

Towards a new understanding of the retro-aldol reaction for oxidative conversion of lignin to aromatic aldehydes and acids

Ajinkya More ^a, Thomas Elder ^b, Zhihua Jiang ^{a,*}

^a Alabama Center for Paper and Bioresource Engineering, Department of Chemical Engineering, Auburn University, Auburn, AL 36849, United States

^b United States Department of Agriculture – Forest Service, Southern Research Station, Auburn, AL 36849, United States

ARTICLE INFO

Article history:

Received 14 April 2021

Received in revised form 9 May 2021

Accepted 15 May 2021

Available online 21 May 2021

Keywords:

Lignin

Vanillin

Vanillic acid

Oxidation

Retro-aldol reaction

Technology

ABSTRACT

The retro-aldol reaction is one of the key steps involved in the oxidative conversion of lignin to aromatic aldehydes and acids. In principle, the retro-aldol reaction can proceed in the absence of oxygen. In this work, a new approach based on the influence of oxygen on the oxidation of lignin was investigated. In this approach, the duration of oxygen charged during the reaction was optimized to, for the first time, improve the yield of aromatic aldehydes and acids. The effect of reaction chemistry, time, temperature, and lignin feedstock plays a key role on the yield of aromatic aldehydes and acids. At 140 °C, oxidation of softwood Lignoboost kraft lignin for 40 min results in combined maximum yield of 5.17% w/w of vanillin and vanillic acid. In comparison, using the new approach in which oxygen was charged for only 20 min during the 40 min reaction improved this yield considerably to 6.95%. Further, yield improvement was obtained when applying this approach to different lignin feedstocks. Oxidation also increased the carboxyl content in lignin from 0.49 mmol/g to 1.41 mmol/g which represents a marked improvement. The current study provides new evidence showing that the oxidation reaction is a crucial pathway for lignin valorization.

© 2021 Elsevier B.V. All rights reserved.

1. Introduction

Lignin is the most abundant natural biopolymer after cellulose and accounts for about 30% of non-fossil carbon on earth [1,2]. The distinct aromatic nuclei representing *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl nuclei (S) in the lignin structure arise from the monomers *p*-hydroxycoumaryl alcohol, coniferyl alcohol, and sinapyl alcohol respectively [3,4]. The ratio of these monomers influences the degree of branching and the reactivity of lignin [5]. During pulping processes, delignification of the wood matrix takes place by chemically degrading the lignin to liberate cellulose. The lignin obtained from technical processes has different structural characteristics based on the origin of wood species, delignification type and recovery process of lignin from pulping liquors [6,7]. The structural details of one fragment of technical kraft lignin are highlighted in Fig. 1.

In an integrated approach, the oxidative conversion of lignin to aromatic aldehydes and acids such as vanillin and vanillic acid is widely proposed [9–12]. Currently, only lignosulfonates are used for the industrial production of vanillin due to higher yields ranging from 5 to 7% in the presence of transition metal catalysts. However, lignosulfonates represent less than 10% of total lignins extracted [13,14]. Kraft lignin represents the majority of lignin produced worldwide and accounts

for 85% of all the lignin produced, corresponding to around 45 million metric tons/year. However, lignin which is an abundant aromatic biopolymer, is underutilized and is considered as a non-valorized waste [15]. Currently, it is mainly used as a source of low grade fuel in the pulping operation and only about 100,000 tons of kraft lignins available are valorized per year [16,17]. Therefore, valorization of kraft lignin into high value-added products is an important goal.

Pine kraft lignin has been commercially available as 'Indulin' from Mead-Westvaco since the 1950s. Indulin represented the main source of commercial kraft lignin for a long time. However, the conventional process of lignin precipitation and separation from kraft black liquors causes serious problems related to complete or partial plugging of the filter cake and of the filter medium. In 2013, the Domtar Plymouth Mill in North Carolina (USA) installed the 'Lignoboost' process to address the problems associated with traditional processes of lignin separation from kraft black liquors. In the following year, West Fraser Company installed the 'Lignoforce' process at the Hinton Pulp Mill in Alberta, Canada. Due to the differences in the process of lignin precipitation and separation from kraft black liquors, these three kraft lignins have quite different properties [18,19].

The oxidation of lignin produces vanillin (4-hydroxy-3-methoxybenzaldehyde, C₈H₈O₃), which is a high value product having applications in a variety of industries. It is useful due to its aromatic nature and reactive functional groups, methoxy, aldehyde and phenol. It is the highest volume aromatic aldehyde produced worldwide [13]. It has

* Corresponding author.

E-mail address: zzj0012@auburn.edu (Z. Jiang).

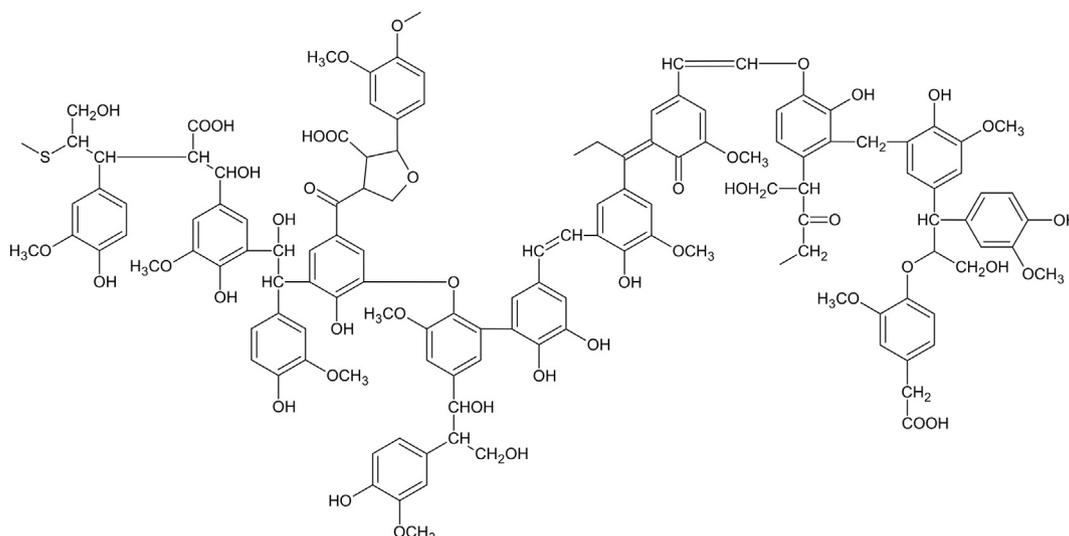


Fig. 1. Structure of kraft lignin [8] (reproduced with permission from Elsevier).

various applications in food, cosmetic, chemical and pharmaceuticals industries. The importance of vanillin is also growing because of its antioxidant and anti-cancer properties and also its involvement in bacterial cell to cell signaling [20,21]. Other applications of vanillin are as a ripening agent to increase the sucrose content in sugar cane or in the preparation of sunscreen [22]. Vanillic acid (4-hydroxy-3-methoxybenzoic acid) is another value added product generated in the process of lignin oxidation which is used as a flavoring agent. Recently, vanillic acid has received considerable attention due to its role in preventing human diseases. It is of importance due to its antibacterial, antimicrobial and chemo-preventive properties [23]. Vanillin and vanillic acid have both been obtained as major products from the alkaline oxidation of softwood and hardwood lignin [24,25].

The oxidative conversion of lignin to vanillin and vanillic acid in an alkaline medium using oxygen has been widely discussed in the literature [14,26–31]. Oxygen is the preferred oxidizing agent due to its environmental friendliness, high atom economy and low price. However, due to the lower yields obtained in this process, catalysts in combination with oxygen are required to improve the yields by a factor of 1.5–2. To produce vanillin and vanillic acid in the kraft pulping process, it is crucial to avoid the use of catalysts to allow for downstream processing of kraft liquor in evaporators and recovery boilers. The high consumption of oxygen – over 10 mol per mol of vanillin obtained is also an unresolved problem [12]. Another limitation of this process is the use of large amount of acids, organic solvents and energy which have environmental consequences [13]. Therefore, the development of technologies which are sustainable and easily integrable in the current pulp and paper industrial processes are needed. The work reported here is focused on addressing this problem in the oxidative conversion of lignin to vanillin and vanillic acid using a sustainable approach. In this approach, more lignocellulosic biomass can be converted to value-added products with lower consumption of oxygen improving the overall economic and environmental viability of this process.

