The Scientific Temper (2023) Vol. 14 (1): 100-105
Doi: 10.58414/SCIENTIFICTEMPER.2023.14.1.12
E-ISSN: 2231-6396, ISSN: 0976-8653
https://scientifictemper.com/

RESEARCH ARTICLE

The effect of oxygen bleaching on reactivity of syringyl and guaiacyl units of Eucalyptus tereticornis pulps lignin

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Abstract

Dioxin lignin of Eucalyptus tereticornis kraft pulps and its oxygen-treated pulps with kappa number 21.32, 12.84; 13.03, 12.18 were isolated and subjected to alkaline nitrobenzene oxidation studies to determine the structural changes during oxygen bleaching. HPLC quantitatively identified eight compounds during nitrobenzene oxidation of dioxin lignin in alkaline media. Lignin obtained from E. tereticornis has syringyl and guaiacyl units. Residual lignin composition in pulps was studied and found that during oxygen delignification, the syringyl unit was degraded more in comparison to guaiacyl and p-hydroxy phenyl unit, i.e., the molar ratio of syringyl unit is lower than guaiacyl and p-hydroxy phenyl unit. The results are adequately described in the research.

Keywords: Kraft lignin, Nitrobenzene oxidation, Syringyl, Guaiacyl and S/G ratio

INTRODUCTION

Lignin is a polymeric organic substance present in the space of inter-cellulosic micro-fibrils in primary and secondary cell walls and the middle lamella of fibers (Gurunathan et al., 2015). It acts as an encrusting and cementing material of fibers, imparts hardening and mechanical strength to xylem tissue, regulates water and nutrient transportation in plants and protects from micro-organisms (Lima et al., 2008). Hardwood has approximately 20–25% lignin and 25–30% in softwood and non-wood plants. In the papermaking industry, lignin is an undesirable component in the conversion of wood into pulp and paper (Campbell & Sederoff, 1996) (Rodrigues et al., 1999). As a major component of plant cell walls, lignin has been studied in the field of papermaking in recent years (Li et al., 2008).

Lignin is biosynthesized from the radical coupling reaction of three primary precursors are trans-coniferyl alcohol, trans-sinapyl and, trans-p-coumaryl (El Mansouri et al., 2011). The three-dimensional polymeric structure of lignin contains phenyl propane units, which consist of syringyl (S), guaiacyl (G), and para-hydroxyphenyl (H) groups (Figure 1) (Grima-Pettenati & Goffner, 1999) Typically, hardwood and non-wood contain both S-unit and the G-unit, whereas only the G-unit is found in softwood. Syringaldehyde (Sa) and vanillin (Va or Ga) in Figure 2 produced by Nitrobenzene oxidation (NBO) are from the S-unit and G-unit, respectively. The molar ratio of the S-unit to G-unit is known as the S/G molar ratio. The high S/G molar ratio confers higher solubility to lignin during alkaline cooking and provides higher pulp yield. According (Akiyama et al., 2005), S-unit lignin decomposed faster than G-unit lignin during alkaline cooking because of strong carbon-carbon bonds at the C-5 position in the aromatic ring of guaiacyl units making them more resistant to depolymerisation during wood pulping.

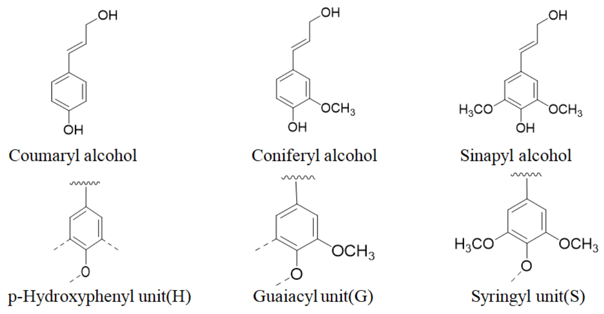

Figure 1: Primary Precursors of Lignin

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How to cite this article: Bhatnagar A. (2023). The effect of oxygen bleaching on reactivity of syringyl and guaiacyl units of Eucalyptus tereticornis pulps lignin. The Scientific Temper, **14**(1):100-105

Doi: 10.58414/SCIENTIFICTEMPER.2023.14.1.12

Source of support: Nil

Conflict of interest: None.

