博士論文

Study on the *erythro* or *threo* preferential degradation of *β-O*-4 type lignin model compounds under oxygen and hydrogen peroxide bleaching conditions

(酸素および過酸化水素漂白条件下における *β-O-4* 型リグニンモデル化合物のエリトロ/トレオ優先的分解に関する研究)

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Chapter 1

Introduction

1.1 Preface

According to the chemical pulping process, it is the process to chemically removed lignin to obtain the fibers from wood chips. [1] Moreover, in order to obtained the high quality paper, the lignin removing is required due to their negatively effect on the paper properties. Therefore, several types of commercial chemical pulping processes were employed including kraft pulping which is so far the most important process. By the way, after the chemical pulping processes, residual lignin, contaminants as well as chromophores were remained in the system. The bleaching process is further used to remove those remaining substances to increase the brightness and cleanliness of the fibers. Several common chemicals were used for bleaching processes including chlorine (Cl_2), chlorine dioxide (ClO_2), ozone (O_3), oxygen (O_2) as well as hydrogen peroxide (H_2O_2). In the past, the most popular bleaching chemical was chlorine (Cl_2) and chlorine dioxide (ClO_2) which is now rarely used due to its toxicity and environmental regulations. Regarding to the environmental issues in present, the environmentally benign chemical bleaching methods including ozone (O_3), oxygen (O_2) as well as hydrogen peroxide (H_2O_2) are received significant attention. Alkaline oxygen treatment was one of the most commercial bleaching chemicals which commonly used in combination with other bleaching chemical due to their low selectivity toward lignin. For the hydrogen peroxide treatment, it is also one of the commercial pulp bleaching chemicals. Under alkaline conditions, the hydroperoxide anion (HO_2^-) was generated which believed to eliminate the chromophores remained after the pulping process. [2] By the way, hydroxyl radicals generated under alkaline condition with the presence of transition metals founded to have a role in delignification. [3]

1.2 Chemistry of bleaching

Due to the wood pulp treatment, the wood chips are firstly get through the cooking process following with bleaching process for lignin removal. Otherwise, after those cooking steps, the residual lignins founded in the unbleached pulp which required further lignin removal for increasing of the brightness and cleanliness of the pulp. Under the bleaching treatment, various oxidation reactions were occurs which can be classified into four categories including nucleophilic reaction, and electrophilic reaction, insertion reaction as well as radical coupling reaction. [4] For the nucleophilic reaction, the major mode of reaction is nucleophilic attacked by peroxide anion on electron deficient carbon in lignin including various types of conjugated carbonyl structure as well as quinomethide group in lignin. For the electrophilic reaction, this reaction showed to have high reactivity against lignin which is easily oxidized due to their electron-rich structure including double bond, aromatic nuclei as well as carbanions. Moreover, the electrophilic and nucleophilic reaction showed to have high reaction selectivity while insertion reaction, and radical coupling reaction have low reaction selectivity.

The chlorine containing bleaching treatment showed to have high reaction selectivity due to their major reaction mechanism, electrophilic and nucleophillic reaction. Under the reaction with chlorine (Cl_2), and chlorine dioxide (ClO_2), the major reaction mode is electrophilic reaction where the radical coupling reaction is also presence in the reaction with chlorine dioxide (ClO_2), and chlorine monoxide (ClO). In contrast, the nucleophilic reaction was presence in the oxidation with chlorous acid ($HClO_2$), and hypochlorous acid (HClO) as the only reaction system. [5-6] Additional, for the bleaching reagent containing oxygen, the electrophilic and insertion reaction were presence

resulting in low reaction selectivity under ozone bleaching treatment. [7] For oxygen treatment, the presence of electrophilic and radical coupling reaction also result in low reaction selectivity. [8] On the other hand, under hydrogen peroxide bleaching treatment, nucleophilic reaction was occurs lead to high reaction selectivity. By the way, under oxygen and hydrogen peroxide treatments, active oxygen species (AOS) formed (hydroxyl radical, superoxide radical, etc.) have an important role in the delignification process. Therefore, the main reaction of these generated species is hydrogen abstraction which has low reaction selectivity. As the result, the treatment of oxygen and hydrogen peroxide as bleaching agent has low reaction selectivity due to the presence of oxygen species.

TABLE 1-1: Conclusion for reaction systems of various bleaching agents

Bleaching agent	Reaction system			
	Nucleophilic	Electrophilic	Insertion	Radical coupling
Reaction selectivity	High		Low	
Chlorine containing agents				
Chlorine (Cl_2)	−	✓	-	-
Chlorine dioxide (ClO_2)	-	✓	-	✓
Chlorous acid ($HClO_2$)	✓	-	-	-
Hypochlorous acid (HClO)	✓	-	-	-
Oxygen containing agents				
Ozone (O_3)	-	✓	✓	-
Oxygen (O_2)	-	✓	-	✓
Hydrogen peroxide (H_2O_2)	✓	-	-	-

1.3 Oxygen species

Under oxygen bleaching treatment, several types of active oxygen species (AOS) was present as shown in Table 1-2. The molecular oxygen is a precursor of these AOS formed by one-electron and proton transfer mechanisms as shown in Figure 1-1.

TABLE 1-2: Oxygen species present under oxygen bleaching treatment

Oxygen species	Conjugate base	pKa value
Hydroperoxyl radical ($HO_2 \bullet$)	Superoxide anion radical ($O_2 \overline{\bullet}$)	4.8
Hydrogen peroxide (H_2O_2)	Hydroperoxyl anion ($HO_2{}^-$)	11.6
Hydroxyl radical ($HO \bullet$)	Oxyl anion radical ($O \overline{\bullet}$)	11.9

$$O_2 \xrightarrow{+ e^-, H^+} HO_2^\bullet \xrightarrow{+ e^-, H^+} H_2O_2 \xrightarrow{+ e^-, H^+} H_2O + HO^\bullet \xrightarrow{+ e^-, H^+} 2H_2O$$

$$pK_a\ 4.8 \updownarrow \qquad pK_a\ 11.6 \updownarrow \qquad pK_a\ 11.9 \updownarrow$$

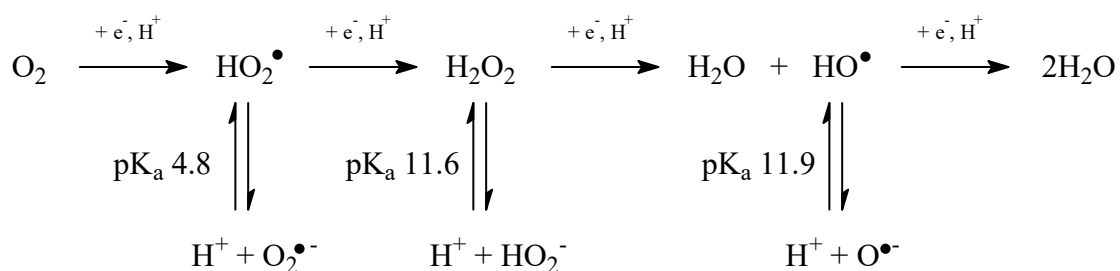$$H^+ + O_2^{\bullet -} \qquad H^+ + HO_2^- \qquad H^+ + O^{\bullet -}$$

FIGURE 1-1: Active oxygen species (AOS) derived from molecular oxygen under oxygen bleaching treatment.

1.3.1 Superoxide anion radical ($O_2\cdot^-$)

The reactivity of superoxide anion ($O_2\cdot^-$) and its conjugate acid (hydroperoxyl radical, $HO_2\cdot$) is highly relying on the reaction conditions including pH and solvent system. For instance, the superoxide anion ($O_2\cdot^-$) suggested to be primary species at high pH, whereas hydroperoxyl radical ($HO_2\cdot$) considered as predominant specie under low pH condition. Hence, the chemistry of superoxide anion ($O_2\cdot^-$) depends on its basicity as well as nucleophilicity in the system.

By the way, as a radical reaction, the superoxide anion ($O_2\cdot^-$) has low possibility of the hydrogen abstraction due to the weakness of the bond ($H-O_2^-$) which formed by the abstraction between proton and hydrogen atom abstraction by superoxide anion ($O_2\cdot^-$). [9] Nevertheless, the hydrogen abstraction reactions by the superoxide anion ($O_2\cdot^-$) in catechol have been published. [10-12] Moreover, according to the proton abstraction mechanism, the hydroperoxyl radical ($HO_2\cdot$) was present in the system before the hydrogen atom abstraction performed due to higher hydrogen abstraction reactivity of hydroperoxyl radical ($HO_2\cdot$) compared with superoxide anion ($O_2\cdot^-$). [13]

1.3.2 Hydrogen peroxide (H_2O_2)

Hydrogen peroxide has two properties including electrophilic and nucleophilic as well as being weak oxidant. Under acidic conditions with the presence of transition metals, the hydrogen peroxide found to decompose to hydroxyl radicals ($HO\cdot$) through Fenton's reaction system. [13-14] Under alkaline condition, the hydrogen peroxide is equilibrium with Hydroperoxyl anion (HO_2^-) which is a strong nucleophile and found to

react with quinone and aldehydes to form epoxides. [15-16] Moreover, under these alkaline conditions, hydrogen peroxide also found to oxidize the aromatic aldehydes to carboxylic acids [15] or lead to Dakin reaction with aromatic aldehydes. [17]

1.3.3 Hydroxyl radical (HO•)

Under alkaline oxygen treatment, hydroxyl radical (HO•) considered being the most reactive among generated active oxygen species (AOS). [13] Numerous studies have indicated that hydroxyl radical (HO•) can react with most organic compounds by hydrogen or electron abstraction, electrophilic addition as well as radical coupling reactions. [18-20] Under strong alkaline conditions, the oxyl anion radical (O•⁻), as the conjugate base, are easily produced by the hydrogen abstraction of hydroxide anions (OH⁻) by hydroxyl radicals (HO•). [21-22] The hydrogen abstraction reactivity of the hydroxyl radical (HO•) depends on the strength of the bonding of substrate (R-H) which can defined by the difference between bond formation energy of the product (HO-H) and the bond dissociation energy of the substrate (R-H). [13, 18] This hydrogen abstraction reaction by hydroxyl radical (HO•) can occur in alkanes, aldehydes, ketones, esters, ethers, alcohols as well as carboxylic acids. [18, 20] By the way, if the bond energy is too high to permit the hydrogen abstraction, the hydroxyl radical (HO•) will precede through the electrophilic addition reactions, for example, the reaction between the hydroxyl radical (HO•) and aromatic compounds. In the previous studies, they suggested that the aromatic compounds can react with hydroxyl radical (HO•) by the addition to aromatic ring. [13, 19, 23]
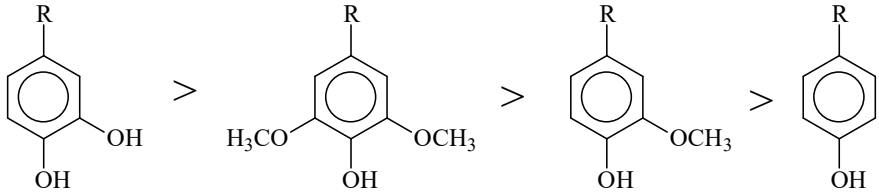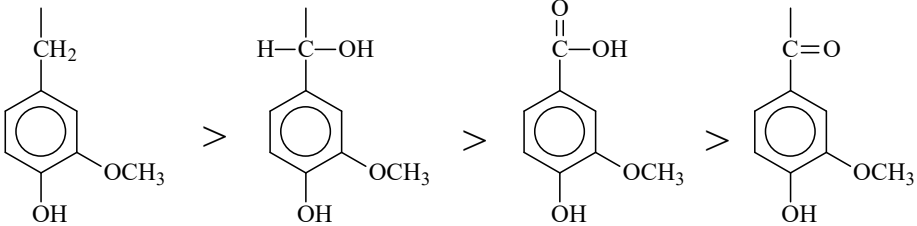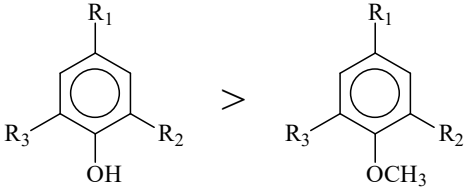
1.4 Chemistry of delignification under oxygen treatment

       Lignin, as one of the three main components of wood together with celluloses and hemicelluloses, is removed from wood during the chemical pulping processes. It has been reported that about 92-94% of lignin can be removed from wood by kraft cooking treatment. [24] Moreover, the residual lignin will be further removed during the subsequent delignification and bleaching stages which normally starting with oxygen stage (O). The residual fiber lignin is structurally heterogeneous after the pulping processes. The free phenolic end-groups considered to be the most reactive toward electrophilic reagent among the various lignin structure units present. Under the oxygen treatment, the typical reaction condition is 60 minutes at 100ºC with an oxygen pressure of 0.6 MPa. The degree of the delignification, measured as the change in kappa number, is in the order of 35-65% depending on the wood species and oxygen treatment conditions. [25]

1.4.1 Effect of the structural of lignin model compound on the reactivity

The reactivity of the substituent group in the lignin model compound under oxygen bleaching conditions is shown in Table 1-3.

TABLE 1-3: Relatively reactivity of functional group with oxygen [8, 26, 32]

Functional group	Relative reactivity
Methoxyl	
Side-chain	
Phenolic hydroxyl	

1. Methoxyl group

Due to the results from several researches, it showed that the catechol containing compounds are the most reactive under alkaline oxygen bleaching condition. In addition, the results are also showed that the degradation of syringyl model compound (S) was found to be approximately ten times faster than guaiacyl compound (G) that was also approximately ten times faster than the ρ–hydroxyphenyl compound (H).

2. Side-chain

The results from various studies indicated that the lignin structure containing methyl groups are quite reactive under alkaline oxygen treatment followed by alcohols, carboxylic acids, aldehydes and ketones. The electron donating ability of these side-chain substituents should affect the reaction rate of these compounds.

3. Phenolic hydroxyl group

According to several previous studies, the results indicates that only the lignin structure containing free hydroxyl group reacts to any extent due to its metal catalyzed formation of phenoxyl radicals from phenoxyl anions at initial step of ring cleavage reaction. However, a non-phenolic model compound showed to be degraded under oxygen treatment in the presence of phenolic compound and excess ion in the reaction system. [27] Moreover, several studies also indicated that the reactivity of the non-phenolic lignin model compound was caused by the generation of active oxygen species between the reaction of phenolic lignin compound and molecular oxygen.

1.4.2 Effect of the lignin linkage on the degradation reactivity

Several dimer lignin model compounds were used to examine the susceptibility of the model compound under oxygen treatment as shown in Figure 1-2.

According to the results from previous studies, it showed that the most reactive compounds were stilbene and vinyl ether which both of them containing double bond in the side chain at α–β position. Moreover, the reactivity of stilbene was hundred time faster than Vinyl ether compound under oxygen treatment. [28] In addition, several linkages were investigated including *β*-1, *β-O*-4 (α-carbonyl), *β-O*-4 (α-hydroxyl), 5-5, and *β*-5. By the way, the different in the reactivity under oxygen treatment among these dimer lignin model compounds was small. The degradability of *β*-1, *β-O*-4 (α-carbonyl) was approximately two times faster than the *β-O*-4 (α-hydroxyl), 5-5, and *β*-5. [29-33]
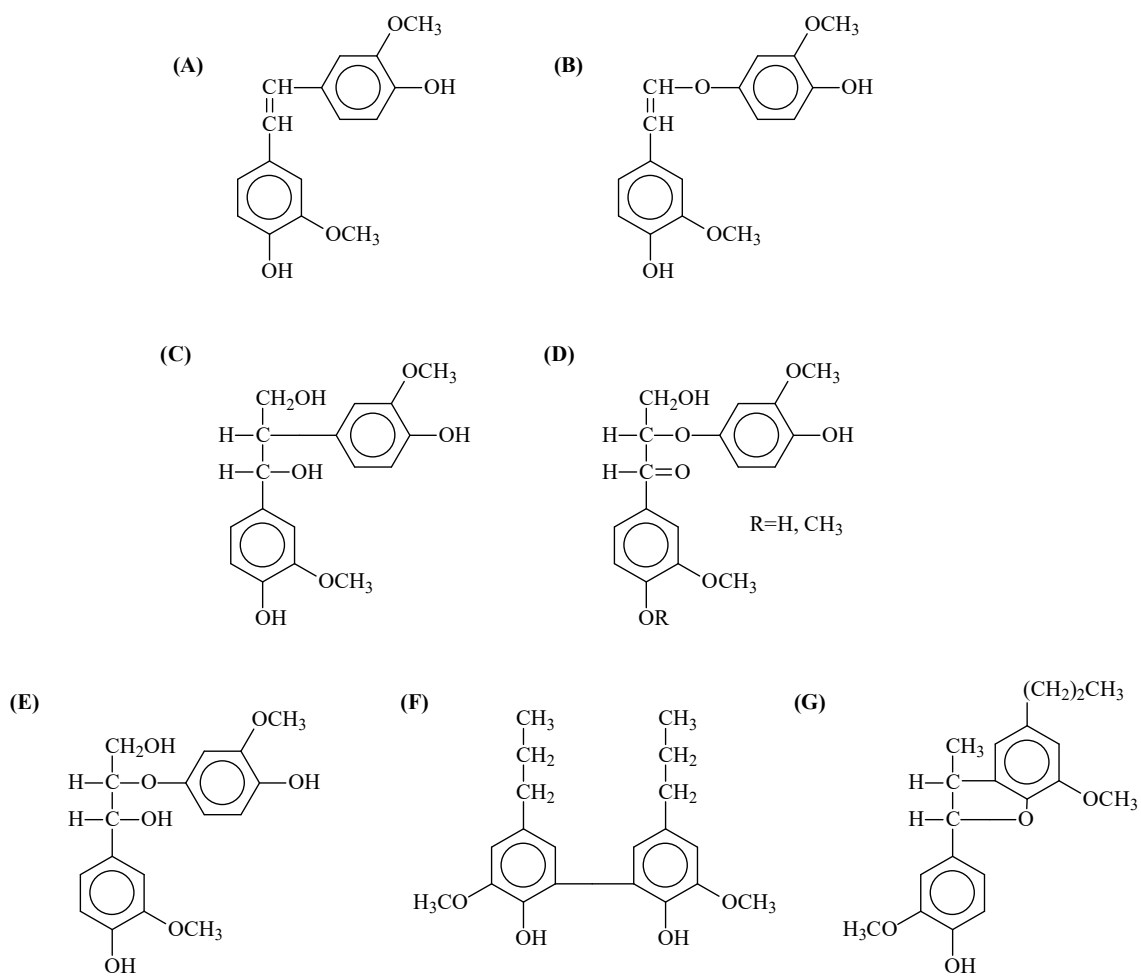
FIGURE 1-2. Dimer lignin model compound used to examine the susceptibility under oxygen treatment. (A) Stilbene, (B) Vinyl ether, (C) *β*-1, (D) *β*-*O*-4 (α-carbonyl), (E) *β*-*O*-4 (α-hydroxyl), (F)5-5, (G) *β*-5

1.4.3 Reaction mechanisms

Generally, the reaction mechanisms have been proposed based on the reaction products obtained from the reaction between lignin model compound and molecular oxygen which generates various types of oxygen radical species involving in the lignin degradation. [34-35] The generation of superoxide anion radical ($O_2^-\bullet$) is resulting in the reaction between lignin model compounds and oxygen under alkaline conditions through one-electron transfer mechanism. This mechanism generally requires the present of metal ion and/or high temperature condition. [36-37] Moreover, the generation also considered as the rate-determining step of the oxidation. By the way, the superoxide anion radical ($O_2^-\bullet$) cannot directly attacked at the lignin structure due to its low oxidation ability. This superoxide anion radical ($O_2^-\bullet$) can form hydroperoxyl anion (HO_2^-) which also undergo forming hydroxyl radical ($HO\bullet$) via metal catalysis. By the way, among the generated oxygen species, only the peroxyl radical ($R\text{-}O\text{-}O\bullet$) and hydroxyl radical ($HO\bullet$) have strong oxidizing ability to involve in the degradation of lignin under alkaline bleaching conditions.

