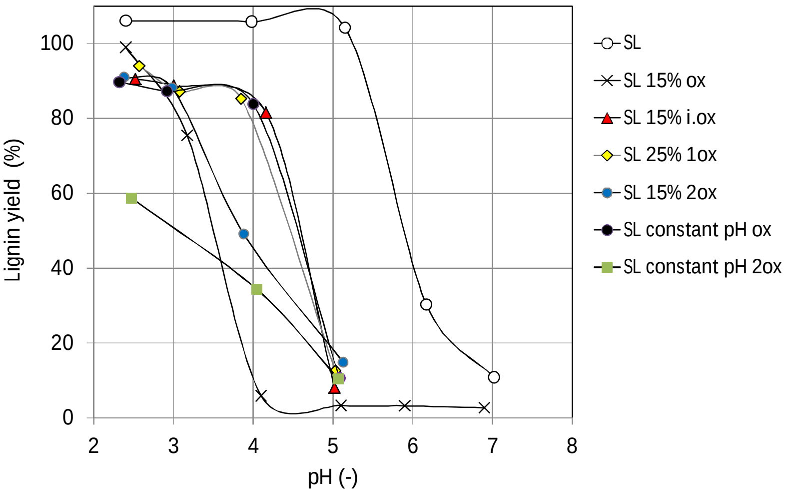


Figure 17. Impact of oxidation on soda lignin (SL) solubility: Lignin precipitation yield as a function of pH determined by an acidification-centrifugation procedure.

4.1.2.2 Results of the application testing

Plasticizing performance of the oxidized lignin solutions was evaluated stepwise, proceeding from the easiest medium to the most demanding. The initial testing was done in cement pastes (Fig. 3 in IV), thereafter in mortar (**Figure 18**), and lastly the performance of selected samples was verified in concrete (**Figure 19**).

Oxidized SL solutions decreased the yield stress in pastes made of two different types of cement, indicating the plasticizing performance. The differences between the oxidized lignin samples became more noticeable when testing their performance in mortar. The high Haegermann flow value (spread of mortar) demonstrates that the production of the oxidized lignin by 2-stage or constant pH oxidation, or the combination thereof, offered a better plasticizing effect when compared to those produced by single-stage oxidation. However, all of the oxidized SLs, and also the un-oxidized, were superior to lignosulfonate, WRDA 90D.

The performance of the three samples, produced by the improved oxidations, was finally evaluated in concrete using a slump test. The results (**Figure 19** a) show that these samples provided an excellent plasticizing effect in concrete, even with a dosage of 0.40 w-% on cement. Their performance was again clearly better than that of WRDA 90D. The 0.40 w-% dosage of the oxidized SL provided roughly the same plasticizing efficiency as a 0.20 w-% dosage of Glenium, which can be considered to represent an excellent performance. In addition, the results of the mortar tests suggest that the performance of these oxidized SLs is very close to that of the other polycarboxylate-based superplasticizer, VB Parmix, in concrete, with an equal dosing. In one of the experiments, defoamer agent (TBF 2.5% on lignin) was used with SL constant pH 2ox. The results indicate that the defoamer does not affect the plasticizing performance of the oxidized SLs. The photographs in **Figure 20** illustrate the concrete flowability in the slump test.

The samples SL constant pH ox and SL constant pH 2ox, showing the best performance as plasticizers, had relatively low M_w (4580, 3200 g mol^{-1}) and posed a high negative charge at a pH of 6 (6.5, 6.9 mmol g^{-1}). Recently, different fractions of soda-AQ lignin isolated from the spent liquor were tested for their plasticizing performance in mortar (Takahashi et al., 2014). Similarly, the results suggested that the fraction with a relatively low M_w (4000–5000 g mol^{-1}) was the most advantageous for adsorption on the cement particles, and showed the highest plasticizing performance of all the tested fractions. The fraction also had a high content of phenolics (2.3–2.5 mmol g^{-1}), which hold charge while being dissociated in the highly alkaline media.

The oxidized SLs were observed to introduce air into mortar. However, the amount was less than in the case of VB Parmix and un-oxidized SL. When evaluating the oxidized lignins in concrete, one of the samples, SL constant pH ox, showed surprisingly good performance while it did not introduce air in the mixture, which was also the behavior with the commercial plasticizer products (**Figure 19** b). The air content in fresh concrete correlates with the density and strength properties of the matured concrete (28 d) (**Figure 19** c), and thus in the case of SL constant pH ox, the compression strength was in line with that of concrete plasticized using commercial products (Table 3 in IV). By using a small amount of defoamer with the other samples, the air content in concrete could also be reduced, ensuring sufficient properties in the matured concrete. Inclusion of defoamer is a common practice with lignosulfonate products, which, without the agent, can cause air entrainment in concrete (Chang and Chan, 1995; Taylor, 1997; Lora, 2008).

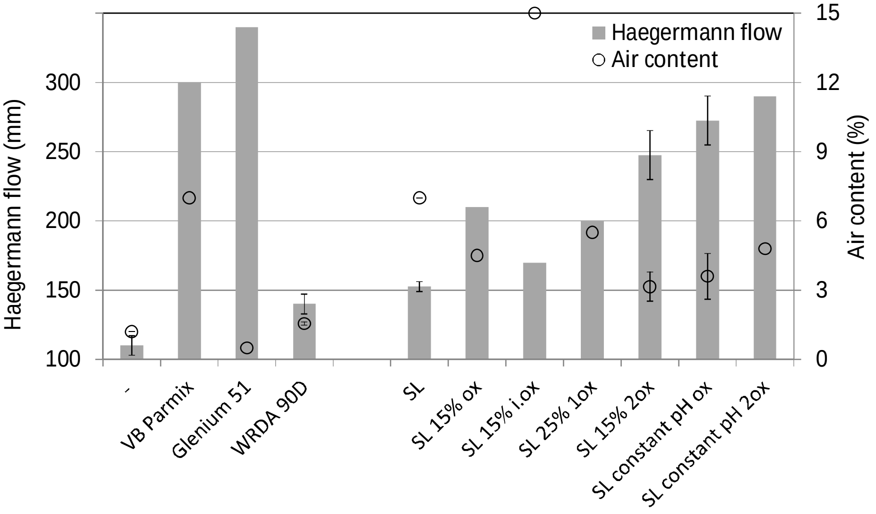


Figure 18. Haegermann flow and air content of fresh mortar. Commercial products or soda lignin (SL) solutions (un-oxidized or oxidized) used as plasticizers with a dosage of 0.40 w-% on cement.

On contact with water, cement undergoes several reactions and forms many hydration products. The hydration is investigated by measuring the heat flow released from the material as a function of time. Typically, it is desirable that the additional agents introduced into the concrete do not slow down the hydration (Ramachandran et al., 1998). However, the hydration kinetics were affected by the lignosulfonate, WRDA 90D. They were also affected by the oxidized SLs to some extent (Table 3 in IV), but there was no correlation between the retarded hydration and the compression strength of the matured concrete, which indicates that the oxidized lignin is already a promising alternative to lignosulfonate products. It was only the increased air content that had an inverse correlation with the compression strength.

The muconic acids, which are assumed to exist mainly in ring form, as lactones, may hydrolyze back to their acid form in highly alkaline concrete (pH 13–14), and thereby increase negative change of the oxidized lignin in situ. This may enhance their plasticizing performance after cement hydration has started.

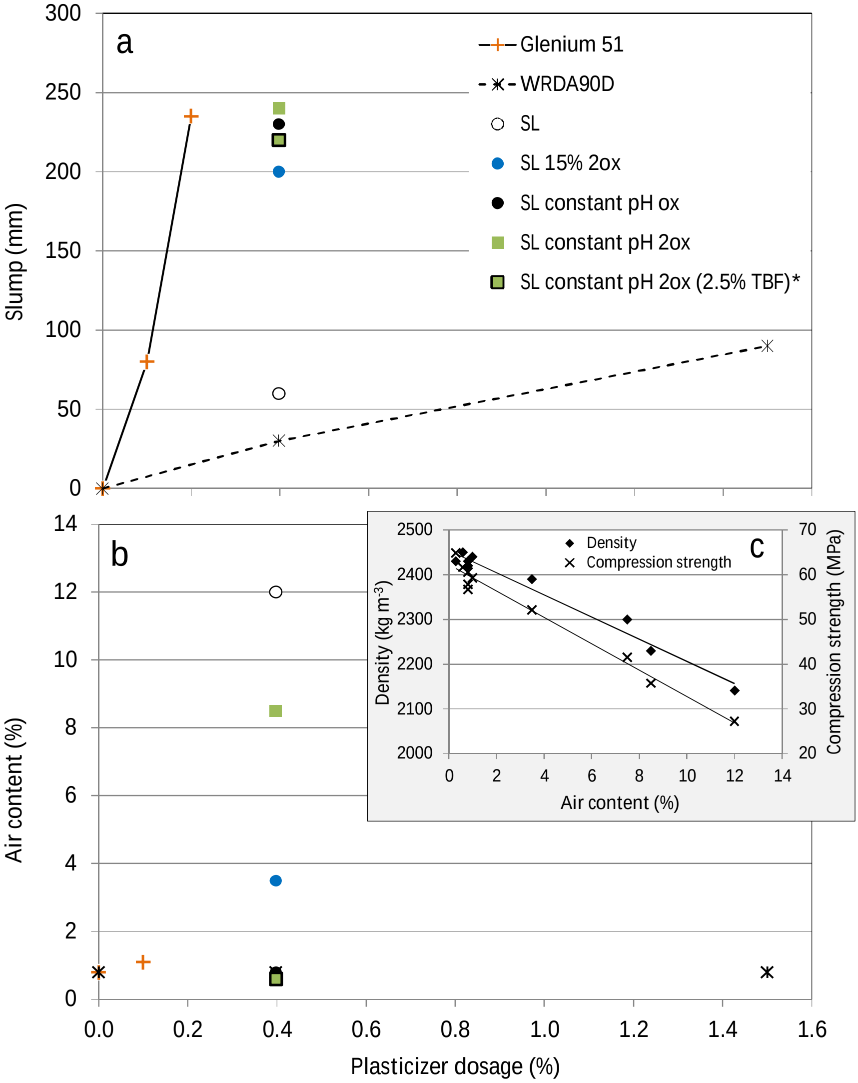


Figure 19. a) Slump and b) air content in fresh stage concrete; c) impact of air content on compression strength and density of matured concrete. Commercial products or a soda lignin (SL) solution (un-oxidized or oxidized) used as plasticizers, with a dosage of 0–1.5 w-% on cement. *Defoamer agent (TBF 2.5% on lignin) used with SL constant pH 2ox.

Figure 20. Photos of the concrete slump test. Commercial products (Glenium 51, WRDA 90D) or an oxidized soda lignin solution (SL constant pH ox) used as plasticizers, with dosages of 0.20 and 0.40 w-% on cement.

4.2 Oxidation of lignin using O_2 and laccase as a catalyst

The potential of lignin to replace oil-based raw materials in material applications, such as in composites, is being actively investigated. However, there are several difficulties in applying lignin. One of them is the volatile organic compounds (VOCs), either present in technical lignin, or formed as they are processed at high temperatures in thermoplastic processes. The other problem is the poor melt-flow, meaning the softening behavior of lignin under the elevated temperatures.

Publications II and III report the studies in which laccase-catalyzed O_2 oxidation was applied to enhance the utilization of softwood kraft lignins for the composite applications. In study II, laccase-catalyzed oxidation was applied to polymerize lignin-derived low-molecular phenolics for the reduction of VOCs. In study III, it was intended to introduce phenolic derivatives with a polyether-type hydrophilic side chain into the lignin structure in order to soften the lignin. In both studies (II and III), the oxidation by O_2 under alkaline conditions was investigated as an alternative method to induce polymerization of phenolics in an analogous manner to the laccase-catalyzed reaction, via the phenoxyl radicals.

4.2.1 Reduction of VOCs in kraft lignin

VOCs in kraft lignin are typically lignin-originated phenolic structures, such as guaiacol (2-methoxyphenol), or reduced sulfur compounds (thiols) formed in cooking. The odor threshold values of these VOCs are extremely low, in the range of a few ppb (values presented in publication II).

Three fungal laccases, ThL (Rittstieg et al., 2002), TaLcc1, and TaLcc2 (**Table 4**), functioning in mild acidic conditions were tested in order to reduce the low-molecular phenolic VOCs of two kraft lignins (SE and Indulin AT) via polymerization. To increase the dissolution and the reactivity of lignin, a fungal laccase, r-MaL (Kiiskinen et al., 2002), functioning at pH 8, was also evaluated. TaLcc1 and TaLcc2 are used, for example, to treat (lighten) denim and for stain removal (Paloheimo et al., 2006a, 2006b).

The potential of a sulfhydryl oxidase, AoSOX1 (Faccio et al., 2010), together with r-MaL was tested as a means to reduce the odor from thiols (e.g. methanethiol, CH_3SH). Sulfhydryl oxidases catalyze the oxidation of thiols into disulfides, with the reduction of molecular oxygen to hydrogen peroxide. These enzymes have been investigated in the food industry to improve the flavor of products (Swaisgood, 1977; Starnes et al., 1986).

As in laccase-catalyzed oxidation, O_2 is reduced to water, and in sulfhydryl oxidase-catalyzed reaction to H_2O_2, monitoring the consumption of dissolved O_2 in the reaction solution serves as a method of evaluating the reactivity of these enzymes. The reactivity of the enzymes toward both lignins was first evaluated on an analytical scale by monitoring the reduction of dissolved O_2 in the reaction solution containing lignin 2.5 or 5 w-%. At pH 6, ThL and TaLcc2 showed higher reactivity toward lignins, indicated by faster O_2 consumption than in the case of TaLcc1 (Fig. 1a in II). At pH 8, lignin already reacted with the dissolved O_2 present in the reaction solution. However, more O_2-consuming reactions occurred in the presence of TaLcc1, TaLcc2, and r-MaL than in their absence (Fig. 1b in II). In addition to the laccase- (and AoSOX1-) catalyzed oxidation, the oxidation of SE was followed in alkaline conditions (pH 10), which causes the dissociation of the phenolic structures. Increasing the pH from 8 to 10 increased O_2 consumption and indicated improved lignin reactivity when compared to r-MaL-catalyzed oxidation (Fig. 2 in II).

Based on the results obtained from the analytical scale experiments, selected laccase treatments and oxidation at alkaline pH were repeated at higher lignin content and under oxygen excess on a larger laboratory scale (2-L pressure batch reactor) (**Table 9**). In two experiments, a mediator (HBT) was used in addition to the laccases. The pH level for the alkaline oxidation was set to 10.7, according to the experimentally determined dissociation constant value of Indulin AT (at 21°C) (Norgren and Lindström, 2000). The effect of the treatments on VOC reduction was determined both by sensing (odorimetry) and chemical (TD-GC/MS, SEC) analyses. The thermal desorption (TD) method was developed in order to simulate the formation of volatile degradation products at the elevated temperatures prevailing in composite processing.

The overall odor intensity of the lignin samples (**Table 9**) was evaluated by ten human sniffers (the odor panel). The odor intensity of the samples varied between 2.9 and 3.9 (4 = disturbing, 3 = clearly perceptible) with rather large standard deviations (**Figure 21** a). Although the odor of the samples differed only slightly, the oxidation by TaLcc2 and r-MaL+AoSOX1 and the oxidation at pH 10.7 indicated reduced VOC content with the lowest odor intensity values. Grading the lignin samples using the numbers from 0 to 10 (0 = the least odor intense, 10 = the most intense odor) supported the same observations (**Figure 21** b).

Volatile degradation products of the freeze-dried lignin samples, namely SE treated with or without r-MaL/r-MaL+AoSOX1, and at pH 10.7, were determined by TD-GC/MS at 150ºC and 190ºC. Lignin softening takes place typically at or below 150ºC, and kraft lignins begin to degrade at temperatures around 160°C. The extrusion temperature in composite processing is generally around 160 to 165°C (Schorr et al., 2014).

The most abundant degradation product of lignin was guaiacol (Fig. 4 in II). The formation of volatiles was clearly stronger at 190ºC than at 150ºC. At both temperatures, clearly fewer volatiles were formed when lignin was oxidized at pH 10.7, than when treated with or without r-MaL/r-MaL+AoSOX1 at pH 8. At least three explanations for the reduced formation of volatiles of alkali-oxidized lignin exist. Firstly, in the freeze-dried alkaline sample, the low-molecular phenolics most likely existed in sodium-salt form, which hindered their volatilization. Secondly, part of the phenolics might have degraded to non-volatile structures. Thirdly, polymerization/radical coupling of small-molecular phenolics, such as guaiacol, could have taken place.

The oxidation at pH 10.7 also reduced the amount of sulfur containing VOCs (Fig. 4 in II). In these conditions, they might have degraded and further oxidized to sulfate. Furthermore, the dissociation of thiols occurs at alkali pH levels for instance, the pK_a value of methanethiol is 10.33 (Shields and Seybold, 2014) – and thus the existence as sodium-salt may have hindered their volatilization.

The quantification of the main volatile, guaiacol, showed that its amount in the alkali-oxidized lignin, SE (pH 10.7), 1.3 ppm at 150ºC and 24 ppm at 190ºC (**Figure 22**), was still high when compared to the odor threshold values, 3 to 21 ppb, measured in water solutions (Fazzalari, 1978; Buttery et al., 1988; Guth, 1997). Thus, generally, the odor threshold values of the main VOC compounds are extremely low, which poses a challenge to VOC reduction. The analyses performed showed no clear effect of sulfhydryl oxidase or the mediator (HBT).

In order to investigate the importance of oxygen excess in the alkaline treatment, the pH 10.7 treatment was repeated for Indulin AT with and without oxygen boosting (0.5 MPa of initial O_2). The results clearly showed that O_2-pressurized alkali treatment decreased the volatile guaiacol formation at 150ºC and at 190ºC, when compared to the alkaline treatment performed without O_2 boosting (**Figure 22**).

Table 9. Experimental set-up of the oxidative treatments of softwood kraft lignins (SE and IndAT) performed with and without an enzyme, under 0.5 MPa initial O_2 pressure at room temperature. Reference samples (ref.) were not O_2 boosted.

Sample	**Substrate**[a]	**Lignin dry solids (%)**	**pH**	**Enzyme**[b]	**Dose**[c] **(nkat g^{-1} or %)**	**HBT**[d] **(%)**	**Treatment (h)**	
1	SE	5	5	-	-	-	-	ref.
2	SE	5	5	-	-	-	2	
3	SE	5	5	ThL	100	-	2	
4	SE	5	5	ThL	100	5	2	
5	SE	5	6	TaLcc2	100	-	2	
6	SE	5	6	TaLcc2	100	5	2	
7	IndAT	5	5	-	-	-	-	ref.
8	IndAT	5	5	-	-	-	2	
9	IndAT	5	5	ThL	100	-	2	
10	SE	10	8	-	-	-	-	ref.
11	SE	10	8	-	-	-	1	
12	SE	10	8	r-MaL	0.1	-	1	
13	SE	10	8	r-MaL, AoSOX1	0.1, 0.1	-	1	
14	SE	10	10.7	-	-	-	1	

[a] Lignins: SE=Stora Enso lignin, IndAT=Indulin AT

[b] Laccases: ThL=*Trametes hirsuta*, TaLcc2=*Thielavia arenaria* Lcc2, r-MaL=*Melanocarpus albomyces*, Sulfhydryl oxidase: AoSOX1=*Aspergillus oryzae*

[c] ThL and TaLcc2 activity measurements with 2,2'-azino-bis-(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS); doses nkat g^{-1}. r-MaL and AoSOX1 doses on protein bases; r-MaL dose in respect to lignin, AoSOX1 dose in respect to sulfur in lignin.

[d] HBT=1-hydroxybenzotriazole, dose with respect to lignin.

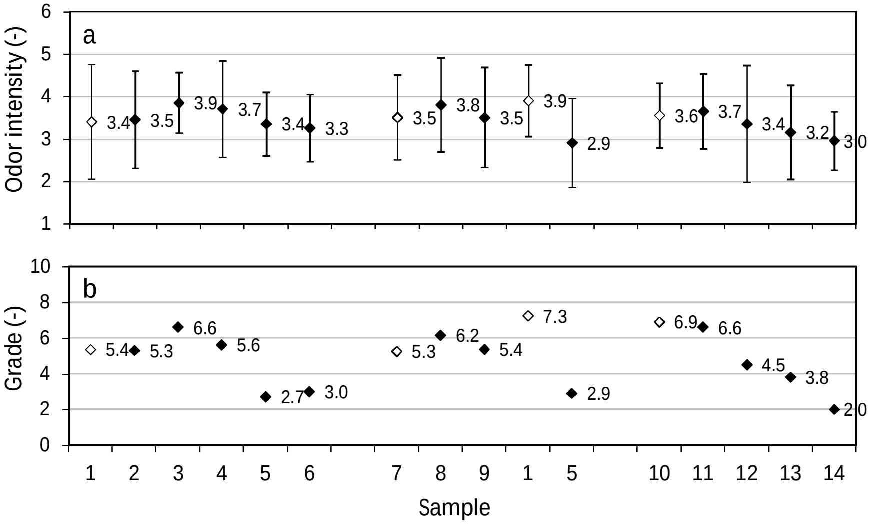


Figure 21. a) Odor intensity and b) grade of the wet kraft lignin (SE and Indulin AT) samples treated with and without laccases (and AoSOX1) at different pH levels. Samples according to Table 9; open dots refer to reference samples that were not O_2 boosted. Evaluation of samples 1 and 5 was conducted in the first and second separate odor panel. ***Odor intensity****: 6=not acceptable, 5=strongly disturbing, 4=disturbing, 3=clearly perceptible, but not disturbing, 2=perceptible, not disturbing, 1=not perceptible;* ***Grade****: 10=most odorous; 0=least.*

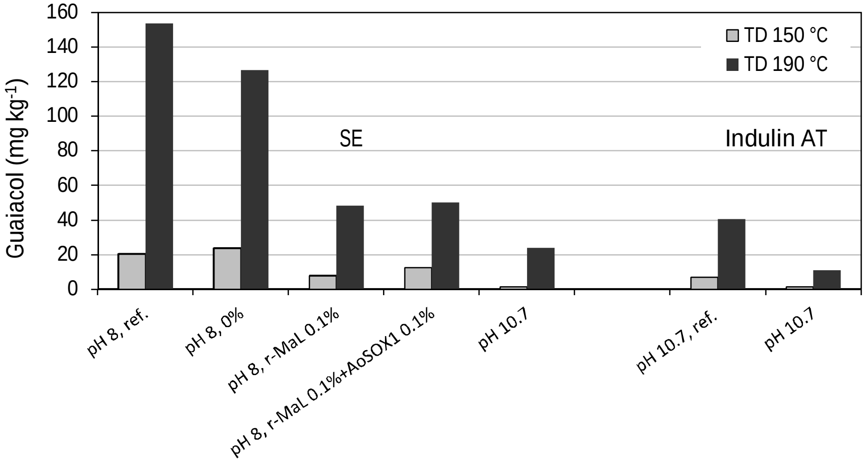


Figure 22. Volatile guaiacol of freeze-dried kraft lignin (SE and Indulin AT) samples at 150°C and 190°C. Reference samples (ref.) were not O_2 boosted. Treatments of SE according to 10–14 in Table 5.

The effect of the treatments on the molecular mass of both kraft lignins was analyzed. The results indicated that the initial oxidation products, phenoxyl radicals, polymerized via radical coupling in the O_2 oxidation performed at pH 10.7 at room temperature (Fig. 6 a and b in II). In addition, r-MaL and r-MaL+AoSOX1-catalyzed oxidation caused minor polymerization of phenolics. Moreover, the experiments done with Indulin AT verified that the O_2-pressurized alkali treatment caused lignin polymerization, when compared to the alkali treatment done without O_2 boosting (Fig. 6 b in II). However, the change in M_w was rather small when compared to the results presented in III. In study III, a higher initial alkaline pH, lignin content, and O_2 load were applied than in study II, and a pH decrease during oxidation was allowed. All these factors probably accelerated lignin condensation and increased M_w.

4.2.2 Experiences of kraft lignin functionalization

The poor softening behavior of lignin is partly due to the crosslinking between lignin macromolecules via intermolecular hydrogen bonding. Polymer blending (Kadla and Kubo, 2004; Kubo and Kadla, 2005) or chemical modifications (Glasser and Jain, 1993; Jain and Glasser, 1993; Lora and Glasser, 2002) of lignin by linear hydrophilic substituents have been studied as means to block this type of crosslinking. This internal plasticization of lignin is often measured as the reduced T_g.

Incorporation of water-soluble phenols into kraft lignin (Indulin AT), using laccase as a catalyst, in aqueous dioxane solutions, has been investigated by Lund and Ragauskas (2001). In study III, vanillic acid poly(ethylene glycol) methyl ester (Ester V-PEG) and ether (Ether V-PEG) were synthesized and applied for this purpose. Laccase-catalyzed oxidation by TaLcc1 and TaLcc2 (pH optimum at 6) and r-MaL (pH optimum at 8) was studied as a plausible method to induce crosslinking between softwood kraft lignin (SE and Indulin AT) and the hydrophilic derivative in water solutions.

Laccase treatments were performed on the two-component systems containing lignin and the derivative with a molar ratio of 3:1 (mass ratio 1:1) and total dry solids of 2.5 w-% in the solution. Laccase treatments were also performed with one-component systems containing only lignin or the derivative as references.

Monitoring the reduction of dissolved O_2 indicated that at a pH of 6, the ester derivative is a substrate for TaLcc2 (Fig. 4 a in III). Without the laccase, there were no O_2-consuming reactions. The O_2 consumption was remarkably enhanced when using the ether derivative as a substrate for TaLcc2. At a pH of 8, in addition to r-MaL (30 nkat g^{-1}), TaLcc2 (100 nkat g^{-1}) was functioning well toward the ester derivative (Fig. 4 b in III).

Because TaLcc2 was detected to be clearly more reactive against the ether than the ester derivative, lignin functionalization at pH 6 was studied only using the ester, to avoid excessive homogeneous polymerization of the derivative. TaLcc1 and TaLcc2 were active toward lignin at a pH of 6 (Fig. 4 c in III). However, they were clearly more reactive in the system when replacing half of the lignin with the ester derivative. At a pH of 8, both r-MaL and TaLcc2 were functioning well toward lignin with or without the ester or ether derivative (Fig. 4 d in III), yet the consumption of O_2 being faster in the presence of the ether derivative.

Monitoring the O_2 consumption in the reaction solution of the two-component system did not reveal if the lignin was actually functionalized, that is, if the radical coupling between the lignin and the derivative had taken place. Therefore, the molecular mass distributions (MMDs) (**Figure 23** a–c) and the average molecular masses (M_n, M_w) (Table 1 in III) of part of the samples were determined.