Received: 05/01/2023 **Accepted:** 06/02/2023 **Published** : 25/03/2023


(Sa) (Va or Ga)

Figure 2: Structures of Syringaldehyde (Sa) and Vanillin (Va or Ga) as the main lignin products by nitrobenzene oxidation (NBO).

The chemical and structural nature of lignin is becoming an important scientific approach to improving delignification/dissolution behavior during bleaching in terms of delignification rate, reagent consumption, and pulp yield (Zhang et al., 2013). Little has been reported regarding establishing the optimum conditions for obtaining the maximum yield. Although several methodologies have been used to study lignin structure, nitrobenzene oxidation in an alkaline medium is the standard method most used to determine S/G ratio. Freudenberg introduced nitrobenzene oxidation (NBO) to confirm the aromatic nature of lignin. During NBO, lignin is oxidative cleaved to form aromatic carbonyl compounds such as syringaldehyde (Sa) and vanillin (Va) as the main products (Schultz & Templeton, 1986). The composition and quantity of the aromatic aldehydes obtained represents the structure of uncondensed lignin because condensed lignins typically do not give aromatic aldehydes.

In present studies, dioxin lignin from Kraft pulps and its oxygen treated pulps were isolated and subjected to nitrobenzene oxidation to assess the structural modification occurred in lignin during oxygen treatment of *Eucalyptus tereticornis* under two different conditions.

Materials and Methods

Sample Preparation

Five-year-old *E. tereticornis* wood logs were collected from the Forest Research Institute campus in Dehradun. Logs were chipped and stored for the experiment. After determining the moisture content (13%), wood chips were pulped in an Air bath pulping unit using 14 & 18% active alkali as Na_2O, 25%-sulfide, 1:4- bath ratio, at a maximum temperature of 165°C and kept constant for 60 minutes in both cases. Pulping schedule corresponds to H-factor, 810. After washing, pulp yield and kappa number of pulp were determined in each case. Kraft pulp produced using 14 and 18% active alkali corresponding to Kappa numbers 44.91 and 26.46, were treated with oxygen 8 Kg/cm^2 oxygen pressure and 1:12 bath ratio using 0.25 $MgSO_4$ at 100 and 120°C temperatures. Kappa number of kraft pulps of 44.91 was reduced up to 21.32 and 12.84 with oxygen treatment at 100 and 120°C temperatures, respectively. Similarly, Kappa number of kraft pulps of 26.46 was decreased to 13.03 and 12.18 with oxygen treatment at 100 and 120°C, respectively(Bhandari & Bhatnagar, 2001) (Figure 3).

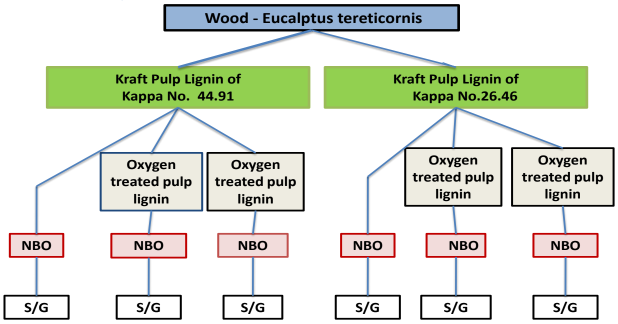

Figure 3: Scheme of NBO analytical methods for *E. tereticornis*.

Isolation and Purification of Lignin

Extractable, Air-dried dust (40–60 mesh) from the pulp of *E. tereticornis* was used for lignin isolation. The pulp was acidified with acidifying solvent (dioxin: water, 9: 1 v/v) under N_2 atmosphere for 48 hours with hydrochloric acid corresponding to 0.2 N standard and allowed to cool. The dust was filtered off and the solvent mixture was concentrated under reduced pressure at 45-50°C until a gummy material containing crude lignin appeared. The gummy material was added to a large excess of water with vigorous stirring. The coagulated lignin was separated by centrifugation and decantation and washed extensively with water three times by centrifugation. Purified lignin was dried over anhydrous sodium sulfate under reduced pressure in vacuum desiccators (Isolation, 1992).