The formation of vanillin and vanillic acid from the oxidation of lignin using oxygen under alkaline conditions depends on reaction chemistry, time, temperature, oxygen partial pressure, lignin concentration, and feedstock. Among the various oxidation pathways proposed in the literature, the retro-aldol reaction is the most promising pathway for explaining the production of vanillin and acetovanillone. In alkaline conditions, the reaction begins with dehydration of the C_{α} - C_{β} bond proceeded by electron abstraction from the phenoxyl anion. The oxygen oxidation then results in single-electron abstraction from phenoxyl anion resulting in the formation of the superoxide anion radical and phenoxyl radical (Step 1) (Fig. 2). The major steps that follow are the

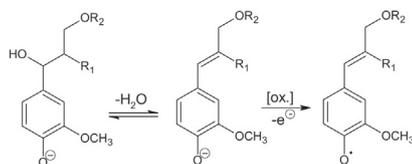
formation of a quinone methide from the phenoxyl radical (Step 2a & 2b), and nucleophilic attack of a hydroxyl ion at γ position which is under thermodynamic control (Step 3). The hydroxyl group at the γ position is oxidized to a γ -carbonyl group (coniferaldehyde type structure) (Step 4). The retro-aldol reaction proceeds by alkaline hydrolysis of this structure to form an α -hydroxy- γ -carbonyl structure (Step 5). Further, alkaline dissociation of this α -hydroxy- γ -carbonyl structure takes place at C_{β} position (Step 6) followed by retro-aldol cleavage to form vanillin as shown in (Step 7). Under kinetic control, nucleophilic attack of hydroxyl ion takes place at the α -position. Subsequent oxidation and eventual retro-aldol reaction of the resulting α -oxo- β -unsaturated structure results in acetovanillone.

In principle, the retro-aldol reaction (Steps 5, 6 and 7) proceeds in the absence of oxygen. Also, the degradation of vanillin and vanillic acid occurs at severe oxidation conditions if oxygen is charged beyond this final phase of product formation [6,14,32]. To confirm the role of the retro-aldol reaction, a model compound study was performed using vanillidenacetone (α - β -unsaturated- γ -carbonyl type structure) in the literature [30]. In this study, the model compound was formed by the aldol condensation of vanillin and acetone as shown in Fig. 3.

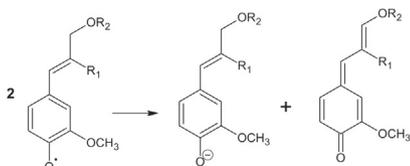
It was observed that under an argon atmosphere, vanillidenacetone is hydrolyzed with the formation of vanillin at pH 10 and 160 °C for 30–50 min, with vanillin selectivity of about 90%. In the presence of oxygen and a catalyst, vanillidenacetone is consumed faster and the vanillin yield decreases by a factor of 3–5. Therefore, the rate of the retro-aldol reaction decreases with the introduction of oxygen which decreased vanillin yield substantially at pH 10. However, increasing the pH to 11, increased the rate of the retro-aldol reaction, since it is directly proportional to the concentration of hydroxide ion. In this case, vanillin yield does not decrease under the action of oxygen prior to reaching its maximum. A decrease in vanillin yield after reaching its maximum is likely due to its subsequent oxidation. Therefore, this study confirms the occurrence of the retro-aldol reaction in the formation of vanillin from the oxidation of lignin model compounds. It can also be deduced from this study that the retro-aldol reaction can proceed in the absence of oxygen and the introduction of oxygen in this phase is likely to contribute negatively to vanillin yield due to its subsequent oxidation after reaching its peak yield.

In another study, vanillin, vanillic acid and traces of acetovanillone were formed from sodium lignosulfonate in the absence of oxygen solely by the hydrolysis reaction [14]. In this study, hydrolysis was observed at much lower temperatures (120 °C) compared to the previous study in which it was carried out at 160 °C. The formation of vanillic acid in this case also suggests that it is not a product of only the oxidation of

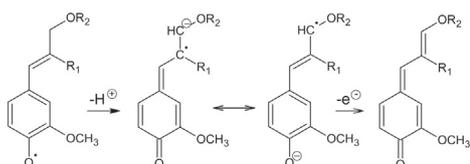
Step 1: Dehydration followed by initiation of phenoxyl anion



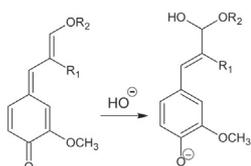
Step 2a: Disproportionation of phenoxyl radical to form quinone methide



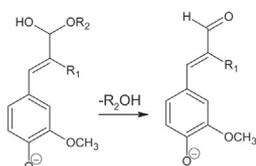
Step 2b: Oxidation of phenoxyl radical preceded by proton detachment



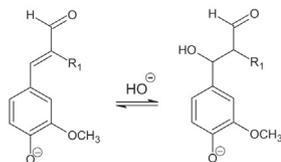
Step 3: Nucleophilic addition of hydroxide ion to form coniferyl alcohol structure



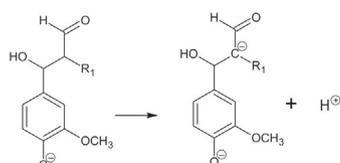
Step 4: Oxidation of coniferyl alcohol structure to γ -carbonyl group



Step 5: Alkaline hydrolysis of γ -carbonyl coniferyl structure



Step 6: Alkaline dissociation of α -hydroxy- γ -carbonyl structure



Step 7: Retro-aldol cleavage of C α -C β bond to form vanillin

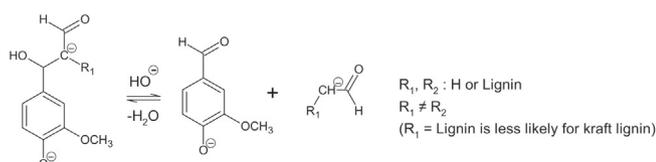


Fig. 2. Mechanism of alkaline oxidation of lignin using oxygen [31].

vanillin. It was also observed in this study that the increase in oxygen partial pressure led to an increase in the vanillin oxidation rate after the maximum vanillin concentration was reached. This study supports the presence of the retro-aldol reaction and the role of oxygen in the oxidative conversion of lignins to aromatic aldehydes and acids. Therefore, yields may be improved in this final phase of the reaction sequence and degradation of high-value added products can be inhibited by controlling the amount of oxygen charged in the reaction after reaching the peak yields from oxidation. The objective of this work is to analyze the effect of controlling the amount of oxygen charged during the reaction on the yield of vanillin and vanillic acid. Since the recently commercialized LignoBoost technology is gaining momentum in the kraft lignin market, softwood kraft lignin from this process was selected as a feedstock for analysis. Also, it contains lower levels of impurities which is advantageous in further chemical processing of this lignin. The optimum conditions based on time and temperature were established and then optimization based on the effect of oxygen addition was performed. Further, based on these experiments for LignoBoost kraft lignin, different lignin feedstocks were then analyzed to determine if similar yield improvements can be observed.

2. Materials and methods

2.1. Chemicals

Oxygen and nitrogen of ultra-high purity were supplied by Airgas, USA. Sodium hydroxide beads (purity $\geq 97\%$), sulfuric acid and hydrochloric acid (1.0 N laboratory reagent grade), HPLC grade acetonitrile (assay $\geq 99.95\%$) were purchased from VWR Chemicals, USA. Sodium chloride (assay $\geq 99\%$) was supplied by Fisher Chemical™, USA. Vanillin and vanillic acid, both with a purity higher than 99% were supplied by Sigma-Aldrich, USA.

