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 (O₂), an environmentally friendly and low-cost oxidizing agent, is active towards phenolic lignin, and is thus a potential choice for lignin valorization. O₂ oxidation of lignin can be aided using alkaline conditions or an enzyme as a catalyst. In this work, a simple alkali-O₂ 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 O₂ oxidation were used to polymerize lignin-derived low-molecular phenolics for the reduction of volatile odoriferous 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-O₂ oxidation provides wide possibilities to modify lignin molecular mass and charge properties.
Chemical and enzymatic oxidation using molecular oxygen as a means to valorize technical lignins for material applications

Anna Kalliola
Process Chemistry and Environmental Engineering
VTT Technical Research Centre of Finland Ltd

Forest Products Chemistry
School of Chemical Technology
Aalto University

Thesis for the degree of Doctor of Science (Technology) to be presented with due permission of the School of Chemical Technology, Aalto University for public examination and criticism in Puu2 auditorium, Tekniikantie 3, Espoo on Friday, 14th of August, 2015 at 12 noon.
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$_2^\cdot$) 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$_2^\cdot$ is not present and thus the reaction paths described above do not function. Therefore, the formed phenoxy radicals couple with each other rather than degrade. O$_2$ has a significantly lower tendency to attach to the phenoxy radical compared to O$_2^\cdot$.

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-O₂ 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


Hapetettuja soodaliigniiniliuoksia käytettiin sellaisenaan tuotteena betonin notkisuksessa. Ne toimivat selvästi paremmin kuin kaupallinen lignosulfonaatti ja olivat vertailukelpoisia suhteessa syntetiseihin tehonotkisiin. Parhaiten toimintun ligniiniiliuos (hapetettu vakio-pH:ssa 11.5) oli lupaava myös muiden betonisovellustestien mukaan.

Sekä lakkaasi että alkalikatalysoiduun O$_2$-hapetusta käytettiin ligniiniperäisten, pienimolekyylisten fenoleiden polymerointiin sulfaattiligniinissä. Näin pyrittiin vähentämään haihtuvien yhdisteiden määrää ja edistämään ligniinin käyttöä komposittisoveltussissa. Aििśi kurustavan ja kemiallisen analyysin mukaan alkalikatalysoidu
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.

I highly appreciate the contribution of my co-authors, Dr. Tiina Liitiä, Dr. Susanna Kuitunen, Mr. Tapio Vehmas, Dr. Stella Rovio, Mrs. Taina Ohra-aho, Dr. Riku Talja, Dr. Martta Asikainen, Mrs. Anne Savolainen, and Dr. Greta Faccio. It has been a pleasure to work with them all! Professor Tapani Vuorinen is acknowledged for his advices and the pre-examiners, Prof. Gunnar Henriksson and Prof. Dimitry Evtuguin for their suggestions concerning this thesis.

I am thankful for the skilful personnel in the laboratory, especially Mr. Juha Kaunisto and Mrs. Tarja Wikström, for their contribution. Special thanks also go to Dr. Petteri Kangas, Dr. Martina Andberg, and all the other colleagues and persons who have helped with this thesis.

Finally, I would like to thank my parents, sister and brother, and my friends for their support, and my dear family, Heikki, Iiris, and Riku for keeping me busy outside the office hours.

June 2015, Espoo, Finland

Anna Kalliola
<table>
<thead>
<tr>
<th>Role</th>
<th>Name and Institution</th>
</tr>
</thead>
</table>
| Thesis advisor       | Doctor Tarja Tamminen  
                         | VTT Technical Research Centre of Finland Ltd  
                         | Espoo, Finland |
| Reviewers            | Professor Dmitry Evtuquin  
                         | Department of Chemistry  
                         | University of Aveiro  
                         | Aveiro, Portugal |
|                      | Professor Gunnar Henriksson  
                         | Division of Wood Chemistry and Pulp Technology  
                         | Royal Institute of Technology  
                         | Stockholm, Sweden |
| Opponent             | Assistant professor Claudia Crestini  
                         | Department of Chemical Sciences and Technologies  
                         | University of Rome Tor Vergata  
                         | Rome, Italy |
| Supervising professor| Professor Tapani Vuorinen  
                         | School of Chemical Technology  
                         | Aalto University  
                         | Espoo, Finland |
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.