Alkaline Nitrobenzene oxidation of lignin: Alkaline nitrobenzene oxidation of lignin (Table 1) was carried out in stainless steel bombs (Chen, 1992) under the following conditions:

Purification of Nitrobenzene oxidation products: Unused nitrobenzene and its reduction products were extracted from a cooled oxidation mixture with diethyl ether (5x20 mL). The remaining aqueous solution was acidified with hydrochloric acid (5N) to pH ˜2 and the oxidation product was extracted with dichloromethane (4×50 mL) and diethyl ether (4×50 mL). Both solvent fractions were mixed. The combined solvent fractions were dried over anhydrous sodium sulfate, concentrated under reduced pressure on a rotary evaporator to a small volume, transferred to a dry vial,

Table 1: Conditions of Alkaline nitrobenzene oxidation of Lignin

1.	Lignin, g	0.10
2.	Nitrobenzene	0.80
3.	Sodium hydroxide (2N), *mL*	0.60
4.	Maximum temperature, 0°C	170
5.	Time, min	
i.	Time to raise the temp to 170°C	60 min
ii.	Time to 170°C	90 min

Table 2: Parameter of High-performance liquid chromatography (HPLC) analysis of Lignin

Column	*Reserve phase pecophere sphere C18 column having 12% loading of bonded monomer*
Elution media	0.5 N acetic acid and acetonitrile (85:15v/v)
Elution rate, mL/min	0.50
Scanning wave length, nm	280
Peak width, nm	25
Band width, nm	8
Chart speed, nm/min	10

and concentrated to near dryness (approximately 0.4 mL) again under a nitrogen atmosphere (Reeves, 1986).

Characterization of Nitrobenzene oxidation products: Alkaline nitrobenzene oxidation products were characterized using a Perkin-Elmer (USA) model 235 high-performance liquid chromatography (HPLC) equipped with a programmable binary LC pump and a model 235 UV diode array detector. Eight compounds were identified by comparing their retention times to those of standard samples and observing the peak enhancement upon adding each standard sample to the oxidation mixture. Chromatography was performed under the following conditions Table 2.

Quantitative estimation of oxidation products: Known amounts of each identified oxidation product were mixed and dissolved in the solvent mixture used for HPLC analysis. The stock solution was diluted to 5 different concentrations and chromatograph under identical analytical conditions matching those of the oxidation products. A standard curve was constructed by calculating the peak area of each compound at a different known concentration and plotting the peak area against the amount of each compound injected for analysis, and a constant was derived for acids, and aldehydes and ketones. Amount of each compound in the oxidation mixture was calculated by multiplying the peak area and the constant obtained for each compound. This data calculated relative percents, relative moles, and relative molar ratios.

Result and Discussion

Comparison of alkaline Nitrobenzene oxidation products of E.tereticornis kraft pulp (produced using 14% alkali during pulping) lignin and its oxygen-treated pulp lignins: Data recorded in Table 3 revealed that in general relative percentage of acid were higher except syringic acid. The p-hydroxyl benzoic acid: vanillic acid ratio was 43.50:17.75 and 46.54:16.84 in oxygen-treated pulp lignin at 100 and 120oC as compared to kraft pulp lignin where it was 25.10: 13.87.

The Relative percentage of aldehyde i.e. vanillin: syringaldehyde: p-hydroxyl benzaldehyde: was 5.39: 7.59:7.96 and 4.69:6.73:7.43 for oxygen-treated pulp lignin at 100 and 120^{o}C lower than the kraft pulp lignin 10.80 : 8.74: 12.64. The decrease of aldehyde unit in the oxidation mixture may be due to the easy cleavage of p-hydroxyl phenyl moieties during the course of bleaching/delignification i.e. prior to oxidation (H'ng, 2016)(Higuchi et al., 1967)(Higuchi et al., 1967)(Higuchi et al., 1967).

The relative molar ratio of vanillin:syringaldehyde:p-hydroxyl benzaldehyde:vanillic acid: syringic acid:p-hydroxy benzoic acid were 11.05: 7.47: 16.13:12.92: 11.36: 28.30 for kraft pulp lignin and 5.37:6.33: 9.89:16.02: 5.84:47.81 and 4.68: 5.59: 9.21:15.13: 6.38: 50.89 for oxygen treated pulp lignin at 100

Table 3: Relative retention time, relative percentage, relative moles and relative molar ratio of alkaline nitrobenzene oxidation products of kraft pulp of kappa number 44.91 and its oxygen treated pulp lignin of *E. tereticornis* at different treatment temperature.