2.2. Raw materials

In this work, four different lignins were used to study the effect of their different processing conditions. They are the following: 1) LignoBoost softwood from Valmet AB LBoostS; 2) Indulin AT from Sigma-Aldrich LlnAT 3) *H*-lignin (enzymatic hydrolysis lignin) from FPIInnovations 4) Maraspense AG from Borregaard Lignotech LMarAG. All lignins were analyzed for moisture content and used without further purification. The structural features of the lignins have been characterized in the literature [18,33] and shown in Table 1 as follows:

2.3. Oxidation of lignin

Oxidation experiments were carried out in a Parr reactor with a capacity of 1 L (Series 4520). The heating and temperature were controlled with a Parr 4848 reactor controller. Temperature and total pressure inside the reactor were measured using a Type J (iron-constantan) thermocouple and a pressure gauge respectively. The agitator speed was kept constant at 400 RPM for all runs. The procedure for oxidation of lignin using oxygen was carried in accordance with literature [3]. 20 g of lignin were dissolved in 200 mL of alkaline solution containing 26.67 g of NaOH. After complete dissolution, the resulting mixture was diluted to a final volume of 333 mL. This concentration of lignin (20 g in 333 mL of solution) was kept constant for all experiments performed. The resulting solution was introduced into the reactor and initially pressurized to 30 psi using nitrogen. The reactor was then heated until the temperature set point was reached. After the temperature set point was reached, oxygen was introduced to the reactor and the reaction time was monitored from the point of admission of oxygen. The reactor was pressurized with 50 psi of oxygen for all experiments based on optimized conditions for oxygen pressure in the literature [3]. The solubility of oxygen in the reaction medium is dependent on the oxygen partial pressure (P_{O_2}), temperature of the reaction (T) and the ionic

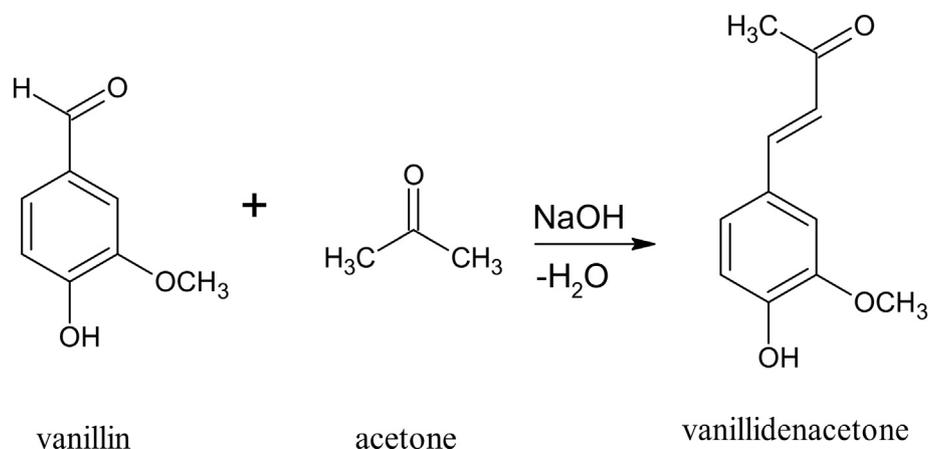


Fig. 3. Aldol condensation of vanillin and acetone resulting in vanillidenacetone* according to the literature [30]. *Note – The term 'vanillidenacetone' used here to be consistent with the literature and it refers to the compound 'dehydrozingerone' (4-(4-hydroxy-3-methoxyphenyl)-3-buten-2-one).

strength of the liquid medium (I). The concentration of dissolved oxygen $[O_2^{liq}]$ can be obtained with the following empirical correlation which is valid for temperatures ≤ 150 °C and oxygen partial pressures ≤ 5 MPa [14,32,34]:

$$[O_2^{liq}] = \left(3.559 - 6.659 \times 10^{-3} \times T - 5.606 \times P_{O_2} + 1.594 \times 10^{-5} \times P_{O_2} \times T^2 + 1.498 \times 10^3 \times \frac{P_{O_2}}{T} \right) \times \left(10^{-0.144 \times I} \right) \times \left(10^{-3} \right)$$

where P_{O_2} is in bar, I and $[O_2^{liq}]$ is in mol per liter, and T is in Kelvin.

The water partial pressure above the NaOH solution, P_{NaOH} (bar) can be calculated as follows:

$$\log P_{NaOH} = a + b \log p^0$$

the parameters a and b depend only on the concentration of NaOH (M) and p^0 (bar) represents the equilibrium partial pressure of water vapor over pure water as a function of temperature

$$a = -0.010986M - 1.461 \times 10^{-3}M^2 + 2.03528 \times 10^{-5}M^3$$

$$b = 1 - 1.34141 \times 10^{-3}M + 7.07241 \times 10^{-4}M^2 - 9.5362 \times 10^{-6}M^3$$

$$\text{and } \log p^0 = 35.4462 - \frac{3343.93}{T} - 10.9 \log T + 0.0041645T$$

where T is in Kelvin [14,35].

Since the reaction is exothermic, the reaction was monitored using the initial temperature (T_i). The effect of temperature on the yield of vanillin and vanillic acid and the carboxyl content of lignin was studied at 120 °C, 130 °C, 140 °C, 150 °C and 160 °C at a constant reaction time of 40 min. The effect of time at 0, 20, 40 and 60 min was studied by keeping the temperature of the reactor constant at 140 °C. The effect of controlling the amount of oxygen charged during the reaction was studied by introducing oxygen for 0, 5, 20 and 30 min out of the 40 min reaction time at 140 °C. *LBoostS*, *LlnAT*, *H-lignin* and *LMarAG* were then used to compare the conventional strategy (oxygen introduction = 40 min)

Table 1
Structural characterization of different lignins.

Lignin properties	Lignoboost	Indulin AT	H-lignin	Maraspense AG
Molecular weight, g/mol	M_w : 6772	M_w : 6549	M_w : 5840–7580	M_n : 534.5
Carboxylic OH, mmol/g	0.35	0.40	0.10–0.15	n.d
Aliphatic OH, mmol/g	2.02	2.19	4.8–6.3	n.d
Total phenolic OH ^a , mmol/g	4.28	3.43	0.62–0.92	1.42

Note^a-includes condensed and uncondensed phenolic OH groups, n.d: not detected.

against a new proposed strategy (oxygen introduction = 20 min out of 40 min).

2.4. Quantification of yield using high-performance liquid chromatography

The low molecular weight aromatic aldehydes and acids produced by lignin oxidation with oxygen were quantified by high performance liquid chromatography (HPLC). The details on the extraction procedure and HPLC conditions were in accordance with the literature [34]. Quantification of all compounds was done based on calibration curves prepared from standard solutions in the required concentration range. The HPLC system was equipped with an Alcott 708 auto-sampler, an on-line degasser, a Lab Alliance Series III pump and a Waters 2487 Dual λ Absorbance Detector. The detection wavelength was set to 280 nm and the injection volume was 10 μ L. Chromatograms were acquired at 60 °C with a flow rate of 0.6 mL/min using a mobile phase composed of two eluents (A) 5 mM sulfuric acid and (B) acetonitrile with a ratio of 4:1 by weight respectively [36]. The analytical column was Aminex HPX 87H ion exclusion column with column size 300 mm \times 7.8 mm, and a particle size of 9 μ m.

2.5. Carboxyl content determination using conductometric titration

The carboxyl content of the precipitated lignin from the oxidation reactions and untreated lignin was determined using the conductometric titration method [37]. After the oxidation treatment, an aliquot of lignin solution was adjusted to pH below 2 by addition of 1 N HCl in the ratio of 1:3 v/v (lignin solution: 1 N HCl). This caused the lignin present in the solution to precipitate out. The precipitated lignin was then recovered by centrifugation and then freeze dried to obtain dry lignin powder with moisture content $\leq 10\%$ by weight. Further, freeze-dried samples of precipitated lignin equivalent to 1.5 g oven dry were added to 300 mL of 0.10 N HCl and stirred for 1 h. The lignin was then filtered and washed with de-ionized water until the conductivity of the effluent water was less than 5 μ S cm^{-1} . The washed lignin was then dispersed in 0.001 N NaCl (250 mL) with further addition of 0.10 N HCl solution (1.5 mL), stirred and titrated with 0.05 N NaOH under continuous bubbling of nitrogen. A Metrohm Autotitrator was used for titration, with an 856 Conductivity module connected to the main module (888 Titrando) coupled with Tiamo™ 2.5 software. The conductivity was plotted against the volume of NaOH resulting in parabolic curve composed of three distinguishable regimes. The first regime shows a decrease in conductivity, which is the neutralization of added HCl. The second regime is a horizontal zone corresponding to the neutralization of weak acid groups, i.e. carboxylic acid groups. Finally, the third regime is associated with the conductivity increase after reaching the equivalence point, due

to the introduction of excess NaOH to the suspension. The carboxyl content of the samples was then determined based on the intersection of the three trend lines drawn for each of these regimes.