The results indicated that at a pH of 6, TaLcc2 catalyzed minor dimerization of the ester (small shoulder in the MMD) and clear polymerization of the ether derivative (a). This behavior was consistent with the detected O_2 consumption. At a pH of 8, dimerization of the ester derivative was induced by both r-MaL and TaLcc2 (b). When comparing the MMDs of the two-component system treated in the presence and absence of TaLcc2 at a pH of 6, it was seen that the signal caused by the ester derivative was present in both distributions. In the presence of TaLcc2, the lignin part of the distribution was wider, representing homogenous polymerization of Indulin AT (c). The results indicate that no or only very minor functionalization took place at a pH of 6, even though TaLcc2 was observed to be active against the components, lignin and Ester V-PEG, separately.

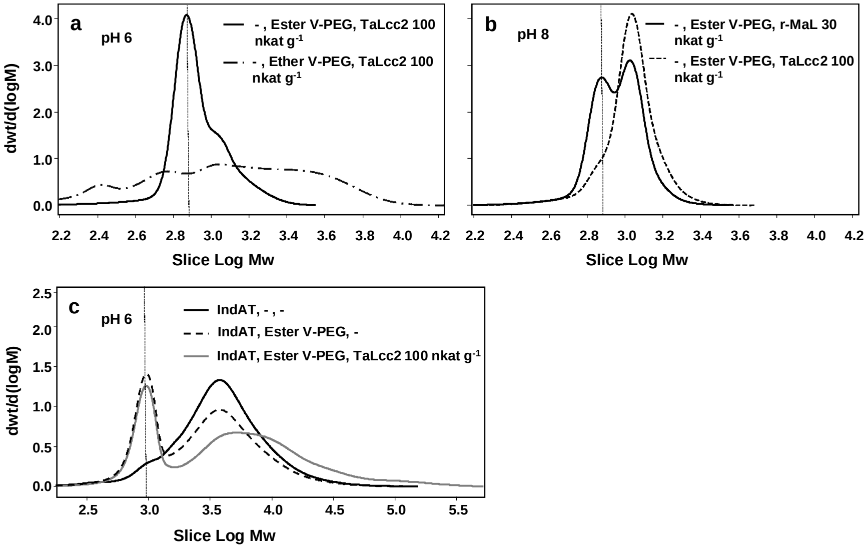


Figure 23. (a) Molecular mass distributions (MMDs) of ester and ether derivatives treated in the presence of TaLcc2 at pH 6; (b) MMDs of ester derivative treated in the presence of r-MaL and TaLcc2 at pH 8; (c) MMDs of kraft lignin (Indulin AT) with or without ester derivative in the presence or absence of TaLcc2 at pH 6.

From the water-soluble phenols (4-hydroxybenzoic acid, 4-hydroxyphenylacetic acid, vanillic acid, coumaric acid, ferulic acid, guaiacol sulfonate, and 4-

hydroxybenzene sulfonic acid) studied in the former investigations, the incorporation of guaiacol sulfonate in lignin was most strongly illustrated (Lund and Ragauskas, 2001). Covalent binding of the derivative to lignin was concluded to take place, since the incorporation made the lignin water-soluble at a pH of 2.4. The amount of guaiacol sulfonate and 4-hydroxyphenylacetic acid incorporation was approximately 10 w-% and 2 w-% (determined by conductometric titration), respectively. Their non-methoxyl and non-methyl-substituted analogs, 4-hydroxybenzene sulfonate and 4-hydroxybenzoic acid, did not show detectable incorporation at all. It was suggested that both the catalytic rate of oxidation and the stability of the generated phenoxyl radical could influence the degree of incorporation. Both these factors are increased in the case of phenols with substituents such as methoxyl and methyl groups. Upon enzymatic oxidation and removal of dioxane, phenols like vanillic acid, coumaric acid, and ferulic acid were found to precipitate over a wide pH range and could not be separated from lignin, and their incorporation was not studied further (Lund & Ragauskas, 2001).

In study III, the competing coupling reactions, namely the polymerization of the derivative to its homopolymer, the polymerization of lignin, and the incorporation of the derivative to lignin, might have been controlled by slowly continuing addition of the derivative to the solution initially containing only lignin and laccase. In particular, the continuous addition might have enhanced the incorporation of the derivative to lignin.

Because the lignin functionalization with the ester derivative by laccase-catalyzed oxidation was not successful, alkali-O_2 oxidation in high ‘substrate’ dry solids (25 w-%) was tested as an alternative chemical method. In the experiments, two-thirds of the substrate content included lignin and one-third ester derivative. Reference oxidations without the derivative were conducted on lignin dry solids of 25 and 16.7 w-% (2/3 of the 25 w-%).

The oxidation reactions on lignin dry solids of 25 w-% (reference) consumed much oxygen and were exothermic, as was seen from the pressure drop and simultaneous temperature increase in the reactor (Fig. 7 in III). The corresponding behavior in the two-component system, wherein 1/3 of the lignin was replaced with the ester derivative, indicated clearly fewer reactions. The behavior of the second reference experiment, which only contained lignin at 16.7 w-%, was similar. The results indicate that only lignin reacted, with the ester derivative being relatively inert.

The computed O_2 consumption was 0.83 mol O_2 mol^{-1} lignin (15 w-% on lignin) in the case of high lignin dry solids (25 w-%), but less than half of that in the case of 16.7 w-%. The initial pH, 13.3, and the increased temperature during the oxidation accelerated the oxidation reactions, leading to higher O_2 uptake in the case of 25 w-% than in the case of 16.7 w-% of lignin. With the two-component system (and the second reference), the initial pH was set to 12.2 to avoid the hydrolysis of the ester derivative.

The MMDs and average molecular masses of lignin, the ester derivative, and the alkali-O_2 oxidized samples were determined (**Figure 24**, **Table 10**). The alkali-O_2 oxidation caused strong condensation of lignin at 25 w-%, most likely via the 5-

5 coupling, and increased lignin molecular mass from 4500 to 9700 g/mol. The signal caused by ester derivative was present in the MMD of the two-component system, indicating that no major functionalization occurred. In addition, there was no or only very minor homogenous polymerization of the ester derivative. These conclusions were also supported by the results of dialysis yield and T_g measurements (below).

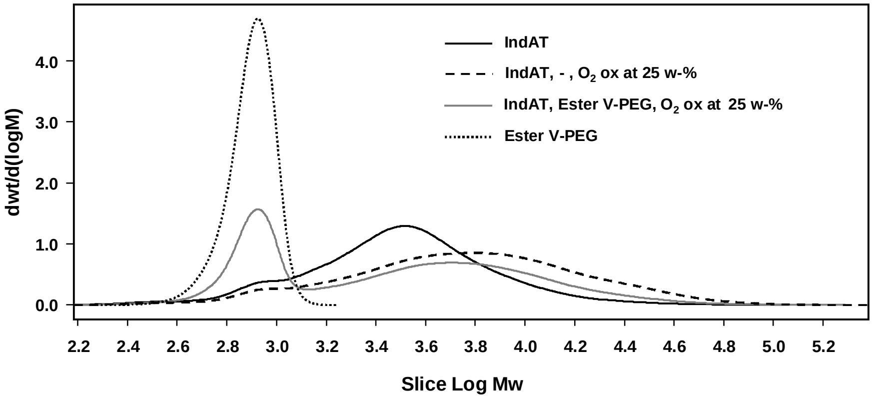


Figure 24. MMDs of softwood kraft lignin (Indulin AT), ester derivative, and alkali-O_2 oxidized lignin without or with the derivative.

The results obtained were negative in terms of lignin functionalization, but they illustrate the fact that chemical O_2 oxidation is a simple method of increasing the molecular mass of kraft lignin via radical polymerization. At an alkaline pH, O_2 is able to react with phenolic lignin without an enzyme or other catalyst. In addition, the results also indicate (as the results of IV) that the most important factors contributing to lignin condensation and increase in molecular mass are lignin density and the prevailing pH conditions in the oxidation. Without pH control the highly alkaline pH drops fast below 12 under the high lignin density and the hydroperoxide intermediates decompose to phenoxyl radicals, which undergo coupling. The competing reaction, leading to the formation of primary oxidation products, is minimized.

The tendency of soda lignin from wheat straw (IV) and kraft lignin from softwood (III) to undergo polymerization by chemical oxidation cannot be directly compared, due to the slightly varying operating conditions in the alkaline-O_2 oxidations. However, by applying a lignin content of 25 w-% combined with $pH_{Init.}$ 13.4, $T_{Init.}$ 50°C, O_2 load of 30 w-% (on lignin) for soda lignin (SL 25% ox in IV) and pH_{Init} 13.3, T_{Init} 40°C, O_2 load 24 w-% for kraft lignin (Indulin AT, - , O_2 ox at 25%) increases in M_w were 2.3 and 2.2-fold, respectively. PD also increased by 1.7 and 1.5-fold, respectively. Most likely, the 5-5 coupling reactions of phenoxyl radicals are more pronounced with softwood kraft lignin than with hardwood kraft lignin, because the latter is rich in S units containing two methoxyl groups in the aromatic ring (lacking the free C5 position).

4.2.2.1 Lowering T_g

After the alkali-O_2 oxidation of the one- and two-component systems (25 w-%), the reaction solutions were dialyzed to remove the free ester derivative from the large lignin macromolecules, which were targeted to be at least partly functionalized during the oxidation. The dialyzed sample solutions were freeze-dried. In addition, un-oxidized lignin as a reference was treated likewise.

The yield after the dialyses in the samples that only contained lignin (un-oxidized and oxidized) was in the range of 74 to 79%, showing that the dialysis membranes passed approximately 25% of the lignin material. In the sample containing 2/3 of lignin and 1/3 of the derivative, the yield was as low as 50 to 54%, indicating that in addition to the 25% of the lignin material, all of the derivative (M_w 800 g mol^{-1}) was also free, meaning that it did not incorporate onto lignin and passed through the membranes (cut-offs 1000 and 3500 Da) (**Table 10**).

Typically, plasticization (functionalization) provides lignin with lower T_g. When comparing the T_g values of the dialyzed, un-oxidized lignin (92; 99°C) to those of the dialyzed, oxidized two-component sample (99; 95°C), there is no clear difference. This indicates that the alkali-O_2 oxidation did not induce functionalization. By DSC, T_g for Indulin AT has been determined to be between 135 and 142°C (Penkina et al., 2012). Here, interestingly, a clear decrease in T_g for Indulin AT, from 144 to 92 to 99°C, was obtained by the reference procedure, including lignin dissolution in NaOH, followed by dialysis purification and freeze-drying. Here, the internal bonding of dissolved lignin, namely the intra-molecular hydrogen bonds, was probably disabled by the freeze-drying. When disabling the (re)arrangement of lignin, the mobility of the molecules is retained, providing lower T_g. This suggests that, if aiming at a moderate reduction of lignin T_g, no modification for the internal plasticization is needed if using an isolation procedure hindering the internal bonding of lignin. However, the contribution of the lowered T_g toward improving the thermoplastic properties of lignin under the composite processing conditions should be verified by experiments.

Although T_g generally increases with increasing molecular mass, it has been established that T_g increases with the increasing degree of condensation involving C–C linkages between phenylpropanoid units (Baumberger et al., 2002). Here, it was also observed that when lignin was more condensed by the alkali-O_2 oxidation, it possessed higher T_g (116; 117°C) (IndAT, - , O2 ox at 25%, dialysis purified; **Table 10**) than the less condensed form. However, in the case of the condensed lignin with M_w higher than 10000 g mol^{-1} (dialysis purified), the T_g was clearly lower than that measured from the un-dialyzed lignin (IndAT).

Table 10. Analysis results of Indulin AT, ester derivative, and alkali-O_2 oxidized (O_2 ox) Indulin AT, with or without the derivative (MMDs shown in Figure 24). Cut-off (CO) of dialysis membrane marked in the case of dialysis purification.

Lignin, derivative, d.s. (w-%)	**Dialysis CO (Da)**	**Dialysis yield (%)**	**Lignin yield, computed (%)**	**M_n (g mol^{-1})**	**M_w (g mol^{-1})**	**PD**	**T_g (°C)**	**ΔC_p (J g^{-1} K^{-1})**
IndAT	-	-	-	2 100	4 500	2.1	144	0.329
IndAT, - , 25% [a]	1000	75	75	3 300	6 200	1.9	92	0.222
	3500	79	79	3 200	5 900	1.8	99	0.126
IndAT, - , O_2 ox at 25%	-	-	-	3 100	9 700	3.1	-	-
	1000	78	78	4 400	10 300	2.3	116	0.108
	3500	74	74	4 400	10 100	2.3	117	0.159
IndAT, ester, O_2 ox at 25%	-	-	-	1 600	5 600	3.5	-	-
	1000	50	75	3 500	7 800	2.2	99	0.19
	3500	54	81	3 300	7 500	2.3	95	0.132
Ester V-PEG [b]	-	-	-	760	800	1.1	-56	0.791

[a] Indulin AT dissolved in NaOH at 25 w-% dry solids, un-oxidized

[b] Melting temperature, T_m 5.4°C; ΔH -62 J g^{-1}

5. Conclusions

Although using lignin as a fuel will save fossil oil, there is great potential to create higher value for lignin by utilizing it as a source for bio-based chemicals and materials. However, to suit these purposes, some modification of the lignin is often needed. In particular, technical lignins other than water-soluble lignosulfonates require increased hydrophilicity if applied as surface active agents. For the composite applications, the softening behavior of lignin should be enhanced. In addition, an increase in molecular mass prior to further modification may be beneficial for polymeric and material applications, since technical lignins typically possess relatively low molecular masses when compared to many commercially available synthetic polymers.

Kraft and soda lignins are especially rich in phenolic hydroxyl groups, which is advantageous from the reactivity point of view. Molecular oxygen (O_2), an environmentally friendly and economically feasible oxidizing agent, is active toward phenolic lignin, and is thus a potential choice for lignin valorization.

Under alkaline conditions, lignin reactions start with the formation of a phenoxyl radical through electron transfer from the ionized phenolic group to O_2. Subsequent steps include reaction with the superoxide ($O_2•^-$), forming an organic hydroperoxide anion structure. Its rearrangement leads to the formation of the primary reaction products, which are vulnerable to further oxidative degradation. The hydroperoxide structure, with a pK_a value of 12–13, plays a crucial role, and has recently been shown to be the key intermediate in the course of phenolic lignin oxidation. Thus, the pH of the reaction solution determines whether the intermediate rearranges, leading to degradation, or induces condensation as its protonated form decomposes back to phenoxyl radical, which spontaneously undergoes coupling.

Laccase catalyzes the four-electron reduction of O_2 to water with concomitant one-electron oxidation of the phenolic hydroxyl groups into phenoxyl radicals, typically under a neutral or mild acidic pH. After this, non-enzymatic reactions cause mainly polymerization. The phenoxyl radicals may also form partly the same oxidation products as O_2 under alkaline conditions, but the reaction of the phenoxyl radical and O_2 proceeds at a much lower rate than with $O_2•^-$, which is not present in the enzymatic system. Even if some $O_2•^-$ should form and attack the phenoxyl radical, non-alkaline pH conditions favor the protonated form of the hydroperoxide intermediate, which does not lead to the oxidation products (ring opening or cleavage of the side chain) typical of alkaline conditions. Consequently, only lignin polymerization and Cα oxidation take place in laccase-catalyzed oxidation.

Oxidation of softwood and hardwood kraft lignins was observed under the conditions of oxygen delignification with a lignin content of 0.75 w-%. According to several indicators, extensive degradation of lignin took place. The study revealed that there is a rather big difference in O_2 consumption between kraft lignin oxidation and industrial pulp oxygen delignification. This can be partly due to the O_2 consumption of sulfide carry-over in industrial applications, which wastes part of the oxidation potential.

A simple alkali-O_2 oxidation method was developed to convert wheat straw soda lignin into a polyelectrolyte with increased water solubility. Lignin characteristics can be controlled by the oxidation parameters, especially by pH. Oxidation under high lignin content (15 and 25 w-%) without controlling the decreasing pH in the reaction solution caused condensation and up to a 2.3-fold increase in lignin M_w. However, lignin solubility was enhanced considerably even though its M_w was increased. Performing oxidation under a constant of pH 11.5 clearly hindered lignin condensation and increased the negative charge. Oxidation at a constant of pH 13 clearly decreased lignin M_w. The results highlight the fact that the course of lignin oxidation is determined by the hydroperoxyl intermediate and its protonation state.

The oxidized soda lignin solutions were applied as ready-to-use products for concrete plasticizing. They showed superior performance to the commercial lignosulfonate and provided plasticizing efficiency in concrete by 0.4 w-% dosing equal to the synthetic polycarboxylate-based superplasticizer by 0.2 w-%. The best performing oxidized lignin solution (oxidized at a constant pH of 11.5) did not even introduce air into concrete. Cement hydration kinetics were affected by the oxidized lignins, but this did not reduce the compression strength of the matured concrete. Previously, modified alkali lignins have not been reported as being tested in comparison to the polycarboxylate-based superplasticizers in concrete. In general, the results were very promising, and the oxidized lignins may provide a sustainable and techno-economically feasible alternative to lignosulfonates or even superplasticizers.

To enhance the utilization of lignin in composite applications, both laccase-catalyzed and alkali-catalyzed O_2 oxidation were used to polymerize lignin-derived low-molecular phenolics for the reduction of VOCs. According to sensing analysis, undesirable odor in kraft lignin suspension could be reduced to a greater extent by alkali-catalyzed than by laccase-catalyzed O_2 oxidation. According to thermal desorption analysis, besides the reduction of guaiacol, the main degradation product of lignin, alkali-O_2 oxidation also reduced the amount of sulfur containing VOCs. However, the odor threshold values of the main VOC compounds are extremely low, in the range of ppb, which poses a challenge to VOC reduction.

Functionalization of lignin with a hydrophilic phenolic compound was attempted to lower the glass transition temperature (T_g) of kraft lignin. However, homogenous polymerization of this compound was favored over coupling to lignin. Again, efficient lignin polymerization under alkali-O_2 conditions (not pH controlled) with a lignin content of 25 w-% was observed. However, it was found that freeze-drying as such induces a clear decrease (from ca. 140 to 90°C) in T_g, probably by hindering the internal bonding of lignin and thus increasing its mobility. This suggests that, in addition to functionalization, there may be alternative means to soften lignin.

In summary, in valorizing the kraft and soda lignins for material uses (II–IV), the alkali-O_2 oxidations were performed under the following range of conditions: $pH_{Init.}$ 10.7–13.4, lignin content 0.75–25.0 w-%, O_2 load 8–36 w-% on lignin, and $T_{Init.}$ 22–80°C. A reaction time of 30 minutes was mainly used. Oxidation in series (2-stage: 1. for inducing polymerization and 2. for charge) and oxidation under a constant alkaline pH were conducted. The laccase-catalyzed O_2 oxidations were performed under pH conditions of 5–8 and lignin content of 1.0–10 w-% at room temperature. O_2 boosting (8 and 16 w-% on lignin) was used in the treatments with the highest lignin contents. Reaction times of 60 and 120 minutes were used. The impact of operating parameters of alkali- and laccase-catalyzed O_2 oxidation on lignin characteristics was revealed, as summarized in **Table 11**.

This indicates that alkali-O_2 oxidation provides wide possibilities to modify lignin molecular mass and charge properties. Laccase-catalyzed oxidation is most applicable when lignin polymerization is desired.

Table 11. The impact of operating conditions of O_2 oxidation on lignin characteristics. + for increasing and - for decreasing impact. 0 indicates that there is no or only very little impact.

	O_2 oxidation					
	Under alkaline conditions					**Laccase-catalyzed**
	High lignin content	**High O_2 load**	**High pH[a] 11-12**	**High pH[a] $\geq$ 12**	**1) High lignin content 2) High pH_{Init}**	**pH ≤ 8**
Molecular mass	++	+	0 / +	- / --	+ / ++	+
Negative charge	0 / +	+	++	++	++	0
Solubility	+	+	+	++	+	0

[a] Constant pH

6. Future prospects

Even if lignin content and pH are crucial for the course of oxidation in alkali-O_2 oxidation, the kinetics and extent of oxidation are also affected by temperature, reaction time, and O_2 load. Thus, it would be beneficial to investigate the whole operating window systematically. Thereby, the operating conditions of alkali-O_2 oxidation could be optimized for targeted lignin characteristics, which would increase the further application potential of technical lignins. In addition, the production of even more concentrated final product-containing solution should be aimed at for reasons of economy, namely to avoid the transportation and dosing of large liquid volumes.

The promising results of applying the alkali-O_2 oxidized lignin for concrete plasticizing encourage continuing the investigations further. In these studies, it is important to emphasize more the structure-function relationship of the oxidized lignin, as well as its impact on cement hydration. Searching for new uses of oxidized lignins is an attractive option. They may also be suitable for dispersing applications in an acidic pH range, due to the increased water solubility.

In future studies, laccase-catalysed oxidation for polymerization of water soluble technical lignins, such as lignosulfonates, could be utilized to provide lignin materials of high molecular mass.

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PUBLICATION I

Lignin oxidation mechanisms under oxygen delignification conditions

Part 1. Results from direct analyses

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Lignin oxidation mechanisms under oxygen delignification conditions. Part 1. Results from direct analyses

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Abstract

Oxidation of softwood and hardwood kraft lignins was observed under conditions of oxygen delignification (90°C and 110°C; 0.6 and 0.9 MPa) as a function of time by means of a number of analysis techniques and quantitative information was obtained on the degradation and formation of various compounds and structures. The decrease in reactor pressure was monitored during a 4-h reaction period. During the first 60 min, lignin reactivity was high, while a very intense stage took place during the first 20 min. The reactions decelerated after the first 60 min and after 120 min the reactions did not significantly advance. The oxygen consumption after 4-h reaction was 1.3–1.5 mole O_2 per 1 mole lignin depending on the conditions. In the first 20 min, 50%–60% of the oxygen was consumed and the consumption increased only slightly after 60 min. At 90°C, the changes in all observed quantities were smaller throughout the whole 4-h reaction period than at 110°C. Under the studied conditions, increasing the reaction temperature, rather than the pressure, had primary significance in the increasing rate of lignin degradation. Hardwood kraft lignin was more reactive than softwood kraft lignin. The results obtained in this study are the basis for the development of a mechanistic model for the oxygen delignification process of pulps to be published in subsequent papers.

Keywords: kraft lignin; lignin; modeling; oxidation; oxygen; oxygen consumption.

Introduction

Lignin, a three-dimensional amorphous polymer consisting of methoxylated phenylpropane structures, is one of the three essential components of wood together with cellulose and hemicelluloses, which form a special supramolecular architecture (Sarkanen and Ludwig 1971; Salmén and Burgert 2008). In chemical pulping processes, lignin is removed from wood and the fibers are separated from each other. About 92%–94% of the fiber lignin can be removed by kraft cooking with reasonable selectivity (Gellerstedt and Lindfors 1991). The residual lignin is removed during the subsequent delignification and bleaching stages, typically starting by oxygen in alkaline medium. During this oxygen stage (O), the degree of delignification increases, depending on the operation parameters, in the range of 35%–50% (Sjöström 1993; Alén 2000; van Heiningen et al. 2003). The average oxygen consumption in industrial oxygen delignification is 0.14% per kappa number for softwoods (SW) and 0.16% for hardwoods (HW) (McDonough 1996).

In oxygen delignification, the most reactive sites in lignin are the phenolic structures, more specifically their phenolate forms in the alkaline solution. Thus, oxidation rate at a given temperature should be controlled by the content of phenolic units, pH, and concentration of oxygen. In the residual lignin of kraft pulp, 25%–40% of the aromatic structures have been reported to be phenolic (Gellerstedt and Lindfors 1984; Lindgren and Lindström 1996; Jiang and Argyropoulos 1999; Moe and Ragauskas 1999). Delignification occurs fast at the beginning of the oxygen stage and decelerates after reaching the 50% reduction level (Lai et al. 1994). The amount of phenolic hydroxyls decreases and the amount of carboxylic acids increases in the O stage (Gellerstedt and Lindfors 1987; Sun and Argyropoulos 1995; Jiang and Argyropoulos 1999; Moe and Ragauskas 1999). Residual lignin isolated from SW kraft pulp (*Picea mariana*, kappa no. 30.9) was oxygen treated by Asgari and Argyropoulos (1998) at elevated temperatures. The quoted authors found that the rate of reduction of phenolic guaiacyl units is considerably slower at 80 and 95°C than at or above 110°C. Furthermore, the degradation of guaiacyl units at temperatures above 100°C proceeds in two distinct phases: an initial rapid decrease observed within 20–40 min is followed by a significantly slower regime.

The changes observed in lignin structure can be explained by the following reaction mechanism. The phenolates react with oxygen: phenoxyl radical is the primary oxidation product and superoxide (radical anion) is the one-electron reduction product of oxygen (Gierer et al. 2001). In secondary reactions, the phenoxyl radicals react with superoxide or oxygen forming quinone and muconic acid type structures (Gierer 1997). Superoxide also undergoes dismutation to oxygen and hydrogen peroxide and hydroxyl radicals are formed via further decomposition reactions. Hydrogen peroxide oxidizes the quinone intermediates further. In the aromatic ring-opening reaction and following hydrolysis, methanol is released from the methoxy group attached to C-3 and/or C-5. The dissociated form of the hydroxyl radical (oxyl anion radical) reacts with the side chain of lignin forming aliphatic acids and α-carbonyl structures (Gierer 1990; Sjöström 1991; Gavrilescu 2005). In summary, all the complex oxidation reactions cause lignin depolymerization and introduce acidic groups in lignin and consequently enhance its dissolution. Oxygen is also consumed in further reactions of the degradation products.

Although oxidation proceeds via ionized phenolic units, only 30%–60% of the phenolic structures are reported to react in oxygen delignification of pulps (Gellerstedt and Lindfors 1991; Sun and Argyropoulos 1995; Moe and Ragauskas 1999; Argyropoulos and Liu 2000; Akim et al. 2001). This was also observed in oxidizing experiments with isolated residual kraft lignin (Asgari and Argyropoulos 1998). One reason for the limited oxidation is the presence of condensed units (e.g., biphenyl units), which are native in wood but which can also be formed during cooking (e.g., diphenylmethane) (Lai et al. 1994; Jiang and Argyropoulos 1999; Argyropoulos and Liu 2000). Such units are fairly stable towards oxidation and are thus enriched in the residual lignin (Hortling et al. 1992; Lai et al. 1994; Argyropoulos and Liu 2000; Akim et al. 2001). The proportion of the condensed phenolic units in SW residual lignin is higher than that in HW residual lignin. The reduction of these structures can be improved by increasing the oxygen partial pressure, and the effect is greater in HW than SW lignin (Sun and Argyropoulos 1995). Syringyl (S) units are believed to be more reactive under conditions of oxygen delignification than guaiacyl (G) units. Yang et al. (2002) suggested that the bleachability of HW kraft pulp with low kappa number is detrimentally influenced by the accumulation of inert G units. Stilbene structures, existing initially in lignin and surviving the cooking, are among the most reactive structures in oxygen delignification (Ljunggren and Johansson 1990; Gellerstedt and Lindfors 1991). In addition to stilbenes, enolether structures are also formed during cooking. The content of enolethers decreases in the O stage, whereas α-carbonyl type conjugated structures are formed (Tamminen and Hortling 2001).