Under oxygen treatment, the most reactive site in lignin is the phenolic structures due to their electron-rich anionic sites (phenolate or ennolate ions) under alkaline treatment. As mentioned above, the only species generated that have an important role in delignification processes under oxygen treatment are peroxyl radical ($R\text{-}O\text{-}O\bullet$) and hydroxyl radical ($HO\bullet$). The generation mechanism can be suggested according to several previous studies as shown in Figure 1-3.

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Reaction 1: The primary reaction in the oxidation of a phenolic structure is the electron transfer from the phenolate anion to oxygen with the formation of superoxide anion radical ($O_2^-\bullet$) and phenoxyl radical. [38] By the way, during oxygen treatment, this generated hydroxyl radical ($HO\bullet$) can also react with phenolate resulting in the formation of phenoxyl radical as well as hydroxide anion. [63, 64]

Reaction 2: The secondary reactions are depending on the oxygen pressure conditions.

- High oxygen pressure

    Under high oxygen pressure condition, the phenoxyl radical, stabilized by resonance, reacts with a second molecule of oxygen to form a peroxyl radical ($R-O-O\bullet$) which is rapidly reduced to hydroperoxide or directly reacts with a superoxide anion radical ($O_2^-\bullet$). [39-40]

- Low oxygen pressure

    The presence of the combination between phenoxyl radical and superoxide anion radical ($O_2^-\bullet$) was observed under low oxygen pressure conditions resulting in the formation of lignin peroxide.

Reaction 3: Under metal-catalyzed condition, superoxide anion radical ($O_2^-\bullet$) also undergoes dismutation into oxygen (O_2) and hydrogen peroxide (H_2O_2) where hydroxyl radicals ($HO\bullet$) are obtained via decomposition reactions.
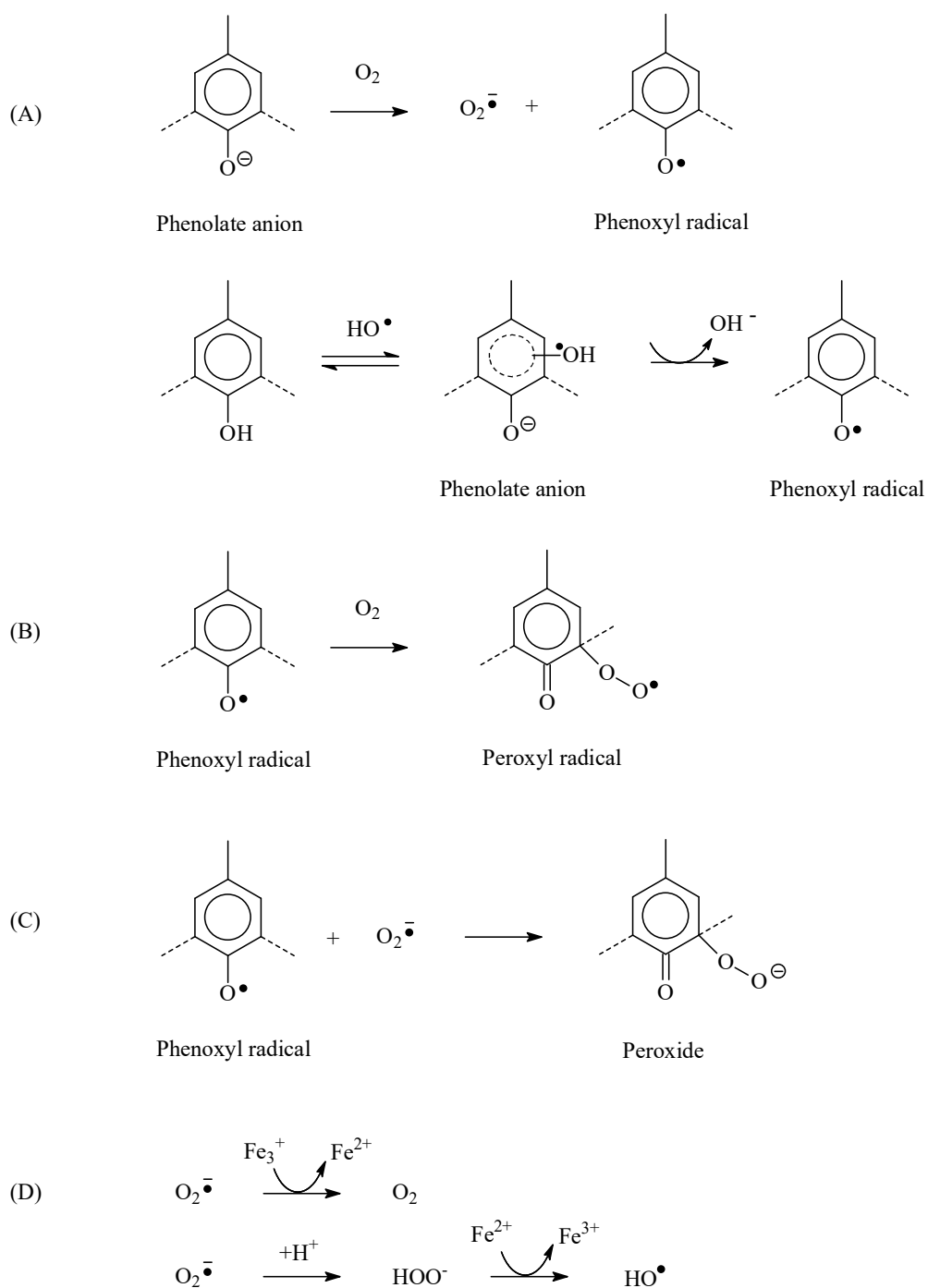
FIGURE 1-3: Outline for generation mechanism of active oxygen species under oxygen treatment (A) Reaction 1, (B) Reaction 2 at high oxygen pressure, (C) Reaction 2 at low oxygen pressure, (D) Reaction 3

These reactions between those lignin phenolic structures and oxygen have been the subject of numerous studies. The detailed degradation pathways as well as the reaction kinetics have been elucidated under oxygen-alkali oxidation. [41-47] Moreover, the reactions of polymeric lignin also have been studied both with isolated lignin preparations and through chemical and spectroscopic analyses of dissolved and residual lignin after an oxygen treatment. [48-51]

The reactivity of the generated peroxyl radical (R-O-O•) and superoxide anion radical (O_2^{-}•) with lignin model compound has been investigated and the reaction mechanism mode can be proposed in several previous studies as shown in Figure 1-4. Under oxygen alkaline treatment, the phenolic units and carbonyl groups, as the main reaction side, oxidized by the molecular oxygen resulting in the production of cabanions. The presence of cabanions can lead to three main reaction mechanisms resulting in demethoxylation, ring opening, and side chain cleavage as shown in Figure 1-5 to Figure 1-7. [52-54] Numerous studies have been examined the reactivity of lignin model compound and hydroxyl radical (HO•). The results suggested that three main reaction mechanisms are presence as following text.

(i) Addition to the aromatic ring

(ii) Electron abstraction by phenolate ions

(iii) Hydrogen abstraction from the side chain

Moreover, it should be noted that hydroxyl radical (HO•) are reacts not only phenolic but also non-phenolic units at about the same rate under oxygen treatment. [38, 55-58, 65]

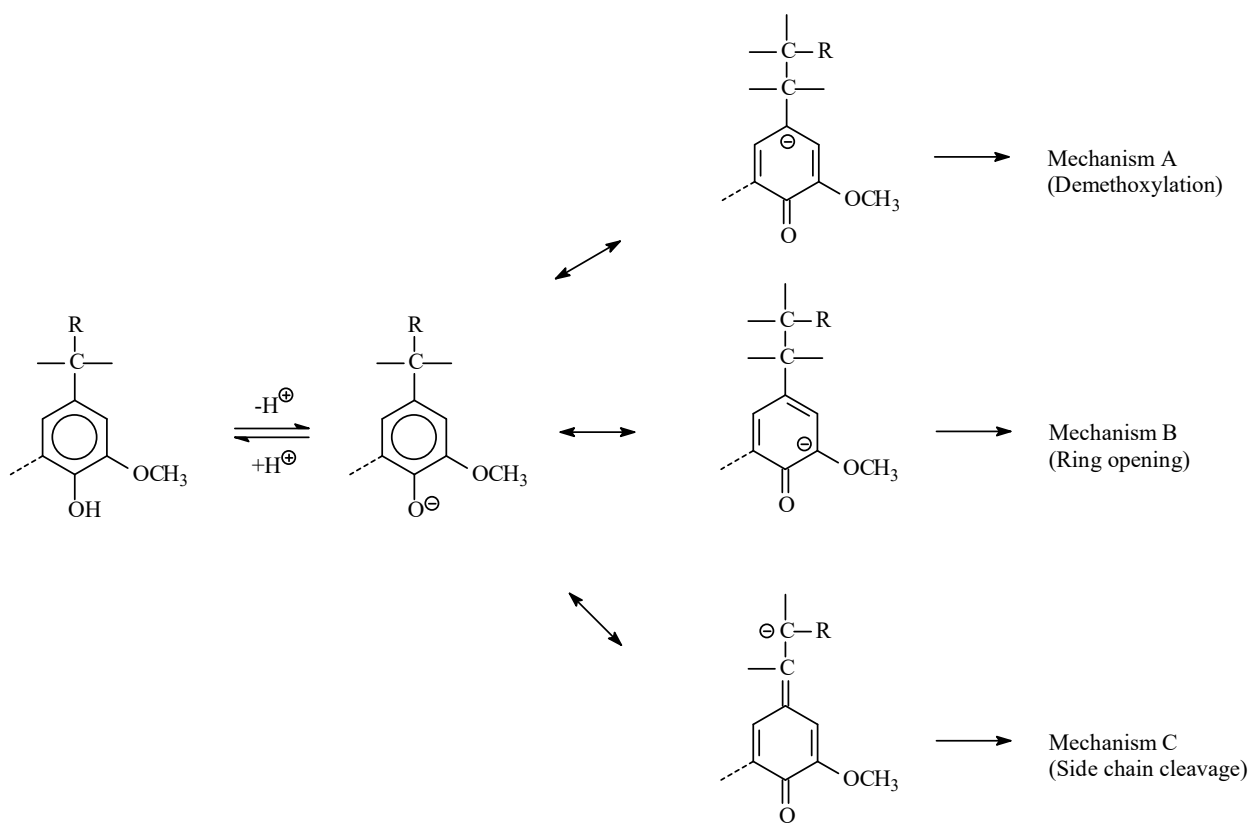**FIGURE 1-4**: Outline for reaction mechanism modes of phenolic units under oxygen alkaline treatment
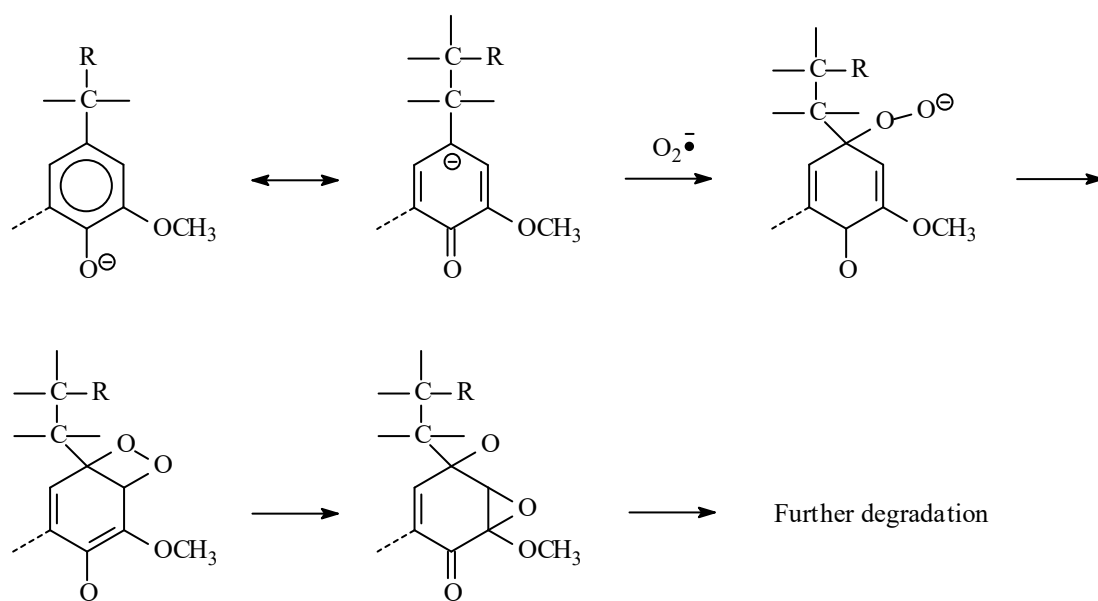
FIGURE 1-5: Reaction mechanism A of phenolic units under oxygen alkaline treatment (Demethoxylation)
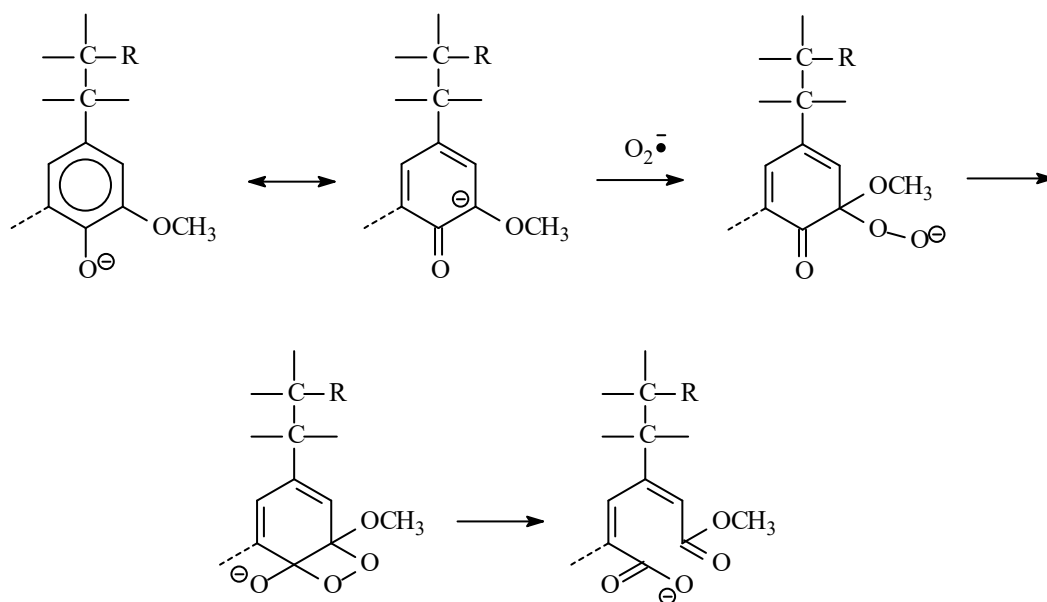

FIGURE 1-6: Reaction mechanism B of phenolic units under oxygen alkaline treatment (Ring opening)
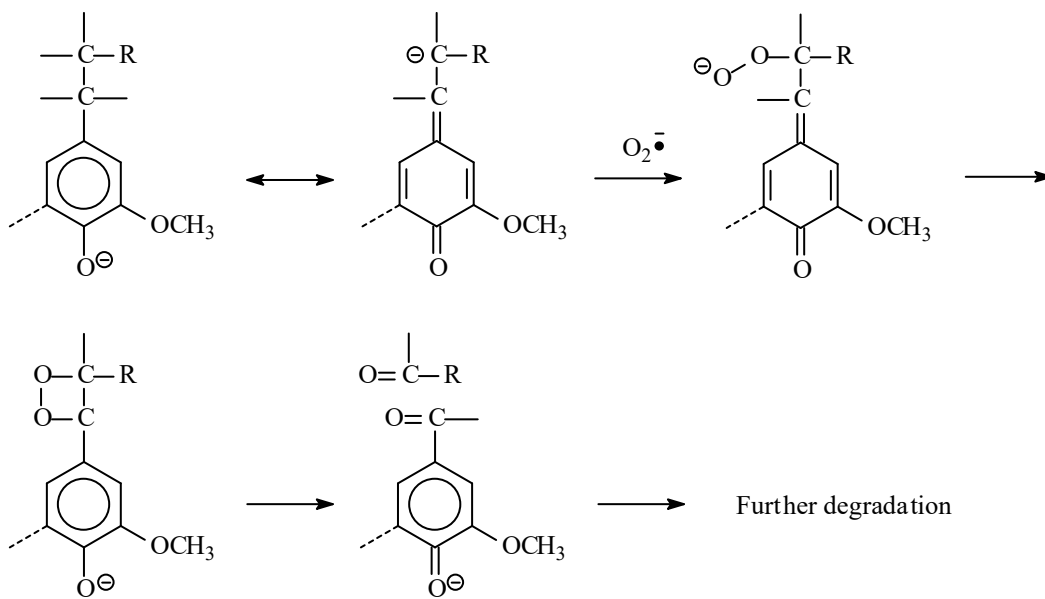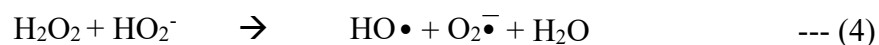
FIGURE 1-7: Reaction mechanism C of phenolic units under oxygen alkaline treatment
(Side chain cleavage)

1.5 Chemistry of delignification under hydrogen peroxide treatment

After the kraft pulping processes, the bleaching treatment with hydrogen peroxide (H_2O_2) as the agents has been used in the alkaline extraction stage where sometimes together treated with oxygen. During treatment, hydrogen peroxide (H_2O_2) readily decomposed into hydroxyl radicals (HO•) which has an important role in delignification under alkaline hydrogen peroxide bleaching condition. Moreover, these hydroxyl radicals (HO•) showed to attack lignin compound both at side-chain and aromatic structure according to previous study result. By the way, under reaction condition with pH higher than 11.6, oxyl anion radical ($O\overline{\bullet}$), oxidized form of hydroxyl radicals (HO•), generate an involving in the delignification processes and found to attack lignin only at side-chain position. [34] These hydroxyl radicals can be formed via two main mechanisms including hemolytic cleavage of hydrogen peroxide (Equation (1)) and metal-catalyzed decomposition as shown in Equation (2)-(3). By the way, the rate of the hydrogen peroxide hemolytic cleavage can be increase by two factor including temperature and presence of alkali (with maximum pH 11.6) according to equation (4).