2.6. Comparing results for statistical significance using Tukey test

The yield changes due to optimization of the proposed strategy were analyzed by performing Tukey's honest significance test using Minitab® 19 software.

3. Results and discussion

The production of vanillin and vanillic acid from kraft lignin is carried out at highly alkaline conditions (pH ~ 14) and at elevated temperature (between 100 and 200 °C). Under such conditions it has been established in the literature that oxidation of lignin to vanillin and vanillic acid is linked to reaction chemistry, temperature, oxygen pressure, pH, reaction time and lignin concentration. The investigation of all these parameters on oxidation of lignin is beyond the scope of this work since the present work is mainly focused on studying the effect of controlling the amount of oxygen charged during the reaction on the yield of vanillin and vanillic acid. In principle, the mechanism of lignin oxidation to vanillin shown in Fig. 2 unites the lignin chemistry principles about phenoxyl radicals and quinone methide intermediates with the concept of the retro-aldol reaction as the final process step. It explains the necessity of using strongly alkaline media for the process and the formation of aceto derivatives as by-products.

It is evident from these studies that the retro-aldol reaction plays a key role in the formation of vanillin and vanillic acid. The amount of oxygen charged during this reaction phase may increase the yield of these compounds. This approach has not been examined in optimizing the yield of vanillin and vanillic acid in literature [3,12,30]. For the first time, such an attempt to understand this effect has been made and results are discussed in the following sections. The experiments in Sections 3.1, 3.2 and 3.3 were carried out based on conventional oxidation strategy whereas the Sections 3.4 and 3.5 were focused on understanding the effect of controlling the amount of oxygen charged during the reaction.

3.1. Effect of temperature on vanillin and vanillic acid yield

In the oxidation of lignin using oxygen, the reaction begins with dehydration (Fig. 2, Step 1) of the C_α-C_β bond followed by electron abstraction from the phenoxyl anion. The oxidation is then initiated by single electron transfer from activated phenoxyl anion species with the formation of a superoxide anion radical and a phenoxyl radical. The formation of phenoxyl radical in this step happens at elevated temperatures. Also, the dissociation constants of phenolic compounds of the type present in the lignin depend on the temperature of the system. The dissociation constant decreases as the temperature increases due to an increase in the formation of a quinone methide intermediate [38]. The effect of temperature on the yield of vanillin and vanillic acid from oxidation of *LBoostS* is shown in Fig. 4. Oxidation of *LBoostS* was carried out by keeping the oxygen pressure at 50 psi, lignin concentration at 60 g/L of solution, pH of 14 and a reaction time of 40 min based on the optimized conditions found in literature in which oxygen was introduced throughout the reaction time [3]. The combined yield of vanillin and vanillic acid shows an increasing trend from 120 °C to 140 °C. The yield increase can be attributed to an equilibrium of depolymerization favoring vanillin under thermodynamic control. The side reaction of phenoxyl radical dimerization is also suppressed with increasing temperature. The maximum yield corresponding to 140 °C was 3.10 g/L (5.17% w/w) comprised of 1.75 g/L (2.92% w/w) vanillin and 1.35 g/L (2.25% w/w) vanillic acid. The results also show that increasing the temperature to 160 °C increased the degradation of vanillin and vanillic acid. It can be observed that the relative rate of vanillic acid degradation

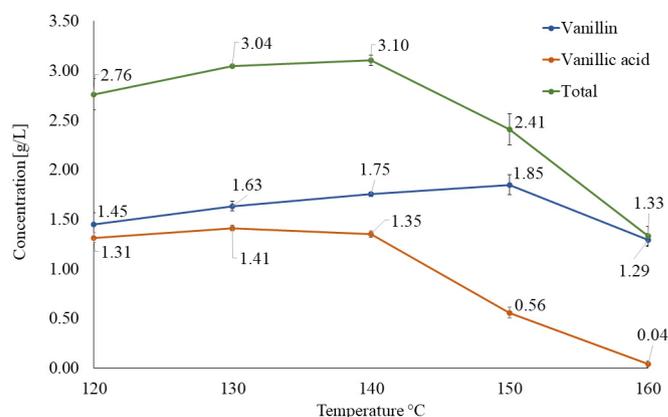


Fig. 4. Effect of temperature on the yield of vanillin and vanillic acid.

is higher compared to vanillin. Therefore, vanillic acid is more reactive and susceptible to degradation which is in accordance with recent literature [32].

3.2. Effect of temperature on carboxyl content of lignin

In an effort to provide additional insights on the structural modification of lignin, the oxidized *LBoostS* sample was isolated and analyzed as a function of temperature. The presence of carboxylic acid groups in lignin is thought to improve lignin solubilization [39–42]. In general, the carboxyl groups can occur in monomeric compounds and in end groups of the lignin macromolecule. The monomeric carboxylic acid derivatives are formed by ring opening reactions of phenolic compounds. Side chain elimination results in the formation of phenolic compounds such as vanillin as described earlier. However, in this study the analysis of carboxyl content was based on the changes in the structure of lignin macromolecule.

The carboxylic acid group formation on the end groups of the lignin macromolecule can arise from electrophilic attack at lignin centers of high electron density causing the formation of a four membered cyclic peroxide intermediate called 'dioxetane' [41,43–45]. This reactive intermediate can further rearrange to induce cleavage of carbon-carbon bonds. The effect of oxidation on carboxyl content formation in *LBoostS* is shown in Fig. 5. The carboxyl content of untreated *LBoostS* increased considerably from 0.49 mmol/g to 1.41 mmol/g after oxidation. In the temperature range studied, two distinguishing regimes can be observed. The rate of carboxyl content increases up to 130 °C. It is likely that temperatures lower than 120 °C will result to even lower carboxyl content in lignin. This is because lower temperatures will result only in minor oxidative changes which is also evidenced by lower yields of

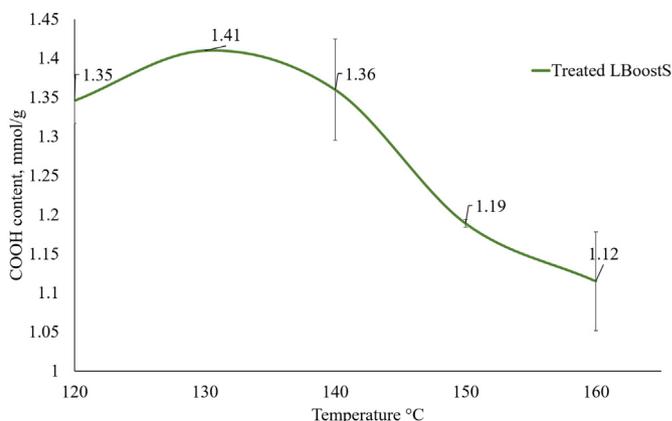


Fig. 5. Effect of temperature on carboxyl content of *LBoostS*.

vanillin and vanillic acid (Fig. 4). The carboxyl content obtained at the peak yield of these compounds was 1.36 mmol/g which is slightly lower compared to the maximum. However, increasing the temperature further to 160 °C decreased the carboxyl content to 1.12 mmol/g. It can be observed that in this temperature range, increasing the reaction severity does not increase the carboxyl content correspondingly.