The objective of the present study was to provide detailed information on alkaline oxygen oxidation of SW and HW lignins for the development of a mechanistic model for the pulp oxygen delignification process. Detailed data in this regard are not provided in the literature, even if lignin reactions during oxygen delignification have been studied extensively. Therefore, experiments were performed by applying delignification conditions for the treatment of lignin, without the interference of carbohydrates or extractives. Kraft lignins isolated from black liquors were selected as starting materials, because of the large amounts of lignins needed for the experiments, which could not be isolated from pulp residual lignins. The structures of the dissolved and residual lignin are supposed to be rather similar (Akim et al. 2001; Fu et al. 2005), even though the structural changes induced by cooking are more pronounced in the dissolved lignin. For example, the content of phenolic hydroxyls in dissolved lignin has been measured to be ca. twofold greater than that in residual lignin (Gellerstedt and Lindfors 1984; Robert et al. 1984). Residual lignin composition in the modeling was based on that reported in literature, although its reactions were based on the data obtained from the kraft lignin experiments.

This paper presents the results from the direct analyses of the kraft lignin oxidation experiments. In order to follow the reaction kinetics and determine the relative importance of different reaction routes for the kinetic model, it was necessary to quantify the degradation products in addition to the detection of structural changes in the polymeric lignin. Therefore, the oxidized lignin samples obtained from this study were further characterized by advanced analysis methods to distinguish carboxylic acids bound to lignin macromolecule and those bound to aliphatic acids.

Materials and methods

Experimental

Indulin AT, a softwood kraft lignin (KL_{SW}) and PC1369, a hardwood kraft lignin (KL_{HW}) obtained from MeadWestvaco Corp. (Appomattox, VA, USA) were dissolved in 0.1 M NaOH solution in concentration of 7.5 g l^{-1} corresponding to the content of fiber-bound lignin in the oxygen delignification of high kappa number pulp (ca. $\kappa = 50$) at 10% consistency.

Reactor: 1-l stainless steel high-pressure batch reactor (Parr Instrumental Co., Moline, IL, USA), which was equipped with baffles and a six-bladed turbine-type impeller stirrer, an electric heating jacket, a gas inlet, a liquid sampling tube, and a gas release valve. Parameters: two temperatures (90 and 110°C) and two initial oxygen pressures (0.6 and 0.9 MPa); for operating conditions see Table 1. The preheating was continued for 30 or 90 min to reach the desired temperature. In the experiments at 110°C, the reactor was flushed with nitrogen for displacing air before raising the temperature above 90°C. After preheating, pure oxygen was introduced in the reactor and this was set as the starting point of the oxidation reaction. In all experiments, the volume of the reaction solution was 0.8 l and the impeller was operated at 600 rpm.

Liquid samples of 50 ml were taken periodically during the whole 4-h reaction time (5, 10, 20, 30, 60, 120, 180 and 240 min after the starting point). The stirring was stopped during the sampling to avoid foaming. As the sampling lowered the reactor pressure, immediately after taking the sample more oxygen was fed to the reactor to recuperate the previous pressure. Pressure and temperature in the reactor were recorded during the whole reaction period. The pH of the samples was measured at room temperature. The preparation of both KL_{SW} and KL_{HW} solutions and preheating were repeated in separate experiments for obtaining the first samples (0 min) of the oxidation experiments. The procedure corresponded to the handling

Table 1 Experimental set-up for oxidation of kraft lignins from softwood (KL_{SW}) and hardwood (KL_{HW}).

Exp. #	Kraft lignin	Initial pressure (MPa)	Temp. (°C)	Code
1	SW	0.94	90	0.9 MPa, 90°C, SW
2	SW	0.94	110	0.9 MPa, 110°C, SW
3	SW	0.64	90	0.6 MPa, 90°C, SW
4	SW	0.64	110	0.6 MPa, 110°C, SW
5	HW	0.64	90	0.6 MPa, 90°C, HW

of the actual reaction conditions right before pressuring the reactor. The rate of oxygen dissolution in 0.1 M NaOH solution was investigated in a separate experiment (90°C, 0.6 MPa, without lignin). Furthermore, the formation/presence of hydrogen peroxide in the oxidation mixture was investigated separately (90°C, 0.6 MPa, KL_{SW}). Samples were taken as a function of time and iodometric titration of hydrogen peroxide was performed right after the sampling.

Analytical methods

Both starting lignins were analyzed for metals and sulfur. For the analysis, 0.3 g of sample was liquefied by concentrated acids (6 ml HNO_3, 2 ml H_2O_2, 1 ml HF) for 28 min in a closed vessel in a microwave oven. After dilution, Na and K were analyzed with FAAS and Ba, Ca, Cu, Fe, Mg, Mn, S, V, and Zn were analyzed with ICP-AES. The elemental analysis (C, H, S, O) and the determination of methoxyl content were conducted at Lindlar Analytische Laboratorien, Germany.

The treatment solutions as such were analyzed for several quantities. Total charge giving the sum of all acids in the solution was measured by titration. In the titration the conductivity of the sample was registered as a function of titrate addition (both 0.1 M HCl and 0.1 M NaOH; pH range 2.5–11.5). The total charge of the sample was calculated using the time difference between the equivalent points of acid and base titration curve. The method is a slight modification of the method described by Zakis (1994a). Ionization difference ultraviolet spectroscopy was used to obtain information on the amount of phenolic lignin and conjugated phenols (Zakis 1994b; Tamminen and Hortling 1999). The UV-Vis measurements were performed in alkaline (0.1 M NaOH) and near neutral conditions (pH 6) with a Perkin Elmer Lambda 900 spectrometer (Perkin Elmer, Waltham, MA, USA). Change of the aromatic lignin content during oxidation was followed as UV-lignin at 280 nm based on absorptivity values of 22.4 l g^{-1} cm^{-1} for SW and 22.2 l g^{-1} cm^{-1} for HW kraft lignin (absorptivities determined in the initial concentration). Also, lignin color was followed by measuring the absorbance at 457 nm and 280 nm (A457/A280) at pH 6. The degree of demethylation was followed by detecting the methanol liberated to the liquid phase by a static headspace-GC/FID method by means of Tekmar 7000 (Teledyne Tekmar, Mason, OH, USA) and HP5890 Series II devices (Hewlett-Packard Co., Palo Alto, CA, USA). Headspace method was conducted in conditions of 60°C and 25 min. Methanol concentrations were calculated in relation to external standard using Agilent Chemstation software (Agilent Technologies, Santa Clara, CA, USA). Average lignin molar masses (Mn, Mw) were measured by size exclusion chromatography (SEC): PSS MCX 1000 column and 0.1 M NaOH eluent ($T=25°C$) with UV detection (280 nm). The molar mass distributions and average molar masses were calculated in relation to polystyrene sulphonate standards with Waters Empower 2 software (Waters, Milford, MA, USA). Several standard analyses were conducted from the 0 min and 240 min samples at KCL Kymen Laboratorio Oy, Finland: chemical oxygen demand, COD(Cr), ISO/DIS 15705; total inorganic carbon, TIC giving the amount of carbon dioxide, SFS-EN 1484:1997; total organic carbon, TOC, SFS-EN 1484:1997.

Capillary electrophoresis (CE) analyses were conducted from the liquid samples to detect small molecular acids formed as lignin degradation products. Structural analyses were performed on the acidified (pH 2.5) and freeze-dried samples as described by Rovio et al. (2011).

Determination of the oxygen consumption

Oxygen consumed in the experiments was calculated from the reactor pressure data by applying the ideal gas law equation and the values of water vapor pressure; the dissolved oxygen was taken into account. It was assumed that lignin oxidation did not occur during the short (0.5–1.5 min) sampling periods. Pressure data from the experiment conducted without lignin were used for estimating the rate constant for the gas-liquid mass transfer model.

Results and discussion

In all experiments, during the first 60 min the lignin reactivity was high, and a very intense stage was observed during the first 20 min. This is readily visible from high oxygen consumption (Figure 1a), from pH decrease (Figure 1b), and fast increase in total charge (Figure 1c). The intense beginning was also clearly seen as degradation of aromatic lignin (Figure 1d) and decreasing content of free phenols (Figure 1e) with a simultaneously increasing proportion of conjugated phenols (Figure 1f). The fast increasing methanol concentration (Figure 1g) correlated with the degradation of aromatic lignin and decreasing content of free phenols. The greatest change in lignin color occurred during the first 30 min, however only in the case of hardwood kraft lignin (Figure 1h). Monitoring the average molar mass of lignin macromolecule (Figure 1i) clearly demonstrates that depolymerization is intense at the beginning of the reaction. According to all observed data, the reactions decelerated after the first 60 min period. After 120 min, the reactions did not significantly advance. Elevating the reaction temperature from 90°C to 110°C increased the oxidation rate of the process, which is in accordance with the general kinetic theory. The effect of pressure was not as significant as that of temperature.

Composition of the starting lignins

The metal analysis results of the starting lignins are presented in Table 2. Elemental compositions and methoxy contents are presented in Table 3. For KL_{SW}, the methoxy content was a bit higher than that reported previously, e.g., by Thring and Griffin (1994), but for KL_{HW}, the methoxy content (26.1%) was surprisingly high when compared both to a recently obtained value of 17.8% (Kubo and Kadla 2006) and also to the S/G ratio (1.28) obtained from the pyrolysis results of the starting KL_{HW} (Rovio et al. 2011). The reason for this is not known. The summative formulae for 100 basic units of KL_{SW} and KL_{HW} (given in Table 3) were computed on the basis of elemental composition and methoxy content. The respective average molecular weights of lignin phenyl propane units were 181 g mol^{-1} and 216 g mol^{-1}, respectively. Metals in the initial KL_{SW} and KL_{HW} were assumed not to react with oxygen and their share (in total 0.85% and 2.37%; computed from the data given in Table 2) was taken into account in estimating the oxygen consumption by molar lignin to correct the results to the actual lignin content.

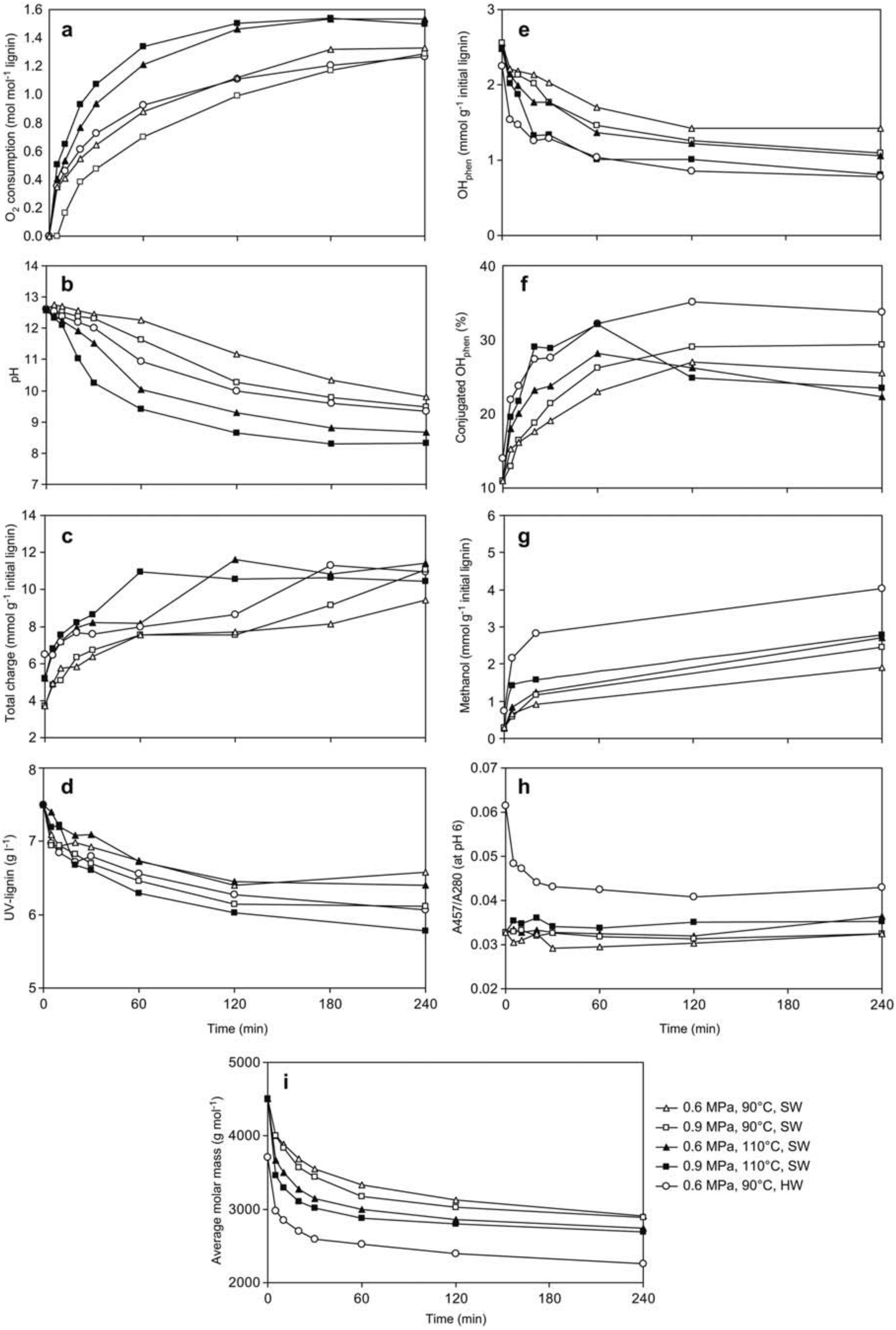

Figure 1 (a–i) Changes of the data indicated on the y-axis as a function of reaction time of oxygen delignification.

Table 2 Metal contents of the initial kraft lignins from softwood (SW) and hardwood (HW).

	SW kraft lignin		HW kraft lignin	
Element	mg kg^{-1}	SD	mg kg^{-1}	SD
Sodium (Na)	7194	±225	20 940	±324
Potassium (K)	894	±106	1336	±17
Barium (Ba)		<2	19	±0.1
Calcium (Ca)	102	±2	659	±20
Copper (Cu)		<2		<2
Iron (Fe)	40	±2	100	±1
Magnesium (Mg)	151	±4	416	±7
Manganese (Mn)	43	±1	130	±2
Vanadium (V)	53	±1	87	±1
Zinc (Zn)	7.6	±0.6	12	±1

Oxygen consumption and pH

About half of the oxygen was consumed during the first 20 min in oxidizing KL_{SW} at 110°C under 0.6 MPa and KL_{HW} at 90°C under 0.6 MPa. In oxidizing KL_{SW} at the harshest conditions (110°C, 0.9 MPa) the relation was almost 60% (Figure 1a). The maximum in oxygen consumption, 1.5 mol mol^{-1} KL_{SW}, was reached in 3 h at 110°C. In the experiments conducted at 90°C, the consumption reached in 4 h was slightly less (1.3 mol for mol^{-1} KL_{SW} and for mol^{-1} KL_{HW}). In computing the consumed O_2 based on the reactor pressure decrement, it was assumed that the progress of the O_2 consuming reactions halted during the short sampling periods. This can explain why the obtained values are slightly smaller than the values obtained by Imai et al. (2008), who measured oxygen consumption of KL_{SW} under oxygen-alkali conditions (95°C, 0.8 MPa) and observed an O_2 consumption of 1.8 moles by 200 g lignin (ca. 1 mol lignin) over 4 h.

Here, the mass stoichiometry of oxygen consumption at the end of the reaction corresponded to 0.23–0.27 g O_2 by 1 g KL_{SW}. In industrial O stage, the oxygen consumption has been estimated to be 1 g O_2 by 1 g residual lignin in the course of modeling the industrial oxygen delignification (van Heiningen et al. 2003; Sixta et al. 2006). Also, the O_2 consumption of organic carry over (0.79 g O_2 by 1 g DOC, dissolved organic carbon) is considered in the model. The inorganic reactions that consume oxygen are likely to be included in these estimates as their contribution could not be considered separately. Untreated white liquor, an alkali source in the oxygen stage, is known to reduce the degree of delignification when compared to the usage of oxidized white liquor or sodium hydroxide. This is reported to be due to the presence of the sulfide (Sixta et al. 2006). Analogously, the residual sulfide that enters the O stage within kraft brownstock reduces the degree of delignification because its oxidation potential is partly wasted for oxidation of sulfide ion (HS^-) to sulfate (SO_4^{2-}) instead of residual lignin. Therefore, the difference in O_2 consumption between KL oxidation and industrial pulp oxygen delignification can be partly due to the O_2 consumption of sulfide carry over caused by insufficient brownstock washing and/or incomplete oxidation of white liquor in industrial applications. For instance, in a survey concerning process and design information of industrial oxygen delignification and the following data analysis (Bennington and Pineault 1999), the black liquor carry over to the O stage remained unquantified because of the lack of data reported by the mills surveyed.

Table 3 Elemental composition and methoxy content of the initial kraft lignins from softwood (KL_{SW}) and hardwood (KL_{HW}).

Sample	C (%)	H (%)	S (%)	O (%)	OCH_3 (%)
KL_{SW}	62.66	5.84	1.33	30.17	14.37
KL_{HW}	57.48	5.59	2.09	34.84	26.14
	Summative formulae				
KL_{SW}	$C_{850}H_{784}S_7O_{270}(OCH_3)_{83}$				
KL_{HW}	$C_{850}H_{652}S_{14}O_{288}(OCH_3)_{182}$				

The summative formulae are calculated for 100 basic units of lignin. The C_{850} basis (instead of C_{900}) takes the partial loss of side-chain carbons during the kraft cooking into account (Marton 1971).

In all experiments, the pH was about 12.6 at the onset of the reaction (0 min). At the end of the 4-h reaction, the pH was 2.8–4.3 units lower than at the beginning (Figure 1b). As the temperature was raised, the oxygen consumption was higher and the final pH lower, indicating higher degradation of lignin macromolecule accompanied by higher generation of aliphatic acids. The dominating aliphatic acids in reaction solutions detected by Rovio et al. (2011) were formic, oxalic, acetic, and glycolic acids. Elevating reaction temperature increased the acid formation, especially during the first 60 min, which covered more than half of the total amount of acids formed (Rovio et al. 2011, Figure 1). The pH decrease ceased after the aliphatic acid formation decelerated. Also, in that stage of the reaction, presumably some of the low molecular weight acids have already oxidized to CO_2 and water. At 90°C, especially during the first half of the reaction period, the pH in KL_{HW} solution was clearly lower than in KL_{SW} solution. Correspondingly, during that period more oxygen was consumed in the reactions (Figure 1a) and more aliphatic acids were formed (Rovio et al. 2011, Figure 1) with KL_{HW} than with KL_{SW}. In previous studies, the magnitude of pH decrease in oxidizing residual KL_{SW} (Asgari and Argyropoulos 1998) or alkaline lignin in waste water treatment (Kindsigo and Kallas 2006; Kindsigo et al. 2009) has been reported to be linearly proportional to the reaction temperature.

Total charge

The total charge developed in a logical manner: raising temperature or raising pressure increased the total charge (Figure 1c). The fast increase in total charge was mainly due to high formation of both lignin macromolecule bound acids and small aliphatic acids (Rovio et al. 2011). The charge formation during the 4-h reaction in the SW case was 5.2–7.3 mmol g^{-1} initial lignin and in the HW case 4.5 mmol g^{-1}. According to the UV method, during the same period the OH_{phen} content decreased 1.1–1.7 mmol g^{-1} and 1.5 mmol g^{-1}, respectively (results discussed hereinafter).

The interpretation is that four carboxylic acid groups were formed from one phenolic KL_{SW} basic unit and three from one KL_{HW} unit. The observation strongly indicates that the muconic acid type structures reacted further.

TOC, COD, and TIC

Lignin degradation was also followed by TOC (total organic carbon), COD (chemical oxygen demand) and TIC (total inorganic carbon) analysis, which are typical for characterizing pulp mill effluents. The analysis results obtained at 0 and 240 min reaction time are shown in Table 4.

Raising temperature reduced TOC. Raising pressure at 90°C reduced TOC, but did not have an impact at 110°C. In the SW case no great differences were detected in COD reduction, although the COD value obtained under the mildest conditions was highest, i.e., material was least oxidized. For KL_{HW} solution, both the TOC and the COD reductions were greater than for KL_{SW} solution under similar conditions.

Carbon dioxide is formed in the further oxidation reactions of lignin degradation products. According to TIC analyses, in the SW case, 7.0%–8.2% of the carbon from the initial lignin ended up in CO_2. For KL_{HW} this ratio was 8.4%. Increasing both temperature and pressure reduced CO_2 formation slightly. This is probably due to the greater reactivity of phenolic lignin under harsher conditions less favoring the oxidation of aliphatic acids and formation of CO_2. Zhang et al. (2007) measured carbonate formation in oxygen delignification of pine kraft pulp (initial kappa 32.5, 0.8 MPa, 60 min residence time) and reported a value of 13.2 mM, which is ca. half of the formation detected here at the end of the 4-h reaction.

Degradation of aromatic lignin and formation of methanol

One good indicator of the degradation of aromatic structures is the formation of methanol because most lignin precursors carry methoxy groups, which are released as methanol in the course of the formation of muconic acid type structures and in the further oxidation of quinones. Therefore, in addition to the detection of aromatic lignin content (Figure 1d) and its functionality (Figure 1e and 1f) by UV-method, the concentration of liquid phase methanol (Figure 1g) was followed.

According to the UV method, 12%–23% of the aromatic structures were degraded during the 4-h reaction period, with the lowest degradation observed for KL_{SW} under the mildest conditions. At the onset of the reaction, the OH_{phen} content was 2.5–2.6 mmol g^{-1} initial lignin (KL_{SW}) and 2.3 mmol g^{-1} initial lignin (KL_{HW}). During the reaction, the OH_{phen} content decreased 44%–67%. The decline is in line with the results obtained by Asgari and Argyropoulos (1998), who oxidized residual KL_{SW}, but detected the OH_{phen} content by ^{31}P NMR. Raising both temperature and pressure reduced the OH_{phen} content. Also, in pulp oxygen delignification, the reduction is related to the severity of the oxygen stage, but even under very harsh conditions, the residual lignin after the stage still contains a considerable amount of phenolic groups (Roost et al. 2003).

Simultaneously with the lowering content of OH_{phen}, the proportion of conjugated OH_{phen} was increased. These conjugated structures were mainly α-carbonyl structures detected by Pyrolysis-GC/MS (Rovio et al. forthcoming). At 110°C, after the first 60 min the proportion of conjugated OH_{phen} clearly decreased and at the end of the reaction the level was lower than in the oxidations conducted at 90°C. This might be due to further reactions of vanillin-like structures at the higher temperature (Gierer and Imsgard 1977). Simultaneous removal of phenolic guaiacyl units and formation of new phenolic α-carbonyl units have been reported in the previous studies (Pasco and Suckling 1998; Yada and Meshitsuka 2007).

For KL_{SW}, 3.1%–4.8% of the carbon from the initial lignin ended up in methanol during the 4-h reaction period. The ratio was 6.9% for KL_{HW}. The molar based reduction of

Table 4 Results of TOC, COD(Cr), and TIC analyses (mg l^{-1}); change (%) between 0 and 240 min.

Parameter of oxigenation	Measured data	TOC, COD, and TIC (mg l^{-1})		$\Delta_{0\text{-}240}$ Change (%)
		At 0 min	At 240 min	
0.9 MPa, 90°C, SW	TOC	4600	3800	-17
	COD	13 000	11 000	-15
	TIC	97	450	364
0.9 MPa, 110°C, SW	TOC	4700	3700	-21
	COD	13 000	11 000	-15
	TIC	100	430	330
0.6 MPa, 90°C, SW	TOC	4600	4100	-11
	COD	13 000	12 000	-8
	TIC	97	480	395
0.6 MPa, 110°C, SW	TOC	4700	3700	-21
	COD	13 000	11 000	-15
	TIC	100	460	360
0.6 MPa, 90°C, HW	TOC	4000	3500	-13
	COD	12 000	9500	-21
	TIC	140	500	257

OH_{phen} was much more modest than the molar based formation of methanol. This might be because the KL was extensively modified so that OH_{phen} could not be properly detected by the UV method, which is not recommended for highly modified lignins (Zakis 1994b). The values given by the UV method are too low for OH_{phen} for modified lignins and for model compounds with more than one aromatic hydroxyl group (Tiainen et al. 1999). However, parallel results obtained by ^{31}P NMR (Rovio et al. 2011) were more plausible, giving ca. 1.5 fold higher concentrations for the initial OH_{phen}, and also giving better agreement with the methanol release. In oxidizing KL_{SW} (Yada and Meshitsuka 2007) under milder conditions than here and in oxygen delignifying residual KL_{SW} (Akim et al. 2001), a slightly smaller formation of methanol or a decrease in methoxy content have been observed. The methanol concentration (1.5 kg t^{-1} pulp) detected during oxygen delignification of SW pulp (kappa no. 37.5) (Pfister and Sjöström 1979) corresponds to formation of 0.83 mmol g^{-1} lignin which is ca. half of the formation measured here.

Lignin color and average molar mass

The main goals of the kraft pulp bleaching sequence are to remove residual lignin and improve pulp brightness. Lignin color observed at pH 6 (Figure 1h) revealed that strong degradation of chromophoric structures in KL_{HW} occured during the intense beginning of the oxidation. The corresponding development was not observed for KL_{SW}, however, nor was darkening.

The average molar masses of the starting lignins were 4500 g mol^{-1} (KL_{SW}) and 3700 g mol^{-1} (KL_{HW}) (Figure 1i). Reduction in the molar mass of KL_{SW} macromolecule represented a 35% decline in the oxidations conducted at 90°C and a 40% decline in the oxidations conducted at 110°C. For KL_{HW}, the decline was 40%. The results clearly demonstrate that oxidation also degrades ether bonds in lignin.

Conclusions

The results correspond well to general theories regarding the lignin oxidation mechanism, and are also in agreement with the knowledge that hardwood kraft lignin is more reactive than softwood kraft lignin. Increasing the reaction time to over 120 min did not further enhance the lignin degradation. Increasing the reaction temperature, rather than the pressure, had primary significance in increasing the rate of lignin degradation. The results indicate that there is a rather big difference in O_2 consumption between kraft lignin oxidation and residual lignin oxidation in industrial delignification. This was interpreted to be partly due to the O_2 consumption of sulfide carry over in industrial applications, which wastes the oxidation potential.

The samples obtained from this study will be further characterized with advanced analysis methods to distinguish carboxylic acids bound to lignin macromolecule and those bound to the small degradation products of lignin (Rovio et al. forthcoming). The data both from the direct measurements and advanced analyses will be used to validate a predictive model for lignin oxidation chemistry on the molecular level (Kuitunen et al. 2011).