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

Abstract .................................................................................................................. 3
Tiivistelmä ........................................................................................................... 5
Preface ................................................................................................................. 7
Academic dissertation ......................................................................................... 8
List of publications .............................................................................................. 9
Author’s contributions ...................................................................................... 10
List of abbreviations ........................................................................................... 13

1. Introduction .................................................................................................. 15

1.1 Technical lignins in the lignocellulosic refinery context ..................... 15
1.2 Lignin ..................................................................................................... 16
    1.2.1 Structure ..................................................................................... 16
    1.2.2 Extraction ................................................................................... 18
    1.2.3 Isolation ....................................................................................... 19
    1.2.4 Properties .................................................................................... 19
    1.2.5 Current and potential commercial applications ....................... 21
1.3 Oxidation of lignin using O_2 under alkaline conditions ..................... 23
    1.3.1 Oxygen delignification ............................................................... 23
    1.3.2 Molecular oxygen ..................................................................... 24
        1.3.2.1 Solubility ........................................................................ 25
        1.3.2.2 Consumption ................................................................. 25
    1.3.3 Reactivity of lignin structures .................................................... 26
    1.3.4 Mechanism ............................................................................... 27
        1.3.4.1 Initiation ......................................................................... 27
        1.3.4.2 Reactions of phenolic lignin ............................................ 28
        1.3.4.3 Rate-determining step and delignification kinetics ......... 29
        1.3.4.4 Secondary reactions ....................................................... 30
        1.3.4.5 Model of oxidation chemistry ...................................... 31
        1.3.4.6 Reactions of non-phenolic lignin .................................. 33
    1.3.5 Applications to technical lignins .................................................. 34
1.4 Oxidation of lignin using O_2 and laccase as a catalyst ...................... 35
1.4.1 Laccases ...................................................................................... 36
1.4.2 Mechanism ................................................................................... 36
1.4.2.1 Laccase-mediator system ....................................................... 37
1.4.3 Applications to technical lignins ..................................................... 38

2. Aims ............................................................................................................. 40

3. Materials and methods ................................................................................. 41

3.1 Technical lignins, enzymes, and derivatives ............................................. 41
3.2 O₂ oxidation of lignin .............................................................................. 43
  3.2.1 Under alkaline conditions .............................................................. 43
   3.2.1.1 O₂ consumption .................................................................. 43
  3.2.2 Using laccase as a catalyst ........................................................... 44
3.3 Characterization methods ....................................................................... 45
3.4 Application testing ................................................................................... 47

4. Results and discussion ................................................................................ 49

4.1 Oxidation of lignin using O₂ under alkaline conditions ....................... 49
   4.1.1 Delignification conditions .............................................................. 49
      4.1.1.1 O₂ consumption ................................................................ 52
   4.1.2 Alkali-O₂ oxidation for producing a soda lignin-based concrete
       plasticizer .................................................................................. 53
      4.1.2.1 Controlling lignin characteristics ...................................... 54
      4.1.2.2 Results of the application testing .................................... 60
4.2 Oxidation of lignin using O₂ and laccase as a catalyst ....................... 63
   4.2.1 Reduction of VOCs in kraft lignin ................................................... 64
   4.2.2 Experiences of kraft lignin functionalization .................................... 68
      4.2.2.1 Lowering Tg ................................................................... 72

5. Conclusions ................................................................................................. 74

6. Future prospects .......................................................................................... 78

References ......................................................................................................... 79

Appendices

Publications I–IV
List of abbreviations

AoSOX1  Aspergillus oryzae sulfhydryl oxidase
BDt  Bone dry ton (dry solids 100%)
CEM II  Cement type Plussementi 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\(^*\)  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
\( O_2\cdot\)  Superoxide anion radical
\( O\cdot\)  Oxyl anion radical
pH\(_{\text{init}}\)  pH, initial
pK\(_a\)  Acid constant (10-base logarithm of dissociation coefficient K\(_a\))
r-MaL  \textit{Melanocarpus albomyces} laccase

S  Syringyl unit

SE  Softwood kraft lignin, provided by Stora-Enso

SL  Soda lignin from wheat straw

SW  Softwood

t  Time

$T_{\text{init.}}$  Temperature, initial

$T_g$  Glass transition temperature

TaLcc1  \textit{Thielavia arenaria} laccase, Lcc1

TaLcc2  \textit{Thielavia arenaria} laccase, Lcc2

TD-MS/GC  Thermal desorption-gas chromatography/mass spectrometer

ThL  \textit{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 \(\beta-O-4\) (aryl alkyl ether) linkage (Figure 1). The other common linkages connecting the phenylpropane units include \(\beta-5\) (phenylcoumaran), 4-O-5, \(\beta-1\), dibenzodioxocin, 5-5 (mainly as part of dibenzodioxocin), and \(\beta-\beta\) 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$_2$S 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, ß-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 C$_6$ 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).
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 re-slurred 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 ($pK_a < 2$) 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).