3.3. Effect of time

The effect of time on the yield of vanillin and vanillic acid is interrelated to the severity of the other processing parameters. In general, a higher severity of reaction due to higher initial temperatures and oxygen pressures can lead to higher vanillin and vanillic acid yields in a shorter reaction time; however, the degradation of vanillin and vanillic acid is also higher [3,6]. The yield profile of these compounds usually follows an increasing trend until the peak yield is obtained at a given time followed by a decreasing trend. Hence, the reaction should be carried out for a certain period of time which optimizes the peak yields. Based on Figs. 4 and 6, it is evident that the yield patterns of vanillin and vanillic acid follow increasing and decreasing regimes. The optimum time for which the reaction equilibrium favors the products was obtained at 40 min.

Under the influence of oxygen, the rate of retro-aldol reaction is lower compared to the rate of oxidation of α -unsaturated coniferaldehyde [30]. Therefore, the introduction of oxygen in the reaction media after the peak yield of vanillin and vanillic acid is reached, should negatively affect the yield of these compounds. Based on this understanding, the experiments in Section 3.4 were carried out to analyze the effect of controlling the amount of oxygen charged during the reaction on the yield of vanillin and vanillic acid.

3.4. Effect of controlling the amount of oxygen charged

In the formation of vanillin and vanillic acid, the rate of the retro-aldol reaction can be improved by controlling the amount of oxygen charged in the reaction. In the conventional oxidation strategy, the oxygen is charged throughout the reaction. In the proposed strategy, the initial t_{max} represents the amount of oxygen charged to favor oxidation reaction, while during the remaining time $t - t_{max}$ the introduction of oxygen into the reactor is stopped to promote the retro-aldol reaction.

In Fig. 7, the increasing trend corresponds to the reaction phase in which there is a scope to improve yield from oxidation reaction. When oxygen was charged for 5 min out of 40 min of reaction, the reaction is principally governed by the hydrolysis reaction (Fig. 2, Step 5) and partially proceeds through the oxidation reaction. Therefore, in this phase sufficient time is available for hydrolysis reaction, however due to minor oxidative changes the peak yields are not obtained. Due to this effect, the yields of vanillin and vanillic acid were 1.22 g/L

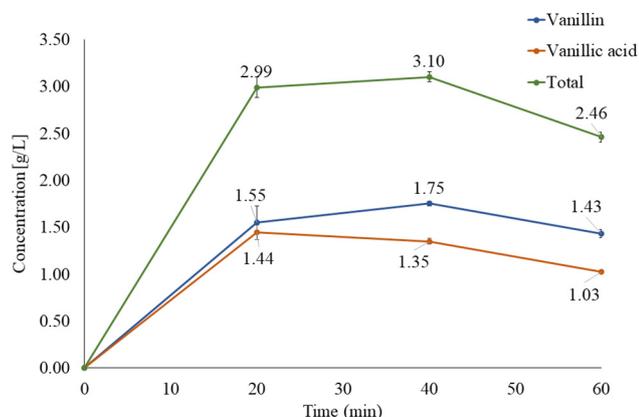


Fig. 6. Effect of time on the yield of vanillin and vanillic acid.

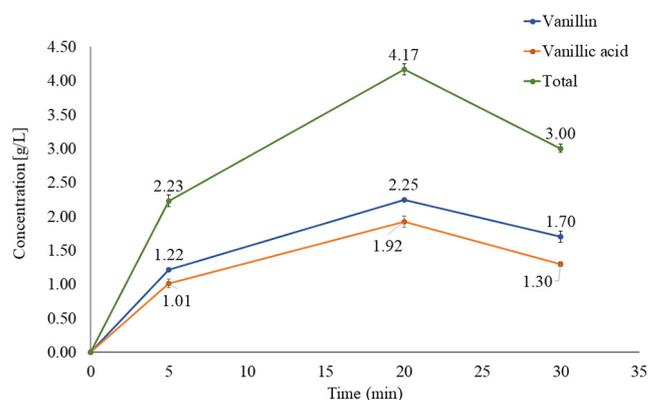


Fig. 7. Effect of controlling the amount of oxygen charged during the reaction on the yield of vanillin and vanillic acid.

(2.03% w/w) and 1.01 g/L (1.69% w/w) respectively. The peak yields of vanillin and vanillic acid were each allowed to proceed for 20 min out of 40 min of reaction. When oxygen was charged for 30 min out of 40 min, the reaction is mainly governed by the oxidation reaction and the hydrolysis is minimized which explains the decreasing trend. The introduction of oxygen into the reactor for 20 out of 40 min of total reaction time resulted in 2.25 g/L (3.75% w/w) vanillin, 1.92 g/L (3.20% w/w) vanillic acid and combined yield of 4.17 g/L (6.95% w/w). Therefore, the contribution from oxidation and hydrolysis on peak yield is likely to be balanced with this duration of oxygen charge. Hence, the proposed strategy is a viable alternative to the conventional strategy for valorization of lignin. The statistical significance of the yields obtained from this strategy was compared to the conventional strategy (in Section 3.3) using the Tukey test. The p -value of 0.008 ($p < 0.05$) was obtained representing significant yield improvement compared to the conventional strategy.

It has been hypothesized that vanillic acid is the product of vanillin oxidation [34], however, from Figs. 4, 6 and 7 one can observe that the reduction in vanillin yield after reaching the maximum does not increase the vanillic acid yield correspondingly, which is consistent with the literature [3,6,14]. Hence, the formation of vanillic acid in lignin oxidation may be a result of a different pathway not solely linked to vanillin.

From the chemistry of the retro-aldol reaction, it is clear that the γ -carbon plays an important role in vanillin formation. In a recent study, it has been found that in the absence of a γ -carbon, the oxidation of lignin model compounds through **Pathway C** favors vanillic acid formation as shown in Fig. 8. In the presence of a γ -carbon, **Pathway A** favored vanillin formation which is in accordance with retro-aldol chemistry. This might explain why the vanillic acid formation is not necessarily linked to vanillin [46].

3.5. Effect of controlling the amount of oxygen charged during the reaction on different lignin feedstocks

The structure of lignin is influenced by the wood species, the wood pulping process and the method used for its recovery from pulping liquors [3,25,39,47–52]. In this part of the study, the effect of controlling the amount of oxygen charged during the reaction was analyzed based on different lignin feedstocks. The yields of vanillin and vanillic acid for *LBoostS* were already optimized based on the parameters discussed in earlier sections. These conditions were then used to analyze other lignin feedstocks. However, it should be noted that the optimized conditions based on different parameters for *LBoostS* cannot be directly used to represent peak yields from other lignin feedstocks. However, the optimization based on the proposed strategy should be universal to other lignin feedstocks and therefore, this section mainly investigates improvements in yields due to this approach. To compare different feedstocks

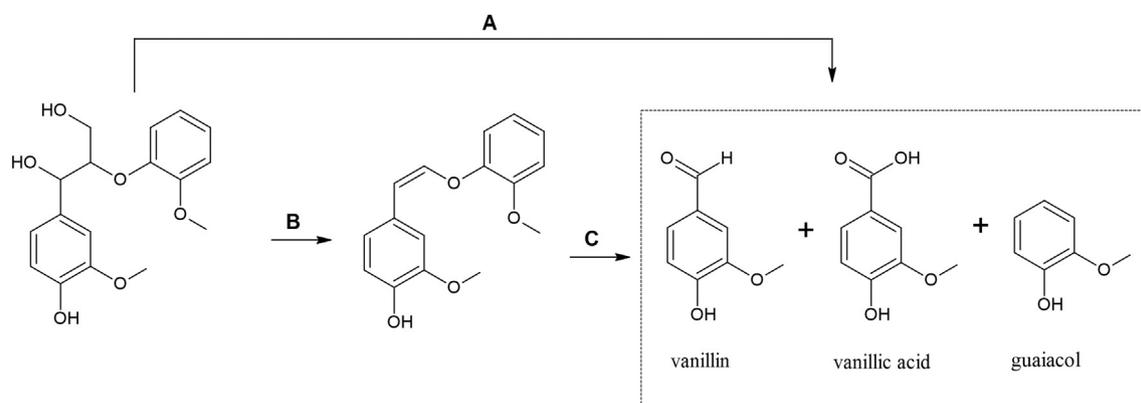


Fig. 8. Proposed pathway of formation of vanillin, vanillic acid and guaiacol [46] (reproduced from an open access article published by Royal Society of Chemistry).