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PUBLICATION II

Reducing the content of VOCs of softwood kraft lignins for material applications

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REDUCING THE CONTENT OF VOCS OF SOFTWOOD KRAFT LIGNINS FOR MATERIAL APPLICATIONS

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Three laccases, functioning in mild acidic, and one in slightly alkaline conditions, were evaluated in order to reduce low-molecular phenolic VOCs of kraft lignins, which could be used in lignin/natural fibers composites. The potential of a sulfhydryl oxidase to catalyze the oxidation of sulfur containing VOCs (thiols) was also tested in combination with the laccase-catalyzed oxidation. In addition, oxidation at alkaline pH at room temperature that may induce polymerization of phenolics in an analogous manner to the laccase-catalyzed reaction was investigated. Enzyme reactivity towards lignin was evaluated as consumption of oxygen in the reaction solution. The effect of treatments on VOC reduction was determined both by sensing (odorimetry) and chemical (TD-GC/MS, SEC) analyses. Laccases, Lcc2, and MaL from *Thielavia arenaria* and *Melanocarpus albomyces*, respectively, showed potential in reducing odors. The most promising results were obtained by oxidizing lignin with O_2 at alkaline pH. However, the odor threshold values of the main VOC compounds are extremely low, which poses a challenge to VOC reduction.

Keywords: VOC; Kraft lignin; Composite; Laccase; Sulfhydryl oxidase; Oxygen; Oxidation

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INTRODUCTION

The potential of lignin to replace oil-based raw materials is being actively investigated for various material applications, such as composites. Based only on renewable resources, the Arboform® composites consist of isolated lignin, natural fibers, and natural additives, which are used in conventional thermoplastic processes such as injection molding (Naegele *et al.* 2002). However, there are several difficulties in applying lignin. One of them is the volatile organic compounds (VOCs), either present in the isolated lignin, or formed as they are processed at high temperature in thermoplastic processes. This drawback reduces the applicability of lignin-based composites, *e.g.* in the inner parts of cars.

VOCs in kraft lignin are typically lignin-originated phenolic structures, *e.g.* guaiacol (2-methoxyphenol), or reduced sulfur compounds formed in cooking. The odor threshold values of these VOCs are extremely low. For example, the odor threshold value for guaiacol in water solutions is reported to vary from 3 to 21 ppb (Fazzalari 1978; Buttery *et al.* 1988; Guth 1997). The great variation is due to different measurement techniques. For reduced sulfur compounds, dimethyl disulfide, and dimethyl trisulfide, the threshold values are reported to be 5 and 0.2 ppb, respectively (Guth 1997; Zoeteman *et al.* 1973).

Recently, small amounts of organically modified montmorillonite (silicate clay-based) dispersed into the lignin/natural fibers system have been shown to improve the mechanical and thermal behaviors. Thermogravimetric analysis results suggested that exfoliated or intercalated layered aluminosilicates acted as a protective barrier against degradation of organic components. The thermooxidative degradation of the organic compounds (lignin, natural fibers, and additives) occurred in the temperature range of 180 to 420°C (Guigo *et al.* 2009).

Laccases catalyze the oxidation of a wide range of aromatic substrates, especially phenols, simultaneously with the reduction of molecular oxygen to water. Phenolic substrates are oxidized to phenoxyl radicals, which, depending on reaction conditions, can spontaneously polymerize via radical coupling or rearrange themselves leading to quinones, alkyl–aryl cleavage, Cα oxidation, cleavage of Cα–Cβ bonds, or aromatic ring. Mediators, acting as intermediate substrates for laccases, enable laccase to indirectly oxidize large molecules, and even nonphenolic substrates (Giardina *et al.* 2010). Polymerization of lignosulfonates by laccases with and without mediators in varying reaction conditions has been confirmed recently (Areskogh *et al.* 2010; Prasetyo *et al.* 2010). Sulfhydryl oxidases catalyze the formation of de novo disulfide bonds from free thiol groups, with the reduction of molecular oxygen to hydrogen peroxide. These enzymes have been investigated in the food industry to improve the flavor of products such as ultraheat-treated milk and aromatic beverages such as coffee, tea, chocolate, wine, and beer (Starnes *et al.* 1986; Swaisgood 1977).

The objective of this study was to evaluate different approaches aiming to reduce VOCs of softwood kraft lignins. Laccase-catalyzed oxidation was tested as a potential means to polymerize the lignin-derived low-molecular phenolics such as guaiacol. Since the solubility of lignin increases significantly as pH increases and favors laccases that are active at higher pH, a laccase with the pH optimum at 8 was evaluated in addition to three other laccases functioning in mild acidic conditions. Sulfhydryl oxidase-catalyzed oxidation was tested as a means to reduce the odor from sulfur-containing compounds, *e.g*. thiols (methyl mercaptan, CH_3SH). As a straightforward way of reducing VOCs, oxidations at alkaline pH and at room temperature were investigated. The idea of the treatment was based on the hypothesis that at ambient temperatures the reaction kinetics will be slower than at elevated temperatures, causing phenoxyl radicals to be relatively stable, which may induce polymerization of phenolics in an analogous manner to the laccase-catalyzed reaction. TD-GC/MS, is presented as a novel method to simulate and quantify the VOC formation in the temperature range of thermoplastic processing.

EXPERIMENTAL

Materials

Two softwood kraft lignins, commercial Indulin AT (Mead WestVaco), and non-commercial SE (Stora Enso) were used as raw materials.

Four laccases were tested: *Thielavia arenaria* Lcc1 (TaLcc1) and Lcc2 (TaLcc2) (Paloheimo *et al.* 2006a and b) produced as recombinant enzymes in *Trichoderma reesei* by Roal Oy (Rajamäki, Finland), *Trametes hirsuta* (ThL) (produced in its native host by VTT), and *Melanocarpus albomyces* (MaL) (overproduced in *Trichoderma reesei* by VTT). TaLcc1, TaLcc2, and ThL are most active in mild acidic conditions, whereas MaL, in slightly alkaline conditions.

The mediator 1-hydroxybenzotriazole (HBT) was used with a dose of 5 percent with respect to lignin, in addition to ThL and TaLcc2 in two experiments. Sulfhydryl oxidase, *Aspergillus oryzae* (AoSOX1) (Faccio *et al.* 2010), produced in *Trichoderma reesei* by VTT was also tested.

Methods

Lignin dissolution

Lignin suspensions for enzyme treatments were prepared by dissolving lignin first into alkaline water, after which pH was decreased using 1 M HCl, and finally set to the target pH using 0.05 M sodium citrate buffer in the case of pH 5 and 6, and 0.05 or 0.2 M sodium phosphate buffer in the case of pH 8. In general terms, the lignin dissolution was performed according to Mattinen *et al.* (2008), however, using NaOH of higher molarities when increasing the lignin dry solids above 0.1 percent. For oxidation at alkaline pH, only a small amount of HCl was needed to decrease pH to 10 or 10.7 after dissolving lignin in NaOH.

Enzyme activity measurements

2,2'-azino-bis-(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS) (Roche) was used as a substrate for laccases in spectrophotometric enzyme activity measurement at pH 5 and 6, and guaiacol (Fluka) at pH 8. Katal units were used to express the catalytic activity of the laccase preparations (nkat/mL).

One katal of laccase is that amount of laccase which converts/oxidizes a mole of substrate per second under the specified conditions. Laccases were dosed on an activity basis with respect to the lignin content (nkat/g). 1 nkatal (10^{-9} mol/s) corresponds to 0.0600 enzyme units (U, 1 μmol/min), another commonly used unit expressing the catalytic activity. AoSOX1 was dosed on protein basis in respect to the sulfur content of lignin (2.2 percent).

Treatments

At the analytical scale, the reactivity of enzymes towards the lignin was evaluated by monitoring oxygen consumption in the reaction solution. Lignin dry solids were 1, 2.5, or 5 percent. Monitoring was performed with an Oxy-10 mini sensor oxygen meter (PreSens, Germany) in a closed 1.9 mL vessel based on dynamic luminescence quenching. Parallel treatments were performed.

Selected treatments with promising enzyme dosages were repeated at a larger laboratory scale in a 2-L Parr reactor to allow the use of sensing analysis (odorimetry). Treatments were done at 5 or 10 percent lignin dry solids, and under 5 bars initial oxygen pressure at room temperature (RT) for 1 or 2 hours (Table 1).

Reference samples (pH 5, 6, and 8, samples 1, 7, 10, and 12, respectively), were prepared according to the dissolution-pH adjustment procedure at room temperature (not O_2 boosted).

Table 1. Experimental Set-up of the Oxidative Treatments Performed with and without Enzyme in 2-L Parr Reactor under 5 Bar Initial O_2 Pressure at RT. Reference samples (ref.) were not O_2 boosted.

Sample	Substrate[a]	Lignin dry solids (%)	pH	Enzyme[b]	Dosage[c] (nkat/g ; %)	HBT (%)	Treatment (h)	
1	SE	5	5	-	-	-	-	ref.
2	SE	5	5	-	-	-	2	
3	SE	5	5	ThL	100	-	2	
4	SE	5	5	ThL	100	5	2	
5	SE	5	6	TaLcc2	100	-	2	
6	SE	5	6	TaLcc2	100	5	2	
7	Ind AT	5	5	-	-	-	-	ref.
8	Ind AT	5	5	-	-	-	2	
9	Ind AT	5	5	ThL	100	-	2	
10	SE	10	8	-	-	-	-	ref.
11	SE	10	8	-	-	-	1	
12	SE	10	8	MaL	0.1	-	1	
13	SE	10	8	MaL, AoSOX1	0.1, 0.1	-	1	
14	SE	10	10.7	-	-	-	1	

[a] Lignins: SE = Stora Enso lignin, Ind AT = Indulin AT

[b] Laccases: ThL = *Trametes hirsuta*, TaLcc2 = *Thielavia arenaria* Lcc2, MaL = *Melanocarpus albomyces*, Sulfhydryl oxidase: AoSOX1 = *Aspergillus oryzae*

[c] ThL and TaLcc2 activity measurements with ABTS; doses nkat/g. MaL and AoSOX1doses on protein bases; MaL dose in respect to lignin, AoSOX1 dose in respect to sulfur in lignin.

Analyses

In the odorimetry procedure, a wet sample volume containing 5 g of lignin dry solids was placed in a small crucible and stabilized inside closed glass containers (500 mL) for 48 hours at 40ºC. Thereafter, the odor panel members (ten qualified experts) evaluated the odor intensity of each sample using a scale of 1 to 6, which was selected according to VDA-270 (1992) recommendations. The stabilized samples were also graded using numbers 0 to 10 by individual perception. The least odor-intense sample got number 0 and the most odor-intense was assigned number 10. The panel was able to evaluate a maximum 6 of the samples at a time. Three separate sessions were conducted. In general terms, the odorimetry method was a modification combining sensing analyses described by Söderhjelm and Pärssinen (1985) and Morvan *et al.* (2003).

The thermal desorption (TD) method was developed based on the earlier used method (Kleen *et al.* 2003) in order to simulate the formation of volatile degradation products at elevated temperatures prevailing in the injection molding. TD measurements were carried out with a Pyrolab pyrolyzer unit (Pyrolab2000®) connected to a Varian 3800 gas chromatography/Varian Saturn 2000 mass spectrometer. About 2.5 mg of the freeze-dried sample was weighed accurately and heated at 150 and 190ºC for 5 minutes in a pyrolysis chamber. Thereafter, volatiles were led to the gas chromatography column (J&W, DB-1701, 30 m x 0.25 mm, film 1 µm) for separation. The oven was programmed as follows: initial temperature 100°C, rate of increase 4°C/min to 265°C and held for 18 min at the final temperature. A constant carrier gas flow of 0.9 ml/L was used. The mass spectra of the products were obtained using an ion trap mass spectrometer (EI 70 eV). The scan range of m/z 46-650 was used. Sulfur and phenol compounds were tentatively identified using commercial mass spectra library Nist05. Quantification of guaiacol was performed with an external standard calibration (guaiacol, 98 percent, Aldrich).

Average lignin molecular size classes (M_n, M_w) of the freeze-dried samples were measured by size exclusion chromatography (SEC), using PSS MCX 1000 and 100 000 columns in 0.1 M NaOH eluent (25°C) with UV detection (280 nm). The molecular size distributions and average molecular sizes were calculated in relation to polystyrene sulfonate standards, using Waters Empower 2 software.

RESULTS AND DISCUSSIONS

Laccase Reactivity towards Lignins

At analytical scale, the reactivity of laccases (ThL, TaLcc1, TaLcc2) functioning in mild acidic conditions was tested towards both kraft lignins by following the reduction of dissolved O_2 in the reaction solutions. At pH 6, ThL and TaLcc2 showed higher reactivity towards lignins, as the curves of O_2 consumption were steeper than in the case of TaLcc1 (Fig. 1a). Although the dissolution-pH adjustment procedure (Mattinen *et al.* 2008) is intended to reduce the agglomerates, lignin is mainly undissolved at pH 6, since the dissociation of guaiacyl- and syringyl-derived phenols occurs at higher pH levels (Ragnar *et al.* 2000). In order to increase the dissolution and the reactivity of lignin, the treatment was performed at pH 8 by inclusion of MaL with the respective pH optimum. At pH 8, lignin already reacted with the dissolved oxygen present in the reaction solution. However, more O_2-consuming reactions occurred in the presence of TaLcc1, TaLcc2, and MaL than in their absence (Fig. 1b). The enzyme dosing was based on guaiacol activity since lignin resembles guaiacol by its chemical structure and has similar behavior in terms of pH. Even if the activity of MaL (to guaiacol) was lowest, *e.g.* MaL 67.1 nkat/mL, TaLcc1 111.2 nkat/mL, TaLcc2 339.6 nkat/mL, it catalyzed lignin oxidation more efficiently than TaLcc1 or TaLcc2 at pH 8. Sulfhydryl oxidase (AoSOX1, 1 percent dose in respect to sulfur in lignin) was tested together with MaL.

However, the O_2 consumption did not increase when compared to MaL treatment done without AoSOX1 (Fig. 1b). From the practical point of view, a 0.1 percent dose of MaL already showed potential in treating lignin.

In addition to the laccase- (and AoSOX1) catalyzed oxidation, the oxidation of SE lignin was followed in alkaline conditions (pH 10), which causes the dissociation of the phenolic structures. Increasing pH from 8 to 10 increased O_2 consumption and indicated improved lignin reactivity when compared to MaL-catalyzed oxidation (Fig. 2).

Lignin Odor and Volatiles Formation

Based on the results obtained from the O_2 consumption measurements, selected laccase treatments and oxidation at alkaline pH were performed at higher lignin dry solids and under oxygen pressure (Table 1). The pH level for the alkaline oxidation was set to 10.7, according to experimentally determined pK_0 value of Indulin AT (at 21°C) (Norgren and Lindström 2000).

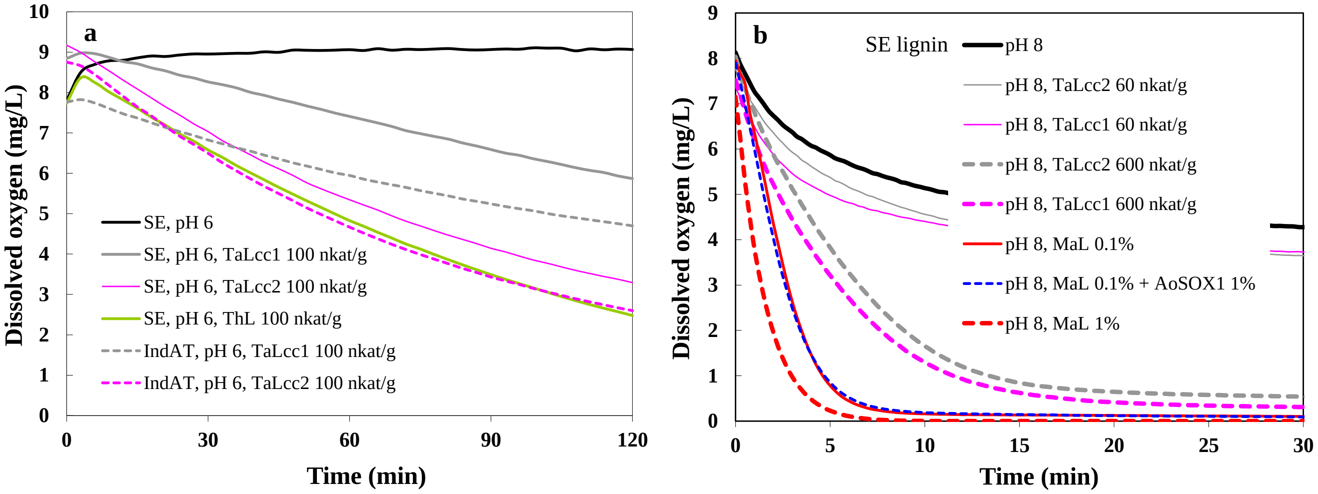


Fig. 1. a) Oxygen consumption of SE lignin and Indulin AT solution (ds. 1%) with and without laccase at pH 6. **b)** Oxygen consumption of SE lignin solution (ds. 2.5%) with and without laccase (and AoSOX1) at pH 8. 0.1% MaL dose equals to 31 nkat/g, and 1% MaL dose equals to 310 nkat/g. 1% AoSOX1 dose with respect to sulfur in lignin

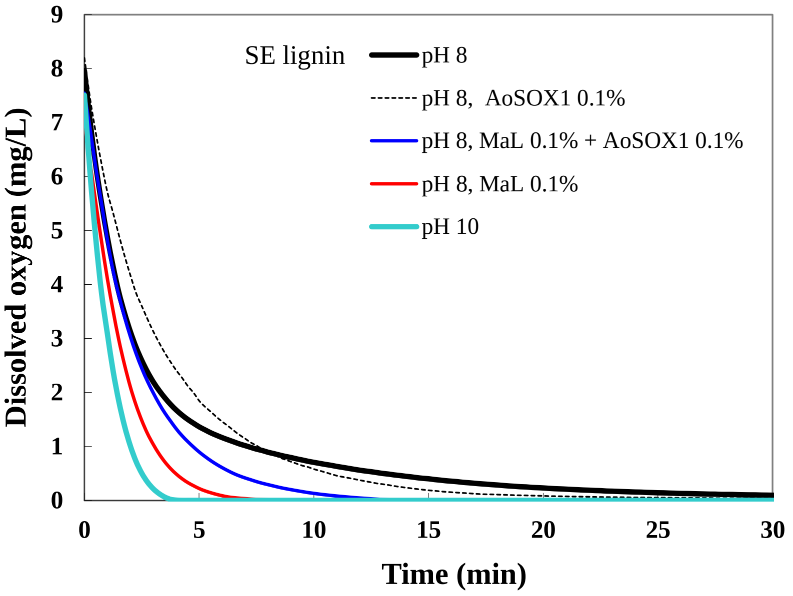


Fig. 2. Oxygen consumption of SE lignin solution (ds. 5%) with and without laccase (and AoSOX1) at pH 8, and oxidation at pH 10. 0.1% MaL dose equals to 57 nkat/g

The overall odor intensity of the lignin samples (Table 1) was evaluated by ten human sniffers (the odor panel). The odor intensity of the samples varied between 2.9 and 3.9 (4 = disturbing, 3 = clearly perceptible), and standard deviations were rather large (Fig. 3 upper panel). Although the odor of the samples differed only slightly, the oxidation by TaLcc2 and MaL+AoSOX1 and the oxidation at pH 10.7, indicated reduced VOC content, with the lowest odor intensity values *e.g.* 2.9 (Sample 5), 3.2 (Sample 13), and 3.0 (Sample 14), respectively. Grading the lignin samples using numbers from 0 to 10 (0 = the least odor intense, 10 = the most intense odor) supported the same observations (Fig. 3 lower panel).

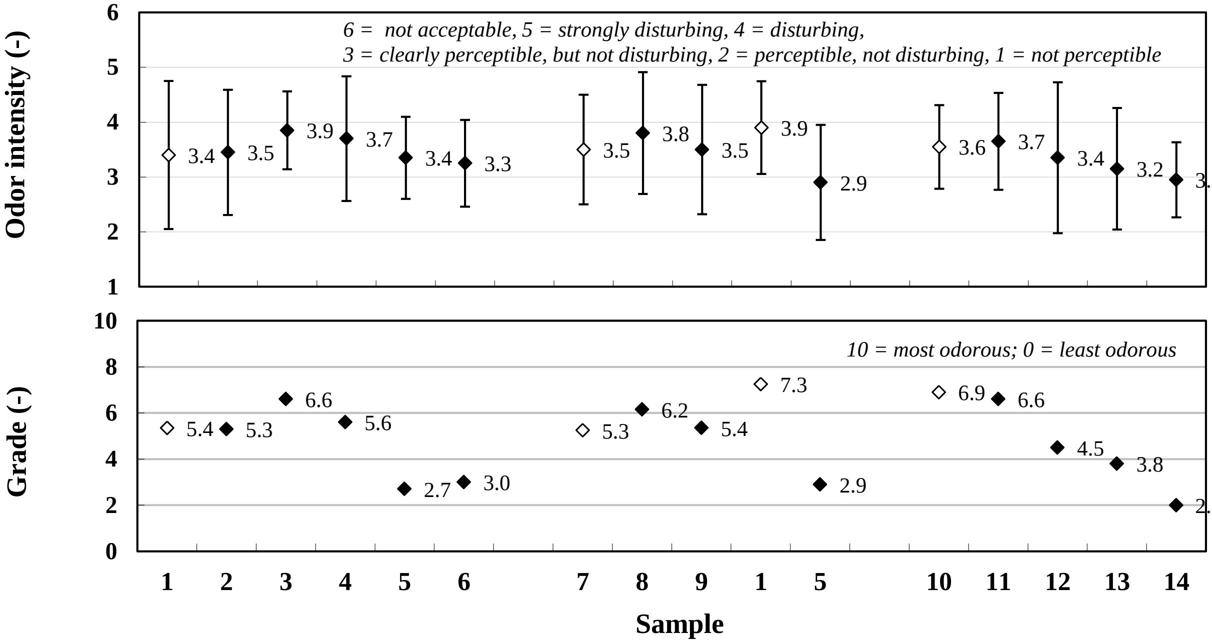


Fig. 3. Odor intensity (upper panel) and grade (lower panel) of the wet SE lignin and Indulin AT samples treated with and without laccases (and AoSOX1) at different pH level. Samples are numbered according to Table 1; open dots refer to reference samples that were not O_2 boosted. Evaluation of samples 1 and 5 was conducted in the first and second separate odor panel

Volatile degradation products of the freeze-dried lignin samples (SE lignin treated with or without MaL/MaL+AoSOX1, and at pH 10.7) were determined by TD-GC/MS at 150 and 190ºC. Lignin softening takes place typically at 150ºC, after which an increase in temperature induces depolymerization and recondensation (Bergmann *et al.* 2005). 190ºC represents the temperature of the latter range, and also resembles the temperature at thermoplastic processing.

The main part of the volatiles had already evaporated within 5 minutes at both temperatures. The most abundant degradation product of lignin was guaiacol (Fig. 4). Clearly more volatiles were formed at 190ºC than at 150ºC. In the case of reference (pH 8, ref.) and O_2 treated (pH 8, 0%) samples, the sum of main volatiles was *ca.* 5-fold more at 190ºC than at 150ºC. In the case of MaL or MaL+AoSOX1 treated lignin, it was *ca.* 3.5-fold more, and in lignin oxidized at pH 10.7 it was less than 2-fold more at 190ºC than at 150ºC.

At both temperatures, clearly less volatiles were formed as SE lignin was oxidized at pH 10.7, than treated with or without MaL/MaL+AoSOX1 at pH 8. At least three explanations for reduced formation of volatiles of alkali-oxidized lignin may exist. Firstly, in the freeze-dried alkaline sample, the low-molecular phenolics most likely existed in sodium-salt form, which hindered their volatilization. Secondly, part of the phenolics might have degraded to non-volatile structures, which is unwanted, since in composite applications the molecular size presumably correlate with strength properties. Thirdly, polymerization/radical coupling of small-molecular phenolics, *e.g.* guaiacol, could have taken place.

Also, the oxidation at pH 10.7 reduced the amount of sulfur containing VOCs. In these conditions, they might have degraded and further oxidized to sulfate. Furthermore, the dissociation of thiols occurs at alkali pH levels, *e.g.* the pK_a value of methanethiol is 10.33 (Steward 1985), and thus the existence as sodium-salt may have hindered their volatilization.

The quantification of the main volatile, guaiacol, showed that its amounts in the SE lignin samples were still rather high when compared to the odor threshold values measured in water solutions, *i.e.* 1.3 ppm at 150ºC and 24 ppm at 190ºC (Fig. 5).