<table>
<thead>
<tr>
<th>Lignin type</th>
<th>Sulfur-lignins</th>
<th>Sulfur-free lignins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspect</td>
<td>Kraft</td>
<td>Lignosulfonate</td>
</tr>
<tr>
<td>Solubility</td>
<td>Alkali Organic solvents</td>
<td>Water</td>
</tr>
<tr>
<td>Number-average molar mass ($M_n \text{ - g/mol}$)</td>
<td>1000-3000 2.5 - 3.5</td>
<td>15,000-50,000 6 - 8</td>
</tr>
<tr>
<td>Polydispersity</td>
<td>140 - 150</td>
<td>120</td>
</tr>
<tr>
<td>$T_g$ (°C)</td>
<td>140 - 150</td>
<td>120</td>
</tr>
</tbody>
</table>
Table 2. Physicochemical properties of commercial kraft (Indulin AT) and soda (Protobind 2400) lignin (adapted from Schorr et al., 2014).

<table>
<thead>
<tr>
<th></th>
<th>Indulin AT</th>
<th>Protobind 2400</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insoluble Klason lignin (%)</td>
<td>92 ± 2</td>
<td>79 ± 1</td>
</tr>
<tr>
<td>Soluble Klason lignin (%)</td>
<td>4.4 ± 0.3</td>
<td>9 ± 1</td>
</tr>
<tr>
<td>Total sugars (%)</td>
<td>1.0 ± 0.2</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>3.59 ± 0.7</td>
<td>1.61 ± 0.06</td>
</tr>
<tr>
<td>C (%)</td>
<td>63.9</td>
<td>61.2</td>
</tr>
<tr>
<td>H (%)</td>
<td>5.6</td>
<td>6.3</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.39</td>
<td>0.93</td>
</tr>
<tr>
<td>S (%)</td>
<td>2.1</td>
<td>2.0</td>
</tr>
<tr>
<td>O (%)</td>
<td>24.4</td>
<td>28.0</td>
</tr>
<tr>
<td>OCH₃ (%)</td>
<td>11.2 ± 0.8</td>
<td>14.8 ± 0.2</td>
</tr>
<tr>
<td>Formula C₉</td>
<td>C₉H₈.₀₈N₀.₀₅S₀.₁₁O₂.₁₃(OCH₃)₀.₆₆</td>
<td>C₉H₄.₄₂N₀.₁₁S₀.₁₂O₂.₃₂(OCH₃)₀.₉₃</td>
</tr>
<tr>
<td>M C₉ (g mol⁻¹)</td>
<td>174.6</td>
<td>182.8</td>
</tr>
<tr>
<td>OHₗhen, (mmol g⁻¹ per C₉)</td>
<td>3.37</td>
<td>2.84</td>
</tr>
<tr>
<td>OHₗhen/OHₗalp (by FT-IR)</td>
<td>0.93</td>
<td>0.94</td>
</tr>
<tr>
<td>H/G/S</td>
<td>2/96/2</td>
<td>6/37/57</td>
</tr>
<tr>
<td>Mₙ</td>
<td>1248</td>
<td>838</td>
</tr>
<tr>
<td>Mₚ</td>
<td>4549</td>
<td>2802</td>
</tr>
<tr>
<td>PD</td>
<td>3.65</td>
<td>3.34</td>
</tr>
<tr>
<td>Dₚₙ</td>
<td>7.1</td>
<td>4.4</td>
</tr>
<tr>
<td>Dₚₚₚ</td>
<td>26.1</td>
<td>14.6</td>
</tr>
<tr>
<td>T1 (°C)</td>
<td>156</td>
<td>185</td>
</tr>
<tr>
<td>T50% (°C)</td>
<td>632</td>
<td>415</td>
</tr>
<tr>
<td>Residues (%)</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>Coal for 1000 °C (%)</td>
<td>31</td>
<td>33</td>
</tr>
<tr>
<td>DTG max (°C)</td>
<td>378</td>
<td>366</td>
</tr>
<tr>
<td>Tₓ (°C)</td>
<td>132</td>
<td>59</td>
</tr>
</tbody>
</table>