$$H_2O_2 \rightarrow 2\,HO\bullet \qquad\qquad \text{--- (1)}$$

$$H_2O_2 + M^{n+} \rightarrow HO^- + HO\bullet + M^{(n+1)+} \qquad\qquad \text{--- (2)}$$

$$HO^- + HO_2^- + M^{(n+1)+} \rightarrow O_2\overline{\bullet} + H_2O + M^{n+} \qquad\qquad \text{--- (3)}$$

$$H_2O_2 + HO_2^- \rightarrow HO\bullet + O_2\overline{\bullet} + H_2O \qquad\qquad \text{--- (4)}$$
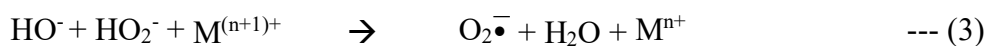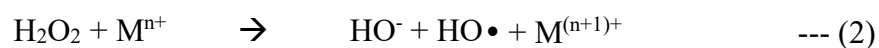
Under alkaline condition, hydroperoxyl anion ($HO_2{}^-$), conjugated base of H_2O_2, attacks at carbonyl groups in lignin structure. [59] As shown in Figure 1-8, the lignin degradation occurs by the addition of hydroperoxyl anion ($HO_2{}^-$) to quinone methide proceeded with the formation of intermediate epoxide and further hydrolyzed resulting in the C_1-C_α bond cleavage followed by the Dakin-like reaction mechanism. [60-62]
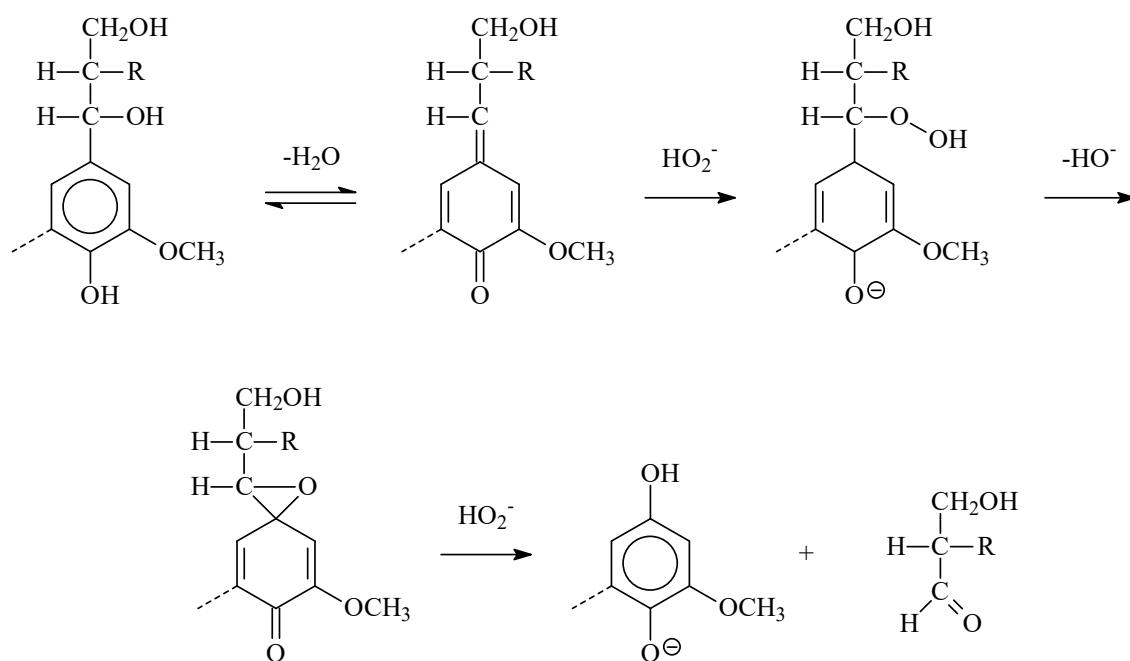
FIGURE 1-8: Reaction mechanism for oxidation of phenolic lignin model compound under alkaline hydrogen peroxide treatment

References

[1] Biermann, C.J. (1996). Handbook of Pulping and Papermaking, 2nd edition, Academic press, Inc., San Diego, 32-33

[2] Pan, X.; Lachenal, D.; Lapierre, C.; Monties, B. (1993). Structure and reactivity of spruce mechanical pulp lignins. Part III. Bleaching and photoyellowing of isolated lignin reactions". *J. Wood Chem. Technol.*, 13(2), 145

[3] Lachenal, D. (1996). Hydrogen peroxide as a delignifying agent. *Tappi* press, 349

[4] Hosoya, S.; Matsumoto, Y. (1997). Wood and forest products science and technology, *Japan Wood Research Society*, II-39

[5] Hoigne, J.; Bader, H. (1944). Kinetics of reactions of chlorine dioxide (OClO) in water I. Rate constants for inorganic and organic compounds, *Water Res.*, 45-55

[6] Sarknen, K.V.; Strauss, R.W. (1970). Demethylation of lignin and lignin models by aqueous chlorine solutions I. Softwood lignin, *Tappi*, 44, 459-464

[7] Hoigne, J.; Bader, H. (1983). Rate constants of reactions of ozone with organic and inorganic compounds in water I. Non-dissociating organic compounds, *Water Res.*, 17, 173-183

[8] Johansson, E.; Ljunggren, S. (1994). The kinetics of lignin reaction during oxygen bleaching, Part 4. The reactivities of different lignin model compounds and the influence of metal ions on the rate of degradation, *J. Wood Chem. Technol.*, 507-525

[9] Afanas'ev, I.B. (1989). In Superoxide ion: Chemistry and Biochemical Implications, Volume I, *CRC Press*, Florida

[10] Miller, R.W. (1970). Reactions of superoxide anion, catechols and cytochrome C", *Can. J. Biochem.*, 48, 935

[11] Lee-Ruff, E.; Lever, A.S.P.; Ringandy, J. (1976). The reaction of catechol and derivatives with potassium superoxide, *Can. J. Chem.*, 54, 1837

[12] Moro-oka, Y.; Foote, C.S. (1976). Chemistry of superoxide ion I. Oxidation of 2, 5-di-tert-butyl catechol with KO_2, *J. Am. Chem. Soc.*, 98, 1510

[13] Sawyer, D.T. (1991). Oxygen Chemistry, Oxford University Press, New York

[14] Cohen, G. (1985). The Fenton reactions" in Handbook of Methods for Oxygen Radical Research, Greenwald, R.A. (ed.), p. 55, CRC Press, Florida

[15] Jones, C.W. (1999). Application of Hydrogen Peroxide and Derivatives, Chapter 2, Clark, J.H. (Ser. ed.), Royal Society of Chemistry, Cambridge, UK

[16] Schumb, W.C., Satterfield, C.N., and Wentworth, R.L. (1955). Hydrogen peroxide, p. 406-412, Reinhold Publishing Corporation, New York

[17] March, J. (1977) Advanced Organic chemistry, Reactions, Mechanisms, and Structure, Second Edition, McGraw-Hill Book Company, New York

[18] Atkinson, R. (1985). Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions, *Chem. Rev.*, 69

[19] Legrini, O.; Oliveros, E.; Braun, A.M. (1993). Photochemical process for water treatment, *Chem. Rev.*, 93,671

[20] Barnes, A.R.; Sugden, J.K., (1986). The hydroxyl radical in aqueous media, *Pharm. Acta Helv.*, 61,218

[21] Buxton, G.V.; Greenstock, C.L.; Helman, W.P.; Ross, A.B. (1988). Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ('OHIO') in aqueous solution, *J. Phys. Chem. Ref. Data*, 17,513

[22] Lee, J.; Grabowski, J. (1992). Reactions of atomic oxygen radical anion and the synthesis of organic reactive intermediates, *Chem. Rev.*, *92,* 1611

[23] Schuchrnann, M.N.; Bothe, E.; von Sonntag, J.; von Sonntag, C. (1998). Reaction of OH radicals with benzoquinone in aqueous solutions. A pulse radiolysis study", *J. Chem. Soc.*, Perkin Trans., 2,791

[24] Gellerstedt, G.; Lindfors, E.-L. (1991). On the structure and reactivity of residual lignin in kraft pulp fibers. In: *Proceeding of the International Pulp Bleaching Conference*, Stockholm, 11-14, 73-88

[25] Van Heiningen, A.; Krothapalli, D.; Genco, J.; Justason, A. (2003). A chemical reactor analysis of industrial oxygen delignification. *Pulp and Paper Canada*, 104, T331-T336

[26] Sultanov, V.S.; Wallis, A.F.A. (1991). Reactivities of Guaiacyl and Syringyl lignin model phenols towards oxidation with oxygen-alkali, *J. Wood Chem. Technol.*, 11 (3), 291-305

[27] Yokoyama, T.; Matsumoto, Y.; Meshitsuka, G. (1999). Reaction selectivity of active oxygen species in alkaline oxygen bleaching. *J. Wood Chem. Technol.*, 19(3), 187-202.

[28] Ljunggren, S.; Johansson, E. (1990). The kinetics of lignin reactions during oxygen bleaching. Part 2. The reactivity of 4,4′-dihydroxy-3,3′-dimethoxy-stilbene andβ-aryl ether structures, *Nord. Pulp Pap. Res. J.*, 5(3), 148-154

[29] Ljunggren, S.; Johansson, E. (1990). The kinetics of lignin reactions during oxygen bleaching. Part 3. The reactivity of 4-n-propylguaiacol and 4,4′-di-n-propyl-6,6′-biguaiacol, *Holzforschung*, 44(4), 291-296

[30] Dence, C.W.; Omori, S. (1981). The reactions of alkaline hydrogen peroxide with

lignin model dimers part 1: Phenacyl α-aryl ethers, *Wood Sci. Technol.*, 15(1), 67-79

[31] Aoyagi, T.; Hosoya, S., Nakano, J. (1979), *J. Japan Wood Res. Soc.*, 25(12), 783-788

[32] Oki, T.; Ishikawa, H.; Okubo, K. (1980), *J. Japan Wood Res. Soc.*, 26(7), 463-470

[33] Oki, T.; Ishikawa, H.; Okubo, K. (1976), *J. Japan Wood Res. Soc.*, 22(9), 518-525

[34] Gierer, J. (1997). Formation and involvement of superoxide ($O_2^-\bullet$/ $HO_2\bullet$) and hydroxyl ($OH\bullet$) radicals in TCF bleaching processes: A Review. *Holzforschung*, 51, 1, 34-46

[35] Gierer, J. (2000). The Interplay between Oxygen-Derived Radical Species in the Delignification during Oxygen and Hydrogen Peroxide Bleaching. Lignin: Historical, Biological, and materials perspectives. American Chemical Society, Washington, D.C., 422-446

[36] Landucci, L.L. (1979). Electrochemical behavior of catalysts for phenoxy radical generation. *Tappi.*, 62 (4), 71-74

[37] Merenyi, G.; Lind, J.; Jonsson, M.(1993). Autoxidation of closed-shell organics: an outer sphere electron transfer. *J. Am. Chem. Soc.*, 115 (11), 4945-4946

[38] Gierer, J.; Reitberger, T.; Yang, E.; Yoon, B.H. (2001). Formation and involvement of radicals in oxygen delignification studies by the autoxidation of lignin and carbohydrate model compounds. *J. Wood Chem.Technol.*, 21, 313-341

[39] Marklund, S.; Marklund, G. (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.,* 47, 469-474

[40] San Clemente, M.R.; Sarkanen, K.V.; Sundin, S.E. (1981). Alkaline autoxidation of 4-ethylguaiacol, *Svensk Papperstidn.*, 84(3), R1-R6

[41] Kratzl, K.; Claus, P.; Lonsky, W.; Gratzl, J.S. (1974). Model studies on reactions occurring in oxidations of lignin with molecular oxygen in alkaline media. *Wood Sci. Technol.*, 8, 35-49

[42] Kratzl, K.; Claus, P.K.; Hruschka, A.; Vierhapper, F.W. (1978). Theoretical fundamentals on oxygen bleaching and pulping. *Cell Chem Technol*, 445-462

[43] Eckert, R.C.; Chang, H.M.; Tucker, W.P. (1973). Oxidative degradation of phenolic lignin model compound with oxygen and alkali. *Tappi*, 56 (6), 134-138

[44] Gierer, J.; Imsgard, F.; Noren, I. (1977). Studies on the degradation of phenolic lignin units of the *β*-aryl ether type with oxygen in alkaline media. *Acta Chem Scand*, B31, 561-572

[45] Gierer, J.; Imsgard, F. (1977). The reactions of lignins with oxygen and hydrogen peroxide in alkaline media. *Svensk Papperstidn*, 80, 510-518

[46] Xu, H.; Lai, Y.Z. (1997). Reactivity of lignin diphenylmethane model dimers under mild alkali-O_2 conditions. *J. Wood. Chem. Technol.*, 17, 223-234

[47] Ljunggren, S. (1986). Kinetics aspects of some lignin reactions in oxygen bleaching. *J. Pulp Pap. Sci.,* 12 (2), J54-J57

[48] Young, R.A.; Gierer, J. (1976). Degradation of native lignin under oxygen-alkali conditions. *Appl. Polym. Symp.*, 28, 1213-1223

[49] Gellerstedt, G.; Gustafsson, K.; Lindfors, E.L. (1986). Structural changes in lignin during oxygen bleaching. *Nordic Pulp Pap. Res. J.*, 1 (3), 14-17

[50] Sun, Y.; Argyropoulous, D. (1995). Fundamentals of high pressure oxygen and low pressure oxygen-peroxide (EOP) delignification of softwood and hardwood kraft pulp: A comparison. *J. Pulp Pap. Sci.*, 21 (6), J185-J190

[51] Asgari, F.; Argyropoulous, D.S. (1998). Fundamental of oxygen delignification. Part

II. Fundamental group formation/ elimination in residual kraft lignin. *Can. J. Chem.,* 76, 1606-1615

[52] Lindgran, B.O.; Kringstad, K.P., Sundin, S.E. (1980). Chemistry of Delignification with Oxygen, Ozone, and Hydrogen Peroxide, *Uni. Publisher Co., Ltd.*, Tokyo, 25

[53] Chang, H.M.; Gratzl, J.S. (1980) Chemistry of Delignification with Oxygen, Ozone, and Hydrogen Peroxide. *Uni. Publisher Co., Ltd.*, Tokyo, 151

[54] Gierer, J.; Imsgard, F. (1980). Chemistry of Delignification with Oxygen, Ozone, and Hydrogen Peroxide. *Uni. Publisher Co., Ltd.*, Tokyo, 137

[55] Gierer, J.; Yang, E.; Reitberger, T. (1992).The reactions of hydroxyl radicals with aromatic rings in lignins, studied with creosol and 4-methylveratrol. *Holzforschung*, 46(6), 495-504.

[56] Gierer, J. Yang, E.; Reitberger, T. (1994). On the significant of the superoxide radical ($O_2\bar{\bullet}$/ HO_2) in oxidative delignification studied with 4-t-butylsyringol and 4-t-butylguaiacol. *Holzforschung*, 48, 405-414

[57] Gierer, J. Yang, E.; Reitberger, T. O. (1996). The reactions of chromophores of the stilbene type with the hydroxyl radical (HO•) and the superoxide radical ($O_2\bar{\bullet}$/ HO_2). Part 1. The cleavage of the conjugated double bond. *Holzforschung*, 50, 342-352

[58] Gierer, J. Yang, E.; Reitberger, T. O. (1996). The reactions of chromophores of the stilbene type with the hydroxyl radical (HO•) and the superoxide radical ($O_2\bar{\bullet}$/ HO_2). Part 2. Reactions other than cleavage of the conjugated double bond. *Holzforschung*, 50, 353-359

[59] Dence, C.W. (1996). Chemistry of mechanical pulping. In: CW Dence and DW Reeve, eds. Pulp Bleaching: Principles and Practice. Atlanta: Tappi press, 160-181

[60] Dence, C.W.; Omori, S. (1981). The reactions of alkaline hydrogen peroxide with lignin model dimers part 2: Phenacyl α-aryl ethers, guaiacylglycerol-*β*-guaiacyl ether. *Wood Sci. Technol.*, 15, 113-123

[61] Kadla, J.K.; Chang, H.M.; Jameel, H. (1997). The reactions of lignin model compounds. *Holzforschung*, 51, 428-434

[62] Heuts, L.; Gellerstedt, G. (1998). Oxidation of guaiacylglycerol-*β*-guaiacyl ether with alkaline hydrogen peroxide in the presence of kraft pulp. *Nordic Pulp Pap. Res. J.*, 13, 107-111

[63] Gierer, J. (1982). The chemistry of delignification. A general concept: Part 2. *Holzforschung*, 36(2), 55-64

[64] Sixta, H., Suess, H., Potthast, A., Schwanninger, M., Krotscheck, A.W. (2006). Pulp bleaching" in: *Handbook of Pulp*, H.Sixta (ed.), Wiley-VCH, Weinheim, 628-734

[65] Kuitunen, S., Kalliola, A., Tarvo, V., Tamminen, T., Rovio, S., Liitia, T., Ohra-aho, T., Lehtimaa, T., Vuorinen, T., Alopaeus, V., (2011). Lignin oxidation mechanisms under oxygen delignification conditions. Part 3. Reaction pathways and modeling. *Holzforschung*, 65, 587-599

Chapter 2

Stereo-preferential degradation of the *erythro* and *threo* isomers of *β-O*-4 type lignin model compounds under oxygen bleaching conditions

2.1 Introduction

Lignin, as one of the main wood component, is a polymer consisting of several phenylpropane units. The most abundant lignin linkage is *β-O*-4 where the cleavage of this bond is a key factor in controlling the delignification under various chemical processes including pulping and bleaching system. Therefore, there are two diastereomers exist in the side-chain of this *β-O*-4 lignin structure named erythro (***E***) and threo (***T***) isomer. These two isomers showed to have different reactivity in various chemical processes, for instance, the degradation rate of ***E*** isomer is more rapid than ***T*** isomer under alkaline pulping conditions. [1-5] Otherwise, there was a few studies observed this stereo-preferential degradation under oxidation conditions. The stereo-preference of ***T*** isomer was observed when oxidized the dimeric non-phenolic lignin model compound with lignin peroxidase and several laccase-mediator systems except the system treated with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) where the reverse tendency was observed. Otherwise, no stereo-preferential degradation was observed under Fenton's reagent system. [6-7]

Owing to the environmental benefits, alkaline oxygen treatment is commercially used for the lignin removal from kraft pulp. Several previous studies have been investigate the delignification reactivity of various lignin model compounds under alkaline oxygen bleaching treatment conditions. [8-11] By the way, the detailed information on the delignification mechanism of the model experiment under various alkaline oxygen treatment conditions need more investigation.

2.2 Objectives of this work

Under the oxygen alkaline treatment, the delignification was occurs not only by only molecular oxygen but also the active oxygen species (AOS) which generated from the reaction between oxygen and phenolic units in lignin. The objectives of this work are shown in the following text.

1. To investigate the effect of the structural of model compound on the stereo-preferential degradation under oxygen alkaline treatment. Three different non-phenolic β-O-4 type lignin model compounds were used in this study.

2. To investigate the effect of the type of co-existing phenolic compound on the stereo-preferential degradation under employed condition. In this study, two different phenolic compounds were used in the reaction system. The results are also suggested the understanding of the active oxygen species reactivity which generated in each system

3. To investigate the effect of the oxygen pressure. The oxygen pressure in this study was adjusted to either 0.4 or 1.1 MPa in order to observe the effect on both stereo-preferential degradation and generation of the AOS.

4. To investigate the alkaline concentration toward the degradation of lignin model compound. Under the oxygen treatment employed, the alkaline concentration was varied using either 0.1 or 0.5 mol/L NaOH to observe their effect on the stereo-preferential degradation.

2.3 Materials and methods

2.3.1 Materials

Semiconductor grades sodium hydroxide (NaOH), Ferric Chloride (FeCl₃) and all the other purchased chemicals including co-existing phenolic compound, 4-hydroxy-3-methoxybenzyl alcohol (vanillyl alcohol, Valc) and 2, 4, 6-trimethylphenol (TMPh) were used without further purification. Therefore, Ultra-high-purity water was used in all the experiments.