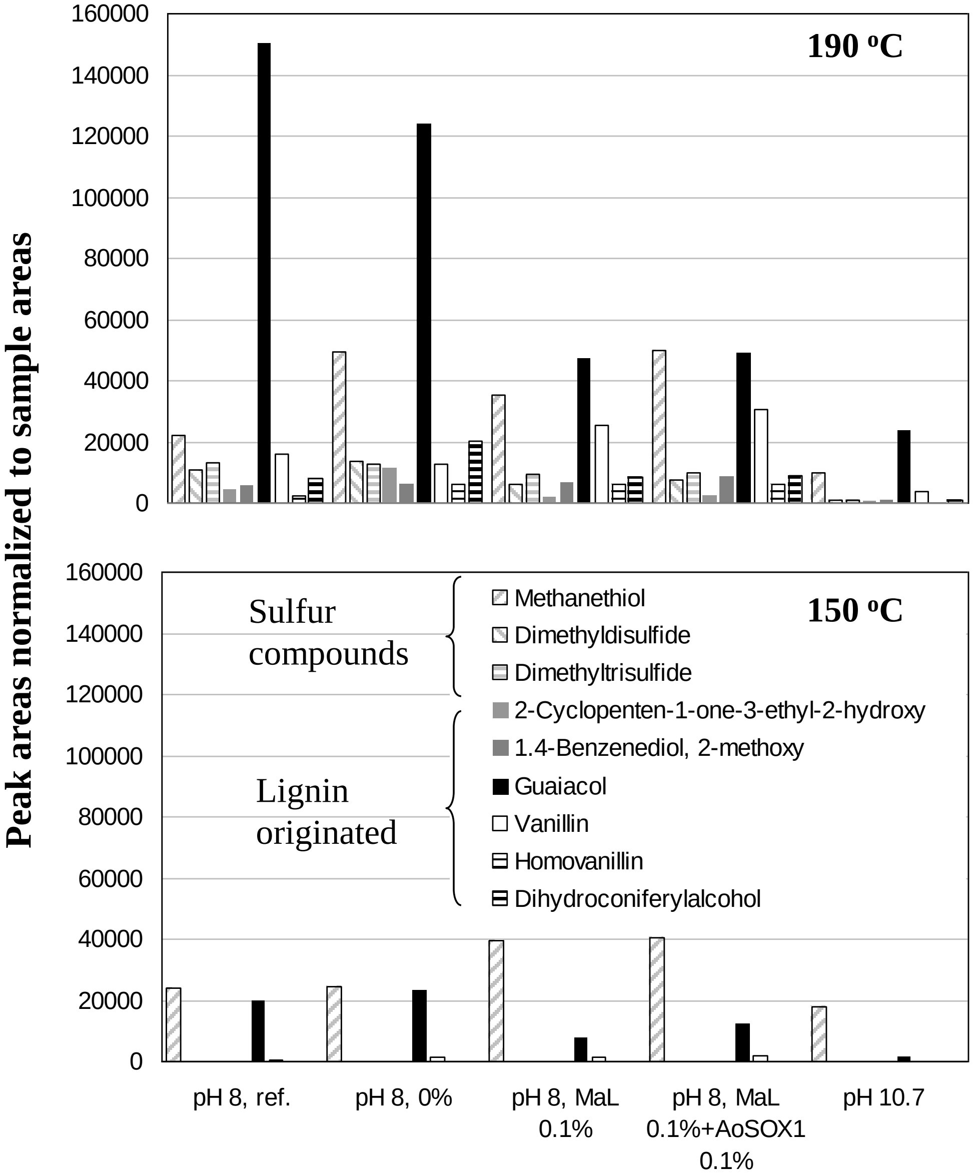


Fig. 4. Main volatiles of freeze-dried SE lignin samples at 190ºC (upper panel) and 150 ºC (lower panel). Reference sample (ref.) was not O_2 boosted

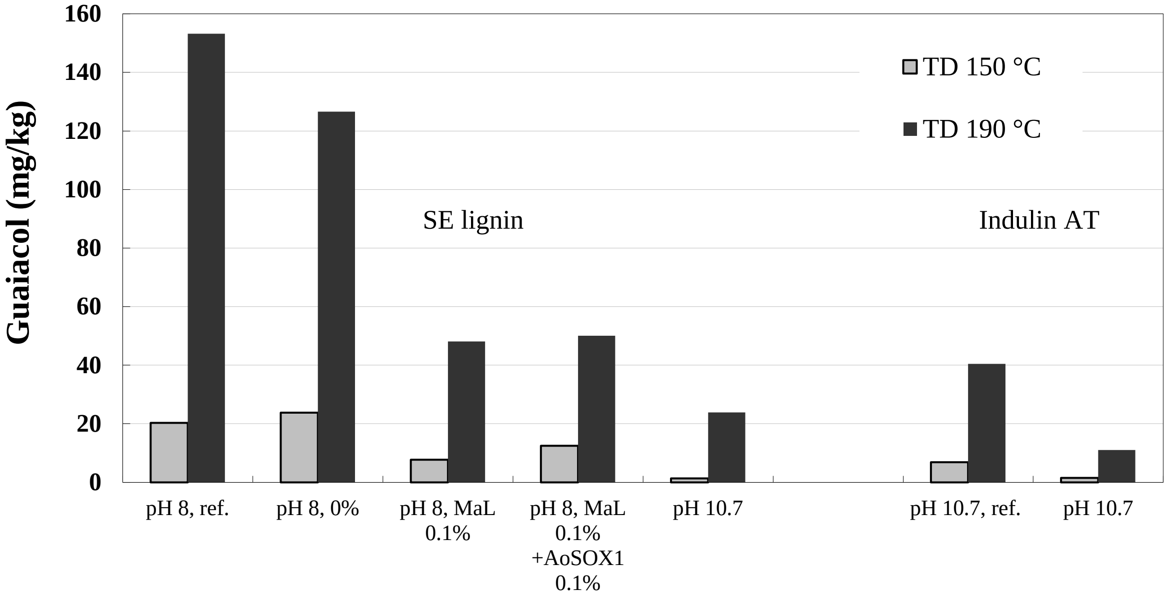


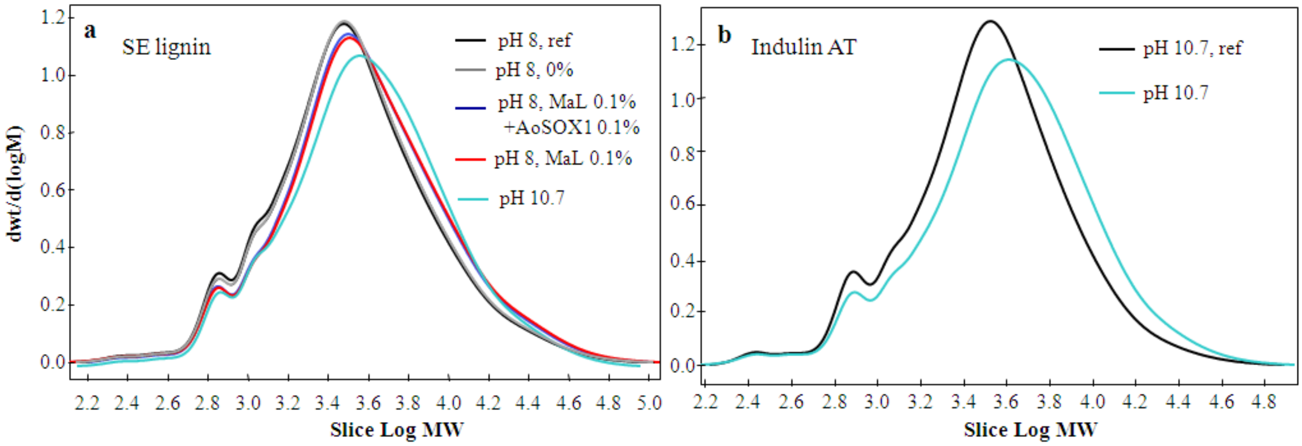
Fig. 5. Volatile guaiacol of freeze-dried SE lignin and Indulin AT samples at 150°C and 190°C. Reference samples (ref.) were not O_2 boosted

In order to investigate the importance of oxygen excess in the alkaline treatment, the pH 10.7 treatment was repeated to Indulin AT with and without oxygen boosting (5 bar initial O_2). The results clearly showed that oxygen-pressurized alkali treatment decreased the volatile guaiacol formation at 150ºC and at 190ºC, when compared to the alkaline treatment performed without O_2 boosting (Fig. 5).

Molecular Size Distribution of Lignin

The effect of the treatments on molecular size of both SE lignin and Indulin AT was analyzed using SEC. The results (molecular size distributions) indicated that the primary oxidation products, phenoxyl radicals, polymerized via radical coupling in the oxidation performed at room temperature and at pH 10.7. Also, MaL and MaL+AoSOX1 catalyzed oxidation caused minor polymerization of phenolics (Fig. 6a). Moreover, the experiments done with Indulin AT verified that at room temperature, the oxygen-pressurized alkali treatment caused lignin polymerization when compared to the alkali treatment done without O_2 boosting (Fig. 6b). However, the change in molecular size was rather small. Yet, the aim of this study was not to polymerize lignin in great extent, but to reduce the formation of low-molecular weight phenolic volatiles.

In alkaline oxygen oxidation (initially 5 bar O_2 excess) performed at elevated temperatures (90°C and 110°C), just the opposite takes place, as the Indulin AT macromolecule degrades fast, partly ending up to carbon dioxide (Kalliola *et al.* 2011). The degradation of lignin is desirable in pulp delignification, but likely unwanted in the composite applications.



Sample	Mn	Mw	PD
SE lignin, pH 8, ref	2 250	5 150	2.3
SE lignin, pH 8, 0%	2 330	5 260	2.3
SE lignin, pH 8, MaL 0.1% + AoSOX1 0.1%	2 470	5 840	2.4
SE lignin, pH 8, MaL 0.1%	2 480	5 990	2.4
SE ligin, pH 10.7	2 570	5 980	2.3

Sample	Mn	Mw	PD
IndAT, pH 10.7, ref	2 250	4 750	2.1
IndAT, pH 10.7	2 640	6 000	2.3

Fig. 6. **a)** Molecular size distributions of freeze-dried SE lignin samples. **b)** Molecular size distributions of freeze-dried Indulin AT samples. Reference samples (ref.) were not O_2 boosted

CONCLUSIONS

1. Laccase-catalyzed oxidation and oxygen oxidation in alkaline conditions were evaluated in order to reduce VOCs of kraft lignins for composite applications. Laccases Lcc2 from *T. arenaria* and MaL from *M. albomyces* with or without sulfhydryl oxidase from *A. oryzae*, as well as the alkaline oxygen oxidation (pH 10.7), showed potential in reducing odors. The importance of the oxygen excess in the alkali treatment was highlighted by the results. The analyses performed showed no clear effect of sulfhydryl oxidase.
2. Volatile degradation products of lignin were determined by TD-GC/MS method at temperatures prevailing in the thermoplastic processing. Also, the quantification of volatile guaiacol was conducted. The analysis method developed a possibility to study the effect of application processing under varying temperature and residence time, as well as distinct volatiles formed from different lignins from each other's.
3. From the practical point of view, the most promising results were obtained by oxygen oxidizing lignin at alkaline pH. The treatment would be straightforward to apply at an industrial scale.
4. Yet, the odor threshold values of the main VOC compounds are extremely low, which makes the reduction of VOCs challenging.

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PUBLICATION III

Experiences of kraft lignin functionalization by enzymatic and chemical oxidation

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Experiences of Kraft Lignin Functionalization by Enzymatic and Chemical Oxidation

Anna Kalliola,* Martta Asikainen, Riku Talja, and Tarja Tamminen

Linear hydrophilic derivatives are expected to soften lignin and improve its utilization in composite applications. Oxidation by means of laccase in the presence of oxygen was employed in an attempt to functionalize commercial kraft lignin by vanillic acid-PEG ester and ether derivatives. *Thielavia arenaria* and *Melanocarpus albomyces* laccases at pH 6 and 8 were used. According to O_2 consumption and the increase in molar mass, the tested laccases were active toward the lignin and the vanillic acid derivatives and also formed corresponding phenoxyl radicals. However, homogenous polymerization instead of cross-coupling and functionalization took place. As an alternative, lignin functionalization by the ester derivative by chemical oxidation under alkali-O_2 conditions was also tested. Efficient lignin polymerization was observed. However, functionalization was not detected. Interestingly, a clear decrease in lignin glass transition temperature was obtained by an isolation procedure that included freeze-drying. This suggests that functionalization may not be necessary to induce the desired softening effect.

Keywords: Kraft lignin; Vanillic acid; Polyethylene glycol; Composite; Glass transition temperature; Laccase; Oxygen; Oxidation

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INTRODUCTION

The production of bio-composites containing lignin as the thermoplastic component is a promising application for this industrially significant biomass side stream material (Gosselink *et al.* 2004; Schorr *et al.* 2014). The problem, however, is that lignin itself has poor thermal stability (Fenner and Lephardt 1981) and melt-flow (softening) behavior under the elevated temperatures in composite processing. The latter is partly due to crosslinking between the macromolecules *via* intermolecular hydrogen bonding. Polymer blending (Kadla and Kubo 2004; Kubo and Kadla 2005) or chemical modifications (Glasser and Jain 1993a, b; Lora and Glasser 2002) of lignin by linear substituents, *e.g.*, alkylene, (poly)ethylene, propylene oxide, and butylene oxide have been studied as means to block this type of crosslinking. This internal plasticization of lignin is often measured as the reduced glass transition temperature (T_g), which indicates the ability of the material to undergo softening at reduced temperature. Lignin plasticization by chemical modification enhances the glass transition and reduces the brittleness of lignin derived polymers, both characteristics desirable *e.g.* in polymer coating and composite applications (Hult *et al.* 2013; Laurichesse and Avérous 2014).

Laccase enzymes catalyze the oxidation of a wide range of aromatic substrates, especially phenols, with the simultaneous reduction of molecular oxygen to water. Phenolic substrates are oxidized to phenoxyl radicals, which, depending on the reaction conditions, can spontaneously polymerize *via* radical coupling or react further leading to quinones, Cα oxidation, or cleavage of Cα–Cβ bonds or aromatic rings (Giardina *et al*. 2010). However, the main reaction pathway of the phenoxyl radicals generated by laccase is considered to be spontaneous polymerization. The other reaction pathways are not that likely to occur due to slow kinetics of oxygen addition to the phenoxyl radical species (Crestini *et al*. 2010).

Laccases have been widely used for lignin modification. Recently, in comparing different types of technical and emerging lignins, higher lignin-laccase (*Trametes hirsute*) reactivity was found to correlate with lower lignin molecular mass and higher amounts of monomeric phenolics (West *et al*. 2014). Laccases have also been applied to provide a route for the addition of desired functionalities onto lignin, as the phenoxyl radicals are potential sites for coupling reactions with the functionalizing reactant (phenolic) radicals. Lund and Ragauskas (2001) investigated the incorporation of water-soluble phenols with carboxylic or sulfonic acid groups onto kraft lignin by laccase (*Trametes villosa*) catalysis in dioxane. The oxidative coupling between lignin and a phenol was most strongly illustrated by the incorporation of guaiacol sulfonate onto lignin, which made the lignin water-soluble at a pH of 2.4. A chemo-enzymatic polymerization pathway has been reported as a means to graft acrylamide and acrylic acid onto lignosulfonates. In this process, the role of laccase (*Trametes versicolor*) appeared to catalyze the formation of phenoxyl radicals in lignin, which further induced the formation of organic peroxide-derived radicals required as initiators in the co-polymerization (Mai *et al*. 2000a,b, 2001, 2002). In the investigations by Hüttermann *et al.* (2000) laccase activated lignosulfonate owing relative high radical density was shown to react as a crosslinking agent after mixing into a kraft lignin solution. The activated lignosulphonate was also postulated to react with nucleophiles such as cellulose and starch via covalent bonds.

The objective of this study was to find ways to soften lignin to enhance its utilization for composite applications. The primary route studied was *via* the introduction of hydrophilic functionality into the lignin structure. Phenolic derivatives with polyether type hydrophilic side chain were synthesized and applied for this purpose. Laccase-catalyzed oxidation was studied as a plausible method to induce crosslinking between lignin and the hydrophilic derivative.

EXPERIMENTAL

Materials

Lignin

A commercial kraft lignin, Indulin® AT (MeadWestvaco; USA), was used in this study.

Laccases

Three laccases were tested: *Thielavia arenaria* Lcc1 (TaLcc1) and Lcc2 (TaLcc2) (Paloheimo *et al.* 2006a, b) produced as recombinant enzymes in *Trichoderma reesei* by Roal Oy (Rajamäki, Finland) and *Melanocarpus albomyces* (r-MaL) (overproduced as recombinant enzyme in *Trichoderma reesei* by VTT).

The TaLcc1 and TaLcc2 are most active in mild acidic conditions, whereas r-MaL, in slightly alkaline conditions. Enzyme activity measurements were carried out according to Kalliola *et al.* (2011).

Methods

Synthesis of vanillic acid derivatives

Vanillic acid was derivatized with polyethylene glycol mono methyl ether (PEG-OMe, Mn 550). Two derivatives were prepared: (1) Ester derivative with vanillic acid and PEG-OMe (Ester V-PEG) was prepared by esterification with *p*-toluenesulfonic acid as a catalyst (Fig. 1) and (2) ether derivative was prepared by first brominating vanillyl alcohol (Fig. 2), followed by etherification with PEG-OMe (Fig. 3).

The analytical data associated with the structures of vanillic acid PEG ester, bromovanillate and vanillic acid PEG ether are shown in Appendix.

1. Ester derivative (Ester V-PEG)

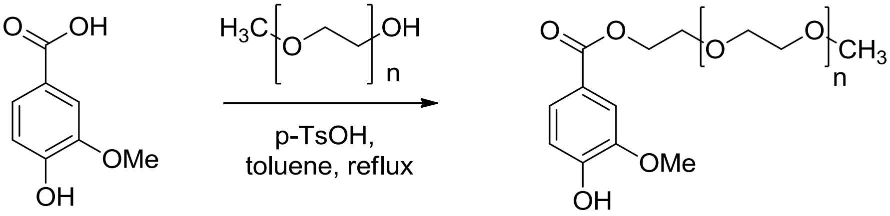

Fig. 1. Synthesis of vanillic acid PEG ester

The reaction flask was charged with vanillic acid (5.0 g, 29 mmol, 100 mol-%) and poly(ethylene glycol) methyl ether (16.3 g, 29 mmol, 100 mol-%, Mn 550). Toluene was added (50 mL), and the reaction mixture was stirred at room temperature until a smooth suspension formed. Then, *p*-TsOH (1.1 g, 6 mmol, 20 mol-%) was added. The reaction mixture was heated up to reflux for 70 h. The solvent was evaporated on a rotary evaporator and the residue was purified with column chromatography (EtOAc to remove the vanillic acid residues, then 5 % MeOH in CH_2Cl_2). The product was obtained as slightly brown oil (12.5 g, 18 mmol, 60 %).

2. Ether derivative (Ether V-PEG)

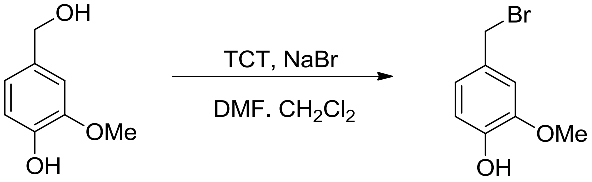

Fig. 2. Synthesis of bromovanillate (Nieddu *et al.* 2008)

Trichlorotriazine (TCT) (0.92 g, 5 mmol, 106 mol-%) was charged in the reaction flask under argon and DMF (1.5 mL, 19.4 mmol, 412 mol-%) was added. After 2 h, TCT had reacted and no solvents remained. CH_2Cl_2 (12 mL) was added, and the reaction mixture was stirred vigorously for 1 h before the addition of NaBr (0.98 g, 9.5 mmol, 202 mol-%). After 20 h, 4-hydroxy-3-methoxy benzyl alcohol (0.72 g, 4.7 mmol, 100 mol-%) was added and the reaction mixture was let to stir at room temperature for another 24 h. The reaction was quenched by adding 10 mL of water and 10 mL of 1N HCl; the resulting mixture was filtrated through a pad of Celite with a CH_2Cl_2 wash, and the organic layer was collected from the filtrate and concentrated in vacuum. The residue was purified with flash column chromatography to yield bromovanillate (0.26 g, 1.2 mmol, 26 %), which was a slightly yellow oil. The product compound decomposed quickly.

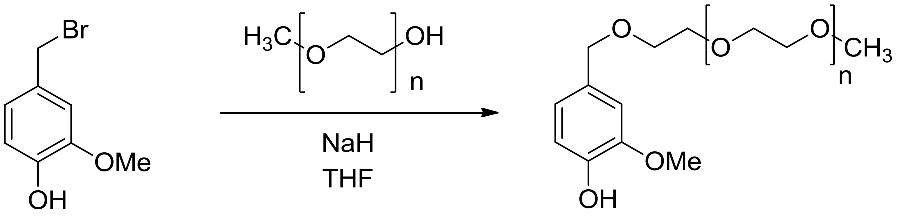

Fig. 3. Synthesis of vanillic acid PEG ether

NaH (60 w-%, 60 mg, 1.4 mmol, 120 mol-%) was charged in the reaction flask under argon and washed twice with 1 mL of hexane to remove any oil. Tetrahydrofuran (THF) (1 mL) was added, followed by the addition of poly(ethylene glycol) methyl ether (0.66 g, 1.2 mmol, 100 mol-%, Mn 550) in 2 mL of THF. After 15 min, bromovanillate was added over 30 min in 3 mL of THF. The reaction was let to stir at room temperature overnight before the addition of water (0.05 mL). The reaction mixture was filtered through a pad of Celite with a THF wash. The filtrate was collected and solvents were evaporated in vacuum to yield the product as brown oil (0.72 g, 1.1 mmol, 87 %).

Laccase-catalyzed oxidation

Lignin was first dissolved in 0.1 M NaOH, followed by gradual adjustment of pH to the desired level of 6 or 8 using 1 M HCl. Thereafter, the derivative (liquid at r.t.) was added to the system, followed by the addition of laccase. The total dry solids of the two-component system, containing lignin and the derivative, were set to 2.5 w-%.

The dosing of the lignin and the derivative was based on the phenolic hydroxyl group (PhOH) content in the substrates, as the PhOHs are the reactive sites in laccase catalyzed oxidation. The content of phenolics in Indulin® AT and in the ester/ether derivative was 4.3 (Hult *et al.* 2013) and 1.4 mmol/g, respectively. The molar ratio of lignin: derivative was set to 3:1, which equals a mass ratio of 1:1. In the experiments, laccases were dosed as nkat/g substrate, *i.e.*, lignin or derivative. Laccase treatments were also performed with one-component systems containing only lignin or the derivative as references.

Alkali-O_2 oxidation

Alkali-O_2 oxidation at high substrate concentration, 25 w-% dry solids, was performed as a chemical method to functionalize the lignin. Two-thirds of the substrate concentration included lignin and 1/3 of Ester V-PEG. Reference oxidations without the derivative were conducted at lignin dry solids of 25 and 16.7 w-% (2/3 of the 25 w-%). Analogously to the laccase experiments, dissolution of lignin in NaOH was done first, followed by the addition of the ester derivative in the case of the two-component system. The initial pH of the two-component system was set to 12.2 to avoid the hydrolysis of the ester derivative. In the case of the reference experiments conducted at lignin dry solids of 25 and 16.7 w-%, the initial pH was set to 13.3 and 12.2, respectively. The oxidation was performed in a 1-L Parr reactor applying 20 bars of initial O_2 excess for the reaction solution (total 300 g). The initial temperature was set to 40 °C, and the reaction time was 30 min.

The oxygen consumed in the experiments was calculated from the reactor pressure data by applying the ideal gas law equation and the values of water vapor pressure. The dissolved oxygen was taken into account. A molar mass (Mw) of 180 g/mol was used for Indulin® AT.

After the oxidation, the reaction solution was dialyzed against ion exchanged water using tubes from the cellulose ester with molecular weight cut-offs of 1000 and 3500 Da (Spectra/Por Biotech) to remove the free Ester V-PEG from the large lignin macromolecules, which was targeted to be at least partly functionalized during the oxidation. The dialyzed sample solution was freeze-dried.

Analysis

The reactivity of laccases toward the one- and two-component systems was evaluated by monitoring oxygen consumption in the reaction solution just after preparing the solutions. Monitoring was performed with an Oxy-10 mini sensor oxygen meter (PreSens, Germany) in a closed 1.9-mL vessel based on dynamic luminescence quenching. Parallel treatments were also performed.

Average molar masses (M_n, M_w) of the material in the reaction solutions and in the dialyzed and freeze-dried samples were measured by size exclusion chromatography (SEC), using PSS MCX 1000 and 100,000 columns in 0.1 M NaOH eluent (25 °C) with UV detection (280 nm). The molar mass distributions and average molar masses were calculated in relation to polystyrene sulfonate standards using Waters Empower 2 software.

The glass transition temperature (T_g) of the dialyzed and freeze-dried samples was measured by differential scanning calorimetry (DSC; Mettler Toledo DSC820, STARe SW 12.10, Mettler Toledo GmbH, Switzerland). First, 5 to 8 mg of sample was weighed into a standard aluminum crucible with a volume of 40 µL, which had been oxidized at 500 °C prior to use. The crucible was closed by cold-pressing and the lid was pricked to allow evaporation of volatile substances during the measurement. The DSC was used at a heating rate of 10 °C/min, and a flow of dry N_2 (50 mL/min) was used to purge the measurement cell. The drying of the lignin sample was carried out at dynamic temperature range from 25 to 105 °C followed by isothermal conditioning at 105 °C for 20 min. The thermogram of the lignin sample was recorded across a temperature range from 25 to 250 °C. The thermogram of the Ester-PEG was recorded twice over the temperature range from -80 to 50 °C. The T_g was determined as a midpoint temperature of the baseline shift at the glass transition region.

RESULTS AND DISCUSSION

Laccase-Catalyzed Oxidation

O_2 consumption

Laccase-catalyzed oxidation forms phenoxyl radicals in substrates simultaneously with the reduction of oxygen to water. Thus, monitoring the consumption of dissolved oxygen in the reaction solution served as a means to evaluate the reactivity of the laccases towards the one- and two-component systems. Figures 4a-e show the dissolved O_2 consumption as a function of time in the various one- and two-component systems experimented with lignin and the derivatives at pH 6 and 8.

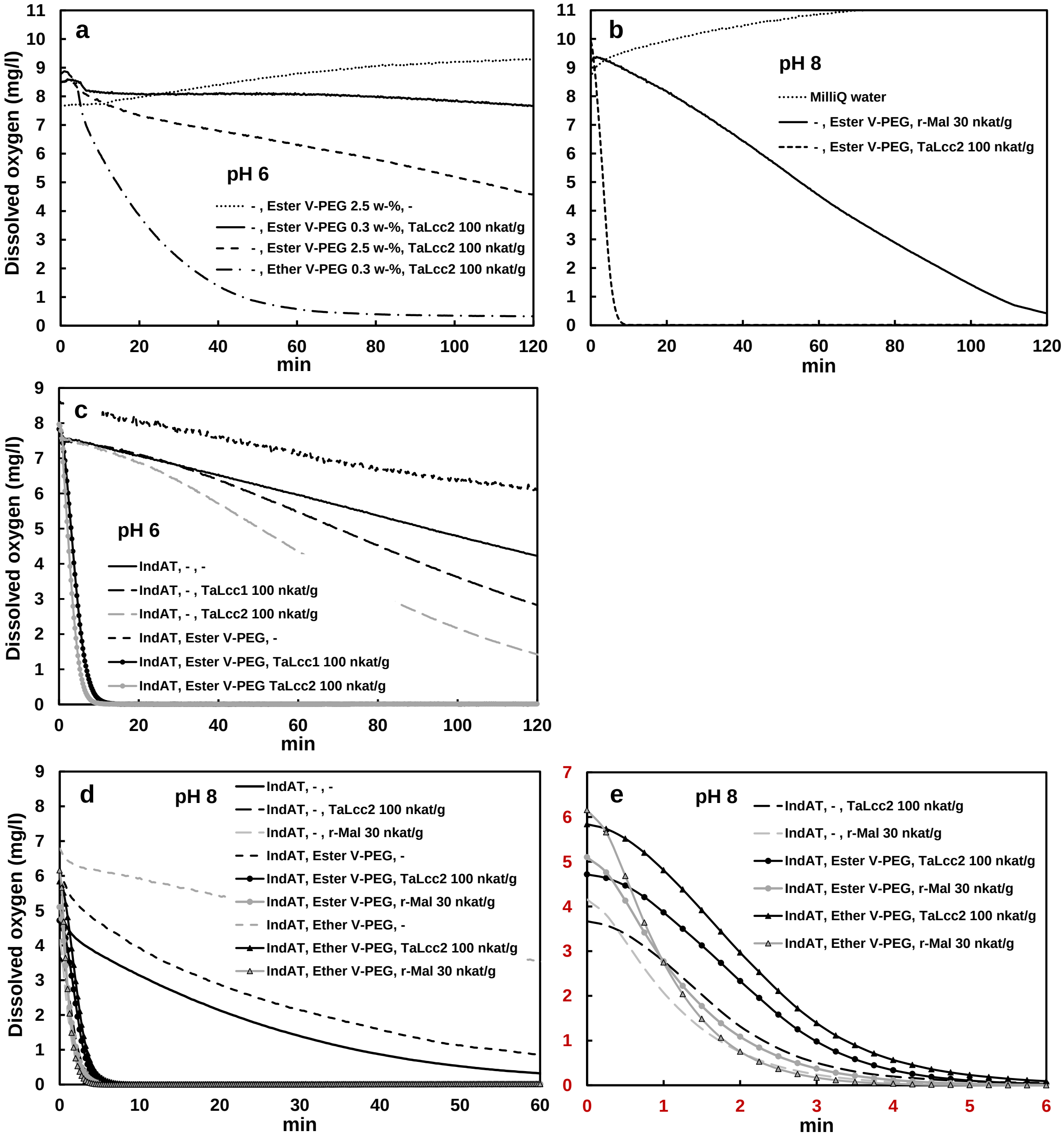

Fig. 4. (a) TaLcc2 reactivity toward ester and ether derivatives at pH 6; (b) r-MaL and TaLcc2 reactivity toward ester derivative at pH 8; (c) TaLcc1 and TaLcc2 reactivity towards Indulin® AT without or with ester derivative at pH 6; (d) r-MaL and TaLcc2 reactivity towards Indulin® AT without or with ester or ether derivative at pH 8; (e) r-MaL and TaLcc2 reactivity toward Indulin® AT without or with ester or ether derivative at pH 8, focus on the first 6 minutes

At pH 6, the reduction of dissolved O_2 in the reaction solution indicated that Ester V-PEG derivative is a substrate for TaLcc2 (Fig. 4a). Without the laccase, there were no O_2 consuming reactions. The O_2 consumption was remarkably enhanced when using the ether derivative as a substrate for TaLcc2 (comparison of the ester and ether derivative experiments conducted at 0.3 w-% dry solids). This was likely because the ether type of the derivative resembles lignin structure more than the oxidized ester type. At pH 8, in addition to r-MaL (30 nkat/g) having optimum activity in slightly alkaline conditions, the TaLcc2 (100 nkat/g) was functioning well towards the ester derivative (Fig. 4b).