The number average (Dₚₚₚ) and mass average (Dₚₚₚ) degree of polymerization were calculated by dividing Mₙ or Mₚ 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; Tₓ = glass transition temperature determined by thermomechanical analysis (TMA) measurements. N₂ 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 hydrophilicity to be applied as surfactants, dispersants, or poly-electrolyte-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” surfac-
tants 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\textsubscript{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$_2^-$) is formed. It can protonate to form a hydroperoxyl radical (HOO$^\cdot$). 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$^\cdot$) that is in equilibrium with an oxyl anion radical (O$^-$). 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$^\circ$ standard reduction potential) (Sixta et al., 2006b initially adopted from Gratzl, 1992).](image)

<table>
<thead>
<tr>
<th>Oxygen species</th>
<th>Hydroperoxyl radical</th>
<th>Hydrogen peroxide</th>
<th>Hydroxyl radical</th>
</tr>
</thead>
<tbody>
<tr>
<td>E$^\circ$ at pH 14</td>
<td>-0.33</td>
<td>0.20</td>
<td>-0.03</td>
</tr>
<tr>
<td>Dioxygen O$_2$</td>
<td>HOO$^\cdot$</td>
<td>HOOH</td>
<td>H$_2$O$^+$</td>
</tr>
<tr>
<td>pK$_a$</td>
<td>4.8</td>
<td>11.6</td>
<td>11.9</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Anionic form</th>
<th>Superoxide anion radical</th>
<th>Hydroperoxy anion</th>
<th>Oxyl anion radical</th>
<th>Hydroxide ion</th>
</tr>
</thead>
<tbody>
<tr>
<td>H$^+$ + O$_2^-$</td>
<td>H$^+$ + HOO$^-$</td>
<td>H$^+$ + O$^-$</td>
<td>HO$^-$</td>
<td></td>
</tr>
</tbody>
</table>
In order to initiate a reaction (reach the activation energy) under the conditions of alkaline O\textsubscript{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\textsubscript{2} and its derivatives (Sixta et al., 2006b).

In O\textsubscript{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\textsuperscript{+}) 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\textsubscript{2}, O\textsubscript{2}\textsuperscript{-}, HO\textsuperscript{•}, and O\textsuperscript{•} function as electrophiles attacking the high electron density sites in lignin, while only HOO\textsuperscript{-} 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\textsubscript{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\textsubscript{2} in the gas phase, other important factors affecting O\textsubscript{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\textsubscript{2} in solutes as a function of O\textsubscript{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\textsubscript{2} has its minimum at 100 °C. The solubility of O\textsubscript{2} in NaOH solution is less than that in water. According to Tromans (1998a, 1998b), at 100°C and under O\textsubscript{2} pressure of 700 kPa, the solubility of O\textsubscript{2} in NaOH solutions of 1.50, 1.00, and 0.50 M is 0.09, 0.12, and 0.16 g l\textsuperscript{-1}, respectively, and in water 0.19 g l\textsuperscript{-1}.

By assuming that the oxygen gas charge is about 2 w-% of pulp, or 2 g l\textsuperscript{-1} at 10% pulp consistency, only about 10% of this charge is dissolved in the alkali solution at saturation level. However, the initial O\textsubscript{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\textsubscript{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 O₂ 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 O₂ charged, was actually consumed in the reactions. Thus, a considerable amount of O₂ 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 than 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.
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/\(\cdot\)O\(^{-}\) and HO\(_2^·\)/O\(_2^·\) 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. \(\text{O}_2\) reacts with phenolate, forming phenoxy radical, the first oxidation product, and a superoxide radical anion (\(\text{O}_2^·\)), the one-electron reduction product of oxygen (Figure 6 A). Optionally, the hydroxyl radical (HO\(^·\)), formed during oxygen treatment, can react with phenolate generating a phenoxy radical and being eliminated as a hydroxide anion (Figure 6 B) (Gierer, 1982; Sixta et al., 2006b).
Phenoxyl radicals exist in different mesomeric forms. Delignification proceeds when a superoxide radical anion (O$_2^-$) 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 $\beta$-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 C-C$\beta$ 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 carbonyl-, carboxyl-, 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).
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-ethylguaiacol gave bisethylguaiacol 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 delignifica-
tion 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 pKₐ 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 Gratzi (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.

![Reaction paths of lignin with oxygen](image)

**Figure 8.** Reaction paths of lignin with oxygen (adopted from Chang and Gratzi, 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). α-Quinone reacts further with hydrogen peroxide and results in an epoxide structure (Chang and Gratzl 1980, Gratzl 1990), or alternatively with a hydroperoxy anion, resulting a muconic acid structure (Figure 9 c) (Gierer and Imsgård 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 phenoxy 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 degrada-
tion, 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.

a) Reactions involving phenolates and phenoxy radicals

b) Reactions involving the aliphatic side chain
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.