Oxidation Products	*RRT*	*Pulp lignin of Kappa Number of 44.91*								
		Kraft pulp lignin			*Oxygen treated pulp lignin(14%/3.75%/100^{O}C)*			*Oxygen treated pulp lignin(14%/3.75%/100^{O}C)*		
		RP	*RM*	*RMR*	*RP*	*RM*	*RMR*	*RP*	*RM*	*RMR*
Vanillin	1.00	10.80	0.071	11.05	5.39	0.035	5.37	4.69	0.031	4.68
Vanillic acid	0.72	13.87	0.083	12.92	17.75	0.106	16.02	16.84	0.100	15.13
Acetovanillione	1.18	9.05	0.054	8.41	6.21	0.037	5.64	5.99	0.036	5.44
Total Guaiacyl units			0.208	32.38		0.178	27.03		0.167	25.23
Syringal- dehyde	1.12	8.74	0.048	7.47	7.59	0.042	6.33	6.73	0.037	5.59
Syringic acid	0.75	14.38	0.073	11.36	7.64	0.039	5.84	8.34	0.042	6.38
Acetosyringone	1.30	5.41	0.028	4.36	3.99	0.021	3.11	3.46	0.018	2.69
Total Syringyl units			0.149	23.19		0.101	15.24		0.97	14.63
p-hydroxy- benzaldehyde	0.845	12.64	0.104	16.13	7.96	0.065	9.89	7.43	0.061	9.21
p-hydroxy- benzoic acid	0.65	25.10	0.182	28.30	43.50	0.32	47.81	46.54	0.337	50.89
Total p-hydroxy units			0.286	44.43		0.380	57.69		0.398	60.13

RRT = Relative retention ratio, RP= Relative percentage,
RM= Relative moles, RMR= Relative molar ratio.

Table 4: Total Relative ratio of alkaline nitrobenzene oxidation products of kraft pulp of kappa number 44.91 and its oxygen treated pulp lignin of E.tereticornis at different treatment temperature.

Oxidation Products	*Pulp lignin of Kappa Number of 44.91 (14%)*		
	Kraft pulp lignin (14%)	*Oxygen treated Pulp lignin (14%/3.75%/100^{O}C)*	*Oxygen treated Pulp lignin(14%/3.75%/120^{O}C)*
Guaiacyl units	1.40	1.77	1.73
Syringyl Units	1.00	1.00	1.00
p-hydroxy phenyl unit	1.92	3.78	4.11
S/G molar ratio	0.72/1.0	0.57/1.0	0.58/1.0
Vanillin	1.48	0.85	0.84
Syringaldehyde	1.00	1.00	1.00
p-hydroxyl benzaldehyde	2.16	1.56	1.65
Sa/Va molar ratio	0.68/1.0	1.17/1.0	1.19/1.0

Table 5 : Relative retention time, relative percentage, relative moles and relative molar ratio of alkaline nitrobenzene oxidation products of kraft pulp of kappa number 26.46 and its oxygen treated pulp lignin of E.tereticornis at different treatment temperature.