Table 2
Ash and carbohydrate content of different lignin feedstocks.

Lignin	Actual lignin content (% w/w)	Ash (% w/w)	Carbohydrate (% w/w)	Literature source
a. <i>LBoostS</i>	96.92	0.78	2.30	[3]
b. <i>LInAT</i>	78.20	16.20	5.60	
c. <i>LMarAG</i>	75.04	24.96	*	[33]
d. <i>H-lignin</i>	58.80	**	22.90	

*Carbohydrate content is negligible, **Free sugars and insolubles in water in *H-lignin* account for 18.3% w/w.

for analysis, it is also critical to understand the amount of other impurities such as ash, carbohydrate, free sugars and water insolubles.

LBoostS and *LInAT* both contain ash and carbohydrates. However, due to the isolation procedure, *LBoostS* contains lower levels of these impurities compared to *LInAT*. The ash content of *LMarAG* is higher compared to *LBoostS* and *LInAT*. Usually, the purity of lignosulfonates is lower compared to technical kraft lignins [53]. *H-lignin* contained the lowest percentage by weight of lignin due to the presence of free sugars, carbohydrates and water insolubles. Table 2 highlights the properties of the technical lignins used in this study.

In the kraft process, significant cleavage of β -O-4 and α -O-4 linkages in native lignin takes place. As a result, the kraft lignin that is isolated from this process contains a significant amount of phenolic OH groups. The structural characteristics of lignins obtained from the same kraft process can be different depending upon the chemical charge and extent of cooking. For example, *LBoostS* and *LInAT* both obtained from the kraft process have significantly different properties. *LInAT* is usually isolated from kraft black liquor from the production of linerboard grade

Table 3
Statistical significance of yield improvements using Tukey honest significance test.

Compound	Lignin feedstock	Reaction time 40 min (unoptimized)	Standard deviation P (40 min trial)	Reaction time 20 min (optimized)	Standard deviation P (20 min trial)	p-Value
Vanillin	<i>LBoostS</i>	2.92	0.04	3.75	0.01	0.002
	<i>H-lignin</i>	1.99	0.05	2.62	0.02	0.007
	<i>LInAT</i>	1.55	0.02	1.66	0.01	0.039
	<i>LMarAG</i>	1.49	0.02	1.79	0.02	0.009
	<i>LBoostS</i>	2.25	0.05	3.20	0.14	0.024
Vanillic acid	<i>H-lignin</i>	1.99	0.07	4.68	0.01	0.001
	<i>LInAT</i>	0.41	0.01	1.78	0.03	0.001
	<i>LMarAG</i>	0.24	0.02	0.78	0.002	0.001
	<i>LBoostS</i>	5.17	0.09	6.95	0.14	0.008
Combined yield	<i>H-lignin</i>	3.98	0.03	7.30	0.01	0.000
	<i>LInAT</i>	1.96	0.03	3.44	0.04	0.001
	<i>LMarAG</i>	1.73	0.04	2.57	0.02	0.003
	<i>LBoostS</i>	5.17	0.09	6.95	0.14	0.008

pulp whereas *LBoostS* is obtained from black liquor from bleachable grade pulp production. Compared to *LInAT*, *LBoostS* is produced with higher chemical charges and double the cooking time. Therefore, *LBoostS* contains more phenolic OH groups than *LInAT* [18]. Another major advantage of *LBoostS* compared to other lignin feedstocks is the lower content of ash, carbohydrates and other impurities which are beneficial for further chemical processing.

From Fig. 9, it can be observed that the yield of vanillin from *LBoostS* before optimization is higher compared to other lignin feedstocks. However, it should be noted that this yield was previously optimized based on time and temperature. Based on the proposed strategy, considerable improvement in this yield was obtained due to the role played by hydrolysis (Fig. 2, Step 5) in enhancing this yield when oxygen introduction was optimized. The yield improvements observed for *H-lignin*, *LInAT* and *LMarAG* post optimization based on the proposed strategy were also statistically significant as evident by the Tukey test results shown in Table 3. In comparison, higher increments in yield for all lignin feedstocks were obtained for vanillic acid post optimization as observed in Fig. 9. As discussed earlier, vanillic acid is the compound more susceptible to degradation due to its higher reactivity. Hence, it is likely that the proposed strategy is able to avoid further degradation and promotion of the yield in the absence of oxygen during the hydrolysis phase. The combined yield of vanillin and vanillic acid from *LBoostS* and *LInAT* was higher than *LMarAG* likely due to the lower phenolic OH of lignosulfonates compared to kraft lignins [54]. It can also be observed that in the absence of catalysts the yield of vanillin and vanillic acid from lignosulfonates is lower compared to kraft lignin [3,14].

H-lignin, which is similar to native lignin resulted in a 3.98% yield before optimization which increased 7.30% post optimization with the major contributions coming from an increase in yield from vanillic acid. Similarly, a significant improvement in the yield of vanillic acid

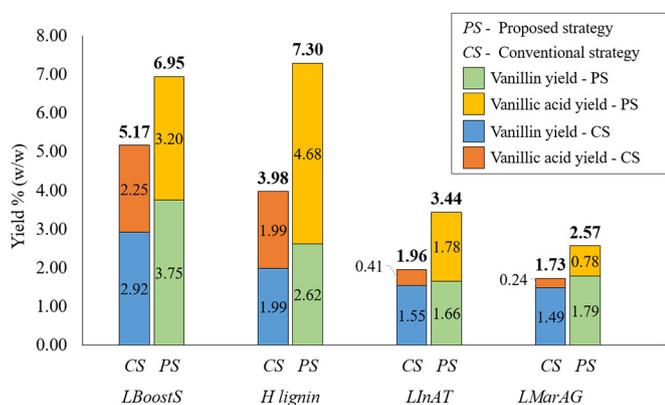


Fig. 9. Effect of controlling the amount of oxygen charged during the reaction on the yield of vanillin and vanillic acid from different lignin feedstocks.

was obtained post optimization for *LlnAT*, *LMarAG* and *LBoostS* with the highest significant improvement obtained from *LlnAT*. The *H-lignin* used in this study, contains 93.19% klason lignin content which has not shown to result in significantly higher vanillin yields, in accordance with literature [12,55]. However, it is evident that the yield of vanillin and vanillic acid can be improved significantly by using the proposed strategy as shown in Table 3. The statistical significance of the proposed strategy compared to conventional strategy showed *p*-values less than 0.05 for all vanillin and vanillic acid yields obtained from different lignin feedstocks. Table 3 compares the yields of vanillin and vanillic acid obtained before and post-optimization using Tukey's honest significance test.

4. Conclusion

The study of the effect of controlling the amount of oxygen charged during the reaction on the yield of vanillin and vanillic acid was the main objective of this work. Based on the results obtained, it was demonstrated for the first time that the efficiency of the retro-aldol reaction can be improved by controlling the amount of oxygen charged, as shown from the yields of vanillin and vanillic acid. This also reduces further degradation of these compounds. The yield improvement using the proposed strategy was validated for different lignin feedstocks, implying a certain level of similarity in the lignin reaction pathways favoring vanillin and vanillic acid. However, in this case, the changes in reaction chemistry due to the presence of reactive sites cannot be eliminated. The results also indicate the degradation of vanillin does not increase the yield of vanillic acid proportionately, supporting the recent reports showing that during oxidation, vanillic acid can be formed from different pathways not solely linked to vanillin. In this regard, the formation of vanillin is linked to the presence of the γ carbon, however in its absence, the preferred product can be vanillic acid. The higher relative degradation rate obtained for vanillic acid can be linked to the reactivity of this compound. Oxidation of lignin also induced the formation of carboxylic acids in the form of functional groups attached at the end of side of lignin macromolecule. The improvement in the amount carboxylic acid groups allows for new utilization strategies for residual lignin after reaction. In conclusion, oxidation strategy can be a crucial pathway to valorize lignin into high value products.