[Diagram of reactions]

**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 O₂ has been studied extensively in order to improve and optimize bleaching techniques. Oxidation of kraft lignins by O₂ (Imai et al., 2007; Kalliola et al., 2011 (publication I); Yada and Meshtsuka, 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 degrada-
tion, 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, Myceliophthora, Pheblia, 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 (pl) 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 O₂ 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 O₂ 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 O₂ 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 O₂ addition to the phenoxyl radical species. For example, the rate constant (k) for the reaction of a tyrosine-derived phenoxyl radical (TyrO•+) with O₂ has been determined to be < 10⁻⁷ M⁻¹ s⁻¹ (Jin et al., 1993). In oxidation under alkaline conditions, a superoxide anion (O₂⁻) is present (Figure 6 A). Phenoxyl radical species react with O₂⁻ at a high rate; for example, k(TyrO•+ + O₂⁻) is 1.5 × 10⁹ (Jin et al., 1993), k(G•+ + O₂⁻) is 1.4 × 10⁹, and k(H•+ + O₂⁻) is 1.7 × 10⁹ 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). O₂ activates laccase, which oxidizes the mediator to form an oxybenzotriazoly 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 O₂ (k < 10⁻¹⁰ M⁻¹ s⁻¹) (Von Sonntag and Schuchmann, 1991; Gierer et al., 1992). Such an intermediate reacts by O₂ addition, yielding a side chain oxidation product with the simultaneous generation of HOC•. Its anion, O₂•-, 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).
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 Figău et al. (2013) that low molecular mass fractions of technical lignins (M<sub>w</sub> < 4000 g mol<sup>-1</sup>), 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<sup>-1</sup> 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 (Figău 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.

---

**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).
(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 phenoxy 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 phenoxy 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 hydrophilic 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.

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

**Table 4.** Laccases and sulfhydryl oxidase.

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

\(^a\) 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 O₂ 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 O₂ 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 O₂ pressure (5–40 bar) were varied in order to set the desired initial O₂ load in respect to lignin. In general, the higher the lignin content, the higher the O₂ 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-O₂ oxidations applied to technical lignins.

<table>
<thead>
<tr>
<th>Lignin (w-%)</th>
<th>pH_int (-)</th>
<th>T (°C)</th>
<th>O₂ load on lignin (w-%)</th>
<th>t (min)</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.75</td>
<td>12.7</td>
<td>90, 110</td>
<td>28, 41</td>
<td>240*</td>
<td>I</td>
</tr>
<tr>
<td>10.0</td>
<td>10.7**</td>
<td>22</td>
<td>8</td>
<td>60</td>
<td>II</td>
</tr>
<tr>
<td>16.7, 25.0</td>
<td>12.2, 13.3</td>
<td>40</td>
<td>36, 24</td>
<td>30</td>
<td>III</td>
</tr>
<tr>
<td>0.75–25.0</td>
<td>12.7–13.4</td>
<td>40–80</td>
<td>12–35</td>
<td>30</td>
<td>IV</td>
</tr>
</tbody>
</table>

*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 O₂ consumption

During the alkali-O₂ oxidations, the reactor pressure and temperature were recorded. In the onset of the reaction (Init), part of the O₂ 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 O₂ (2). The dissolved O₂ starts to consume in lignin reactions. Simultaneously new O₂ is dissolved in the solution causing a pressure decrease. The oxygen consumed in the experiments
(O₂ 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).

\[
n(O_2)_{\text{init}} = n(O_2)_{\text{init}, L} + n(O_2)_{\text{init}, G}
\]

\[
n(O_2)_L = (p - p_{H_2O}) \times k_{eq,O_2} \times V_L
\]

\[
n(O_2)_G = \frac{(p-p_{eq}) \times V_G}{RT}
\]

\[
n(O_2) = \frac{(n(O_2)_{\text{init}} - n(O_2)_{\text{final}}) \times M(lignin)}{m(lignin)}
\]

Where

\[n(O_2) = \text{molar amount of } O_2 \text{ [mol]}\]
\[p = \text{reactor pressure [Pa]}\]
\[p_{H_2O} = \text{water vapor pressure (T dependent) [Pa]}\]
\[k_{eq,O_2} = \text{equilibrium constant for dissolved } O_2 (\text{T dependent}) [\text{M Pa}^{-1}]\]
\[V = \text{volume [m}^3\text{]}\]
\[R = \text{universal gas constant, 8.3144 [m}^3\text{ Pa K}^{-1}\text{ mol}^{-1}]\]
\[T = \text{temperature [K]}\]
\[n(lignin) = \text{molar amount of lignin [mol]}\]
\[m(lignin) = \text{mass of initial lignin in liquid phase [g]}\]
\[M(lignin) = \text{molecular mass of phenyl propane unit (C9), 180 [g mol}^{-1}\text{]}\]

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 O₂ 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.