		Pulp lignin of Kappa Number of 26.46								
Oxidation Products	*RRT*	*Kraft pulp lignin*			*Oxygen treated Pulp lignin(18%/2.50%/100^{O}C)*			*Oxygen treated Pulp lignin(18%/2.50%/120^{O}C)*		
		RP	*RM*	*RMR*	*RP*	*RM*	*RMR*	*RP*	*RM*	*RMR*
Vanillin	1.00	10.66	0.070	10.89	6.59	0.043	6.57	4.92	0.032	4.91
Vanillic acid	0.72	12.86	0.077	11.98	15.74	0.094	14.19	17.73	0.106	16.03
acetovanillione	1.18	9.79	0.059	9.19	7.56	0.045	6.85	5.35	0.032	4.86
Total Guaiacyl units			0.206	32.01		0.182	27.63		0.170	25.79
Syringal dehyde	1.12	10.95	0.060	9.34	8.10	0.045	6.74	7.04	0.039	5.88
Syringic acid	0.75	14.59	0.074	11.52	7.14	0.036	5.47	8.70	0.044	6.67
acetosyringone	1.30	6.33	0.035	5.45	4.04	0.021	3.14	3.55	0.018	2.77
Total Syringyl units			0.169	26.31		0.101	15.34		0.101	15.31
p-hydroxy benzaldehyde	0.845	12.53	0.103	15.99	8.77	0.072	10.89	7.00	0.063	9.59
p-hydroxy benzoic acid	0.65	22.28	0.165	25.69	42.04	0.305	46.14	44.79	0.325	49.29
Total p-hydroxy units			0.268	41.48		0.377	57.03		0.388	58.88

RRT = Relative retention ratio, RP= Relative percentage,
RM= Relative moles, RMR= Relative molar ratio.

and 120^{o}C, respectively. Relative moles of acetovanillione and acetosygringone for oxygen-treated pulp lignin at 100 and 120^{o}C were 5.64:3.11 and 5.44:2.69, respectively, were increased in kraft pulp lignin 8.41:4.36 moles respectively.

Table 4 revealed that the molar ratio of syringaldehyde: vanillin for kraft pulp lignin was 0.68 : 1.00 and increased to 1.18:1.00 and 1.19:1.00 oxygen-treated pulp lignin at 100 and 120^{o}C respectively. The molar ratio of total syringyl unit to total guaiacyl unit was 0.72: 1.00 for kraft pulp lignin and decreased to 0.57 : 1.00 and 0.58 : 1.00 for oxygen treated pulp lignin at 100 and 120^{o}C.

The syringaldehyde: p-hydroxy benzaldehyde ratio in pulp lignin was 0.46:1.00 and 0.64:1.00:0.61:1.00 for oxygen-treated pulp lignin at 100 and 120^{o}C. The ratio of total syringyl: p-hydroxyl phenyl units for kraft pulp lignin was 0.52 :1.00 and 0.26:1.00; 0.24:1.00 for oxygen-treated pulp lignin at 100 and 120^{o}C.

These observations indicated that the syringaldehyde generating moieties were lower than that of vanillin and p-hydroxy benzaldehyde moieties in kraft pulp lignin in comparison to oxygen-treated pulp lignin at 100 and 120°C. However, a comparison of the molar ratio of the total of each class of moieties suggested that the frequency of syringyl unit-generating moieties was lower in oxidation product than that of guaiacyl and p-hydroxyl phenyl unit-generating moieties (Table 4). This is due to the cleavage of β-aryl ether bond in syringyl lignin occuring more easily than in the guaiacyl lignin under alkaline delignification conditions, contributing to an increase the rate of delignification (Tsutsumi et al., 1995).

The molar ratio of the total syringyl/guaiacyl unit was 0.72 for kraft pulp lignin decreased to 0.57 and 0.58 for oxygen-treated pulp lignin at 100 and 120oC. It observed that syringyl units appear to solubilise at a much higher rate

Table 6: Total Relative ratio of alkaline nitrobenzene oxidation products of kraft pulp of kappa number 26.46 and its oxygen treated pulp lignin of E.tereticornis at different treatment temperatures.

Oxidation Products	*Pulp lignin of Kappa Number of 26.46*		
	Kraft pulp lignin (18%)	*Oxygen treated Pulp lignin (18%/2.50%/100^{O}C)*	*Oxygen treated Pulp lignin(18%/2.50%/120^{O}C)*
Guaiacyl units	1.22	1.80	1.68
Syringyl Units	1.00	1.00	1.00
p-hydroxy phenyl unit	1.58	3.72	3.85
S/G molar ratio	0.82/1.0	0.55/1.0	0.60/1.0
Vanillin	1.17	0.98	0.84
Syringaldehyde	1.00	1.00	1.00
p-hydroxyl benzaldehyde	1.72	1.62	1.63
Sa/Va molar ratio	0.86/1.0	1.02/1.0	1.19/1.0

than guaiacyl units during oxygen bleaching. The higher value for a total guaiacyl unit in oxygen-treated pulp lignin per syringyl unit as compared to pulp lignin, thus nearer to grassed lignin rather than hardwood lignin.