In this study, 3 different lignin model compound, 2-(2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) propane-1, 3-diol (*I*), 2-(4-hydroxymethyl-2-methoxy-phenoxy)-1-(3, 4-dimethoxyphenyl)propane-1,3-diol (*2*), or 2-(4-methoxymethyl-2-methoxypheno-xy)-1-(3, 4-dimethoxyphenyl)propane-1, 3-diol (*3*), were synthesized. The synthesizing method for non-phenolic *β*-O-4 type lignin model compounds used in this research was followed Adler *et al.*[12] The compounds were obtained as the combination of corresponding of two isomers (*E* and *T*) which further converted into boron complexes for the separation *via* anion exchange chromatography.[13-15] After the separation, the ozonation method were used to confirmed the purification of isomers produced where the Erythronic or Threonic acid is obtained as the degradation product from *E* or *T* isomer, respectively, of lignin model compound.[16]
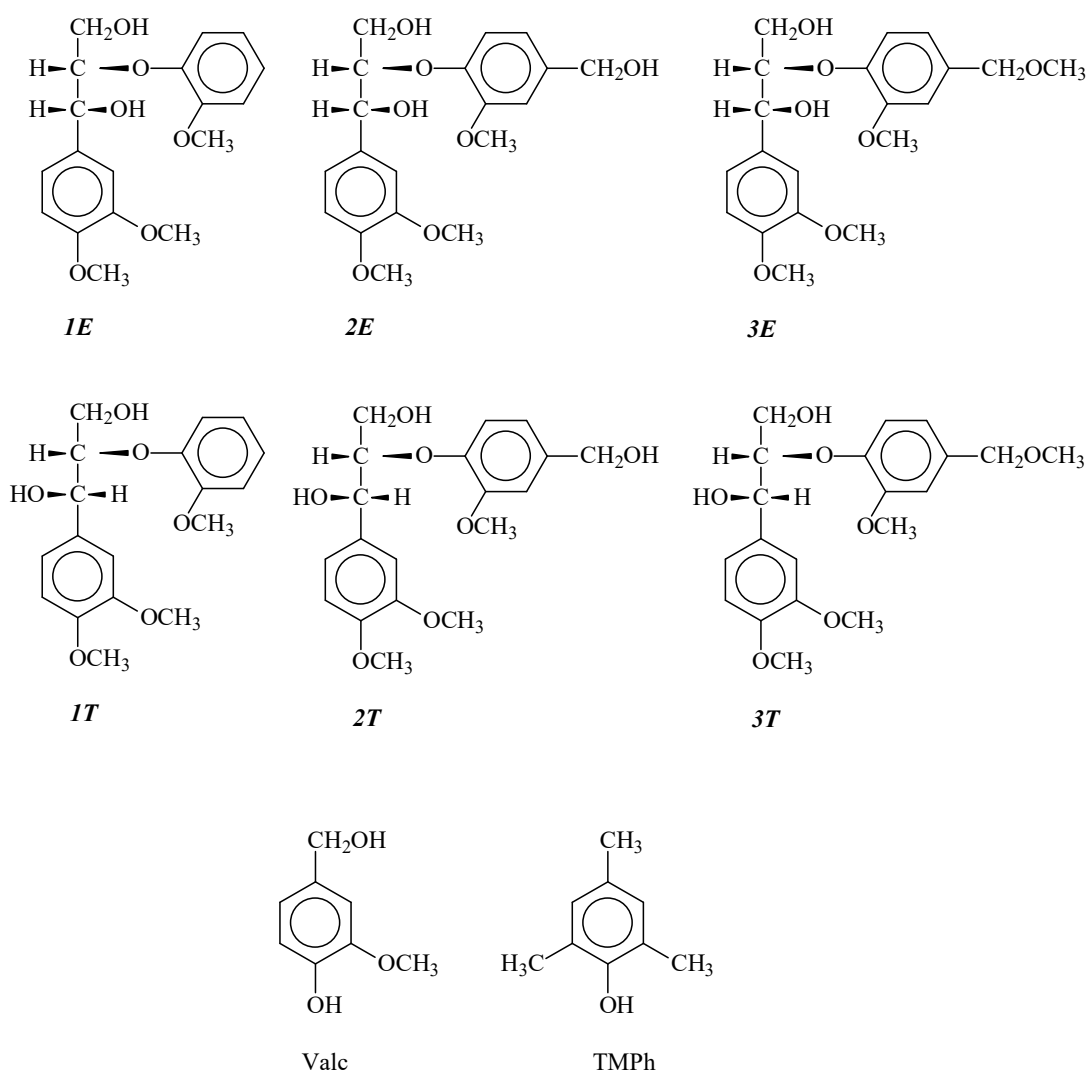
FIGURE 2-1. Chemical structure of dimeric non-phenolic *β-O*-4 type lignin model and co-existing phenolic compounds employed in this study

Synthesis of model compound *1*
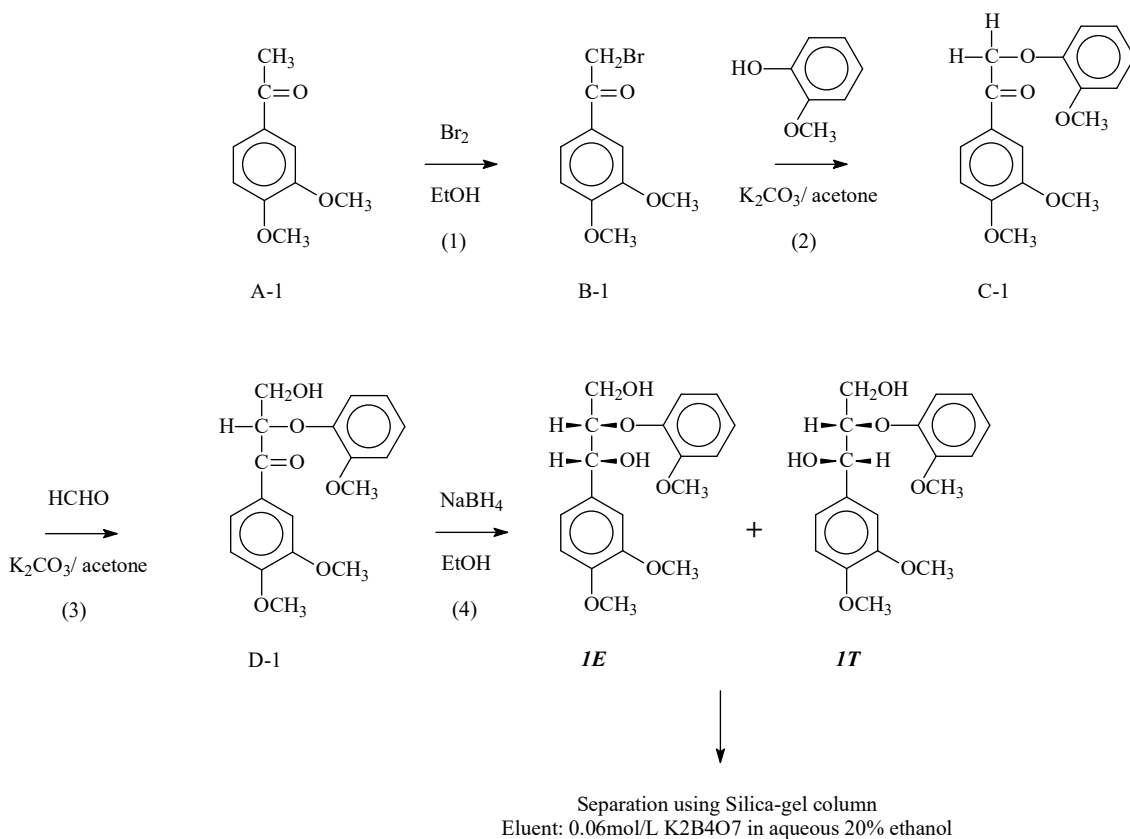
FIGURE 2-2. Synthesis flow diagram for model compound *1*

(1) Bromization of 3, 4-dimethylacetonephenone

The 3, 4-dimethylacetonephenone (A-1) (20.0 g (110 mmol)) and ethanol (110 ml) were stirred together in the round bottom flask, then the Br$_2$ (21.0 g (132 mmol)) added in the solution carefully. The reaction took about 25 minutes until white precipitate was formed. The 4-bromoacetyl-1, 2-dimethoxybenzene (B-1) product yields are 85.45, 80.67 mol% respectively.

(2) Addition of β-O-4 bond

4.59 g (37.03 mmol), 4.60 g (37.03 mmol), 4.60 g (37.03 mmol) of 2-methoxyphenol (guaiacol) and 8.54 g (61.79 mmol),   8.53 g (61.79 mmol), 8.54 g (61.79 mmol) of K_2CO_3 were dissolved in 44 ml of acetone and then placed in a water bath at 40℃ with stirring. Then, 8.00 g (30.85 mmol), 7.89g (30.44 mmol), 7.98 g (30.78 mmol) of 4-bromoacetyl-1, 2-dimethoxybenzene (B-1) were added into the reaction mixture. Thin layer chromatography (TLC) was used to monitor the progress of the reaction and determine the purity of the reaction mixture. The reaction took about 40 minutes. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction solution until all the bubbles are gone for the neutralization. Secondly, extractions were done 4 times by adding the dichloromethane and deionized water respectively. The corrected organic layer was father extracted with NaOH solution. This extraction repeated 4 times to remove the excess guaiacol. The organic layer was dried with dehydrate Na_2SO_4. After removing Na_2SO_4, the organic layer was concentrated using rotary evaporation. The obtained crystal was recrystallized with Ethyl Acetate. The yield of the 2-(2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) ethanone (C-1) got after the reactions are 62.88, 56.97, 61.95 mol%.

(3) Addition of C_γ position

4 g (13.25 mmol), 4 g (13.25 mmol), 4.01 g (13.25 mmol) of 2-(2-methoxyphenoxy)-1-(3,4-dimethoxyphenyl) ethanone (C-1) were dissolved in 130 ml of acetone and then add 16ml of Formaldehyde in the flask and placed in a water bath at 30℃ with stirring. Then, 0.8 g (5.78 mmol), 0.798 g (5.77 mmol), 0.799 g (5.78 mmol) of K_2CO_3 was added into the reaction mixture. The thin layer chromatography was also used to check the progress and the purification of the reaction. Acetic acid was added in order to quench the reaction solution. The reaction took about 20 minutes. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction solution until all the bubbles are gone for the neutralization. Secondly, extractions were done 4 times by adding the dichloromethane and deionized water respectively. Then, the organic layer was dried dehydrate overnight with Na_2SO_4. Therefore, after the recrystallization by Ethyl Acetate, the additional purification by Silica Gel Chromatography has been done and the yields of product (D-1) are 66, 66.64, 69 mol%.

(4) Reduction of carbonyl

The 0.34 g (9.036 mmol) of $NaBH_4$ in 34 ml of ethanol and 1 g (3.012 mmol) of the 2-(2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) propane-1, 3-diol (D-1) were dissolved in ethanol and stirred overnight. The thin layer chromatography was also used to check the progress and the purification of the reaction. Acetic acid was added in order to quench the reaction solution. Then the organic layer was washes with 10 ml of deionized water 3 times, 30, 30, 50 ml of dichloromethane 3 times respectively and 10 ml

of saturated NaCl solution. The organic layer was dried dehydrate with Na_2SO_4. After removing Na_2SO_4, the organic layer was concentrated using rotary evaporator, and remaining CH_3COOH was removed azeotropicly with toluene.

(5) Separation of *E* and *T* isomer via anion exchange chromatography

The mixture of *E* and *T* isomer of the compound *1* were injected into the silica gel chromatography after being converted into the boron complexes in an aqueous ethanol solution containing potassium borate. The HPLC was also used to check the progress and the purification of the reaction. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction for the neutralization. For the extraction, it was done 4 times using dichloromethane and deionized water, respectively. Then, leave the solution overnight after adding Na_2SO_4. After Na_2SO_4 was removed by filtration, the solution was further evaporated.

Silica-gel separation conditions

➢ Silica-gel column: Length 500mm, Diameter 25mm, Volume $220cm^3$, QAE Sephadex A-25 particle size 40-120μm

➢ Separation eluent: 0.06mol/L $K_2B_4O_7$ (Solvent: Ethanol 20%, Water 80%)

(6) NMR characterization

The purity and the structure of each isomer were confirmed using ^{1}H- and ^{13}C-NMR (JNM-A500, 500 MHz, JEOL Ltd., Tokyo, Japan). The spectra of each isomer were recorded using acetone-d_6 and aliquots of D_2O as the solvents. Compound *1E*: ^{1}H-NMR δ 3.68 (dd, 1H, *J*=3.8, *J*=12.0, Cγ-$\underline{H}_a$), 3.74, 3.75, 3.78 (s, 9H, -OC$\underline{H}_3$), 3.81 (dd, 1H, *J*=5.5, *J*=12.0, Cγ-$\underline{H}_b$), 4.33 (m, 1H, Cβ-$\underline{H}$), 4.89 (d, 1H, *J*=5.5, Cα-$\underline{H}$), 6.77-7.09 (m, 7H, aromatic); ^{13}C-NMR δ 55.9, 56.0, 56.2 (-OCH$_3$), 61.4 (Cγ), 73.3 (Cα), 85.7 (Cβ), 111.7, 112.0, 113.3, 118.5, 112.0, 121.8, 122.9, 135.3, 148.7, 149.3, 149.7, 151.4 (aromatic). Compound *1T*: ^{1}H-NMR δ 3.47 (dd, 1H, *J*=5.5, *J*=11.5, Cγ-$\underline{H}_a$), 3.71 (dd, 1H, *J*=4.5, *J*=11.5, Cγ-$\underline{H}_b$), 3.73, 3.74, 3.82 (s, 9H, -OC$\underline{H}_3$), 4.30 (m, 1H, Cβ-$\underline{H}$), 4.92 (d, 1H, *J*=5.0, Cα-$\underline{H}$), 6.78-7.09 (m, 7H, aromatic); ^{13}C-NMR δ 55.8, 55.9, 56.1 (-OCH$_3$), 61.2 (Cγ), 73.0 (Cα), 86.4 (Cβ), 111.4, 111.9, 113.1, 118.0, 119.8, 121.8, 122.8, 134.8, 149.0, 149.2, 149.6, 150.9 (aromatic).
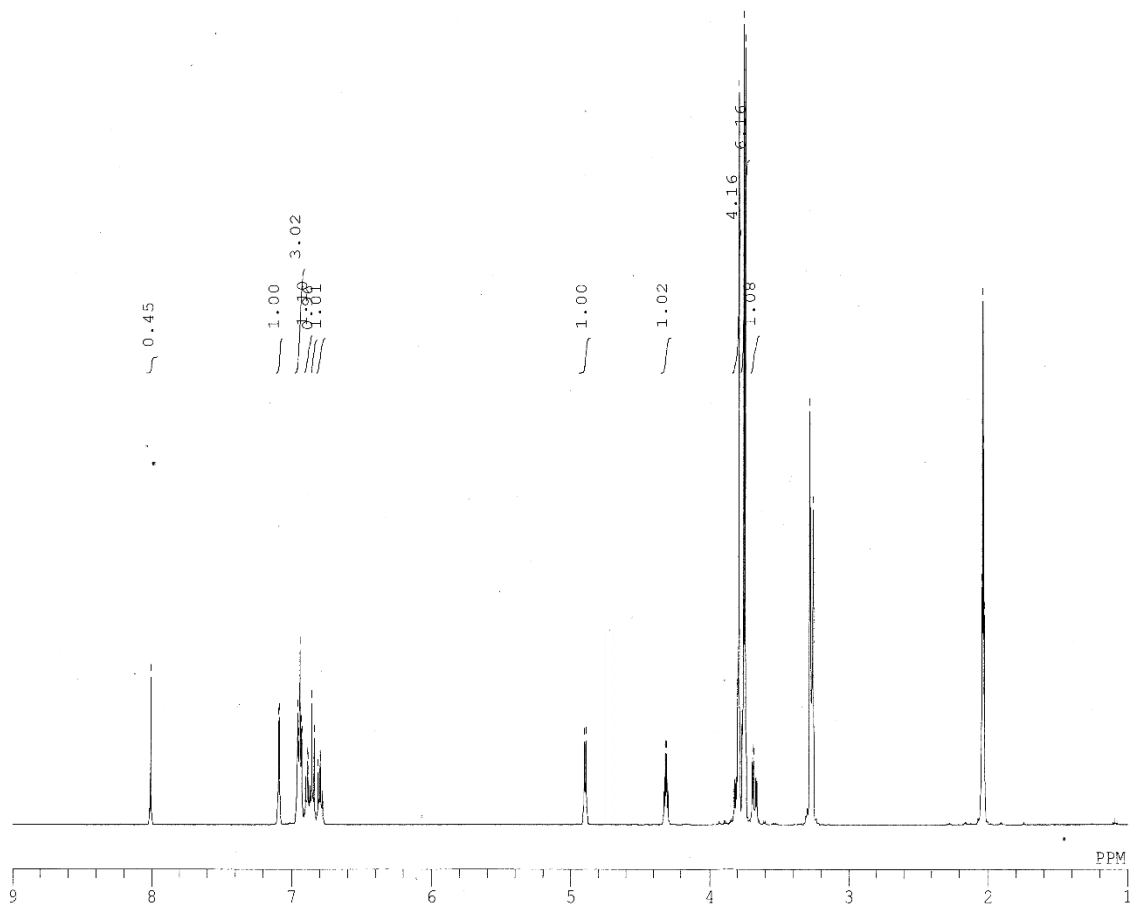
FIGURE 2-3. ¹H-NMR spectrum of model compound *1E*

41
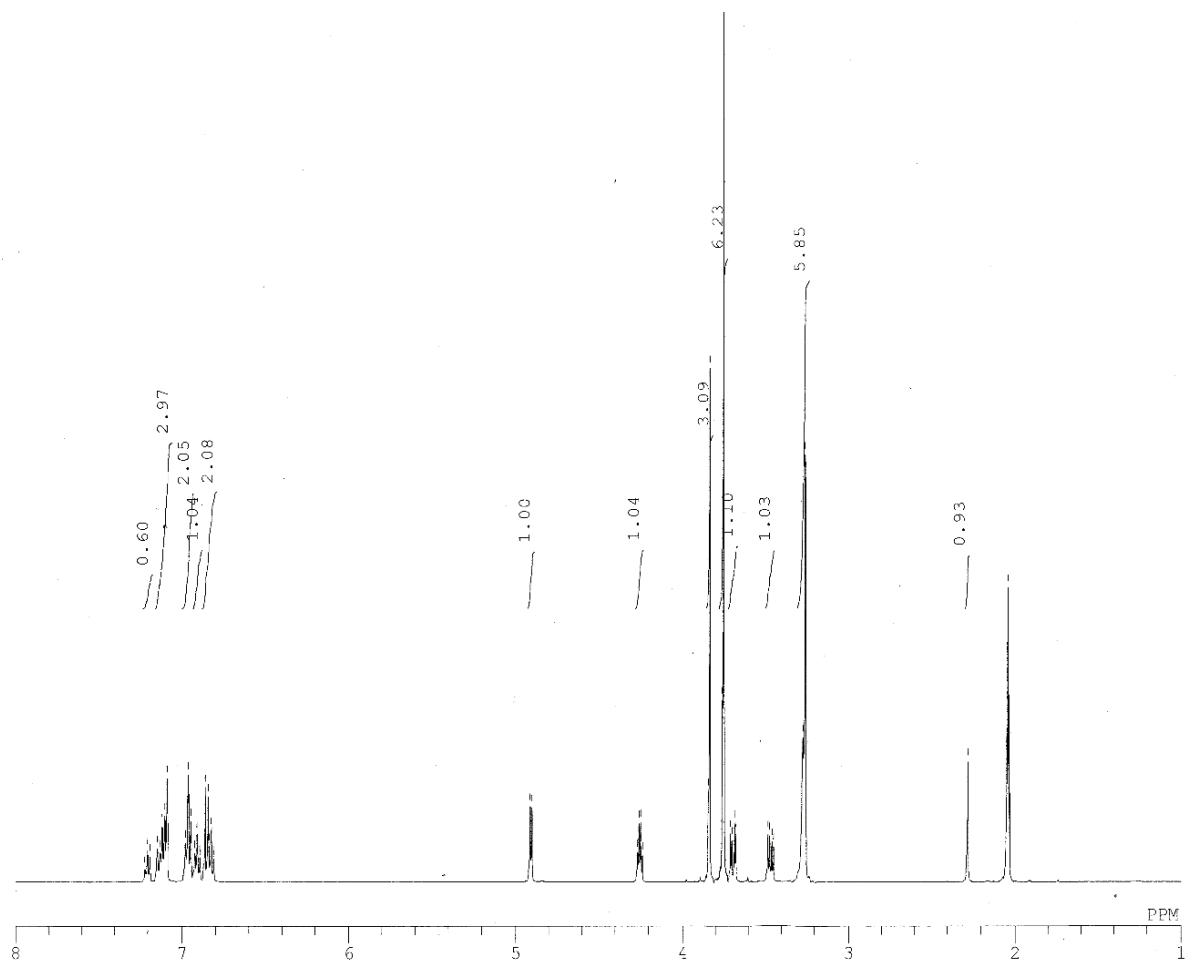
FIGURE 2-4. ^{1}H-NMR spectrum of model compound *1T*