<table>
<thead>
<tr>
<th>Lignin (w-%)</th>
<th>pH (-)</th>
<th>T (°C)</th>
<th>O(_2) load on lignin (w-%)</th>
<th>t (min)</th>
<th>Publication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0, 2.5, 5.0</td>
<td>5, 6, 8</td>
<td>22</td>
<td>-</td>
<td>120</td>
<td>II</td>
</tr>
<tr>
<td>5.0, 10.0</td>
<td>5, 6, 8</td>
<td>22</td>
<td>16, 8</td>
<td>60, 120</td>
<td>II</td>
</tr>
<tr>
<td>2.5**</td>
<td>6, 8</td>
<td>22</td>
<td>-</td>
<td>120</td>
<td>III</td>
</tr>
</tbody>
</table>

*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 CO₂ 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 CO₂ species-free NaOH, the solution was titrated with 0.1 M HCl under N₂ 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, pHᵢ (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 pHᵢ), was subtracted from that consumed during the sample solution titration, V(HCl_sample at pHᵢ). The corresponding negative charge of lignin (mmol g⁻¹) at pHᵢ was computed using the information on the volume difference, V(HCl at pHᵢ), 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 N₂ 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 20x (2.5% TBF)).

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.

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

4.1 Oxidation of lignin using O\textsubscript{2} under alkaline conditions

Depending on the aim of each study, the oxidation by O\textsubscript{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.

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Kraft lignin</th>
<th>Initial pressure (MPa)</th>
<th>T (°C)</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SW</td>
<td>0.94</td>
<td>90</td>
<td>0.9 MPa, 90°C, SW</td>
</tr>
<tr>
<td>2</td>
<td>SW</td>
<td>0.94</td>
<td>110</td>
<td>0.9 MPa, 110°C, SW</td>
</tr>
<tr>
<td>3</td>
<td>SW</td>
<td>0.64</td>
<td>90</td>
<td>0.6 MPa, 90°C, SW</td>
</tr>
<tr>
<td>4</td>
<td>SW</td>
<td>0.64</td>
<td>110</td>
<td>0.6 MPa, 110°C, SW</td>
</tr>
<tr>
<td>5</td>
<td>HW</td>
<td>0.64</td>
<td>90</td>
<td>0.6 MPa, 90°C, HW</td>
</tr>
</tbody>
</table>

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 (l). 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 \( \text{O}_2 \) consumption between kraft lignin oxidation and industrial pulp oxygen delignification can be partly due to the \( \text{O}_2 \) consumption of sulfide carry-over, caused by insufficient brownstock washing and/or incomplete oxidation of white liquor in industrial applications.

### 4.1.2 Alkali-\( \text{O}_2 \) 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-\( \text{O}_2 \) 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 ($pH_{\text{final}}$), molecular mass ($M_w$), and O$_2$ uptake are plotted as a function of lignin content. Figure 15 d shows the relation between $pH_{\text{final}}$ and $M_w$.

It is seen that the higher the lignin content, the lower the $pH_{\text{final}}$ 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$_{\text{final}}$ 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 molecu-
lar 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-O₂ oxidation of soda lignin (SL) from wheat straw and O₂ 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Lignin (w-%)</th>
<th>NaOH on lignin (w-%)</th>
<th>pHInit. (-)</th>
<th>TInit. (°C)</th>
<th>O₂ load on lignin (w-%)</th>
<th>O₂ uptake on lignin (w-%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preliminary oxidations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL 0.75%60C ox</td>
<td>0.75</td>
<td>53</td>
<td>13.1</td>
<td>60</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>SL 0.75%80C ox</td>
<td>0.75</td>
<td>53</td>
<td>13.1</td>
<td>80</td>
<td>23</td>
<td>11</td>
</tr>
<tr>
<td>SL 2.5%60C ox</td>
<td>2.5</td>
<td>31</td>
<td>12.7</td>
<td>60</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>SL 2.5%80C ox</td>
<td>2.5</td>
<td>31</td>
<td>12.7</td>
<td>80</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>SL 5% ox</td>
<td>5</td>
<td>38</td>
<td>13.3</td>
<td>80</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td><strong>SL 15% ox</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL 15% i.ox</td>
<td>15</td>
<td>23</td>
<td>13.1</td>
<td>55</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>SL 25% ox</td>
<td>25</td>
<td>30</td>
<td>13.4</td>
<td>50</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td><strong>SL 25% 1ox a</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Improved oxidations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SL 15% 2ox</td>
<td>15</td>
<td>17 (41)*</td>
<td>13.0</td>
<td>75</td>
<td>34 (49)*</td>
<td>8 (17)*</td>
</tr>
<tr>
<td>SL constant pH ox b</td>
<td>15, 11.4Final</td>
<td>65</td>
<td>13.1</td>
<td>70</td>
<td>35</td>
<td>23</td>
</tr>
<tr>
<td>SL constant pH 2ox c</td>
<td>15, 10.5Final</td>
<td>75 (99)*</td>
<td>13.0</td>
<td>70</td>
<td>35 (50)*</td>
<td>18 (27)*</td>
</tr>
</tbody>
</table>