Similarly, the molar ratio of Sa/Va for kraft pulp and oxygen-treated pulp lignin at 100 and 120oC was 0.68, 1.17 and 1.19, respectively. Generally, higher syringaldehyde yield is attributed to a lower value of uncondensed syringyl units in the kraft pulp, and the high Sa/Va ratio results from the condensation of guaiacyl units. The above observation also revealed that the Sa/Va ratio was slightly increased with oxygen treatment temperature from 100 to 120°C. On the basis of the data obtained, 100°C oxygen treatment temperature was found to be optimum. It has also been reported that the Sa/Va ratio has been correlated with the proportion of the condensed type lignin (Jin et al., 2012).

comparison of alkaline Nitrobenzene oxidation products of E.tereticornis kraft pulps (produced using 18% alkali during pulping) lignin and its oxygen-treated pulp lignins: The result recorded in Table 5 revealed that the formation of acid, vanillic acid & p-hydroxy benzoic acid increased as the oxygen treatment temperature raised from 100 and 120°C , except for syringic acid. The relative percentage of syringic acid was 14.59 for kraft pulp lignin decreased to 7.14 and 8.70 for oxygen-treated pulp lignin at 100 and 120°C.

On basis of above data, the formation of aldehyde decreased during oxygen bleaching of kraft pulps of kappa number 26.46. The relative molar ratio of vanillin : syringaldehyde : p-hydroxyl benzaldehyde was 10.89:9.34:15.99 for kraft pulp lignin and 6.57:6.74:10.89; 4.91:5.88:9.59 in oxygen treated pulp lignin at 100 and 120°C, respectively. The syriangaldehyde:vanillin molar ratio in kraft pulp lignin was 0.86:1.00 and increased to 1.02:1.00 and 1.19:1.00 oxygen-treated pulp lignin at 100 and 120°C, respectively (Table 6). The total syringyl to total guaiacyl unit ratio was decreased, i.e. 0.82 from pulp lignin to 0.55 and 0.60 for in oxygen treated pulp lignin at 100 and 120°C, respectively. These observations revealed that the S/G ratio was slightly increased with oxygen treatment temperature raised from 100 to 120°C.

Conclusion

In alkaline nitrobenzene oxidation, eight compounds namely p-hydroxyl benzoic acid, vanillic acid, syringic acid, p-hydroxy benzaldehyde, vanillin, syringaldehyde, acetovanillione, and acetosyringone were identified by HPLC, quantitatively.

The lignin S/G ratio was determined by nitrobenzene oxidation, using the following substances as markers: vanillic acid, vanillin, acetovanillione, syringic acid, syringaldehyde, and acetosyringone. The S/G ratio, obtained by nitrobenzene oxidation, for *E. tereticornis* karft pulps lignin of two different kappa numbers 44.91 and 26.46 were compared to its oxygen-bleached pulps lignin at 100 and 120°C in each case.

The syringyl/guaiacyl unit ratio was 0.72 for kraft pulp lignin of kappa number 44.91, decreased to 0.57 and 0.58 for oxygen-treated pulp lignin at 100 and 120°C. S/G ratio was slightly increased with oxygen treatment temperature raised from 100 to 120°C. Similar results were found in pulp lignin of higher kappa number i.e. 26.46.

The lower molar ratio of total syringyl units (S) to total guaiacyl(G) and p-hydroxy benzyl units(H) indicated that syringyl units suffered more degradation or demethyoxylation during the course of oxygen treatment than that of the guaiacyl and p-hydroxyl benzyl units. This high reactivity of syringyl lignin may contribute to the faster delignification rate. On the basis of the above data, oxygen treatment at 100°C temperature was found to be optimum. Consequently, the residual lignin in pulps was nearer to softwood lignin as compared to wood lignin. Further studies with other *E.* species are required to establish the optimum conditions of oxygen delignification.

ACKNOWLEDGEMENT

The author acknowledges to the Director, Forest Research Institute, Dehradun and the Supervisor for their support and encouragement in carrying out this work.

Conflicts of Interest

Nil

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