Because TaLcc2 was detected to be clearly more reactive against the ether than the ester derivative, lignin functionalization at pH 6 was studied only using the ester to avoid strong homogeneous polymerization of the derivative. TaLcc1 and TaLcc2, both favoring mild acidic conditions, were active towards Indulin® AT at pH 6 (Fig. 4c). However, they were clearly more reactive in the system when replacing half of the lignin with the Ester V-PEG derivative.

Auto-oxidation of Indulin® AT took place already at pH 8, as a clear reduction of dissolved O_2 was detected (Fig. 4d). The auto-oxidation of kraft lignin under slightly alkaline conditions has also been observed in previous studies (Kalliola *et al*. 2011). Again, at pH 8, both r-MaL (30 nkat/g) and TaLcc2 (100 nkat/g) were functioning well towards Indulin® AT with or without the ester or ether derivative (Fig. 4d). When observing the first minutes of the reaction period more closely, it was seen that the two-component system containing the ether derivative consumed O_2 faster than the system containing the ester derivative (Fig. 4e).

Molar mass distribution

Monitoring the O_2 consumption in the reaction solution of the two-component system did not reveal if lignin actually was functionalized, *i.e.*, if the radical coupling between lignin and the derivative had taken place. Therefore, the molar mass distributions and the average molar masses (M_n, M_w) of part of the samples were determined (Fig. 5a-c, Table 1).

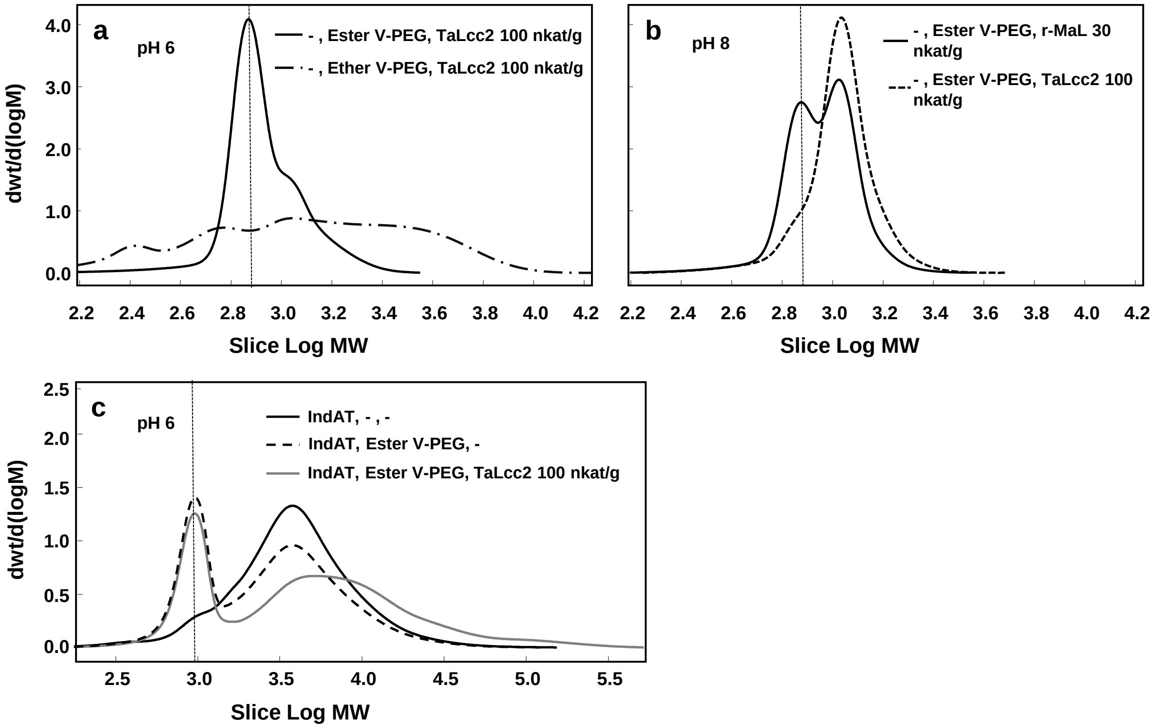

Fig. 5. (a) Molar mass distributions (MMDs) of ester and ether derivatives treated in the presence of TaLcc2 at pH 6; (b) MMDs of ester derivative treated in the presence of r-MaL and TaLcc2 at pH 8; (c) MMDs of Indulin® AT with or without ester derivative in the presence or absence of TaLcc2 at pH 6

Table 1. Average Molar Masses of One- or Two-Component Systems Treated in the Presence or Absence of Laccases at pH 6 and pH 8 (MMDs Shown in Fig. 5)

Lignin	Derivative	pH conditions	Laccase, dose (nkat/g)	M_n (g/mol)	M_w (g/mol)	PD
-	Ester V-PEG	-	-	780	865	1.1
-	Ester V-PEG	6	TaLcc2, 100	775	895	1.2
-	Ether V-PEG	6	TaLcc2, 100	790	1730	2.2
-	Ester V-PEG	8	r-MaL, 30	795	910	1.2
-	Ester V-PEG	8	TaLcc2, 100	905	1035	1.1
Indulin® AT	-	-	-	2 600	5 500	2.1
Indulin® AT	Ester V-PEG	6	-	1 700	4 400	2.6
Indulin® AT	Ester V-PEG	6	TaLcc2, 100	2 100	13 600	6.5

The results indicated that at pH 6, TaLcc2 catalyzed minor dimerization of the ester and clear polymerization of the ether derivative (Fig. 5a). The behavior was consistent with the detected O_2 consumption. At pH 8, dimerization of the ester derivative was induced by both r-MaL and TaLcc2 (Fig. 5b).

When comparing the molar mass distributions of the 2-component system treated in the presence and absence of TaLcc2 at pH 6, it was seen that the signal caused by the ester derivative was present in both distributions. In the presence of TaLcc2, the lignin part of the distribution was wider, representing homogenous polymerization of Indulin® AT (Fig. 5c). The results indicate that no or only very minor functionalization took place at pH 6 even though TaLcc2 was observed to be active against both components, lignin and Ester V-PEG, separately.

Alkali-O_2 Oxidation

Because the lignin functionalization by laccase-catalyzed oxidation was not successful, alkali-O_2 oxidation was tested as an alternative chemical method. Oxygen is an environmentally friendly oxidizing agent and widely utilized in modern pulp bleaching technology to degrade residual lignin. At an alkaline pH, molecular oxygen is able to react with phenolic lignin without an enzyme or other catalyst. Recently, odor of kraft lignin, originating from small-molecular volatile phenolics, was reduced by oxygen oxidation at alkaline pH at room temperature under conditions that induce polymerization of phenolics in an analogous manner to the laccase-catalyzed reaction (Kalliola *et al.* 2012).

Lignin reactions with oxygen in alkaline conditions start with the formation of a phenoxyl radical through an electron transfer from the ionized phenolic group (pKa 10 to 11) to molecular oxygen (Fig. 6). The subsequent steps include a reaction with the superoxide anion radical ($O_2\cdot^-$) forming a hydroperoxide anion structure and its rearrangement to the primary oxidation products (oxirane, muconic acid ester, or carbonyl structures) (Chang and Gratzl 1980; Gierer 1982; Sixta *et al.* 2006). The hydroperoxide structure, with a pKa value of 12 to 13, plays a crucial role, and has lately been shown to be the key intermediate in the course of phenolic lignin oxidation (Ji *et al.* 2009a,b). Below pH 12, the hydroperoxide intermediates tend to protonate and decompose homolytically back to phenoxyl radicals, which may spontaneously combine without re-oxidation caused by $O_2\cdot^-$. Thus, the pH in the reaction solution determines whether the intermediate reacts further, leading to degradation (Route A in Fig. 6), or induces radical coupling (Route B in Fig. 6). The primary reaction products arising from the hydroperoxide anion can react further to acidic low molecular weight reaction products (Gierer and Imsgard 1977a,b;

Chang and Gratzl 1980; Gellerstedt and Agnemo 1980), which have contribution to pH (Kalliola *et al.* 2011).

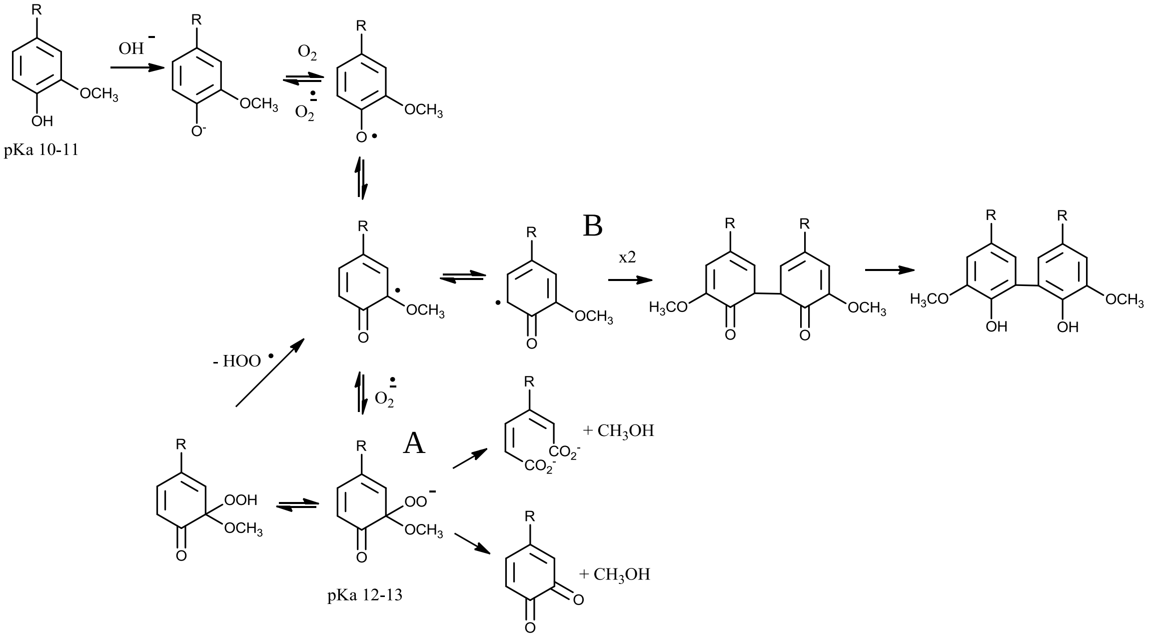

Fig. 6. Reaction mechanism of lignin with oxygen (adopted from Chang and Gratzl 1980; Ji *et a*l. 2009a,b). Route A: degradation, Route B: coupling

High lignin concentration in alkali-O_2 oxidation may favor coupling reactions because the phenoxyl radicals are close to each other. Secondly, the dissolution and diffusion of oxygen is hindered in viscous lignin-alkali solutions, which restricts the availability of oxygen species taking part in the degradation reactions.

O_2 consumption

According to the pressure drop in the reactor during the alkali-O_2 oxidation, the oxidation reactions at lignin dry solids of 25 w-% consumed much oxygen (Fig. 7a, solid, black curve). The reactions were exothermic, as can be seen from the temperature increase (Fig. 7, solid, grey curve). The corresponding behavior in the experiment, wherein 1/3 of the lignin was replaced with the ester derivative, indicated clearly less reactions. The behavior of the second reference experiment, which only contained lignin at 16.7 w-% (2/3 of 25 w-%) was similar. The results indicate that only Indulin® AT reacted, Ester V-PEG being relatively inert. Only the ester derivative was studied by the chemical oxidation route.

The computed oxygen consumption was 0.83 mol O_2/ mol lignin in the experiment with high lignin content, 25 w-%, but less than half of that in the experiments having 16.7 w-% of lignin (Fig. 7b). The initial pH, 13.3, and the increased temperature during the oxidation accelerated the oxidation reactions leading to higher O_2 uptake in the case of 25 w-% than in the case of 16.7 w-% of lignin. With the 2-component system (and its reference), the initial pH was set to 12.2 to avoid the hydrolysis of the ester derivative. The final pH after the oxidations conducted at 25 w-% and 16.7 w-% of lignin (2-component system and its reference) were 9.9 and 10.2, respectively. The greater pH drop during the oxidation in the case of 25 w-% also indicated higher reactivity and formation of acidic degradation products than in the case of 16.7 w-%.

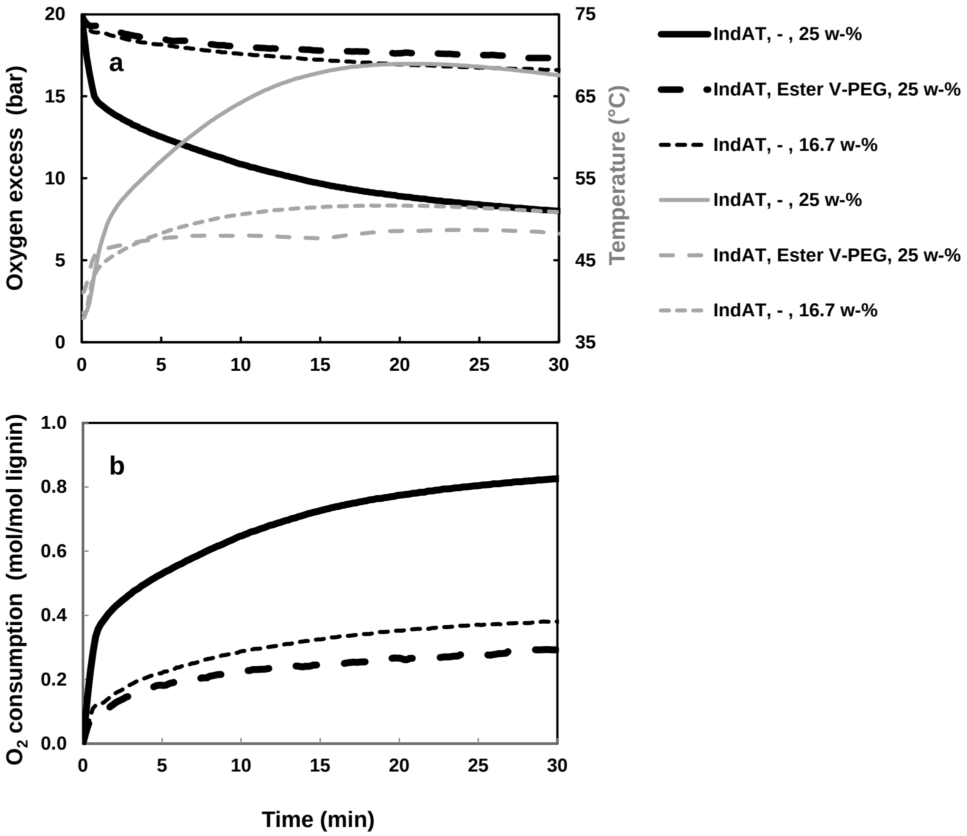

Fig. 7. (a) Pressure drop (black curves) and increase in the solution temperature (grey curves) during the alkali-O_2 oxidations of Indulin® AT without or with ester derivative; (b) Consumed oxygen during the alkali-O_2 oxidations

Molar mass distribution and Tg

The MMDs of Indulin® AT, the ester derivative, and the alkali-O_2 oxidized samples are shown in Fig. 8.

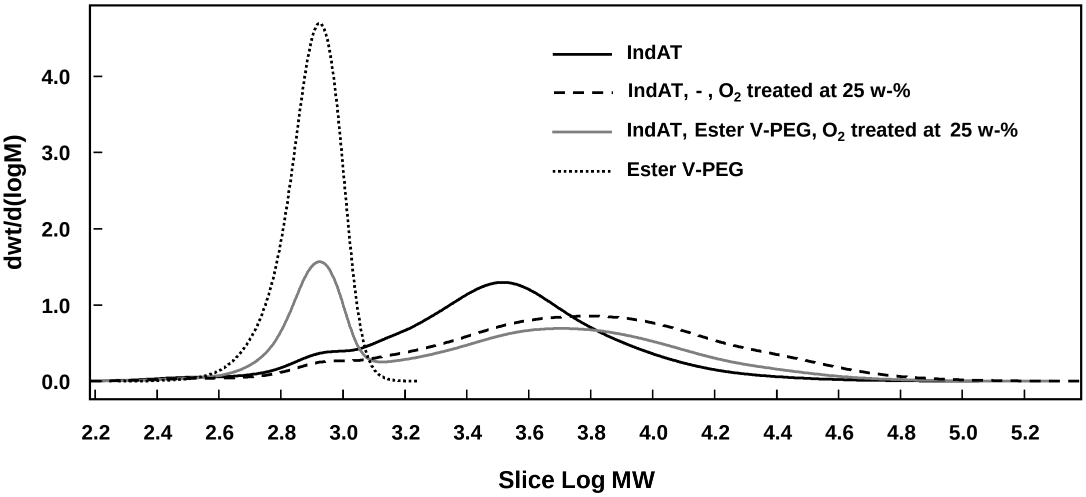

Fig. 8. MMDs of Indulin® AT, ester derivative, and alkali-O_2 oxidized Indulin® AT without or with the derivative

The alkali-O_2 oxidation caused strong condensation of Indulin® AT at 25 w-%, most likely via the 5-5 radical coupling, and increased the molar mass from 4500 to 9700 g/mol (Table 2). Hence, chemical oxidation is a promising, simple method to increase the molar mass of lignin via polymerization. The 5-5 coupling reactions are probably more pronounced with softwood kraft lignin than with hardwood kraft lignin because the latter is rich in syringyl type lignin units containing two methoxyl groups in the aromatic ring.

The signal caused by Ester V-PEG was present in the MMD of the two-component system (Fig. 8), indicating that no major functionalization occurred. In addition, there was no or only very minor homogenous polymerization of the ester derivative. These conclusions are also supported by the results of dialysis yield and glass transition temperature (Table 2).

Table 2. Analysis Results of Indulin® AT, Ester Derivative, and Alkali-O_2 Oxidized Indulin® AT Without or With the Derivative (MMDs Shown in Fig. 8). Cut-Off of Dialysis Membrane Marked in the Case of Dialysis Purification

Lignin, derivative, d.s. (w-%)	Dialysis CO, Da	Yield after dialysis	Computed lignin yield	M_n (g/mol)	M_w (g/mol)	PD	T_g (°C)	ΔC_p (Jg^{-1} K^{-1})
IndAT	-	-	-	2 100	4 500	2.1	144	0.329
IndAT, - , 25%*	1000	75	75	3 300	6 200	1.9	92	0.222
	3500	79	79	3 200	5 900	1.8	99	0.126
IndAT, - , O_2 at 25%	-	-	-	3 100	9 700	3.1	-	-
	1000	78	78	4 400	10 300	2.3	116	0.108
	3500	74	74	4 400	10 100	2.3	117	0.159
IndAT, ester, O_2 at 25%	-	-	-	1 600	5 600	3.5	-	-
	1000	50	75	3 500	7 800	2.2	99	0.19
	3500	54	81	3 300	7 500	2.3	95	0.132
Ester V-PEG**	-	-	-	760	800	1.1	-56	0.791
* Indulin® AT dissolved in NaOH at 25 w-% dry solids, un-oxidized								
** Melting temperature, Tm 5.4 °C; ΔH -62 J g^{-1}								

Alkali-O_2 oxidation for the conversion of softwood black liquor (Mathias *et al.* 1995) and softwood kraft lignins (Fargues *et al.* 1996; Araújo *et al.* 2010; Rodrigues Pinto *et al.* 2011) to phenolic aldehydes, especially to vanillin has been carried out at very aggressive pH conditions. In these batch oxidations, high yield on vanillin, up to 10% of the initial lignin has been obtained using lignin content of *ca.* 6 w-%, pH close to 14, temperatures near 150 °C, and O_2 partial pressure of 3 to 5 bars (constant). It has been observed that independent on the lignin source, the control of pH is the most important variable of the vanillin production. High alkaline pH is required both for the production of vanillin and to remain its yield, since at lower values of pH (<11.5) the vanillin oxidizes *i.e.* degrades at a considerable rate. These findings and the results of the present study highlight the importance of the hydroperoxide intermediate (pKa value of 12 to 13) and the prevailing pH conditions in the formation of the primary oxidation products during the alkali-O_2 oxidation. By maintaining the pH below the pKa value of the hydroperoxide intermediate mostly condensation (by radical coupling) or secondary oxidation reactions of the already formed structures take place.

The yield after the dialyses in the samples that only contained Indulin® AT (un-oxidized and oxidized) was in the range of 74 to 79 %, showing that the dialysis membranes passed *ca.* 25 % of the lignin material. In the sample containing 2/3 of indulin® AT and 1/3 of the derivative, the yield was as low as 50 to 54 %, indicating that in addition to the 25% of the lignin material also all of the derivative (M_w 800 g/mol) was free, *i.e.*, un-grafted to lignin and passed through the membranes with cut-offs of 1000 and 3500 Da.

Typically, plasticization (functionalization) provides lignin with lower T_g. When comparing the T_g values of the dialyzed, un-oxidized Indulin® AT (92; 99 °C) to that of dialyzed, oxidized two-component sample (99*;* 95 °C), there is no clear difference. This indicates that the alkali-O_2 oxidation did not induce functionalization.

By DSC, T_g for Indulin® AT has been determined to be between 135 and 142 °C (Penkina *et al.* 2012). Here, interestingly, a clear decrease in T_g for Indulin® AT, from 144 to 92 to 99 °C, was obtained by the reference procedure, including lignin dissolution in NaOH, followed by dialysis purification and freeze-drying. Here, the internal bonding of dissolved lignin, namely the intra-molecular hydrogen bonds, was probably disabled by the freeze-drying. When disabling the (re)arrangement of lignin, the mobility of the molecules is retained, providing lower T_g. This suggests that if aiming at a moderate reduction of lignin T_g, no modification for the internal plasticization is needed if using an isolation procedure hindering the internal bonding of lignin. The results of thermogravimetric analyses (Schorr *et al.* 2014) have shown that kraft lignins begin to degrade at temperatures around 160 °C, the extrusion temperature being generally around 160 to 165 °C. By using lignin with lower T_g, the composite processing temperatures might also be reduced, which hinders the thermal degradation of lignin.

Although T_g generally increases with increasing molar mass, it has been established that T_g increases with the degree of condensation, expressed as the fraction of phenylpropanoid units involved in C–C linkages (Baumberger *et al.* 2002). Also, in the present study, it was observed that when lignin was more condensed by the alkali-O_2 oxidation, it possessed higher T_g (116; 117 °C) than the less condensed form. However, in the case of the condensed Indulin® AT with M_w higher than 10,000 g/mol (dialysis purified), the T_g was clearly lower than that measured from the un-dialyzed Indulin® AT.

CONCLUSIONS

1. Functionalization of Indulin® AT with vanillic acid-PEG ester- and ether-type derivatives was studied using laccases from *Thielavia arenaria* and *Melanocarpus albomyces*, to soften lignin. Functionalization could not be verified. However, homogenous polymerization of lignin and the derivatives took place, indicating that the laccases were active in the reaction system. The ether derivative was a more preferable substrate for laccase than the ester derivative.
2. Molecular oxygen reacts with lignin and other phenolic compounds that are in a form of phenolates, generating phenoxyl radicals. The formed phenoxyl radicals were shown to either degrade or condensate by radical coupling analogously to the reactions taking place after the laccase catalyzed oxidation.
3. Alkali-O_2 oxidation was tested as an alternative method to functionalize Indulin® AT by the ester derivative. No functionalization was detected, nor did the alkaline oxidation have any effect on the ester derivative. However, significant condensation of

Indulin® AT occurred under the selected conditions of alkali-O_2 oxidation, suggesting that chemical oxidation is a potential means to increase lignin molecular weight prior to further modification for polymeric and material applications.

4. The results also indicate that if aiming at a moderate reduction of lignin T_g, functionalization of lignin is not necessary. Alternatively, a procedure including freeze-drying, whereby the internal bonding in lignin is suppressed, can be applied. However, the contribution of the lowered T_g toward improving the thermoplastic properties of lignin under the composite processing conditions should be verified by experiments.

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APPENDIX

Vanillic acid PEG ester had the following analytical data associated with its structure: ^{1}H NMR (500 MHz, DMSO-d6) δ 9.98 (s, 1H, OH), 7.48 (dd, 1H, J=8.3, 2.0 Hz), 7.44 (d, 1H, J=2.0 Hz), 6.89 (d, 1H, J=8.3 Hz), 4.34-4.33 (m, 2H), 3.82 (s, 3H), 3.74-3.72 (m, 2H), 3.60-3.58 (m, 2H), 3.52-3.49 (m, 45H), 3.44-3.42 (m, 2H); ^{13}C NMR (125 MHz, DMSO-d6) δ 165.6, 151.6, 147.4, 123.5, 120.5, 115.2, 112.5, 72.4, 71.3, 69.9, 69.6, 68.4, 63.6, 58.0, and 55.6; FT-IR (thin film, cm^{-1}) ν_{max} of 3310, 2871, 1710, 1284, and 1103.

From the PEG-OMe (Mn 550) distribution, the main vanillic ester products were expected to be $C_{33}H_{58}O_{16}$ and $C_{31}H_{54}O_{15}$. HRMS analysis gave $C_{31}H_{55}O_{15}$ [M+H] with accuracy of 4.5 ppm.