Synthesis of model compound *2*
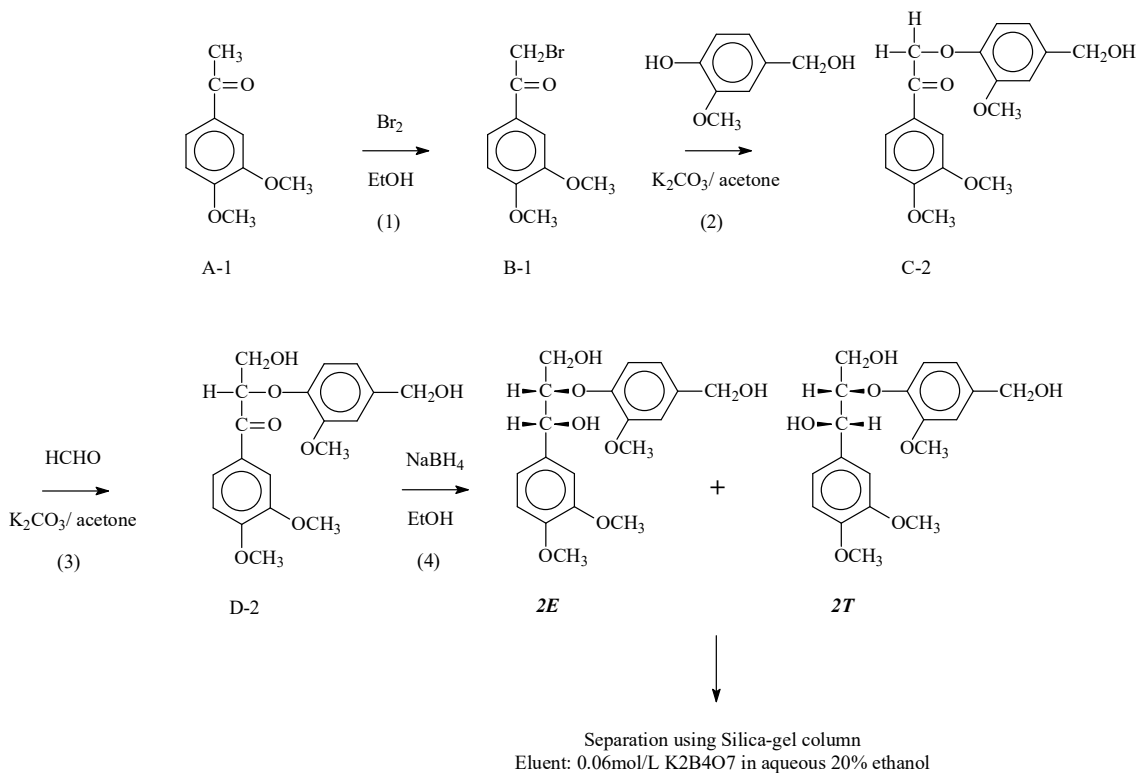
FIGURE 2-5. Synthesis flow diagram for model compound *2*

(1) Bromization of 3, 4-dimethylacetonephenone

The 3, 4-dimethylacetonephenone (20.0 g (110 mmol)) and ethanol (110 ml) were stirred together in the round bottom flask, then the Br_2 (21.0 g (132 mmol)) added in the solution carefully. The reaction took about 25 minutes until white precipitate was formed. The 4-bromoacetyl-1, 2-dimethoxybenzene product yields are 85.45, 80.67 mol% respectively.

(2) Addition of β-O-4 bond

    2.85 g (18.5 mmol), 2.86 g (18.57 mmol), 2.85 g (18.5 mmol) of 4-(hydroxymethyl)-2-methoxyphenol (Vanillyl alcohol) and 4.27 g (30.89 mmol), 4.26 g (30.82 mmol), 4.26 g (30.82 mmol) of K_2CO_3 were dissolved in 22 ml of acetone and then placed in a water bath at 40℃ with stirring. Then, 4.02 g (15.48 mmol), 4.12 g (15.90 mmol), 4.09 g (15.79 mmol) of 4-bromoacetyl-1, 2-dimethoxybenzene were added into the reaction mixture. Thin layer chromatography (TLC) was used to monitor the progress of the reaction and determine the purity of the reaction mixture. The reaction took about 120 minutes. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction solution until all the bubbles are gone for the neutralization. Secondly, extractions were done 4 times by adding the dichloromethane and deionized water respectively. The corrected organic layer was further extracted with NaOH solution. The organic layer was dried with dehydrate Na_2SO_4. After removing Na_2SO_4, the organic layer was concentrated using rotary evaporation. The obtained crystal was recrystallized with Ethyl Acetate. The yield of the 2-(4-(hydroxymethyl)-2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) ethanone got after the reactions are 52.33, 51.14, 50.25 mol%.

(3) Addition of Cγ position

2.1 g (6.3 mmol), 2.0 g (6 mmol), 2.01 g (6.03 mmol)   of 2-(4-(hydroxymethyl)-2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) ethanone were dissolved in 60 ml of ethanol and then add 6ml of Formaldehyde in the flask and placed in a water bath at 35º C with stirring. Then, 1.42 g (10.29 mmol), 1.48 g (10.72 mmol), 1.41 g (10.22 mmol) of K_2CO_3 was added into the reaction mixture. The thin layer chromatography was also used to check the progress and the purification of the reaction. Acetic acid was added in order to quench the reaction solution. The reaction took about 35 minutes. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction solution until all the bubbles are gone for the neutralization. Secondly, extractions were done 4 times by adding the dichloromethane and deionized water respectively. Then, the organic layer was dried dehydrate overnight with Na_2SO_4. Therefore, after the recrystallization by Ethyl Acetate, the additional purification by Silica Gel Chromatography has been done and the yields of product are 60, 61.6, 62.0 mol%.

(4) Reduction of carbonyl

The 0.31 g (8.19 mmol) of $NaBH_4$ in 34 ml of ethanol and 1 g (2.74 mmol) of the 2-(4-(hydroxymethyl)-2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) propane-1, 3-diol were dissolved in ethanol and stirred overnight. The thin layer chromatography was also used to check the progress and the purification of the reaction. Acetic acid was added in order to quench the reaction solution. Then the organic layer was washes with 10 ml of deionized water 3 times, 30, 30, 50 ml of dichloromethane 3 times respectively and 10 ml

of saturated NaCl solution. The organic layer was dried dehydrate with Na_2SO_4. After removing Na_2SO_4, the organic layer was concentrated using rotary evaporator, and remaining CH_3COOH was removed azeotropicly with toluene

(5) Separation of **E** and **T** isomer via anion exchange chromatography

The mixture of **E** and **T** isomer of the compound **2** were injected into the silica gel chromatography after being converted into the boron complexes in an aqueous ethanol solution containing potassium borate. The HPLC was also used to check the progress and the purification of the reaction. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction for the neutralization. For the extraction, it was done 4 times using dichloromethane and deionized water, respectively. Then, leave the solution overnight after adding Na_2SO_4. After Na_2SO_4 was removed by filtration, the solution was further evaporated.

Silica-gel separation conditions

➢ Silica-gel column: Length 500mm, Diameter 25mm, Volume 220cm^3, QAE Sephadex A-25 particle size 40-120μm

➢ Separation eluent: 0.06mol/L $K_2B_4O_7$ (Solvent: Ethanol 20%, Water 80%)

(6) NMR characterization

The purity and the structure of each isomer were confirmed using ^{1}H- and ^{13}C-NMR (JNM-A500, 500 MHz, JEOL Ltd., Tokyo, Japan). The spectra of each isomer were recorded using acetone-d_6 and aliquots of D_2O as the solvents. Compound **2E**: ^{1}H-NMR δ 3.68 (dd, 1H, J=3.8, J=12.0, Cγ-$\underline{H}_a$), 3.74, 3.75, 3.78 (s, 9H, -OC$\underline{H}_3$), 3.81 (dd, 1H, J=5.5, J=12.0, Cγ-$\underline{H}_b$), 4.33 (m, 1H, Cβ-$\underline{H}$), 4.49 (s, 2H, -C$\underline{H}_2$OH (benzylic)), 4.89 (d, 1H, J=5.5, Cα-$\underline{H}$), 6.77-7.09 (m, 6H, aromatic); ^{13}C-NMR δ 55.9, 55.9, 56.1 (-OCH$_3$), 61.3 (Cγ), 64.1 (-CH$_2$OH (benzylic)), 73.2 (Cα), 85.8 (Cβ), 111.6, 111.9, 112.0, 118.2, 119.8, 119.9, 135.3, 137.2, 147.5, 149.2, 149.6, 151.1 (aromatic). Compound **2T**: ^{1}H-NMR δ 3.46 (dd, 1H, J=5.5, J=12.0, Cγ-$\underline{H}_a$), 3.71 (dd, 1H, J=4.5, J=11.5, Cγ-$\underline{H}_b$), 3.73, 3.74, 3.82 (s, 9H, -OC$\underline{H}_3$), 4.26 (m, 1H, Cβ-$\underline{H}$), 4.49 (s, 2H, -C$\underline{H}_2$OH (benzylic)), 4.91 (d, 1H, J=5.5, Cα-$\underline{H}$), 6.78-7.09 (m, 6H, aromatic); ^{13}C-NMR δ 55.8, 55.9, 56.1 (-OCH$_3$), 61.2 (Cγ), 64.1 (-CH$_2$OH (benzylic)), 73.0 (Cα), 86.7 (Cβ), 111.5, 111.8, 112.0, 117.9, 119.8, 119.9, 134.9, 137.1, 147.9, 149.3, 149.6, 150.8 (aromatic).
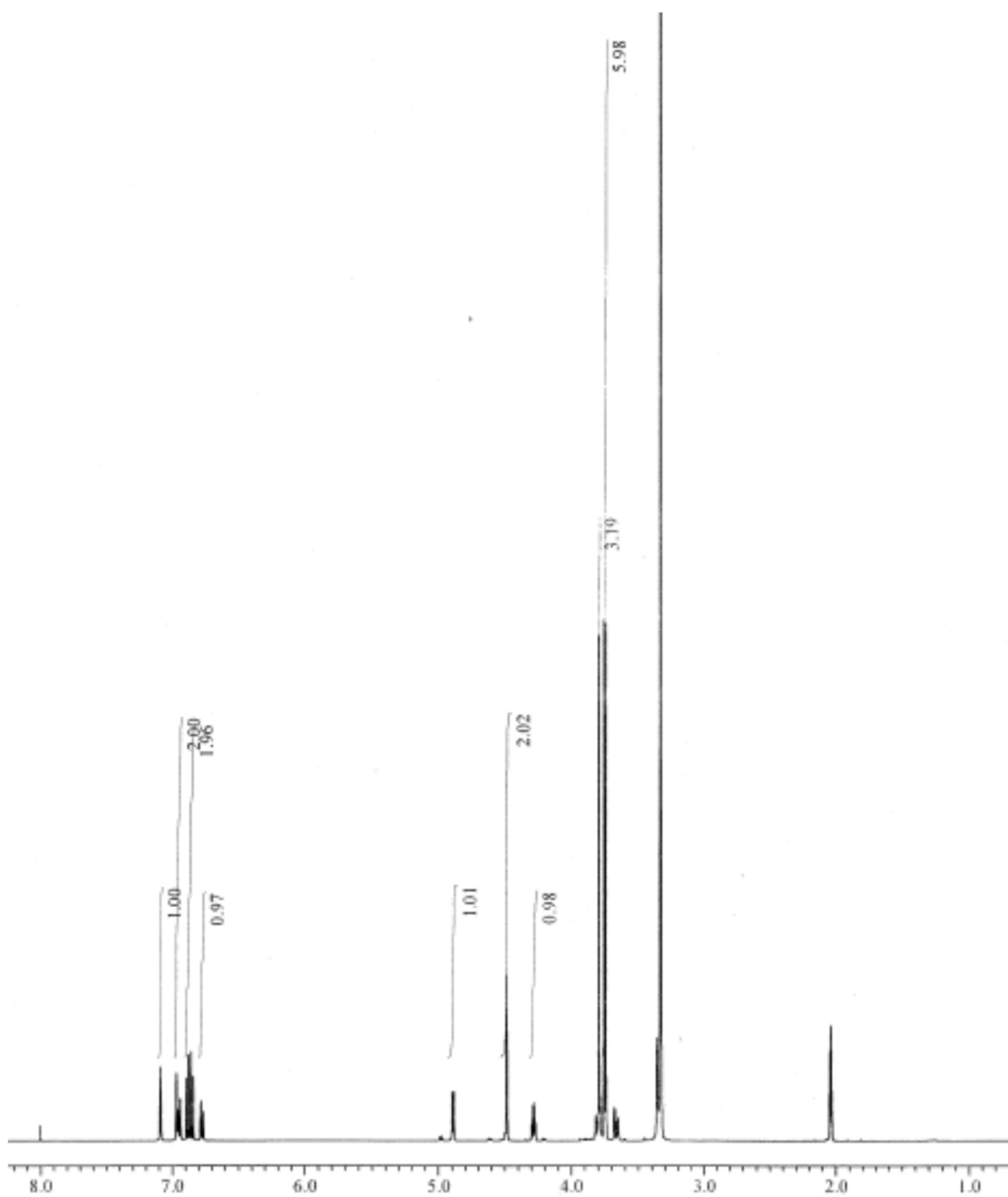
FIGURE 2-6. ^{1}H-NMR spectrum of model compound *2E*
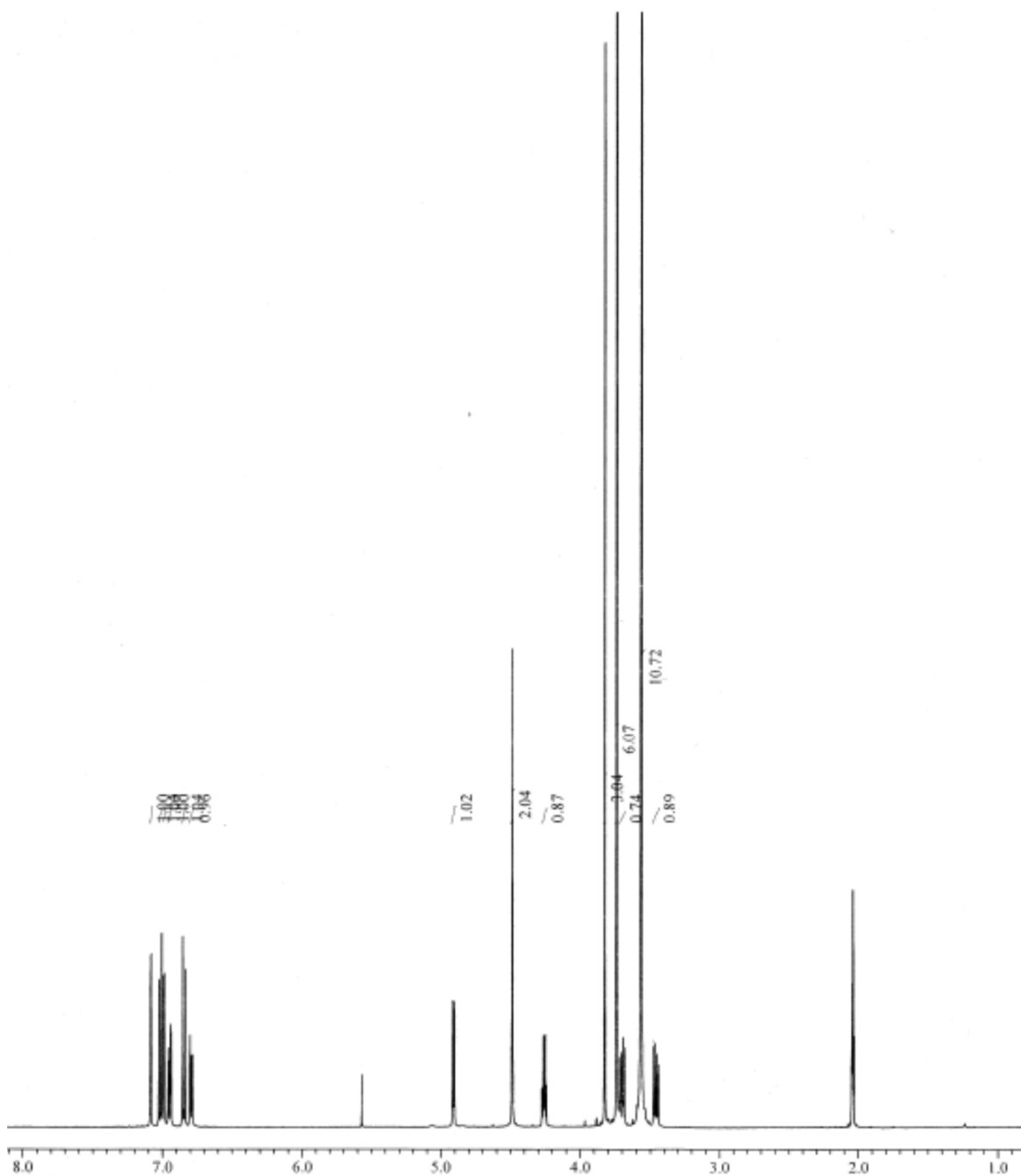
FIGURE 2-7. ^{1}H-NMR spectrum of model compound *2T*

Synthesis of model compound *3*
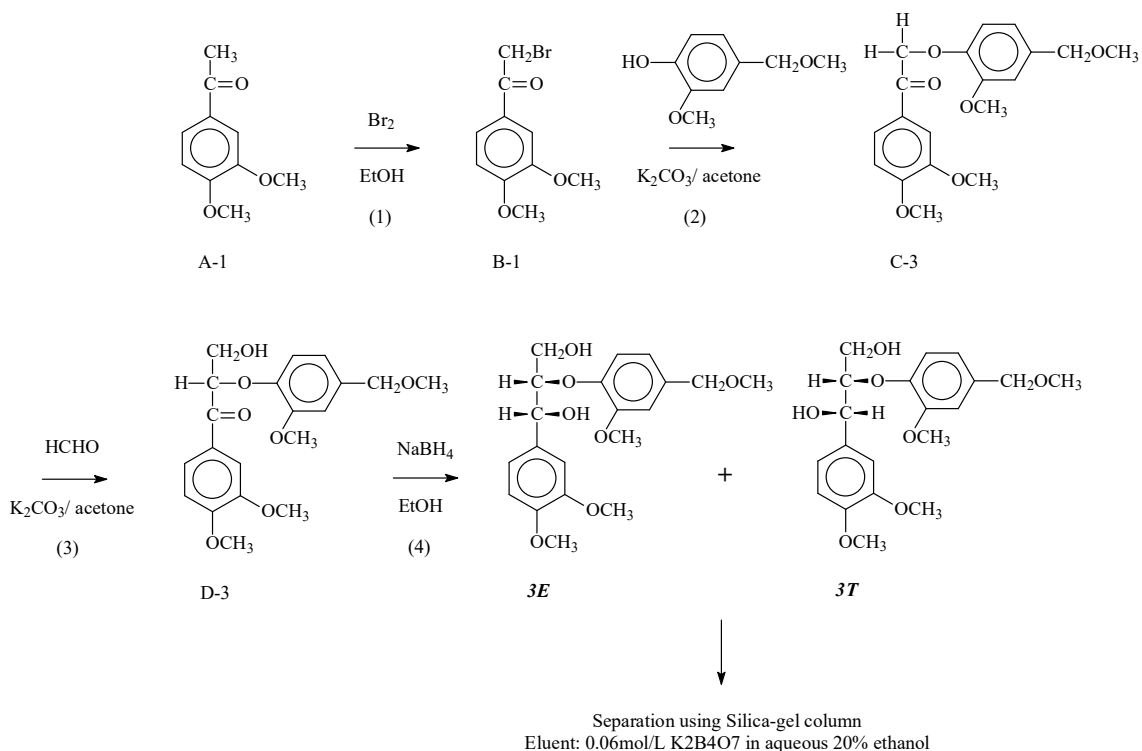
FIGURE 2-8. Synthesis flow diagram for model compound *3*

(1) Bromization of 3, 4-dimethylacetonephenone

The 3, 4-dimethylacetonephenone (20.0 g (110 mmol)) and ethanol (110 ml) were stirred together in the round bottom flask, then the Br_2 (21.0 g (132 mmol)) added in the solution carefully.   The reaction took about 25 minutes until white precipitate was formed. The 4-bromoacetyl-1, 2-dimethoxybenzene product yields are 85.45, 80.67 mol% respectively.