CRediT authorship contribution statement

Ajinkya More – Conceptualization, Methodology, Visualization, Writing – Original draft preparation.
 Thomas Elder – Supervision, Visualization, Reviewing, Editing.
 Zhihua Jiang – Conceptualization, Supervision, Reviewing and Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Valmet AB, FPInnovations, Borregaard and Sigma Aldrich for the supply of lignin samples. The authors gratefully acknowledge the financial support provided by Alabama Center for Paper and Bioresource Engineering.

References

- [1] W. Boerjan, J. Ralph, M. Baucher, Lignin biosynthesis, *Annu. Rev. Plant Biol.* 54 (1) (2003) 519–546, <https://doi.org/10.1146/annurev.arplant.54.031902.134938>.
- [2] G. Cazacu, M.C. Pascu, L. Profire, A. Kowarski, M. Mihaes, C. Vasile, Lignin role in a complex polyolefin blend, *Ind. Crop. Prod.* 20 (2) (2004) 261–273, <https://doi.org/10.1016/j.indcrop.2004.04.030>.
- [3] P.C. Rodrigues Pinto, E.A. Borges da Silva, A.E. Rodrigues, Insights into oxidative conversion of lignin to high-added-value phenolic aldehydes, *Ind. Eng. Chem. Res.* 50 (2) (2011) 741–748, <https://doi.org/10.1021/ie102132a>.
- [4] F.S. Chakar, A.J. Ragauskas, Review of current and future softwood kraft lignin process chemistry, *Ind. Crop. Prod.* 20 (2) (2004) 131–141, <https://doi.org/10.1016/j.indcrop.2004.04.016>.
- [5] M. Culebras, M. Pishnamazi, G.M. Walker, M.N. Collins, Facile tailoring of structures for controlled release of paracetamol from sustainable lignin derived platforms, *Molecules* 26 (6) (2021) 1593, <https://doi.org/10.3390/molecules26061593>.
- [6] P.C.R. Pinto, E.A.B. da Silva, A.E. Rodrigues, Lignin as source of fine chemicals: vanillin and syringaldehyde, in: C. Baskar, S. Baskar, R.S. Dhilon (Eds.), *Biomass Conversion*, Springer, Berlin Heidelberg 2012, pp. 381–420, https://doi.org/10.1007/978-3-642-28418-2_12.
- [7] D. Bajwa, G. Pourhashem, A. Ullah, S. Bajwa, A concise review of current lignin production, applications, products and their environmental impact, *Ind. Crop. Prod.* 139 (2019), 111526, <https://doi.org/10.1016/j.indcrop.2019.111526>.
- [8] J. Huang, S. Fu, L. Gan, Structure and characteristics of lignin, *Lignin Chemistry and Applications*, Elsevier, Cambridge, MA, USA, 2019, <https://doi.org/10.1016/B978-0-12-813941-7.00002-3>.
- [9] G. Chatel, R.D. Rogers, Oxidation of lignin using ionic liquids: an innovative strategy to produce renewable chemicals, *ACS Sustain. Chem. Eng.* 2 (3) (2014) 322–339, <https://doi.org/10.1021/sc4004086>.
- [10] A. Das, A. Rahimi, A. Ulbrich, M. Alherech, A.H. Motagamwala, A. Bhalla, L. da Costa Sousa, V. Balan, J.A. Dumesic, E.L. Hegg, Lignin conversion to low-molecular-weight aromatics via an aerobic oxidation-hydrolysis sequence: comparison of different lignin sources, *ACS Sustain. Chem. Eng.* 6 (3) (2018) 3367–3374, <https://doi.org/10.1021/acssuschemeng.7b03541>.
- [11] O.Y. Abdelaziz, K. Ravi, F. Mittermeier, S. Meier, A. Riisager, G. Lidén, C.P. Hultberg, Oxidative depolymerization of kraft lignin for microbial conversion, *ACS Sustain. Chem. Eng.* 7 (13) (2019) 11640–11652, <https://doi.org/10.1021/acssuschemeng.9b01605>.
- [12] V.E. Tarabanko, N. Tarabanko, Catalytic oxidation of lignins into the aromatic aldehydes: general process trends and development prospects, *Int. J. Mol. Sci.* 18 (11) (2017) 2421, <https://doi.org/10.3390/ijms18112421>.
- [13] M. Fache, B. Boutevin, S. Caillol, Vanillin production from lignin and its use as a renewable chemical, *ACS Sustain. Chem. Eng.* 4 (1) (2016) 35–46, <https://doi.org/10.1021/acssuschemeng.5b01344>.
- [14] A.W. Patek, P. Ding, M. Garrett, G. Sheldrake, A.W. Nienow, Catalytic conversion of sodium lignosulfonate to vanillin: engineering aspects. Part 1. Effects of processing conditions on vanillin yield and selectivity, *Ind. Eng. Chem. Res.* 52 (25) (2013) 8361–8372, <https://doi.org/10.1021/ie4007744>.
- [15] M. Culebras, A. Barrett, M. Pishnamazi, G.M. Walker, M.N. Collins, Wood-derived hydrogels as a platform for drug-release systems, *ACS Sustain. Chem. Eng.* 9 (6) (2021) 2515–2522, <https://doi.org/10.1021/acssuschemeng.0c08022>.
- [16] T.Q. Hu, *Chemical Modification, Properties, and Usage of Lignin*, Kluwer Academic/Plenum Publisher, New York, 2002 291.
- [17] D. Schorr, P.N. Diouf, T. Stevanovic, Evaluation of industrial lignins for biocomposites production, *Ind. Crop. Prod.* 52 (2014) 65–73, <https://doi.org/10.1016/j.indcrop.2013.10.014>.
- [18] Z. Hu, X. Du, J. Liu, H.-m. Chang, H. Jameel, Structural characterization of pine kraft lignin: BioChoice lignin vs Indulin AT, *J. Wood Chem. Technol.* 36 (6) (2016) 432–446, <https://doi.org/10.1080/02773813.2016.1214732>.
- [19] P. Tomani, The lignoboost process, *Cellul. Chem. Technol.* 44 (1) (2010) 53.
- [20] D.P. Bezerra, A.K.N. Soares, D.P. de Sousa, Overview of the role of vanillin on redox status and cancer development, *Oxidative Med. Cell. Longev.* (2016), 9734816, <https://doi.org/10.1155/2016/9734816>.
- [21] J. Choo, Y. Rukayadi, J.K. Hwang, Inhibition of bacterial quorum sensing by vanilla extract, *Lett. Appl. Microbiol.* 42 (6) (2006) 637–641, <https://doi.org/10.1111/j.1472-765X.2006.01928.x>.
- [22] S. Buddoo, Process for the Preparation of Vanillin from a Mixed M-Cresol/P-Cresol Stream, MSc Thesis, Nelson Mandela Metropolitan University, Port Elizabeth,