Bromovanillate had the following analytical data associated with its structure: ^{1}H NMR (500 MHz, DMSO-d6) δ 8.50 (s, 1H, OH), 7.26 (d, 1H, J=0.95 Hz), 7.17 (d, 1H, J=8.1 Hz), 7.06 (dd, 1H, J=8.1, 0.95 Hz), 4.77 (s, 2H), 3.80 (s, 3H); 13C NMR (125 MHz, DMSO-d6) δ 160.6, 138.4, 137.0, 122.7, 121.3, 113.5, 55.9, and 45.8.

Vanillic acid PEG ether had the following analytical data associated with its structure: ^{1}H NMR (500 MHz, DMSO-d6) δ 8.53 (s, 1H), 6.64-6.63 (m, 1H), 6.54-6.52 (m, 1H), 6.44-6.43 (m, 1H), 4.24 (s, 2H), 3.67 (s, 3H), 3.50-3.48 (m, 47H), 3.44-3.40 (m, 4H); ^{13}C NMR (125 MHz, DMSO-d6) δ 121.7, 117.3, 115.8, 113.3, 112.2, 72.9, 72.4, 71.3, 69.8, 69.6, 68.2, 60.2, 58.1, 55.2; FT-IR (thin film, cm^{-1}) ν_{max} of 3461, 2870, 1457, 1106, and 1036.

PUBLICATION IV

Alkali-O_2 oxidized lignin – A bio-based concrete plasticizer

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Alkali-O_2 oxidized lignin – A bio-based concrete plasticizer

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ABSTRACT

Commercial wheat straw soda lignin was used to develop an alkali-O_2 oxidation method to produce a ready-to-use solution for concrete plasticizing (cement dispersing). Lignin characterization was done directly from the solution. Depending on conditions, oxidation primarily induced either condensation or degradation of lignin. Thus, both the negative charge and the molar mass of the lignin were controlled by the oxidation parameters, and especially by pH.

The oxidized lignins were evaluated for their plasticizing performance in cement pastes, mortar, and concrete. Also, their effect on cement hydration was followed and several other properties of fresh and matured concrete were tested. A commercial lignosulfonate and petroleum-based superplasticizers were used as reference products.

The results indicated that alkali-O_2 oxidation at moderately high lignin dry solids is a promising method to produce a novel bio-based concrete plasticizer. The oxidized lignins were superior to the lignosulfonate and showed good performance in comparison to the superplasticizers.

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1. Introduction

Concrete, one of the most used materials in the world is a mixture of cement, water, and fine and coarse stone aggregate. Means of improving its strength, durability, and fresh stage flow-properties for better workability are constantly under research. Plasticizers, such as water-soluble lignosulfonates, or synthetic superplasticizers (organic polyelectrolytes) are often added into the concrete mixture to disperse cement particles to enhance flow-properties while using low water contents. By using less water, it is possible to have stronger concrete. Especially, the use of superplasticizers is growing in all types of concrete applications (Ramachandran et al., 1998). Annual demand of the plasticizing admixtures can be estimated to be 1.5–15 million tons if assuming that half of the annual concrete production of 18,000 million tons (GEreports, 2014) is prepared using a plasticizer dosage of 0.06–0.6 wt-% on cement (Ramachandran et al., 1998).

By far, the largest utilization of lignosulfonates, including the traditional sulphite pulping by-product lignin and the post-sulfonated alkali lignins, is in concrete admixtures with an estimated volume of 700,000 tons annually (Plank, 2004; Lora, 2008). Lignosulfonates are forecasted to have strong demand in regions that are expected to invest in significant infrastructure building in the next few years (Lora, 2008; IHS Chemical, 2012). However, in last decades, polycarboxylate-based superplasticizers specially developed for concrete plasticizing purposes have been displacing lignosulfonates especially in Western Europe and other developed regions (Stern and Schwarzbauer, 2008; IHS Chemical, 2012). The polycarboxylates offer superior flow-properties without affecting much of the other qualities of concrete (Plank, 2004). Lignosulfonates tend to cause some retardation of concrete setting (i.e., cement hydration) particularly when their sugar content is high, and they may also cause air entrainment (Chang and Chan, 1995; Lora, 2008).

In highly alkaline concrete mixture, the working mechanism of the superplasticizers is based on both electrostatic and steric repulsion between individual cement particles to achieve a full dispersion. The negatively charged functional groups of the superplasticizer, sulfonic and carboxylic groups, adsorb on the active sites (calcium aluminate) of the cement particles giving them a negative charge, which leads to the electrostatic repulsion between the particles. The branched structure of the superplasticizer achieves steric repulsion by forcing the cement particles apart (Ramachandran et al., 1998 Yamada et al., 2000). In the case of lignosulfonates, it is postulated that the dispersing effect is mainly due to the electrostatic repulsion between the cement particles (Taylor, 1997).

Alkaline pulping originated lignins are industrially significant biomass side components (Gosselink et al., 2004) whose commercial utilization is constantly searched for (Doherty et al., 2011). It is well known that soda and kraft lignins are soluble in water only at a high pH mainly due to the ionization of the phenolic

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groups (Norgren and Lindström, 2000; Ragnar et al., 2000) and often require altering of the physico-chemical properties prior to potential applications (Lora, 2008). For the substitution of lignosulfonates by applying alkali lignin other than post-sulfonated lignin only few studies concerning cement dispersing exist. Ozonation of kraft lignin (Detroit, 1973) has been stated to result in a water-soluble product that has shown better dispersing performance than lignosulfonate products in a wide variety of organic and inorganic suspensions including cement paste. Unmodified alkali lignins (Nadif et al., 2002) or fractions thereof (Takahashi et al., 2014) have shown dispersing performance of cement particles in mortar accompanied with satisfying strength properties of the cured material. Alkali lignins have also been reacted with epoxylated polyethylene glycol derivatives and tested in cement to yield slightly better dispersing performance and bending strength than when using lignosulfonates (Uraki et al., 2012; Aso et al., 2013). However, the commercial utilization of a new plasticizer is always a combination of price and performance.

Oxygen is an environmentally friendly oxidizing agent and widely utilized in modern pulp bleaching technology to degrade residual lignin. At alkaline conditions, molecular oxygen is able to react with phenolic lignin (phenolate) to form phenoxyl radical. The radical is further oxidized to form a short living key intermediate, hydroperoxide anion structure (pKa 12–13) that rearranges to primary oxidation products (i.e., oxirane, muconic acid ester, or carbonyl structures). This leads to lignin depolymerization and introduction of acidic groups in lignin, both of which increase its water solubility (Chang and Gratzl, 1980; Sixta et al., 2006; Ji et al., 2009). The secondary oxidation of the primary oxidation products also takes place leading to small molecular degradation products (Gierer and Imsgard, 1977; Chang and Gratzl, 1980; Kuitunen et al., 2011). Below pH 12, the hydroperoxide intermediates tend to protonate and decompose homolytically back to phenoxyl radicals, which without further oxidation may spontaneously combine leading to lignin condensation (Chang and Gratzl, 1980; Kalliola et al., 2014).

Alkali-oxidation of lignins by O_2 under conditions favoring lignin condensation has not been widely studied as a means to valorize lignin. On the contrary, the studies have mainly been conducted from the lignin degradation point of view in solution with rather low lignin content, very aggressive pH conditions, and high temperatures (Fargues et al., 1996; Araújo et al., 2010). Yet three old patents wherein lignin O_2 oxidation was done at lignin contents up to 20 wt-%, beneficial for down-stream processing, exist. One of them reports production of polycarboxylic lignin rich in carboxylic functionality in lignin backbone (Lin, 1975). The other two report polymerization of lignin using O_2 and formaldehyde (Benko and Daneault, 1976; Lin, 1980). The modified lignins were mainly tested to have good dye dispersing properties.

In the present study, oxidation by O_2 in alkaline conditions was applied to increase the water solubility of a commercial wheat straw soda lignin by introduction of acidic groups in the polymer. The aim was to use the oxidized lignin solution as such for concrete plasticizing, and thus the oxidation treatment was performed at moderately high lignin content. The feasibility of the oxidized lignin solutions for concrete application was evaluated using several testing methods in comparison to commercial lignosulfonate and synthetic polycarboxylate-based superplasticizers.

2. Methods

2.1. Alkali-O_2 oxidation

Protobind 1000, a wheat straw soda lignin (SL) purchased from GreenValue SA (Alpnach Dorf, Switzerland) was used as raw material. Prior to the oxidation, lignin was dissolved in a mixture of 5 M NaOH and preheated de-ionized water.

Preliminary oxidation experiments were done to understand the impact of the oxidation parameters, especially lignin content on the molar mass and charge. As the aim was to produce a ready-to-use plasticizer with a substantially high active matter content, a moderately high lignin content was intended. The experiments were done using lignin content 0.75–25.0 wt-% of the reaction solution. Under higher lignin content the solution becomes more and more viscous, which makes dispersing of O_2 gas harder. The quantity of NaOH was adjusted to obtain an initial pH ($pH_{Init.}$) of 13 (12.7–13.4) in the solution.

The oxidation was performed in a 1-L stainless steel pressure batch reactor (Parr Instrumental Co., Moline, IL, USA) equipped with baffles and a six-bladed turbine-type impeller stirrer, an electric heating jacket, a gas inlet, and a gas release valve.

The lignin solution was preheated in the reactor to reach a target initial temperature ($T_{Init.}$). The head space was flushed with pure oxygen to replace air. Thereafter, the reactor was pressurized and this was set as the starting point of the oxidation reaction. The quantity of the lignin solution in the reactor (300–800 g) and the partial O_2 pressure (5–40 bar) were varied in order to control the O_2 load (12–35 wt-%) on lignin. In general, the higher the lignin content the higher the O_2 pressure. The impeller was operated at 600 rpm. Reaction period of 30 min was used. Pressure and temperature in the reactor were recorded during the whole reaction period. After the oxidation, pH of the reaction solution (pH_{Final}) was measured at room temperature.

The oxygen consumed in the experiments was computed from the reactor pressure data by applying the ideal gas law equation and the values of water vapor pressure for each data point recorded. The dissolved oxygen was taken into account accordingly. A molar mass (M_w) of 180 g mol^{-1} was used for the lignin phenyl propane (C9) unit. The operating conditions and the total O_2 uptake (O_2 uptake) of the oxidations are presented in Table 1. The oxidized lignin solutions were determined for molar mass and negative charge at pH 6 (the former in the case of selected samples). Also their ability to plasticize cement paste was tested (method described below).

Based on the results of the preliminary oxidation experiments, the alkali-O_2 oxidation was further developed to optimize the properties of the oxidized lignin for cement plasticizing performance. Here, sequential oxidation was performed to first polymerize and then oxidize. Both stages (1ox and 2ox) of this 2-stage oxidation were carried out using the 1-L batch reactor. The initial pH of both stages was adjusted to 13. Thus, after the 1ox the oxidized lignin solution with the initial SL content of 25 wt-% was diluted to 15 wt-% using a mixture of 5 M NaOH and preheated de-ionised water. Also, a constant pH oxidation of SL at pH 11.5 was carried out in a 1.8-L batch reactor, iControl RC1e^{TM} HP100Hastelloy (Mettler-Toledo GmbH, Switzerland) equipped with baffles, gas stirrer, a pH control loop and a pump to introduce 5 M NaOH to the reaction solution under the O_2 excess. Initial SL content was set to 15 wt-%. SL content in the solution was decreased (Table 1) during the oxidation due to the addition of 5 M NaOH, which neutralized the acidity generated in the reactions and kept the pH at desired level. The last modifications of the alkali-O_2 oxidations included the initial oxidation at lignin content of 25 wt-% (1ox) in the 1-L reactor followed by a constant pH oxidation at pH 13 in the 1.8-L reactor, iControl RC1e^{TM} HP100 Hastelloy.

2.2. Lignin characterization

2.2.1. Molar mass

Average molar masses (M_n and M_w) of the lignin material in the solutions were measured by size exclusion chromatography (SEC), using PSS MCX 1000 and 100,000 columns in 0.1 M NaOH eluent

Table 1
Operating conditions for the alkali-O_2 oxidation of soda lignin (SL) and O_2 uptake of SL. Upper panel: preliminary oxidations, lower panel: improved oxidations. Reaction period of 30 min was used in all oxidations. Samples marked in bold were selected for further characterization and evaluation.

Sample	Lignin (wt-%)	NaOH on lignin (wt-%)	$\mathrm{pH}_{\mathrm{Init.}}$ (–)	$T_{\mathrm{Init.}}$ (°C)	O_2 load on lignin (wt-%)	O_2 uptake on lignin (wt-%)
	Preliminary oxidations					
SL 0.75%60C ox	0.75	53	13.1	60	20	8
SL 0.75%80C ox	0.75	53	13.1	80	23	11
SL 2.5%60C ox	2.5	31	12.7	60	14	8
SL 2.5%80C ox	2.5	31	12.7	80	14	9
SL 5% ox	5	38	13.3	80	12	10
SL 15% ox	15	23	13.1	55	17	8
SL 15% i.ox	15	23	13.1	60	35	10
SL 25% ox	25	30	13.4	50	30	13
SL 25% 1ox[a]	25	24	13.0	40	15	9
	Improved oxidations					
SL 15% 2ox	15	17 (41)*	13.0	75	34 (49)*	8 (17)*
SL constant pH ox[b]	15, 11.4_{Final}	65	13.1	70	35	23
SL constant pH 2ox[c]	15, 10.5_{Final}	75 (99)*	13.0	70	35 (50)*	18 (27)*

*In parenthesis the total NaOH load, O_2 load and uptake in the case of sequentially oxidized SL. The letter i within SL 15% i.ox refers to increased temperature and O_2 load in comparison to the other experiment (SL 15% ox) done at same lignin content.

[a] Initial oxidation stage prior to SL 15% 2ox and SL constant pH 2ox.

[b] Oxidation at pH 11.5.

[c] Oxidation at pH 13.

(25 °C) with UV detection (280 nm). M_n and M_w were calculated in relation to polystyrene sulfonate standards using waters empower 2 software.

2.2.2. Charge

A modification of a potentiometric acid-base titration method used for dry lignin samples (Rahikainen et al., 2013) was applied to determine the charge of the oxidized lignin solutions (or SL in alkaline solution in the case of un-oxidized lignin). In the modified method, a given quantity of the oxidized lignin solution containing initially 75 mg of lignin (e.g., 0.5 g in the case of solution containing initially 15 wt-% lignin) was diluted to 5 g using 0.2 M NaOH. Thereafter, the CO_2 species were removed from the sample by acidification and the procedure was continued as described by Rahikainen et al. (2013). The blank sample included both the alkali quantity initially existing in the lignin solution and the additional quantity of the 0.2 M NaOH. The blank was prepared and titrated similarly to the oxidized lignin solution. Linear estimation was used to obtain a titrant volume, V(HCl) for a given pH level, pH_i (i = 11.7, 11.6, 11.5, ..., 2.0). The titrant volume consumed during the blank titration at a given pH level, V($\mathrm{HCl}_{\mathrm{Blank}}$ at pH_i), was subtracted from that consumed during the sample solution titration, V($\mathrm{HCl}_{\mathrm{Sample}}$ at pH_i). The corresponding negative charge of lignin (mmol g^{-1}) at pH_i was computed using the information of the volume difference, V(HCl at pH_i), titrant concentration, and lignin mass in the sample solution. Negative charge at pH 6 is reported as an indication of anionic character.

2.2.3. Aromatic lignin

Aromatic lignin content of selected samples was detected as UV-lignin at 280 nm based on absorptivity value of 25.0 L g^{-1} cm^{-1} determined for SL.

2.2.4. Solubility

A simple procedure was used to evaluate the water solubility of the oxidized lignins. In the procedure lignin recovery (yield, %) from the solution by acidification and centrifugation was quantified as a function of pH range from 2.5 to 7. The acidification was done using 1 M HCl, where after the precipitate was separated by centrifugation at 10,000 g for 30 min and dried overnight at 105 °C. The recovery yield was computed based on the initial lignin content in solution.

2.3. Application testing

2.3.1. Flow properties

Two cement types, Megasementti CEM I 42,5 R (Finnsementti) and Plussementti CEM II B-M (S-LL) 42,5 N (Finnsementti) (shortly CEM I and CEM II) were used. CEM II is a blended cement containing 21–35% of limestone and slag whereas, CEM I does not contain any of those.

As a reference for the oxidized lignins the following commercial plasticizer products were used: WRDA 90D (Grace construction products), a conventional lignosulfonate plasticizer with dry solids of 30 wt-%; Glenium C151 or Glenium 51 (BASF) (shortly Glenium) and VB-Parmix (Finnsementti), all three polycarboxylate-based superplasticizers with dry solids of active matter of 15, 17 and 12 wt-%, respectively. A defoamer agent, tributylphosphate (TBF) with dosage of 2.5 wt-% on oxidized lignin was used in one of the concrete tests.

Plasticizing performance of the oxidized lignin solutions was evaluated by testing the flow properties of cement pastes, mortar and concrete. Cement pastes, containing 66 or 75 wt-% of CEM II or CEM I in water, respectively, were prepared with the oxidized lignin solutions or the commercial plasticizer products using efficient mixing by Heidolph DIAX 600 for 2+2 min. Lignin solutions or the commercial products were added last in the paste. Several dosages, in a range of 0.06–0.24 wt-% of the active matter on cement were used. The fluidity of cement pastes was studied by measuring the shear stress as a function of shear rate by using a rheometer, Bohlin Visco. Mortar was prepared using a standard sand mixture, CEM II, and water with the following mass ratio; 48.6:36.0:15.4. The fluidity of the mortar was studied using the Haegermann flow table (Ø 300 mm) method (DIN 1060) and a mold with a diameter of 100 mm. Concrete, 30-L (approx 60 kg) for each experimental point, was prepared according to EN1766 type C(0,40) with CEM II. This type of concrete has low content of fines, which makes the plasticizing challenging. Slump test was applied to evaluate the workability of the concrete (SFS-EN 12350-2).

2.3.2. Air content, hydration, and compression strength

Air content of fresh mortar and concrete was determined according to SFS-EN 12350-7. Cement hydration kinetics were followed by measuring the heat flow of the plasticized cement paste by a conduction calorimetry (TAM-Air). Compression strength and density of the matured concrete was measured after 28 days

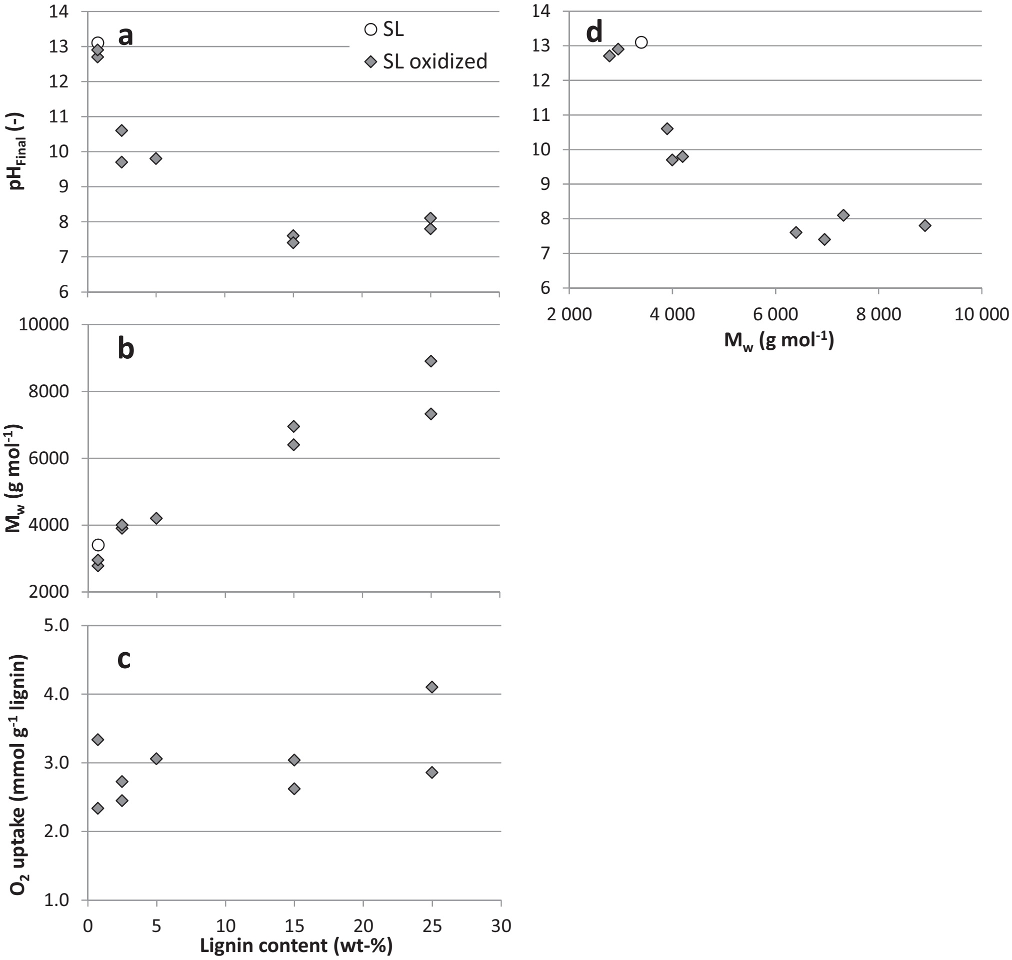

Fig. 1. (a) pH after the oxidation (pH_{Final}), (b) molar mass (M_w), and (c) O_2 uptake as a function of lignin content in oxidation. (d) Relation between pH_{Final} and M_w.

according to EN 196-1. The air content of fresh concrete should be at minimum, since it reduces the density and strength properties of the matured concrete. The impact of one of the samples (SL constant pH 2ox), with and without a defoamer (TBF), on the air content and fluidity of concrete was tested.

3. Results and discussion

3.1. Alkali-O_2 oxidation

The effect of the reaction parameters, lignin content (0.75–25 wt-%), O_2 load (12–35 wt-% on lignin), and temperature (50–80 °C) was first screened by the preliminary experiments. Table 1 shows data of the preliminary oxidations. In Fig. 1a–c pH after the oxidation (pH_{Final}), molar mass (M_w), and O_2 uptake are plotted as a function of lignin content. Fig. 1d illustrates the relation between pH_{Final} and M_w.

From Fig. 1a it can be seen that the higher the lignin content the lower the pH_{Final} until it is settled to a near neutral level (7.5–8) in the case of oxidation done at 15 and 25 wt-%. The decrease in pH is caused by the degrading reactions starting from the phenolates and consuming the major part of the oxygen in formation of the main products, muconic acid and oxirane structure, which are also very vulnerable to further oxidative degradation (Kuitunen et al., 2011). The decrease in pH is more pronounced the higher the concentration of the acidic products in a given volume of the lignin reaction solution.

In addition to the degrading reactions indicated by the pH drop, it was noted that the higher the lignin content the more condensed the lignin after oxidation (Fig. 1b). Increase in the molar mass was stronger the greater the decrease in pH, yet, the increase being further accelerated by the 25 wt-% lignin content (Fig. 1d). Only oxidation at very low lignin content (0.75 wt-%), typical for pulp oxygen delignification (Kalliola et al., 2011), led to actual depolymerization of lignin measured as reduced molar mass.

Despite the rather varying conditions applied to the preliminary oxidations, O_2 consumption was fairly constant, 8–13 wt-% on lignin, which corresponds to uptake of 2.3–4.1 mmol g^{-1} lignin (0.42–0.74 mol O_2 mol^{-1} lignin). Under the selected oxidation conditions there was no clear relation between the O_2 uptake and lignin content (Fig. 1c) or accordingly between the O_2 uptake and pH_{Final} or molar mass.

It appears that the most important factors contributing to lignin condensation and increase in molar mass are lignin concentration and the prevailing pH conditions in the oxidation. This is also supported by the fact that below pH 12, the hydroperoxide intermediates tend to protonate and decompose homolytically back to phenoxyl radicals, which can spontaneously combine by 5–5 coupling reaction (Chang and Gratzl, 1980; Kalliola et al., 2014). Already, moderately high lignin concentration favors the coupling reactions because the phenoxyl radicals are close to each other. In addition, the dissolution and diffusion of oxygen is hindered in viscous lignin–alkali solutions, which restricts the availability of oxygen species taking part in the degradation reactions. Condensation in viscous lignin solution by O_2 under alkali conditions was

Table 2
Impact of oxidation on soda lignin (SL) characteristics. Molar mass characteristics of commercial plasticizers.

Sample	M_w (g mol^{-1})	M_n (g mol^{-1})	M_w/M_n	O_2 uptake (mmol g^{-1})	Negative charge at pH 6 (mmol g^{-1})	UV-lignin (%) $a = 25$[a]
SL	3880	2070	1.9	0.0	1.5	100
SL 15% ox	6400	3000	2.1	2.6	3.2	–
SL 15% i.ox	6950	2690	2.6	3.0	3.5	–
SL 25% 1ox	7320	2600	2.8	2.9	3.4	–
SL 15% 2ox	6270	2390	2.6	5.5	5.1	82
SL constant pH ox	4580	2020	2.3	6.9	6.5	64
SL constant pH 2ox	3200	1650	1.9	5.7	6.9	71
WRDA 90D	3000	1700	1.8	–	–	–
Glenium C151	15900	5100	3.1	–	–	–

[a] Absorptivity value of 25.0 L g $^{-1}$ cm $^{-1}$ determined for SL.

already reported in an old patent (Lin, 1975) and could be partly reduced by optimizing the O_2 load and reaction temperature.

It has also been observed that pH is the most important variable when producing vanillin from kraft lignin (Araújo et al., 2010). High alkaline pH, close to 14 is required both for the formation of vanillin and to maintain its yield, since at lower values of pH (<11.5) the vanillin oxidizes and degrades at a considerable rate. This observation also shows that it is crucial to maintain a highly alkaline pH to ensure the dissociation of the hydroperoxide intermediate. Only the hydroperoxide anion structure (pKa 12–13) rearranges to primary oxidation products, phenolic aldehyde/carbonyl structure (e.g., vanillin) being one of those (Chang and Gratzl, 1980; Sixta et al., 2006).

Since the negatively charged functional groups of the plasticizers induce the electrostatic repulsion between the cement particles, the alkali-O_2 oxidation was further developed in order to increase the negative charge, most preferable in a form of muconic acid structures in lignin polymer. Constant pH oxidation at pH 11.5 and sequential oxidation without or with the constant pH control in the second stage were applied for this purpose. The aim in the sequential oxidations was to first induce lignin condensation at 25 wt-% content during the initial stage (SL 25% 1ox) and thereafter at 15 wt-% content during the following more aggressive stage (SL 15% 2ox or SL constant pH 2ox) to increase the negative charge. Condensation by 5–5 coupling does not consume the phenolic groups (PhOH) in lignin, which are thus able to react with O_2 in the next oxidation stage while being dissociated.