(2) Addition of β-O-4 bond

(2.1) Methylation of Vanillyl alcohol

6.09 g (39.55 mmol) of 4-hydroxymethyl-2-methoxyphenol (Vanillyl alcohol) and 0.5 mol/L of hydrochloric acid (HCl) was mixed in methanol. The reaction was done at 50°C for 30 minutes. After the reaction, the solution was neutralize using sodium carbonate solution (Na_2CO_3) and then remove methanol by evaporation. The extraction was done after evaporation using dichloromethane and deionized water. Na_2SO_4 was added into the extracted solution and leaved overnight. After removing Na_2SO_4, the organic layer was concentrated using rotary evaporation. The obtained crystal was recrystallized with Ethyl Acetate. The yield of the 4-methoxymethyl-2-methoxyphenol obtained after the reaction is 72.09 mol%

(2.2) Addition of β-O-4 bond

6.22 g (37.06 mmol) of 4-methoxymethyl-2-methoxyphenol and 8.52 g (61.76 mmol) of K_2CO_3 were dissolved in 44 ml of acetone and then placed in a water bath at 40°C with stirring. Then, 8.02 g (30.88 mmol) of 4-bromoacetyl-1, 2-dimethoxybenzene were added into the reaction mixture. Thin layer chromatography (TLC) was used to monitor the progress of the reaction and determine the purity of the reaction mixture. The reaction took about 120 minutes. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction solution until all the bubbles are gone for the neutralization. Secondly, extractions were done 4 times by adding the dichloromethane and deionized

water respectively. The corrected organic layer was further extracted with NaOH solution. The organic layer was dried with dehydrate Na_2SO_4. After removing Na_2SO_4, the organic layer was concentrated using rotary evaporation. The obtained crystal was recrystallized with Ethyl Acetate. The yield of the 2-(4-(methoxymethyl)-2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) ethanone got after the reactions are 54.12 mol%.

(3) Addition of Cγ position

2.02 g (5.76 mmol) of 2-(4-(methoxymethyl)-2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) ethanone were dissolved in 60 ml of ethanol and then add 6ml of Formaldehyde in the flask and placed in a water bath at 35º C with stirring. Then, 1.4 g (10 mmol) of K_2CO_3 was added into the reaction mixture. The thin layer chromatography was also used to check the progress and the purification of the reaction. Acetic acid was added in order to quench the reaction solution. The reaction took about 35 minutes. For the extraction part, the acetic acid (CH_3COOH) was firstly added in to the reaction solution until all the bubbles are gone for the neutralization. Secondly, extractions were done 4 times by adding the dichloromethane and deionized water respectively. Then, the organic layer was dried dehydrate overnight with Na_2SO_4. Therefore, after the recrystallization by Ethyl Acetate, the additional purification by Silica Gel Chromatography has been done and the yields of product are 63 mol%.

(4) Reduction of carbonyl

The 0.3 g (7.95 mmol) of NaBH$_4$ in 34 ml of ethanol and 1.05 g (2.65 mmol) of the 2-(4-(methoxymethyl)-2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) propane-1, 3-diol were dissolved in ethanol and stirred overnight. The thin layer chromatography was also used to check the progress and the purification of the reaction. Acetic acid was added in order to quench the reaction solution. Then the organic layer was washes with 10 ml of deionized water 3 times, 30, 30, 50 ml of dichloromethane 3 times respectively and 10 ml of saturated NaCl solution. The organic layer was dried dehydrate with Na$_2$SO$_4$. After removing Na$_2$SO$_4$, the organic layer was concentrated using rotary evaporator, and remaining CH$_3$COOH was removed azeotropicly with toluene

(5) Separation of *E* and *T* isomer via anion exchange chromatography

The mixture of *E* and *T* isomer of the compound *2* were injected into the silica gel chromatography after being converted into the boron complexes in an aqueous ethanol solution containing potassium borate. The HPLC was also used to check the progress and the purification of the reaction. For the extraction part, the acetic acid (CH$_3$COOH) was firstly added in to the reaction for the neutralization. For the extraction, it was done 4 times using dichloromethane and deionized water, respectively. Then, leave the solution overnight after adding Na$_2$SO$_4$. After Na$_2$SO$_4$ was removed by filtration, the solution was further evaporated.

Silica-gel separation conditions

➢ Silica-gel column: Length 500mm, Diameter 25mm, Volume 220cm^3, QAE Sephadex A-25 particle size 40-120μm

➢ Separation eluent: 0.06mol/L $K_2B_4O_7$ (Solvent: Ethanol 20%, Water 80%)

(6) NMR characterization

The purity and the structure of each isomer were confirmed using ^{1}H- and ^{13}C-NMR (JNM-A500, 500 MHz, JEOL Ltd., Tokyo, Japan). The spectra of each isomer were recorded using acetone-d_6 and aliquots of D_2O as the solvents. Compound **3E**: ^{1}H-NMR δ 3.26 (s, 3H, -CH$_2$OC$\underline{H}_3$), 3.68 (dd, 1H, J=3.5, J=11.75, Cγ-$\underline{H}_a$), 3.73, 3.75, 3.78 (s, 9H, -OC$\underline{H}_3$), 3.81 (dd, 1H, J=5.5, J=11.75, Cγ-$\underline{H}_b$), 4.30 (s, 2H, -C$\underline{H}_2$OCH$_3$), 4.32 (m, 1H, Cβ-$\underline{H}$), 4.89 (d, 1H, J=5.5, Cα-$\underline{H}$), 6.73-7.09 (m, 6H, aromatic); ^{13}C-NMR δ 55.9, 56.0, 56.2, 57.7 (-OCH$_3$), 61.4 (Cγ), 73.3 (Cα), 74.6 (-$\underline{C}$H$_2$OCH$_3$), 85.9 (Cβ), 111.7, 112.0, 112.8, 118.2, 119.9, 121.0, 133.4, 135.3, 148.1, 149.3, 149.7, 151.3 (aromatic). Compound **3T**: ^{1}H-NMR δ 3.28 (m, 3H, -CH$_2$OC$\underline{H}_3$), 3.48 (dd, 1H, J=6.0, J=12.0, Cγ-$\underline{H}_a$), 3.69 (dd, 1H, J=4.5, J=11.5, Cγ-$\underline{H}_b$), 3.75, 3.84, 3.85 (s, 9H, -OC$\underline{H}_3$), 4.25 (m, 1H, Cβ-$\underline{H}$), 4.33 (s, 2H, -C$\underline{H}_2$OCH$_3$), 4.90 (d, 1H, J=6, Cα-$\underline{H}$), 6.78-7.10 (m, 6H, aromatic); ^{13}C-NMR δ 55.8, 55.9, 56.1, 57.7 (-OCH$_3$), 61.3 (Cγ), 73.0 (Cα), 74.6 (-$\underline{C}$H$_2$OCH$_3$), 86.8 (Cβ), 111.5, 112.0, 112.7, 117.9, 119.8, 121.1, 133.2, 134.9, 148.6, 149.3, 149.7, 150.9 (aromatic).
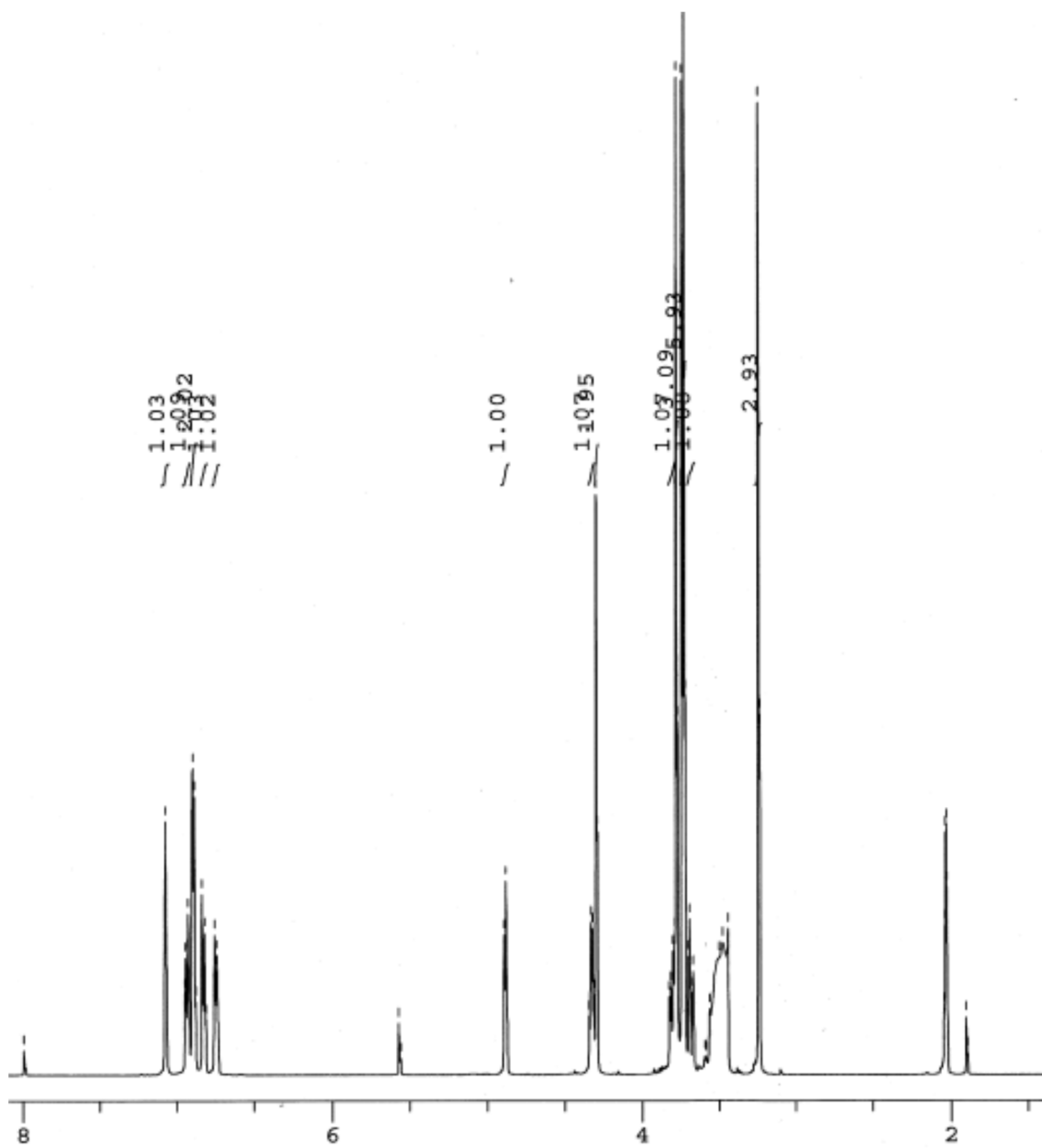
FIGURE 2-9. ^{1}H-NMR spectrum of model compound *3E*
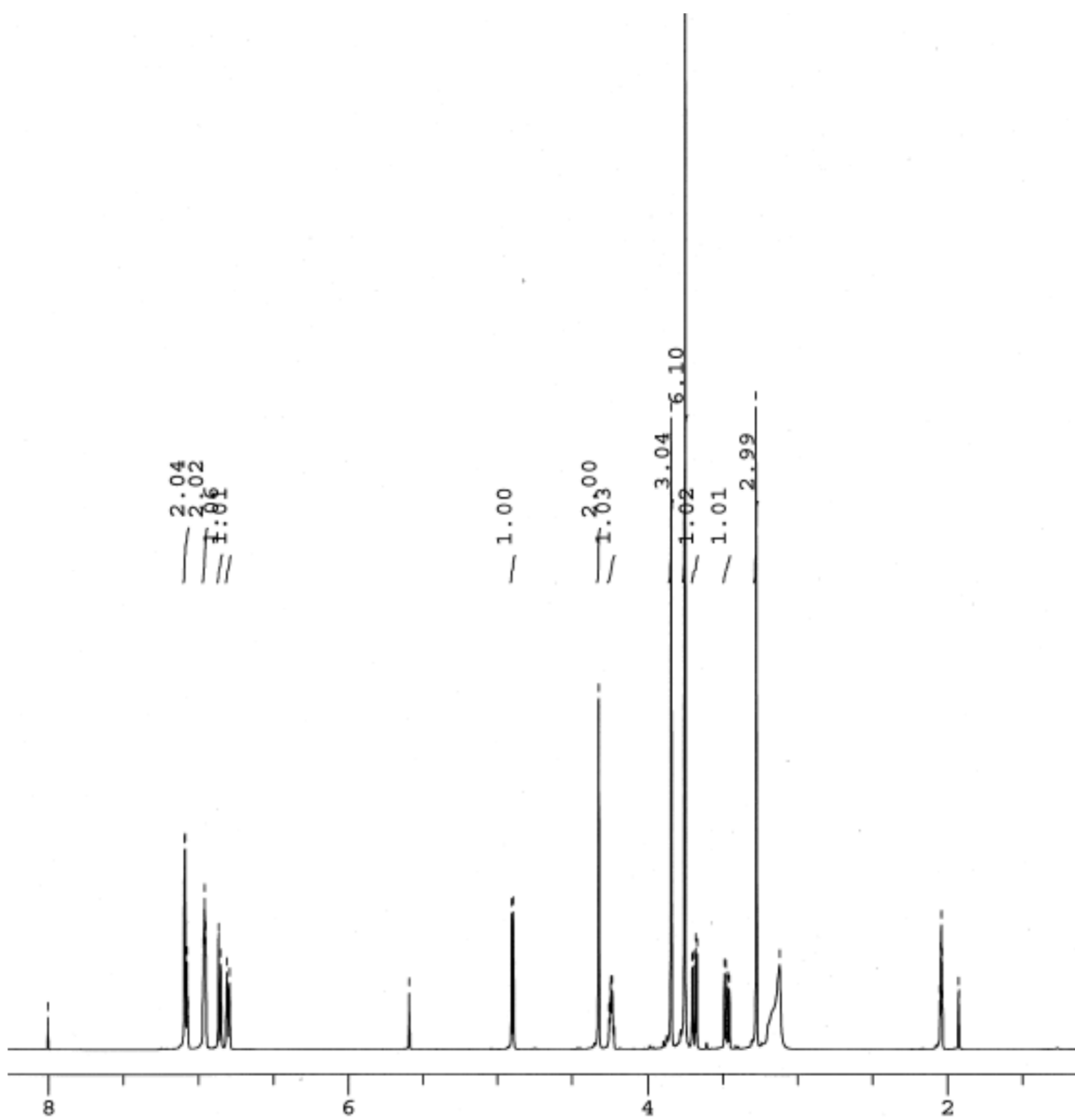
FIGURE 2-10. ^{1}H-NMR spectrum of model compound *3T*

2.3.2 Methods

Alkaline Oxygen Treatment

300 mL of a reaction solution including 0.50 mol/L sodium hydroxide (NaOH), 0.36 mmol/L ferric chloride, 10 mmol/L of co-existing phenolic compound (TMPh or Valc), and 1.0 mmol/L of lignin model compound (*1E*, *1T*, *2E*, *2T*, *3E*, or *3T*) was prepared. It should be noted that only compound *1E* and *1T* was reacted under the TMPh condition. Moreover, a 0.10 mol/L sodium hydroxide (NaOH) was employed as a lower alkaline concentration conditions in some runs of compound *1* and *2T* under Valc system. After the preparation, the reaction solution then transferred into Teflon-coated stainless steel vessel (500 mL, Taiatsu Techno$^®$ Co., Tokyo, Japan), and adjusted oxygen pressure of 1.1 MPa (1.0 MPa as the gauge level). Low oxygen pressure (0.4 MPa or 0.3 MPa as the gauge level) was employed in some runs under TMPh system. The vessel then heated to 95℃ for 10 min and maintained for 360 min while stirring. The reaction time was defined as zero when the temperature reached 95℃. At prescribed reaction times, a portion of the reaction solution was withdrawn for quantification. Each kind of reaction was run three times to confirm the reproducibility.

Quantification of Lignin Model and Phenolic Compounds

The withdrawn reaction solution was acidified with acetic acid. Then, 4-chlorophenol solution as an internal standard compound was added into the reaction solution. After filtration, the solution mixture was injected into HPLC (LC-10A, Shimadzu Co., Kyoto, Japan) equipped with an SPD-M10A detector (280 nm, Shimadzu Co.) to quantify the residual lignin model compound and Valc on the basis of a calibration line prepared for each compound.

The conditions of HPLC were as follows. Column: Luna 5u C18 (2) 100A (150 mm x 4.6 mm, Phenomenex Inc., Torrance, CA, USA); oven temperature: 40℃; flow rate: 1.0 mL/min; solvent system for the analysis of compound *1E* in the reaction with TMPh: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 40/60 for 27.5 min and maintained for 20 min, gradient to 15/85 immediately and maintained for 5 min, total time 60 min; solvent system for the analysis of compound *1T* in the reaction with TMPh: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 35/65 for 27.5 min and maintained for 20 min, gradient to 15/85 immediately and maintained for 5 min, total time 60 min; solvent system for the analysis of compound *1E*, *1T*, or *3T* in the reaction with Valc: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 44/56 for 27.5 min and maintained for 10 min, gradient to 15/85 immediately and maintained for 5 min, total time 50 min; solvent system for the analysis of compound *2E* or *2T*: gradient CH_3OH/H_2O (v/v) from 15/85 to 30/70 for 35 min and maintained for 15 min, gradient to 15/85 immediately and maintained for 5 min, total time 55 min; solvent system for the analysis of compound *3E*: gradient CH_3OH/H_2O (v/v) from 15/85 to 35/65 for 35 min and maintained for 5 min, gradient to 15/85 immediately

and maintained for 10 min, total time 50 min.

For TMPh quantification, the withdrawn reaction solution was immediately cooled also acidified with acetic acid and extracted with dichloromethane containing another internal standard compound, 2, 3, 6-trimethylphenol. The extracted water layer was further extracted with dichloromethane twice. The combined organic layer was dried over anhydrous sodium sulfate, and subsequently injected into GC.