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, O₂ 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 de-
graded. 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$^{-1}$ (Liitä 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; Pleken...
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 Haagermann 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 plast-
cized 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 re-
duced, 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).

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 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.
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.
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 phenoxy 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$_3$SH). 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$_2$O$_2$, 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+AsOx1 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+AsOx1, 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 160ºC. The extrusion temperature in composite processing is generally around 160 to 165ºC (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+AsOx1 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\textsubscript{2} pressure at room temperature. Reference samples (ref.) were not O\textsubscript{2} boosted.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Substrate\textsuperscript{a}</th>
<th>Lignin dry solids (%)</th>
<th>pH</th>
<th>Enzyme\textsuperscript{b}</th>
<th>Dose\textsuperscript{c} (nkat g\textsuperscript{-1} or %)</th>
<th>HBT\textsuperscript{d} (%)</th>
<th>Treatment (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SE</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ref.</td>
</tr>
<tr>
<td>2</td>
<td>SE</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>SE</td>
<td>5</td>
<td>5</td>
<td>ThL</td>
<td>100</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>SE</td>
<td>5</td>
<td>5</td>
<td>ThL</td>
<td>100</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>SE</td>
<td>5</td>
<td>6</td>
<td>TaLcc2</td>
<td>100</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>SE</td>
<td>5</td>
<td>6</td>
<td>TaLcc2</td>
<td>100</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>IndAT</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ref.</td>
</tr>
<tr>
<td>8</td>
<td>IndAT</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>9</td>
<td>IndAT</td>
<td>5</td>
<td>5</td>
<td>ThL</td>
<td>100</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>SE</td>
<td>10</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>ref.</td>
</tr>
<tr>
<td>11</td>
<td>SE</td>
<td>10</td>
<td>8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>12</td>
<td>SE</td>
<td>10</td>
<td>8</td>
<td>r-MaL</td>
<td>0.1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>13</td>
<td>SE</td>
<td>10</td>
<td>8</td>
<td>r-MaL, AoSOX1</td>
<td>0.1, 0.1</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>14</td>
<td>SE</td>
<td>10</td>
<td>10.7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Lignins: SE=Stora Enso lignin, IndAT=Indulin AT

\textsuperscript{b} Laccases: ThL=Trametes hirsuta, TaLcc2=Thielavia arenaria Lcc2, r-MaL=Melanocarpus albomyces

\textsuperscript{c} Sulfhydryl oxidase: AoSOX1=Aspergillus oryzae

\textsuperscript{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 O₂ 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 O₂ 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, phenoxy 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 incorpora-
tion 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 phenoxy radical could influence the degree of incor-
poration. 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 incorpora-
tion 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 particu-
lar, the continuous addition might have enhanced the incorporation of the deriva-
tive to lignin.

Because the lignin functionalization with the ester derivative by laccase-
catalyzed oxidation was not successful, alkali-O₂ 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 O₂ consumption was 0.83 mol O₂ 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 oxida-
tion accelerated the oxidation reactions, leading to higher O₂ 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-O₂ oxidized samples were determined (Figure 24, Table 10). The alkali-
O₂ 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.](image)

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-O₂ 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 ($M_w 800 \text{ g mol}^{-1}$) 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-O₂ 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-O₂ 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-O₂ oxidized (O₂ 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.