- (2003) Online accessed 1 April 2021 Retrieved from <http://hdl.handle.net/10948/138>.
- [23] C.S. Calixto-Campos, T.T. Carvalho, M.S. Hohmann, F.A. Pinho-Ribeiro, V. Fattori, M.F. Manchope, A.C. Zarpelon, M.M. Baracat, S.R. Georgetti, R. Casagrande, Vanillic acid inhibits inflammatory pain by inhibiting neutrophil recruitment, oxidative stress, cytokine production, and NFκB activation in mice, *J. Nat. Prod.* 78 (8) (2015) 1799–1808, <https://doi.org/10.1021/acs.jnatprod.5b00246>.
- [24] S.G. Yao, J.K. Mobley, J. Ralph, M. Crocker, S. Parkin, J.P. Selegue, M.S. Meier, Mechanochemical treatment facilitates two-step oxidative depolymerization of kraft lignin, *ACS Sustain. Chem. Eng.* 6 (5) (2018) 5990–5998, <https://doi.org/10.1021/acssuschemeng.7b04597>.
- [25] J. Villar, A. Caperos, F. García-Ochoa, Oxidation of hardwood kraft-lignin to phenolic derivatives with oxygen as oxidant, *Wood Sci. Technol.* 35 (3) (2001) 245–255, <https://doi.org/10.1007/s002260100089>.
- [26] M.B. Hocking, Vanillin: synthetic flavoring from spent sulfite liquor, *J. Chem. Educ.* 74 (9) (1997) 1055, <https://doi.org/10.1021/ed074p1055>.
- [27] E.B. da Silva, M. Zabkova, J. Araújo, C. Cateto, M. Barreiro, M. Belgacem, A. Rodrigues, An integrated process to produce vanillin and lignin-based polyurethanes from kraft lignin, *Chem. Eng. Res. Des.* 87 (9) (2009) 1276–1292, <https://doi.org/10.1016/j.cherd.2009.05.008>.
- [28] J.D. Araújo, C.A. Grande, A.E. Rodrigues, Vanillin production from lignin oxidation in a batch reactor, *Chem. Eng. Res. Des.* 88 (8) (2010) 1024–1032, <https://doi.org/10.1016/j.cherd.2010.01.021>.
- [29] A. Mathias, A. Rodrigues, Production of vanillin by oxidation of pine kraft lignins with oxygen, *Holzforschung* 49 (3) (1995) 273–278, <https://doi.org/10.1515/hfsg.1995.49.3.273>.
- [30] V.E. Tarabanko, D.V. Petukhov, Study of mechanism and improvement of the process of oxidative cleavage of lignins into the aromatic aldehydes, *Chem. Sustain. Dev.* 11 (2003) 655–667.
- [31] A. More, T. Elder, Z. Jiang, A review of lignin hydrogen peroxide oxidation chemistry with emphasis on aromatic aldehydes and acids, *Holzforschung* (2021) <https://doi.org/10.1515/hf-2020-0165>.
- [32] F.M. Casimiro, C.A. Costa, C.I.M. Botelho, M.F. Barreiro, A.E. Rodrigues, Kinetics of oxidative degradation of lignin-based phenolic compounds in batch reactor, *Ind. Eng. Chem. Res.* 58 (36) (2019) 16442–16449, <https://doi.org/10.1021/acs.iecr.9b02818>.
- [33] M. Jablonský, J. Kočíš, A. Ház, J. Šima, Characterization and comparison by UV spectroscopy of precipitated lignins and commercial lignosulfonates, *Cell. Chem. Technol.* 49 (3–4) (2015) 267–274.
- [34] J.D.P. Araújo, Production of Vanillin from Lignin Present in the Kraft Black Liquor of the Pulp and Paper Industry, Ph.D. thesis. University of Porto, Porto, Portugal (2008) (Online accessed 1 April 2021) Retrieved from <https://repositorio-aberto.up.pt/bitstream/10216/11944/2/Texto%20integral.pdf>.
- [35] J. Balej, Water vapour partial pressures and water activities in potassium and sodium hydroxide solutions over wide concentration and temperature ranges, *Int. J. Hydrog. Energy* 10 (4) (1985) 233–243, [https://doi.org/10.1016/0360-3199\(85\)90093-X](https://doi.org/10.1016/0360-3199(85)90093-X).
- [36] Guide to Aminex HPLC columns: http://www.hplc.sk/pdf/Biorad/Guide_to_Aminex_HPLC_columns.pdf, in: BIO-RAD (Ed.). Online accessed 1 April 2021.
- [37] S. Katz, R.P. Beatson, The determination of strong and weak acidic groups in sulfite pulps, *Sven. Papperstidn* 87 (6) (1984) 48–53.
- [38] J. Kadla, H.M. Chang, H. Jameel, The reactions of lignins with hydrogen peroxide at high temperature. Part I. The oxidation of lignin model compounds, *Holzforschung* 51 (1997) 428–434, <https://doi.org/10.1515/hfsg.1997.51.5.428>.
- [39] G. Gellerstedt, K. Gustafsson, E.L. Lindfors, Structural changes in lignin during oxygen bleaching, *Nord. Pulp Pap. Res. J.* 1 (3) (1986) 14–17.
- [40] Y. Sun, D. Argyropoulos, Fundamentals of high-pressure oxygen and low-pressure oxygen-peroxide (Eop) delignification of softwood and hardwood kraft pulps: a comparison, *J. Pulp Pap. Sci.* 21 (6) (1995) J185–J190.
- [41] F. Asgari, D.S. Argyropoulos, Fundamentals of oxygen delignification. Part II. Functional group formation/elimination in residual kraft lignin, *Can. J. Chem.* 76 (11) (1998) 1606–1615.
- [42] W. Denissen, J.M. Winne, F.E. Du Prez, Vitrimers: permanent organic networks with glass-like fluidity, *Chem. Sci.* 7 (1) (2016) 30–38, <https://doi.org/10.1039/C5SC02223A>.
- [43] J. Gierer, F. Imsgard, The reactions of lignins with oxygen and hydrogen peroxide in alkaline media, *Sven. Pap.* 80 (16) (1977) 510–518.
- [44] H. Chang, J. Gratzl, Ring cleavage reactions of lignin models with oxygen and alkali, *Chemistry of Delignification with Oxygen, Ozone and Peroxide 1980*, pp. 151–163.
- [45] M.R. Fernandes, X. Huang, H.C. Abbenhuis, E.J. Hensen, Lignin oxidation with an organic peroxide and subsequent aromatic ring opening, *Int. J. Biol. Macromol.* 123 (2019) 1044–1051, <https://doi.org/10.1016/j.ijbiomac.2018.11.105>.
- [46] T. Hosoya, K. Yamamoto, H. Miyafuji, T. Yamada, Selective production of bio-based aromatics by aerobic oxidation of native soft wood lignin in tetrabutylammonium hydroxide, *RSC Adv.* 10 (33) (2020) 19199–19210, <https://doi.org/10.1039/D0RA03420G>.
- [47] E. Sjostrom, *Wood Chemistry, Fundamentals and Applications*, Academic Press, New York, 1981.
- [48] P.C. Pinto, D.V. Evtuguin, C.P. Neto, Effect of structural features of wood biopolymers on hardwood pulping and bleaching performance, *Ind. Eng. Chem. Res.* 44 (26) (2005) 9777–9784, <https://doi.org/10.1021/ie050760o>.
- [49] G. Vázquez, G. Antorrena, J. González, S. Freire, The influence of pulping conditions on the structure of acetosolv eucalyptus lignins, *J. Wood Chem. Technol.* 17 (1–2) (1997) 147–162, <https://doi.org/10.1080/02773819708003124>.
- [50] D.R. Robert, M. Bardet, G. Gellerstedt, E.L. Lindfors, Structural changes in lignin during kraft cooking part 3. On the structure of dissolved lignins, *J. Wood Chem. Technol.* 4 (3) (1984) 239–263, <https://doi.org/10.1080/02773818408070647>.
- [51] A. Marques, D. Evtuguin, S. Magina, F. Amado, A. Prates, Structure of lignosulphonates from acidic magnesium-based sulphite pulping of Eucalyptus globulus, *J. Wood Chem. Technol.* 29 (4) (2009) 337–357, <https://doi.org/10.1080/02773810903207762>.
- [52] J.-Y. Kim, H. Hwang, S. Oh, Y.-S. Kim, U.-J. Kim, J.W. Choi, Investigation of structural modification and thermal characteristics of lignin after heat treatment, *Int. J. Biol. Macromol.* 66 (2014) 57–65, <https://doi.org/10.1016/j.ijbiomac.2014.02.013>.
- [53] J.E. Holladay, J.F. White, J.J. Bozell, D. Johnson, Top Value-Added Chemicals from Biomass—Volume II—Results of Screening for Potential Candidates from Biorefinery Lignin, Pacific Northwest National Lab. (PNNL), Richland, WA (United States), 2007.
- [54] S.E. Lebo Jr., J.D. Gargulak, T.J. McNally, Lignin, *Kirk-Othmer Encyclopedia of Chemical Technology*, 2000 <https://doi.org/10.1002/0471238961.12090714120914.a01.pub3>.
- [55] K. Kurshner, The difficulties in the production of vanillin from sulphite liquors, *Russ. J. Appl. Chem.* 28 (1955) 957–968.