The GC analyses were performed on a GC-17A instrument (Shimadzu Co., Ltd., Kyoto, Japan) equipped with a flame ionization detector using He as the carrier gas. The temperatures of the injector and detector were 270℃ and 280℃, respectively. The separations were achieved on a capillary column of IC-1 (30 m x 0.25 mm x 0.25 μm, GL Sciences Inc., Tokyo, Japan). The temperature program was from 100℃ to 140℃ at a rate of 4℃/min with an initial time delay of 5 min.

2.4 Results and Discussion

2.4.1 Reaction system description

Disappearance of the co-existing phenolic compound

According to the previous study, the results was confirmed that the non-phenolic β-O-4 lignin model compound do not directly degraded by the oxygen but the active oxygen species (AOS) generated under the oxygen treatment. Under the reaction employed, these AOS are generated *in situ* by the reaction between oxygen and co-existing phenolic compound. It was shown that no clear degradation of lignin model compound was observed under oxygen alkaline treatment without presence of co-existing phenolic compound. [17] In this study, two different phenolic compound were used under this oxygen alkaline treatment including 4-hydroxy-3-methoxybenzyl alcohol (vanillyl alcohol, Valc) and 2, 4, 6-trimethylphenol (TMPh) (abbreviated as TMPh or Valc system, respectively).   Thus, the addition of the Valc and TMPh into the reaction system faithfully simulates the oxygen delignification process. Under the reaction condition employed in this study, two different co-existing phenolic compound (TMPh or Valc) showed different disappearance time from the system with 45 or 60 minutes, respectively, in any runs under the reaction condition where the oxygen pressure was adjusted at 1.1 MPa. Otherwise, longer the reaction time were used for the TMPh to disappear from the reaction system under lower oxygen pressure system (0.4 MPa) at the reaction time 120 minutes. This disappearance time were mentioned to observe the difference in the reactivity before and after the disappearance of the co-existing phenolic compound from the reaction system. The results showed that the degradation of the lignin model

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compound (Compound **1**, **2**, or **3**) was severe at the beginning of the reaction and became moderate after the disappearance of the phenolic compound for both TMPh and Valc system. This phenomena were always observed not only the treatment with non-phenolic lignin model compound but also various carbohydrate model compounds in several previous studies. [17-25] The possible explanation is that the AOS are readily generated before the disappearance of the phenolic compound (TMPh or Valc). [20, 21] On the other hand, various kinds of AOS are generated by the propagation of the chain-type reactions after the disappearance of the phenolic compound (TMPh or Valc) which will also further degraded both lignin model compound (compound **1**, **2**, or **3**) as well as degradation products generated. [20, 21]

Addition of Ferric chloride

There are two reasons for the addition of the ferric chloride ($FeCl_3$) into the reaction system in all runs under this employed reaction. Firstly, $FeCl_3$ will slightly enhance the generation of AOS. Moreover, it also overshadowed the effect of other metals possibly present in reaction system. By the way, under the employed conditions, almost all of the Fe elements formed precipitates and/or aggregated as oxides and/or hydroxides. Due to the our previous study, the results showed that, under the condition using the same NaOH and $FeCl_3$ concentration under nitrogen atmosphere, Fe^{3+} could directly oxidize some phenol derivatives where the degradation of TMPh was not detectable when treated as a sole organic compound. [20] As a result, it can confirmed that the precipitates and/or aggregates consisting of Fe^{3+} do not directly oxidize phenolic compound (TMPh or Valc).

Generation of the AOS

As mentioned above, under the condition employed in this study, the AOS are generated *in situ* by the reaction between oxygen and co-existing phenolic compound which should be operative before the disappearance of the phenolic compound. Among several possible AOS generated in the system, oxyl anion radical ($O^{\cdot-}$) and an alkoxyl radical (R-O$\cdot$) are the AOS that degrade compound *1*, *2*, or *3* as shown in Figure 2-11. Therefore, another possible AOS that has potential to degrade compound *1*, *2*, or *3* is a peroxyl radical (R-O-O$\cdot$), although this peroxyl radical is commonly weaker oxidant than oxyl anion radical and alkoxyl radical. The oxidation ability of both oxyl anion radical as well as peroxyl radical was reported in our previous study. [25] Thus, several researches were focused on whether or not a Phenoxyl radical reacts with oxygen to be a peroxyl radical. [24-30] Although, some studies suggest that the occurrence of this reaction was being denied. [26-30] Due to the results from our previous report, it was suggested that the phenoxyl radical that derived from TMPh can reacted with oxygen to converted into peroxyl radical while the phenoxyl radical from Valc system cannot. [24, 25] It was suggested that oxyl anion radical considered to play the most important role in the Valc system while the peroxyl radical radicals are also the main AOS in the TMPh system before the disappearance of the phenolic compound. [24, 25] By the way, after the disappearance, there were no other specific species can be described as AOS, although oxyl anion radical should still work as an AOS.
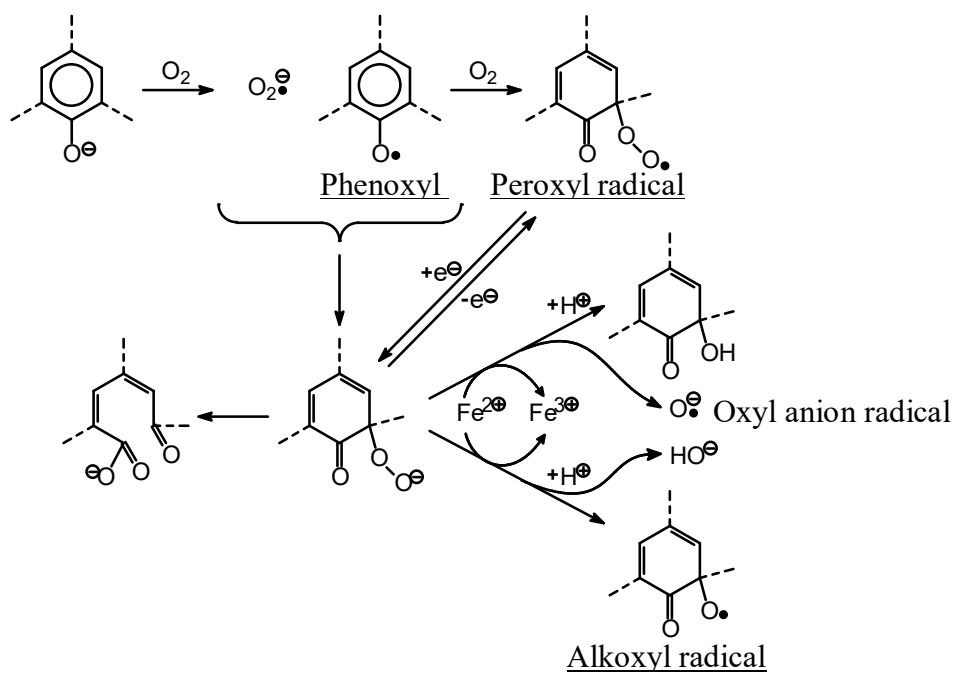
FIGURE 2-11. Possible reaction scheme for a phenolic compound under reaction conditions employed in this study

2.4.2 Results

Degradation of Compound *1* in the TMPh system

As shown in Figure 2-12, it shows the degradation of the compound *1E* and *1T*, when each isomer was individually treated in the TMPh system using 0.5 mol/L NaOH solution under oxygen pressure 1.1 MPa. In this study, the stereo-preferential degradation activities were observed before the disappearance of the phenolic compound (45 minutes in case of 1.1MPa TMPh system). As the results, the *E* isomers was degraded more than *T* isomer. By the way, after the disappearance of the TMPh, the degradation difference between *E* and *T* isomer gradually smaller suggesting the slightly stereo-preferential degradation of *T* isomer. This results can be attributed to the fact that the degradation of TMPh accompanying the profile of AOS generated. The results from our previous report are also confirmed this fact when the reaction between TMPh and oxygen under oxygen treatment accompanying the AOS generation before the disappearance of TMPh. On the other hand, the chain type reaction of lignin model compound, degradation product, and oxygen after the disappearance of TMPh resulting in the AOS profiles changed into radical. [20] Moreover, according to the previous studies, we can suggested that the oxyl anion radical considered as the main AOS generated before the disappearance of the TMPh. [24, 25]

In this study, we also investigate the effect of the oxygen pressure on the stereo-preferential degradation. As shown in Figure 2-13, it shows the yield of the degradation of compound *1E* and *1T* after individually treated under TMPh system using low oxygen pressure condition (0.4 MPa). The degradation before the disappearance of TMPh showed that *T* isomer was degraded more than the corresponding *E* isomer in which it reversed

when comparing to the high oxygen pressure condition. This results showed the possibility of the different AOS profile generated between low and high oxygen pressure condition. Moreover, the difference in the degradation between two isomer becomes larger which resulting in the stereo-preferential degradation of T isomer after the disappearance of TMPh.

FIGURE 2-12. Change in the yield of compound *1E* or *1T* when each compound was individually treated in the TMPh system using 0.5 mol/L NaOH under an oxygen pressure of 1.1 MPa. Each compound was run three times.

FIGURE 2-13. Change in the yield of compound *1E* or *1T* when each compound was individually treated in the TMPh system using 0.5 mol/L NaOH under an oxygen pressure of 0.4 MPa. Each compound was run three times.

Degradation of Compound *1* in the Valc system

Figure 2-14 shows the degradation of the compound *1E* or *1T* when it individually treated under Valc system using 0.5mol/L NaOH with oxygen pressure 1.1 MPa. As a result, the *T* isomer was degraded more than the corresponding *E* isomer where the difference between two isomer was smaller than in TMPh system. The results obtained from Valc system can support the suggestion in our previous studies about the different in AOS profile generated in TMPh and Valc system before the disappearance of TMPh and Valc. It was suggested that the main AOS in Valc system is oxyl anion radical where some other major AOS (presumably peroxyl radicals) are also generated in the TMPh system. [24, 25]

Therefore, under Valc system, low alkaline concentration conditions (0.1 mol/L NaOH) was after done in order to observe the effect on the stereo-preferential degradation. The stereo-preferential degradation of the *E* isomer was observed as shown in Figure 2-15. This reverse stereo-preference was effected by decreasing of the alkaline concentration in the Valc system. The possible explanation for this result could be the change in the alkaline concentration in Valc system varies the profile of the AOS generated. By the way, the change in the alkaline concentration should not affect largely on the generation of AOS where the oxyl anion radical still considered to be the main AOS in both high and low alkaline system (0.5 and 0.1 mol/L NaOH) which also suggested in our previous report. [24, 25] Therefore, the degradation of the model compound was greater in 0.5 mol/L than in 0.1 mol/L NaOH. This result can suggested that the amount of the AOS generated should be larger in 0.5 mol/L system than in 0.1 mol/L system.
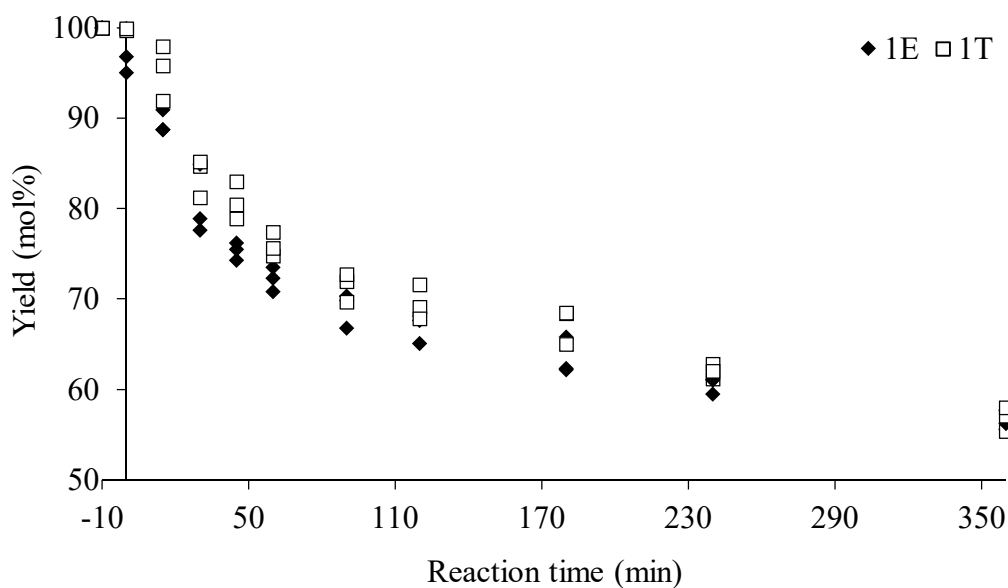
FIGURE 2-14. Change in the yield of compound *1E* or *1T* when each compound was individually treated in the Valc system using 0.5 mol/L NaOH under an oxygen pressure of 1.1 MPa. Each compound was run three times.
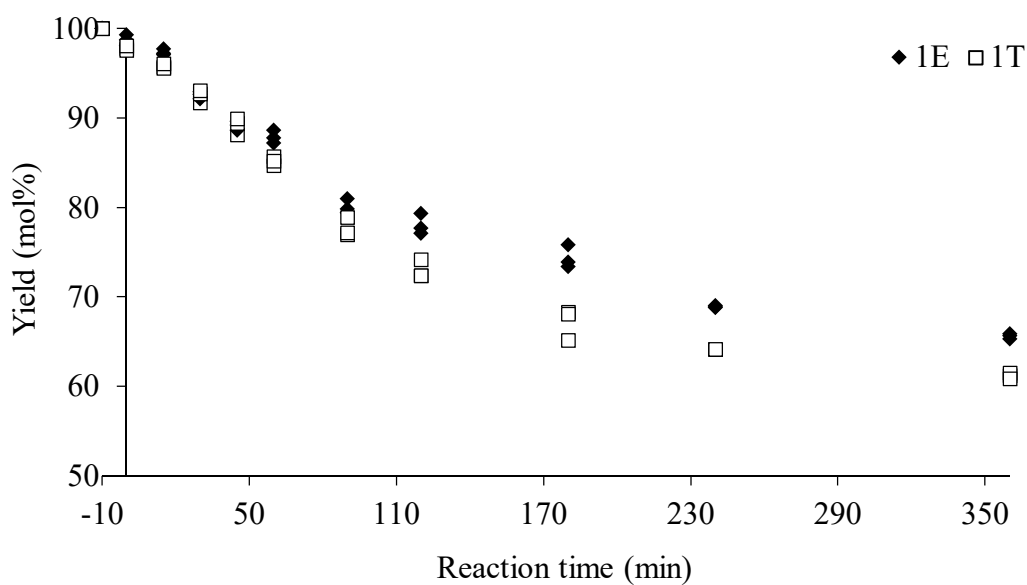

FIGURE 2-15. Change in the yield of compound *1E* or *1T* when each compound was individually treated in the Valc system using 0.1 mol/L NaOH under an oxygen pressure of 1.1 MPa. Each compound was run three times.

<u>Degradation of Compound *2* and *3* in the Valc system</u>

Compound *2* and *3* was employed in this study to observed structural effect of the model compound on the stereo-preferential degradation.

■ Compound *2*

Before the disappearance of Valc, the *E* isomer was degraded slightly more than the corresponding *T* isomer in the degradation of compound *2* using 0.5 mol/L NaOH and 1.1 MPa oxygen pressure. On the other hand, the clear reverse tendency was observed after the disappearance which may cause from the different in AOS profile generated in the system before and after disappearance of co-existing phenolic compound. (Figure 2-16) By the way, when considering the stereo-preference before the disappearance, the results clearly confirmed the degradation difference between compound *1* and *2* which indicate the dependence of the stereo-preference on the structure of model compound. The dependence of the stereo-preference on the change in alkaline concentration was also observed using model compound *2*. In this system using 0.1 mol/L NaOH, the degradation of *E* isomer was clearly greater than the corresponding *T* isomer. (Figure 2-17) This result obtained was the same tendency as compound *1* under the same condition employed. The difference in the degradation between *E* and *T* gradually decreased after the disappearance of Valc. Moreover, the degradation of compound *2* for both *E* and *T* isomer was greater in the reaction using 0.5 mol/L than in 0.1 mol/L NaOH condition. This tendency can be explain by the larger amount of AOS generated in the system.
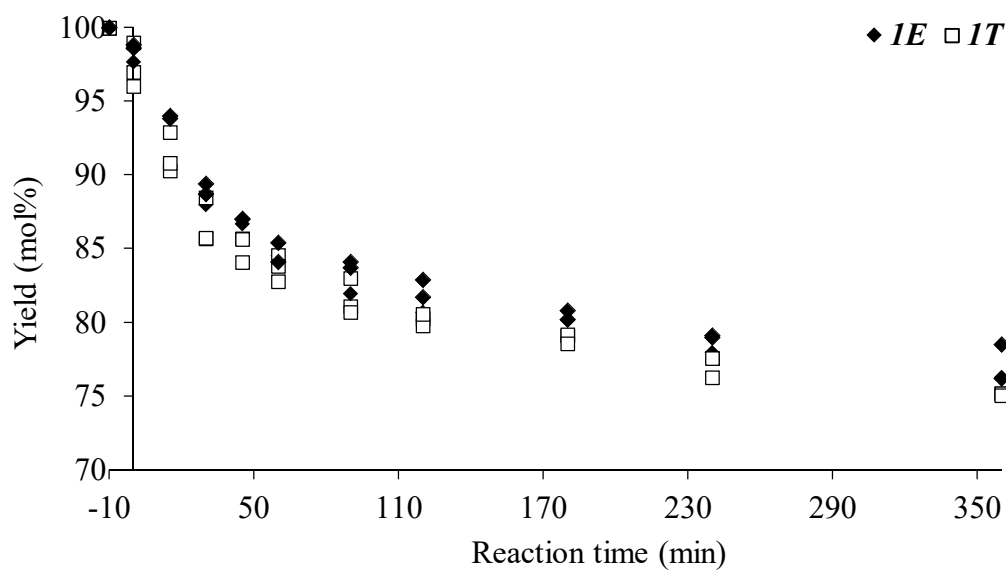
FIGURE 2-16. Change in the yield of compound *2E* or *2T* when each compound was individually treated in the Valc system using 0.5 mol/L NaOH under an oxygen pressure of 1.1 MPa. Each compound was run three times.
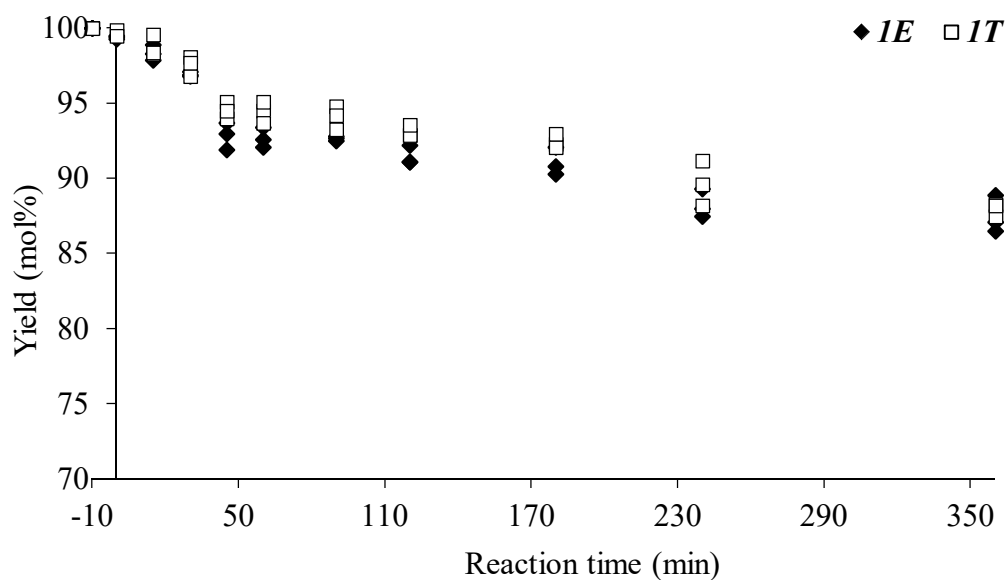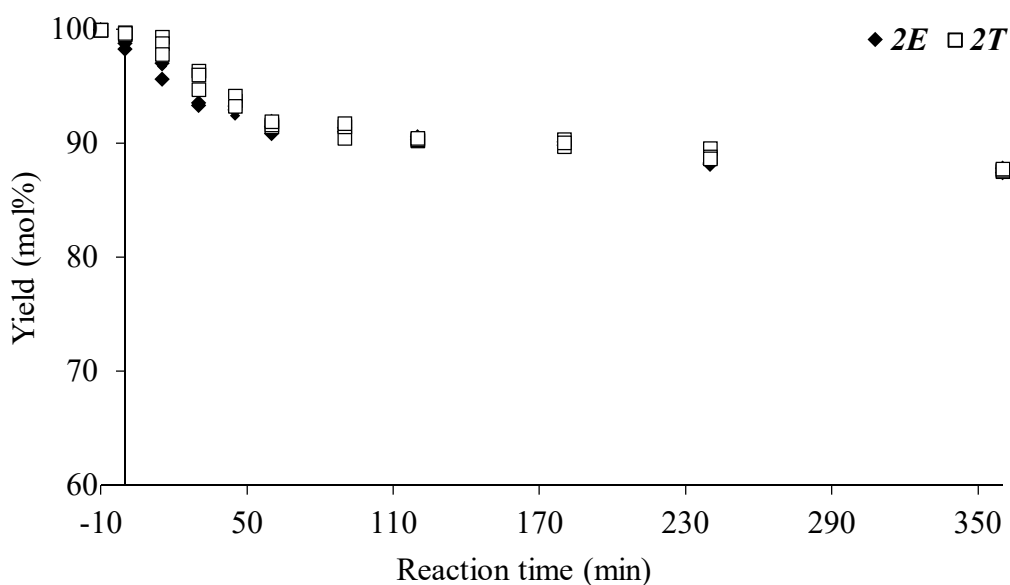

FIGURE 2-17. Change in the yield of compound *2E* or *2T* when each compound was individually treated in the Valc system using 0.1 mol/L NaOH under an oxygen pressure of 1.1 MPa. Each compound was run three times.