Data of the improved oxidations is shown in Table 1. In the case of SL 15% 2ox the total consumption of NaOH increased up to 41 wt-% on lignin when considering the NaOH requirements of both stages. The consumption of NaOH during the constant pH oxidations performed at 11.5 (SL constant pH ox) and at 13 after the initial oxidation (SL constant pH 2ox) was surprisingly high. The consumption of NaOH does not; however, necessarily reflect the real formation of carboxylic acids in the lignin polymer. Acidic, secondary oxidation products including CO_2 are formed and they contribute to pH (Kalliola et al., 2011; Kuitunen et al., 2011) and consume NaOH. It is probably impossible to totally avoid the further oxidation of the primary oxidation products. The O_2 uptake on lignin was also increased by the constant pH oxidations as the highly alkaline pH favored the oxidation of lignin rather than the secondary reactions.

3.2. Lignin characteristics

3.2.1. Molar mass and charge

Selected oxidized lignin solutions, which showed good plasticizing performance in cement paste (results below) were characterized more carefully. Molar masses of the two commercial plasticizers were also determined (Table 2).

The results indicate that the characteristics of SL can be controlled by the oxidation parameters, and especially by pH. Thus, different kind of SL based plasticizer solutions in terms of molar mass (M_w 3200–7320 g mol^{-1}) and negative charge (3.2–6.9 mmol g^{-1}) were produced (Table 2). All the other oxidation treatments except the 2-stage oxidation wherein the second oxidation was performed at constant pH 13 increased the molar mass (M_w 3880 g mol^{-1}) and polydispersity (1.9) of SL. Oxidation at constant pH 11.5 hindered the condensation reactions if compared to those oxidations performed without pH control. Increase in polydispersity indicates that although the condensation reactions were prevailing some of the lignin fragments were also degraded. Molar masses of the commercial plasticizers differed greatly from each other, lignosulfonate being small (M_w 3000 g mol^{-1}) and polycarboxylate being fairly large (M_w 15,900 g mol^{-1}) with wide molecular weight distribution. Yet the copolymer polycarboxylates are more linear in nature than the lignosulfonates (Uchikawa et al., 1997).

The total negative charge at pH 6 in the oxidized lignin solutions determined by potentiometric titration was more than doubled when comparing to that of un-oxidized SL solution (1.5 mmol g^{-1}). As described above the negative charge can be arisen from the carboxylic functionality in lignin polymer or from the acidic, small molecular reaction products of lignin. In addition, part of the negative charge may be originated from the reduction products of O_2. Protonation of superoxide anion ($O_2^{\bullet-}$) to form hydroperoxyl radical ($HOO^{\bullet}$) takes place under pH 6, as the superoxide anion has a pKa value of 4.8 (Gratzl, 1992).

According to UV280 measurements, 82–71% of the aromatic structures in SL remained after the improved oxidations, thus 18–29% degraded. The content of aromatic hydroxyl groups in SL was recently quantified by ^{31}P NMR to be 3.4 mmol g^{-1} (Liitiä et al., 2014). Thus, assuming that the decrease in UV absorbance is due to degradation of phenolic structures, the quantity of the degraded PhOHs in SL would correspond to 0.6–1.0 mmol g^{-1}. If the degradation only leads to the formation of muconic acid structures in lignin polymer, it would increase the negative charge by 1.2–2.0 mmol g^{-1}. Yet the increase in negative charge measured directly from the treatment solutions of the improved oxidations was almost twofold more. This indicates that part of the primary oxidation products were further oxidized to secondary products, thus increasing the overall amount of the carboxylic acid functionalities. Since, a clear correlation is seen between the O_2 uptake and negative charge at pH 6, a significant part of the charge may originate from other acidic moieties than carboxylic acids bound to lignin.

3.2.2. Solubility

Fig. 2 illustrates the recovery yield of SL and oxidized SL as a function of pH. The oxidation increased lignin solubility, which is desirable for the dispersing and plasticizing applications. SL is

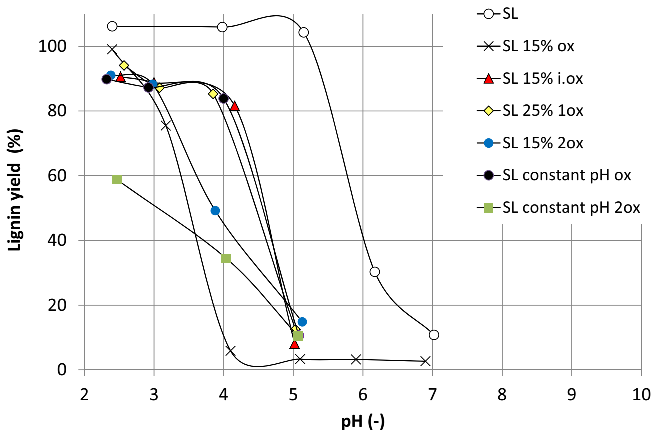

Fig. 2. Impact of oxidation on soda lignin (SL) solubility: lignin yield as a function of pH.

soluble only above pH 7, while the oxidized SL with increased molar mass is soluble above pH 4–5. Part of the lignin material in sample SL constant pH 2ox could not be recovered at pH 2.5.

It seems that the increase in charge in lignin polymer is enough to enhance its solubility considerably even though the molar mass of the polymer is high. The results also indicate that oxidized lignins would be suitable for dispersing application in slightly acidic pH range.

Recovery of lignin material by acidification may alter its structure, for example muconic acids are reported to form lactones via a ring closure reaction under neutral or acidic conditions (Gierer and Imsgard, 1977; Chang and Gratzl, 1980; Pieken and Kozarich, 1990). More specified characterization of the functionalities in oxidized lignin material was considered not to be necessary, since the solutions were to be used as such for the plasticizing applications.

3.3. *Application testing*

3.3.1. *Flow properties*

Plasticizing performance of the oxidized lignin solutions was evaluated stepwise proceeding from the easiest media to the most demanding. The initial testing was done in cement pastes (Fig. 3), thereafter in mortar (Fig. 4) and finally the performance of selected samples was verified in concrete (Fig. 5).

Oxidized SL solutions decreased the yield stress in pastes made of both cement types, CEM I and CEM II (Fig. 3a and b) demonstrating the plasticizing performance. With a dosing of 0.20 wt-% on cement, most of the oxidized lignins provided roughly the same yield stress than the commercial polycarboxylate, Glenium with dosing of 0.06–0.07 wt-%. Differences between the oxidized SLs seemed rather small, but the constant pH oxidation indicated to be a means of producing efficient plasticizer for both cement types. The commercial lignosulfonate, WRDA 90D required approximately the same dosage than SL constant pH ox to yield an equal effect. Un-oxidized SL solution functioned as well, yet, with a twofold dosing when compared to SL constant pH ox.

The evaluation of the oxidized SLs versus the commercial products was continued in mortar (mixture of CEM II, sand, and water) using a constant dosing of 0.40 wt-% on cement. Fig. 4 presents the Haegermann flow value and air content of the fresh mortar. Now the differences between the samples are more noticeable. A higher value represents better flow properties. The production of the oxidized lignin by the 2-stage or constant pH oxidation or the combination thereof offered better plasticizing effect when compared to those produced by a single-stage oxidation. Yet all of the oxidized SLs and also the un-oxidized were superior to WRDA 90D.

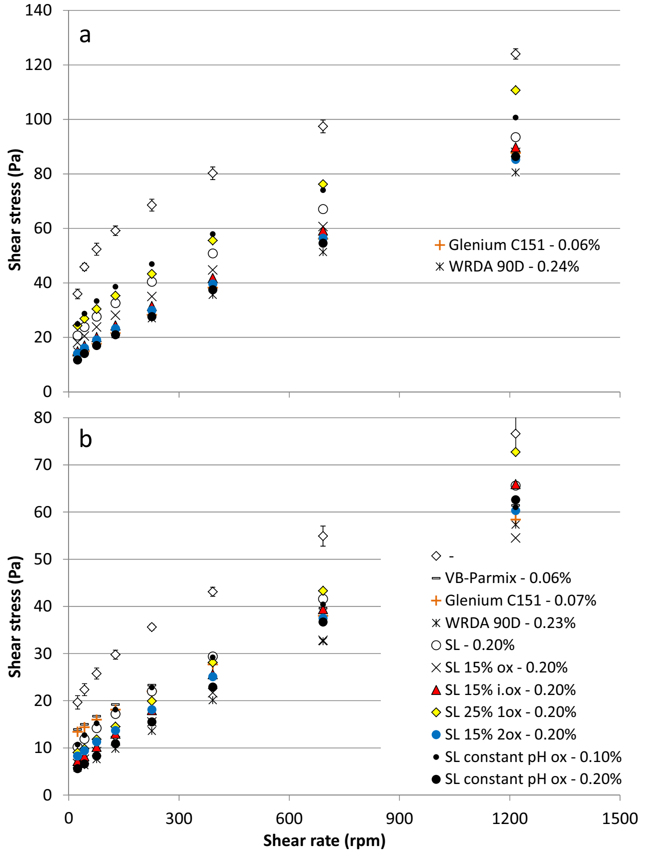

Fig. 3. Shear stress of cement pastes made of (a) CEM I (70 wt-%) and (b) CEM II (66 wt-%) as a function of shear rate. Commercial products or soda lignin (SL) solutions (un-oxidized or oxidized) used as plasticizers with dosages of 0.06–0.23 wt-% on cement. VB-Parmix tested only in the case of CEM II (b).

In addition, the performance of SL constant pH ox and SL constant pH 2ox was close to that of the other polycarboxylate-type superplasticizer, VB Parmix. The impact of the plasticizers on the air content of the testing medium is discussed below.

The performance of the three samples, produced by the improved oxidations, was finally evaluated in concrete using a slump test. The higher the slump the better the flow properties. Fig. 5a shows that these samples provided excellent plasticizing effect in concrete even with the dosage of 0.40 wt-% on cement. Their performance was again clearly better than that of WRDA 90D. The 0.40 wt-% dosage of the oxidized SL provided roughly the same

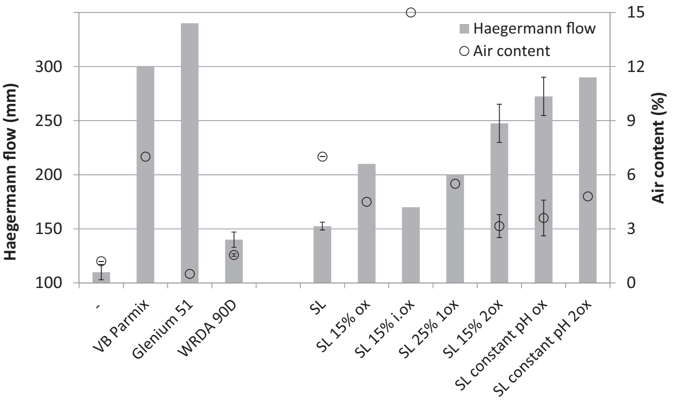

Fig. 4. Haegermann flow and air content of fresh mortar. Commercial products or soda lignin (SL) solutions (un-oxidized or oxidized) used as plasticizers with dosage of 0.40 wt-% on cement.

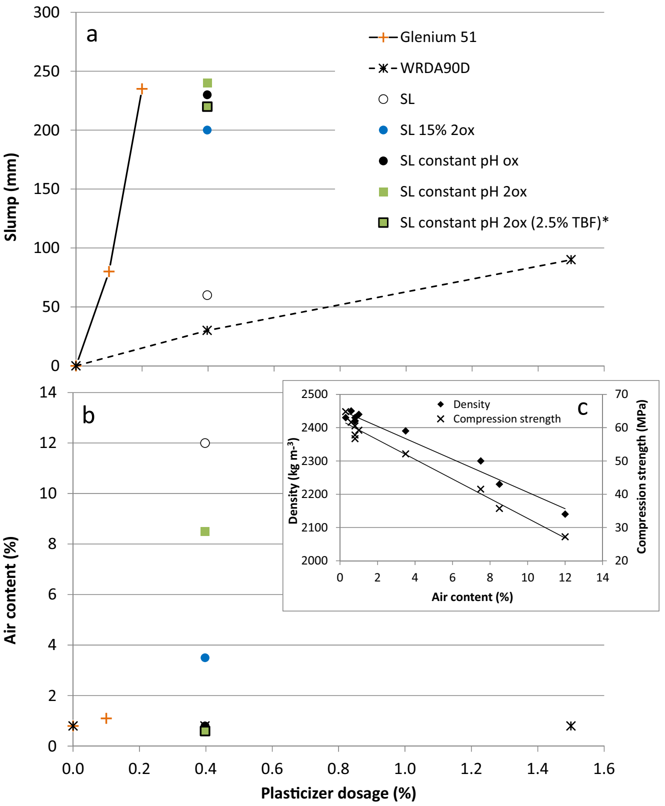

Fig. 5. (a) Slump and (b) air content in fresh stage concrete. (c) Impact of air content on compression strength and density of matured concrete. Commercial products or soda lignin (SL) solution (un-oxidized or oxidized) used as plasticizers with dosage of 0–1.5 wt-% on cement. *Defoamer agent (TBF 2.5% on lignin) used with SL constant pH 2ox.

plasticizing efficiency as 0.20 wt-% dosage of Glenium, which can be considered to stand for an excellent performance. In one of the experiments, defoamer agent (TBF 2.5% on lignin) was used with SL constant pH 2ox. It was seen that the plasticizing effect of SL constant pH 2ox did not change even though the air introduction was prevented. This result suggests that the defoamer does not affect the plasticizing performance of the oxidized SLs, and the plasticizing performance of the oxidized SLs is not due to the increased air content.

The samples SL constant pH ox and SL constant pH 2ox showing the best performance as plasticizers had relative low M_w (4580, 3200 g mol^{-1}) and posed high negative charge at pH 6 (6.5, 6.9 mmol g^{-1}). Recently, different fractions of soda-AQ lignin isolated from the spent liquor were tested for their plasticizing performance in mortar (Takahashi et al., 2014). Similarly, the results suggested that the fraction having a relatively low M_w (4000–5000 g mol^{-1}) was the most advantageous to bring adsorption on the cement particles and showed the highest plasticizing performance. The fraction also had a high content of PhOH (2.3–2.5 mmol g^{-1}), which hold charge while being dissociated in the highly alkaline media.

3.3.2. *Air content, hydration, and compression strength*

The oxidized SLs were observed to introduce air in mortar (Fig. 4). However, the amount was less than in the case of VB Parmix and un-oxidized SL. When evaluating the oxidized lignins in concrete, one of the samples, SL constant pH ox showed surprisingly good performance while it did not introduce air in the mixture, which was the behavior also with the commercial plasticizer products (Fig. 5b). Typically, and as well here, the air content in fresh concrete correlates with the density and strength properties of the matured concrete (28 d) (Fig. 5c). Thus, in the case of SL constant pH ox (no extra air), the compression strength was in line with that of concrete plasticized using the commercial products (Table 3). By using a small amount of a defoamer also with the other samples, the air content in concrete could be reduced ensuring sufficient properties in the matured concrete. Inclusion of defoamer is a common practice with lignosulfonate products (Taylor, 1997) which without the agent can cause air entrainment in concrete (Chang and Chan, 1995; Lora, 2008).

On contact with water, cement undergoes several reactions and forms many hydration products via complicated mechanism depending on the composition of the concrete mixture (Stark, 2011). The hydration is typically investigated by measuring the heat flow released from the material as a function of time. Typically, it is desirable that the additional agents introduced in concrete do not slow down the hydration. However, now the hydration kinetics were affected by the lignosulfonate, WRDA 90D. It was also affected by the oxidized SLs to some extent (Table 3), but there was no correlation between the retarded hydration and the compression strength of the matured concrete, which indicates that the oxidized lignin is already a promising alternative to lignosulfonate products. It was only the increased air content that had the inverse correlation with the compression strength.

The setting time of cement paste has been reported to depend roughly on the ionic functional group concentration (carboxylic and sulfonic) in the aqueous phase of the cement paste (Ramachandran et al., 1998 Yamada et al., 2000). The carboxylic functionality rich secondary oxidation products of the oxidized SL solutions likely remain more easily in the liquid phase than the polymeric lignin. In the liquid phase these small compounds could interact with the forming hydrate particles, and thus alter the cement hydration.

At the moment, any application wherein lignin has techno-economic potential is extremely interesting. Previously, modified

Table 3
Test results of concrete plasticized using commercial products or un-oxidized or oxidized soda lignin (SL) solution.

Sample	Dose (%)	Heat (J g^{-1} cement)			Density (kg m^{-3})	Compression strength, 28 d (MPa)
		24 h	72 h	168 h		
–	0	196	275	305	2420	56.7 ± 1.0
Glenium 51	0.20	162	258	283	2430	64.8 ± 1.1
WRDA 90D	0.40	45	76	86		
WRDA 90D	1.50				2430	60.5 ± 0.6
SL	0.40	53	108	119	2140	27.2 ± 1.7
SL 15% 2ox	0.40	22	106	123	2390	52.1 ± 0.8
SL c. pH ox	0.40	65	215	279	2420	57.8 ± 0.3
SL c. pH 2ox	0.40	53	207	263	2230	35.7 ± 1.4
SL c. pH 2ox (2.5% TBF)[a]	0.40	39	187	247	2450	61.6 ± 0.9

c: Refers to constant.
[a] Defoamer agent (TBF 2.5% on lignin) used with SL constant pH 2ox.

alkali lignins have not been reported of being tested in comparison to the polycarboxylate-based superplasticizers in concrete. The method developed in this study to produce a lignin-based plasticizer is rather simple and the results of the application testing are very promising. Yet in further investigations it is important to emphasize more on the structure-function relationship of the oxidized lignin as well as on its impact on the cement hydration.

4. Conclusions

A simple alkali-O_2 oxidation method to control the polymerization and solubilization of soda lignin was developed. The oxidized lignin solutions were applied as ready-to-use products for concrete plasticizing. They showed superior performance to the commercial lignosulfonate and provided equal plasticizing efficiency by 0.4 wt-% dosing than the synthetic superplasticizer by 0.2 wt-%. The best performing oxidized lignin solution did not even introduce air in concrete. Hydration kinetics was affected by the oxidized lignins, but this did not reduce the compression strength of the matured concrete. Oxidized lignins may provide a sustainable and techno-economically feasible option for future superplasticizer technology.

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Title	**Chemical and enzymatic oxidation using molecular oxygen as a means to valorize technical lignins for material applications**
Author(s)	Anna Kalliola
Abstract	Oxidation by molecular oxygen (O_2) is one of the lignin modification methods. O_2 is active towards phenolic groups, which are particularly abundant in kraft and soda lignins. The main aim of this thesis was to apply oxidation by O_2 to modify technical lignins to enhance their utilization for polymeric chemicals and material applications. O_2 oxidation was aided by using either alkaline conditions or laccase enzyme as a catalyst. In addition, oxygen delignification of pulp was studied using kraft lignin as a model substrate to provide data for a mechanistic model for the process. Lignin oxidation mechanisms by O_2 under alkaline conditions and laccase catalysis are discussed. A simple alkali-O_2 oxidation method under high lignin content was developed to increase the water solubility of soda lignin, desirable for dispersing applications. Lignin characterization was done directly from the reaction solution. Both the negative charge and the molecular mass of the lignin were controlled by the oxidation parameters, and especially by pH. Oxidation without controlling the pH decrease caused condensation and an increase in molecular mass. Oxidation under a constant pH of 11.5 clearly hindered the condensation and increased the negative charge. Oxidation at constant pH of 13 decreased molecular mass. The results indicate that the organic hydroperoxide formed via coupling of a phenoxyl radical with superoxide ($O_2^{\bullet -}$) is the key intermediate. The course of further reactions is dependent on the degree of protonation of this intermediate (pKa 12–13) and is thus pH dependent. The hydroperoxide anion rearranges leading to degradation. Below pH 12, the protonated form decomposes back to the phenoxyl radical, which spontaneously undergoes coupling and thus induces condensation. Under laccase catalysis conditions, $O_2^{\bullet -}$ is not present and thus the reaction paths described above do not function. Therefore, the formed phenoxyl radicals couple with each other rather than degrade. O_2 has a significantly lower tendency to attach to the phenoxyl radical compared to $O_2^{\bullet -}$. The oxidized soda lignin solutions were applied as ready-to-use products for concrete plasticizing. They were superior to commercial lignosulfonate and good in comparison to synthetic superplasticizers. The best performing lignin solution (oxidized at a constant pH of 11.5) also showed promising results in other concrete application tests. To enhance the utilization of kraft lignin in composite applications, both laccase- and alkali-catalyzed O2 oxidation were used to polymerize lignin-derived low-molecular phenolics for the reduction of VOCs. According to sensing and chemical analysis, the undesirable odor and the formation of VOCs under elevated temperatures were reduced to a greater extent by alkali- than by laccase-catalyzed oxidation. However, neither method led to adequate odor removal. In order to lower the glass transition temperature of lignin, functionalization with a hydrophilic phenolic compound was attempted. However, homogeneous polymerization of this compound was favored over coupling to lignin. The operating conditions of alkali-O_2 oxidation could probably be optimized for targeted lignin characteristics, which would increase the further application potential of technical lignins. Laccase-catalyzed oxidation is best applied when lignin polymerization is desired.
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Nimeke	**Kemiallinen ja entsymaattinen happihapetus teknisen ligniinin valorisoinnissa materiaalisovelluksia varten**
Tekijä(t)	Anna Kalliola
Tiivistelmä	Hapetus molekyylisellä hapella (O_2) on yksi ligniinin muokkausmenetelmistä. O_2 reagoi fenolisten ryhmien kanssa, joita on runsaasti erityisesti sulfaatti- ja soodaligniineissä. Tässä työssä päätavoitteena oli soveltaa O_2-hapetusta teknisten ligniinien muokkaukseen, jotta niiden käyttö polymeerisenä kemikaalina ja materiaalisovelluksissa helpottuisi. O_2-hapetus toteutettiin joko alkalisissa olosuhteissa tai entsyymiavusteisesti lakkaasilla. Lisäksi työssä tutkittiin massan happidelignifiointia käyttäen malliaineena sulfaattiligniinia. Näin tuotettiin tietoa kyseisen prosessin mekanismimallinnusta varten. Työssä käsitellään ligniinin O_2-hapetusmekanismeja alkalisissa olosuhteissa ja lakkaasikatalysoidusti. Soodaligniinille kehitettiin yksinkertainen korkeassa ligniinipitoisuudessa tapahtuva alkali-O_2 -hapetusmenetelmä, joka nostaa ligniinin vesiliukoisuutta dispergointisovelluksia varten. Ligniiniä luonnehdittiin suoraan reaktioliuoksesta. Sekä ligniinin negatiivista varausta että moolimassaa hallittiin hapetusparametreilla, erityisesti pH:n avulla. Hapetus ilman pH-laskun hallintaa aiheutti kondensaatiota ja kasvatti moolimassaa. Hapetus vakio-pH:ssa 11.5 esti selvästi kondensaatiota ja kasvatti negatiivista varausta. Hapetus vakio-pH:ssa 13 pienensi moolimassaa. Tulokset osoittavat, että orgaaninen hydroperoksidi, joka muodostuu fenoksiradikaalin ja superoksidin ($O_2^{\bullet-}$) yhdistyessä, on oleellinen välituote. Jatkoreaktioiden suunta on riippuvainen välituotteen protonoitumisasteesta (pKa 12–13) ja siten pH:sta. Hydroperoksidianionin uudelleenjärjestäytyminen johtaa pilkkoutumiseen. pH:ssa alle 12 sen protonoitunut muoto hajoaa takaisin fenoksiradikaaliksi, joka yhdistyy spontaanisti toiseen vastaavaan ja siten aikaansaa kondensaatiota. Lakkaasikatalysoiduissa olosuhteissa $O_2^{\bullet-}$ ei esiinny, ja siksi yllä esitetyt reaktiopolut eivät toimi. Tämän takia muodostuneet fenoksiradikaalit yhdistyvät keskenään sen sijaan, että hajoaisivat. O_2 reagoi fenoksiradikaalin kanssa merkittävästi hitaammin kuin $O_2^{\bullet-}$. Hapetettuja soodaligniiniliuoksia käytettiin sellaisenaan tuotteena betonin notkistuksessa. Ne toimivat selvästi paremmin kuin kaupallinen lignosulfonaatti ja olivat vertailukelpoisia suhteessa synteettisiin tehonotkistimiin. Parhaiten toiminut ligniiniliuos (hapetettu vakio-pH:ssa 11.5) oli lupaava myös muiden betonisovellustestien mukaan. Sekä lakkaasi- että alkalikatalysoitua O_2-hapetusta käytettiin ligniiniperäisten, pienimolekyylisten fenoleiden polymerointiin sulfaattiligniinissä. Näin pyrittiin vähentämään haihtuvien yhdisteiden määrää ja edistämään ligniinin käyttöä komposiittisovelluksissa. Aistiin perustuvan ja kemiallisen analyysin mukaan alkalikatalysoitu hapetus vähensi hajua ja haihtuvien yhdisteiden muodostumista korkeissa lämpötiloissa enemmän kuin lakkaasikatalysoitu. Kumpikaan menetelmistä ei kuitenkaan johtanut riittävään hajun poistoon. Ligniiniin yritettiin liittää vesiliukoinen fenolinen yhdiste, jotta sen lasinsiirtymälämpötila alenisi. Yhdiste kuitenkin homopolymeroitui sen sijaan, että olisi liittynyt ligniiniin. Alkali-O_2 -hapetuksen toimintaolosuhteita optimoimalla saavutetaan hyvin todennäköisesti haluttuja ligniiniominaisuuksia, mikä edistäisi teknisten ligniinien sovelluspotentiaalia entisestään. Lakkaasikataloitu hapetus soveltuu parhaiten ligniinin polymerointiin.
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Chemical and enzymatic oxidation using molecular oxygen as a means to valorize technical lignins for material applications

Large quantities of lignin are available from chemical pulping and emerging biorefineries. Although using lignin as a fuel will save fossil oil, there is great potential to create higher value products from lignin. The main technical lignins, kraft and soda lignins are especially rich in phenolic hydroxyl groups. Molecular oxygen (O_2), an environmentally friendly and low-cost oxidizing agent, is active towards phenolic lignin, and is thus a potential choice for lignin valorization. O_2 oxidation of lignin can be aided using alkaline conditions or an enzyme as a catalyst. In this work, a simple alkali-O_2 oxidation method was developed to convert soda lignin into a polyelectrolyte with increased water solubility. Lignin characteristics could be controlled by the oxidation parameters, especially pH. The oxidized lignin solutions were applied as ready-to-use plasticizer product to enhance the workability of fresh concrete. They showed superior plasticizing performance to the commercial lignosulfonate and were comparable to the synthetic superplasticizer. To enhance the utilization of kraft lignin in composites, both laccase- and alkali-catalyzed O_2 oxidation were used to polymerize lignin-derived low-molecular phenolics for the reduction of volatile odorous compounds (VOCs). Indeed, the treatment reduced the undesirable odor and the formation of VOCs under elevated temperatures. However, the odor threshold values of the main VOCs are extremely low, which poses a challenge to their reduction. Kraft lignin functionalization with a hydrophilic phenolic compound was tried to lower the glass transition temperature of lignin. Unfortunately, homogeneous polymerization of this compound was favored over coupling to lignin. The results indicate that alkali-O_2 oxidation provides wide possibilities to modify lignin molecular mass and charge properties.

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