<table>
<thead>
<tr>
<th>Lignin, derivative, d.s.</th>
<th>Dialysis CO (Da)</th>
<th>Dialysis yield (%)</th>
<th>Lignin yield, computed (%)</th>
<th>$M_n$ (g mol⁻¹)</th>
<th>$M_w$ (g mol⁻¹)</th>
<th>PD</th>
<th>$T_m$ (°C)</th>
<th>$\Delta c_p$ (J g⁻¹ K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IndAT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 100</td>
<td>4 500</td>
<td>2.1</td>
<td>144</td>
<td>0.329</td>
</tr>
<tr>
<td>IndAT, - , 25% a</td>
<td>1000</td>
<td>75</td>
<td>75</td>
<td>3 300</td>
<td>6 200</td>
<td>1.9</td>
<td>92</td>
<td>0.222</td>
</tr>
<tr>
<td></td>
<td>3500</td>
<td>79</td>
<td>79</td>
<td>3 200</td>
<td>5 900</td>
<td>1.8</td>
<td>99</td>
<td>0.126</td>
</tr>
<tr>
<td>IndAT, - , O₂ ox at 25%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 100</td>
<td>9 700</td>
<td>3.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>78</td>
<td>78</td>
<td>4 400</td>
<td>10 300</td>
<td>2.3</td>
<td>116</td>
<td>0.108</td>
</tr>
<tr>
<td></td>
<td>3500</td>
<td>74</td>
<td>74</td>
<td>4 400</td>
<td>10 100</td>
<td>2.3</td>
<td>117</td>
<td>0.159</td>
</tr>
<tr>
<td>IndAT, ester, O₂ ox at 25%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 600</td>
<td>5 600</td>
<td>3.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>50</td>
<td>75</td>
<td>3 500</td>
<td>7 800</td>
<td>2.2</td>
<td>99</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>3500</td>
<td>54</td>
<td>81</td>
<td>3 300</td>
<td>7 500</td>
<td>2.3</td>
<td>95</td>
<td>0.132</td>
</tr>
<tr>
<td>Ester V-PEG b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>760</td>
<td>800</td>
<td>1.1</td>
<td>-56</td>
<td>0.791</td>
</tr>
</tbody>
</table>

a Indulin AT dissolved in NaOH at 25 w-% dry solids, un-oxidized
b Melting temperature, $T_m$ 5.4°C; $\Delta H$ -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 pH 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 hydro-
peroxide 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 C-O 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, effi-
cient 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 O₂ oxidation on lignin characteristics. + for increasing and - for decreasing impact. 0 indicates that there is no or only very little impact.

<table>
<thead>
<tr>
<th></th>
<th>O₂ oxidation</th>
<th>Under alkaline conditions</th>
<th>Laccase-catalyzed</th>
<th>pH ≤ 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High lignin content</td>
<td>High O₂ load</td>
<td>High pH&lt;sup&gt;a&lt;/sup&gt; 11-12</td>
</tr>
<tr>
<td>Molecular mass</td>
<td>++</td>
<td>+</td>
<td>0 / +</td>
<td>- / --</td>
</tr>
<tr>
<td>Negative charge</td>
<td>0 / +</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Solubility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
</tr>
</tbody>
</table>

<sup>a</sup> 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.
References


82


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 (O₂) is one of the lignin modification methods. O₂ 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 O₂ to modify technical lignins to enhance their utilization for polymeric chemicals and material applications. O₂ 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 O₂ under alkaline conditions and laccase catalysis are discussed. A simple alkali-O₂ 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 phenoxy radical with superoxide (O₂•⁻) 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 phenoxy radical, which spontaneously undergoes coupling and thus induces condensation. Under laccase catalysis conditions, O₂•⁻ is not present and thus the reaction paths described above do not function. Therefore, the formed phenoxy radicals couple with each other rather than degrade. O₂ has a significantly lower tendency to attach to the phenoxy radical compared to O₂•⁻. 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-O₂ 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.

---|---
Date | June 2015
Language | English, Finnish abstract
Pages | 92 p. + app. 52 p.
Name of the project | 
Commissioned by | 
Keywords | lignin, oxidation, oxygen, laccase, composite, cement, concrete, plasticizer
Publisher | VTT Technical Research Centre of Finland Ltd P.O. Box 1000, FI-02044 VTT, Finland, Tel. 020 722 111
Kemiaallinen ja entsymaathtinen happihapetus teknisen ligniinin valorisoinnissa materiaalisovelluksia varten

Tekijä(t)
Anna Kalliola

Tiivistelmä


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 (O₂), an environmentally friendly and low-cost oxidizing agent, is active towards phenolic lignin, and is thus a potential choice for lignin valorization. O₂ oxidation of lignin can be aided using alkaline conditions or an enzyme as a catalyst. In this work, a simple alkali-O₂ 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 O₂ 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-O₂ oxidation provides wide possibilities to modify lignin molecular mass and charge properties.