■ Compound *3*

The effect of the structural difference on the stereo-preferential degradation was further observed using compound *3*. The same reaction conditions was employed where 0.5 mol/L NaOH were used to treat the compound at pressure 1.1 MPa. As shown in Figure 2-18, the degradation of *T* isomer was greater than the corresponding *E* isomer before the disappearance of Valc. This tendency was clearly different with compound *2* (Figure 2-16) but the same as compound *1* (Figure 2-14) under the same condition using 0.5 mol/L NaOH at 1.1 MPa. Moreover, the stereo-preference was still *T* isomer after the disappearance of Valc.

By the way, it is not plausible that oxyl anion radical, as the main AOS in Valc system, spontaneously varies its reactivity toward the stereo-structurally difference. It should be noted that another mechanism might be operative and control the stereo-preference of AOS in this employed system.

Due to the results obtained, the differences in the degradation reactivity between those *E* and *T* isomers were small. However, these differences were certainly exists confirmed by three duplicated runs for each condition. Generally, the structure difference between the *E* and *T* isomers were small compare with between lignin and carbohydrate. By the way, the differences between these two isomers may not actually small when treated under oxygen treatment where highly reactive AOS were generated and played an important role in delignification processes
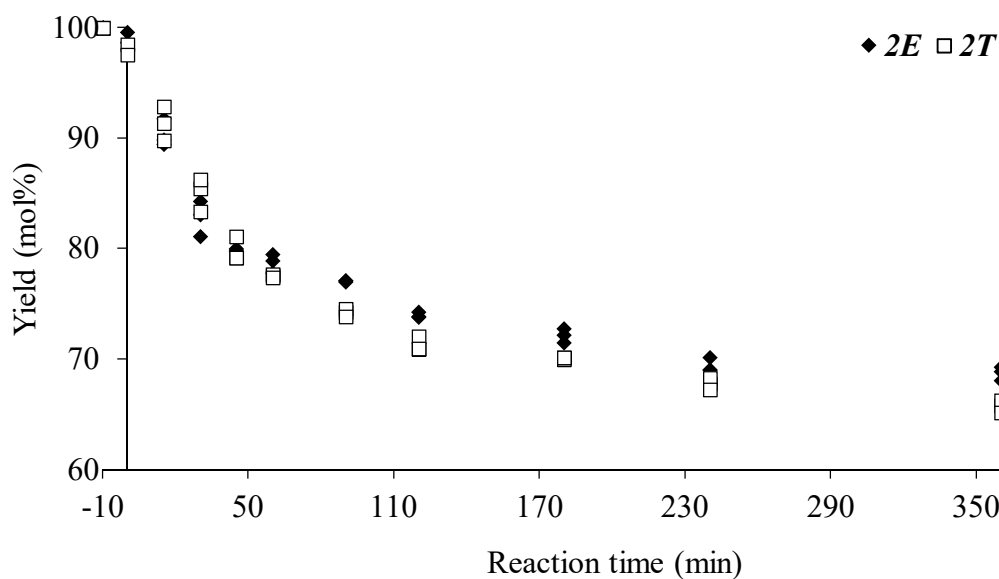
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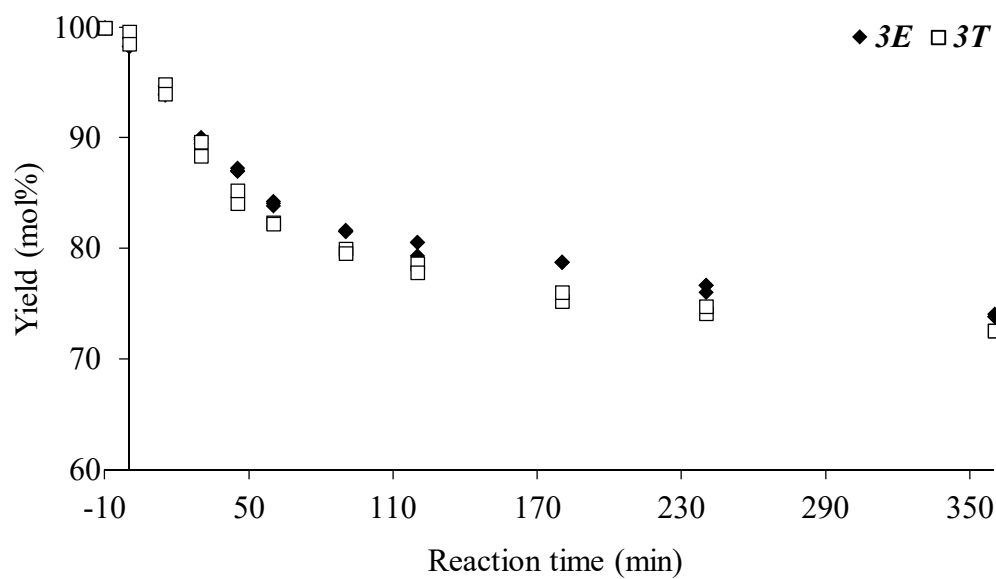
FIGURE 2-18. Change in the yield of compound *3E* or *3T* when each compound was individually treated in the Valc system using 0.5 mol/L NaOH under an oxygen pressure of 1.1 MPa. Each compound was run three times.

TABLE 2-1: Summary of the stereo-preferences observed in this study.

Compound	Reaction System	Oxygen pressure (MPa)	NaOH conc. (mol/L)	Stereo-preference	
				Before[a]	**After**[b]
1	TMPh	1.1	0.5	*E*	*T*
		0.4		*T*	*T*
	Valc	1.1	0.5	*T*	*T*
			0.1	*E*	*T*
2	Valc	1.1	0.5	*E*[c]	*T*
			0.1	*E*	*T*
3	Valc	1.1	0.5	*T*	*T*

[a] Before the disappearance of TMPh or Valc.
[b] After the disappearance of TMPh or Valc.
[c] The stereo-preferences are not very clear.

Degree of the degradation comparison

Under the same reaction conditions using 0.5 mol/L NaOH at 1.1 MPa, the degradation of compound *1* was greater in TMPh system comparing with Valc system in both before and after the disappearance of the co-existing phenolic compound. This result can be explained by the difference in the AOS profile between these two systems. Higher reactivity of generated AOS should be obtained in the reaction in TMPh system compared with Valc system. However, there are also several possible reasons that can be used to explain this observation. For example, the possibility that the amount of the AOS generated in the TMPh system might be larger than in the Valc system. In our previous study, the carbohydrate model compound was also treated under both TMPh and Valc system. The results showed that the degradation in TMPh system was the same or less than that in the Valc system before the disappearance while after the disappearance the degradation of carbohydrate model compound in TMPh system was greater than in Valc system. [22] This result suggested the different in the reactivity of AOS toward the carbohydrate and lignin model compound before the disappearance of the TMPh or Valc. On the other hand, after the disappearance of TMPh or Valc, the AOS reactivity toward the carbohydrate model compound should not be different from the compound *1*.

Under Valc system using 0.5 mol/L NaOH at 1.1 MPa, the degree of the degradation of compound *1*, *2*, or *3* were in the order *2> 3> 1* in the reaction of either *E* or *T* isomer. This result suggested the presence of the benzyl hydroxymethyl or methoxymethyl group in compound *2* and *3*, respectively, enhances the reaction with AOS in the condition employed.

2.4.3 Discussion

There are two suggestions from the previous studies can be used to discuss the observed stereo-preference obtained in this study. [19, 20, 24, 25]

Suggestion 1:   Under Valc system, the main AOS is negatively charged oxyl anion radical while not only oxyl anion radical but also neutral peroxyl radical play an important role under TMPh system at high oxygen pressure condition (1.1 MPa).

Suggestion 2:   Negatively charged oxyl anion radical prefer to attack the lignin model compound at side-chain rather than the aromatic nuclei

The following three hypotheses were also used to further explain the observed results.

Hypothesis 1:   The high acidity of the α–hydroxy group of *1E* resulting in the dissociation in the system using 0.5mol/L NaOH which is not the case for compound *1T*. After the dissociation, the negatively charged α– alkoxide was generated at the side-chain of the model compound.
By the way, this effect is not very significant in low alkaline concentration (0.1 mol/L NaOH) system. This hypothesis has already been introduced in previous report. [31]

Hypothesis 2:   For compound *2*, the dissociation mainly occurs at benzyl hydroxy group due to higher acidity of benzyl hydroxy group compare with the α–hydroxy group in the side-chain. Moreover, the dissociation at the side-chain of compound *2* is also being suppressed.

Hypothesis 3:   The acidity of α–hydroxy group of compound *1E* and *3E* is similar because no benzyl hydroxymethyl is exist.

According to the result, the stereo-preference of *E* isomer was shown before the disappearance of TMPh under high oxygen pressure (1.1 MPa). Under this condition, the not only negatively charged oxyl anion radical but also neutral peroxyl radical considered to play an important role in the system. In this case, if the peroxyl radical has an effect on the stereo-preference cause the *E* isomer to degraded greater than the corresponding *T* isomer, the tendency should be changed under low oxygen pressure condition (0.4 MPa) where negatively charged oxyl anion radical is the main AOS. Under 0.4 MPa condition, the neutral peroxyl radical, derived from the combination reaction between oxygen and phenoxyl radical, should not be presence due to the pressure not high enough to promote the reaction. As the result obtained, the reverse tendency was observed under low pressure condition. This results suggested the mechanism of the presence of the electrostatic-repulsion between two negatively charged oxyl anion radical and dissociated side-chain of *E* isomer.

Due to the Valc system, the stereo-preferential degradation of *T* isomer was observed under high alkaline condition (0.5 mol/L NaOH) for model compound *1* and *3*. As mentioned above, the main AOS generated in this system is negatively charged oxyl anion radical which preferably attack the compound at the side-chain rather than aromatic

position. Under this employed condition, the side-chain of compound *1E* and *3E* was dissociated generate the negatively charged α–alkoxide. Thus, the presence of electrostatic-repulsion further suppressed the degradation resulting in the stereo-preferential degradation of *T* isomer. By the way, under Valc system using 0.1 mol/L NaOH, the electrostatic-repulsion is not strong enough to be accompanied by the stereo-preferential degradation of compound *1E*.

In case of model compound *2*, the α–hydroxy group at the side-chain does not significantly dissociate owing to the presence of the benzyl hydroxymethyl group. Thus, the electrostatic-repulsion in compound *2E* should not strong enough resulting in the slightly stereo-preferential degradation of *E* over *T* isomer. Moreover, under low alkaline condition where the dissociation of the α–hydroxy group is further suppressed, the stereo-preferential degradation became more clear where *E* isomer degraded greater than corresponding *T* isomer. The schematic description of electrostatic-repulsion described is shown in Figure 2-19.
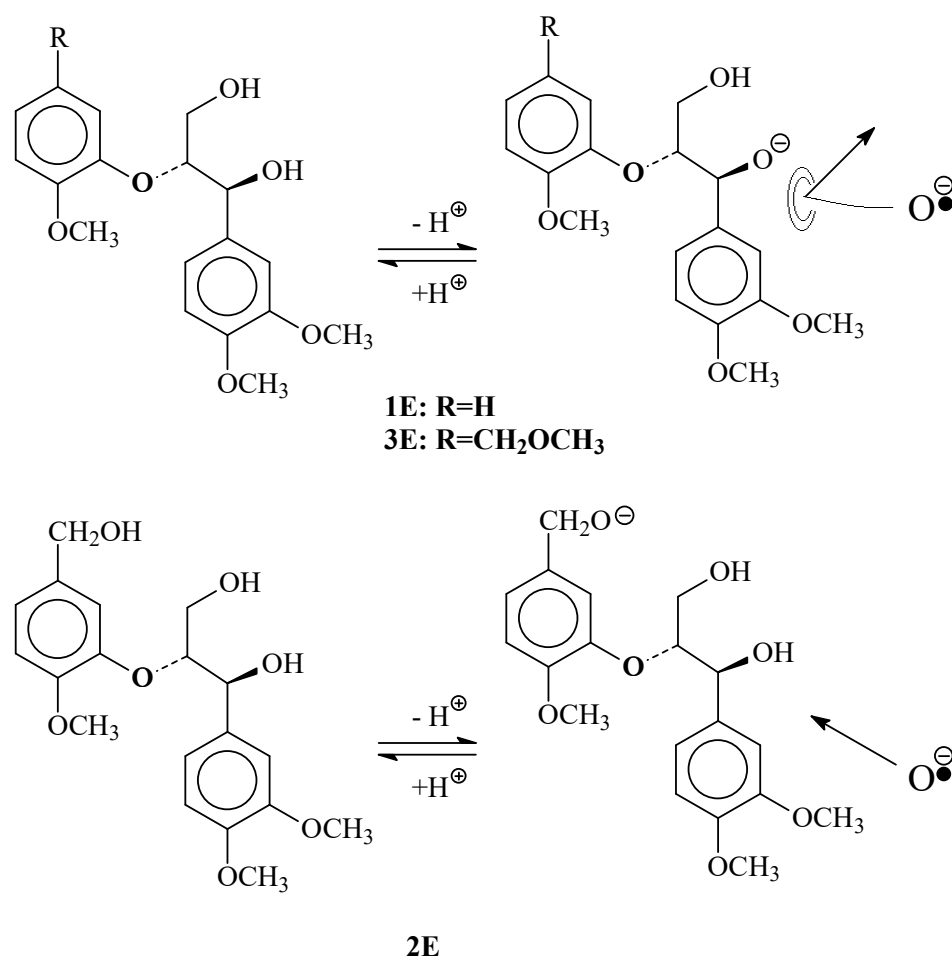
FIGURE 2-19. Schematic description of the hypothetic reaction of the negatively charged oxyl anion radical with the *E* isomers of compound *1*, *2*, or *3*.

2.5 Conclusion

According to the observed stereo-preference, higher acidity of the α–hydroxy group at the side chain of *1E* compared to *1T* resulting in the dissociation occurs at the side-chain. The negatively charged α–alkoxide which presence after the dissociation can promote the generation of electrostatic-repulsion with negatively charged oxyl anion radical which also caused to suppressed the degradation of *1E*. As a result, *T* isomer degraded greater than *E* isomer where the same tendency also observed in the reaction with compound *3*. In case of compound *2*, the benzyl hydroxy group considered to be the dissociated rather than at the side chain position due to its high acidity. Thus, the electrostatic-repulsion in compound *2* should be weak caused *E* isomer slightly degraded greater than the corresponding *T* isomer. The neutral peroxy radical in high pressure TMPh system attack directly at the side-chain of compound *1E* resulting in stereo-preferential degradation of *E* isomer.

All the results obtained in this study confirmed that various factors including structure of the lignin model compound as well as the reaction conditions has an effect on the stereo-preferential degradation in the reaction of AOS under oxygen treatment.

References

[1] Miksche, G. E. (1972). Zum alkalischen abbau der *p*-alkoxy-arylglycerin-*β*-arylätherstrukturen des lignins: Versuche mit erythro-veratrylglycerin-*β*-guajacyläther. Acta Chem. Scand., *26*(8), 3275-3281.

[2] Tsutsumi, Y.; Kondo, R.; Imamura, H. (1993). Reaction of syringylglycerol-*β*-syringyl ether type of lignin model compounds in alkaline-medium. J. Wood Chem. Technol., *13*(1), 25-42.

[3] Criss, D. L.; Elder, T.; Fisher, T. H.; Schultz, T. P. (2002). Effect of the *α*-and *γ*-hydroxyls on the alkaline hydrolysis rate of nonphenolic *β-O*-4 lignin diastereomers. Holzforschung, *56*(1), 67-72.

[4] Shimizu, S.; Yokoyama, T.; Akiyama, T.; Matsumoto, Y. (2012). Reactivity of lignin with different composition of aromatic syringyl/guaiacyl structures and *erythro*/*threo* side chain structures in *β-O*-4 type during alkaline delignification: As a basis for the different degradability of hardwood and softwood lignin. J. Agric. Food Chem., *60*(26), 6471-6476.

[5] Shimizu, S.; Posoknistakul, P.; Yokoyama, T.; Matsumoto, Y. (2013). Quantitative difference in the rates of the *β-O*-4 bond cleavage between lignin model compounds with and without *γ*-hydroxymethyl groups during the alkaline pulping process. BioResources, *8*(3), 4312-4322.

[6] Jonsson, L.; Karlsson, O.; Lundquist, K.; Nyman, P. O. (1990). Stereospecificity in enzymic and non-enzymic oxidation of *β-O*-4 lignin model compounds. FEBS Lett., *276*(1&2), 45-48.

[7] Bohlin, C.; Andersson, P.-O.; Lundquist, K.; Jonsson, L. J. (2007). Differences in

stereo-preference in the oxidative degradation of diastereomers of the lignin model compound 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol with enzymic and non-enzymic oxidants. J. Mol. Catal., B Enzym., *45*(1&2), 21-26.

[8] Ljunggren, S.; Johansson, E. (1990). The kinetics of lignin reactions during oxygen bleaching. Part 2. The reactivity of 4,4′-dihydroxy-3,3′-dimethoxy-stilbene and*β*-aryl ether structures, *Nord. Pulp Pap. Res. J.*, 5(3), 148-154

[9] Ljunggren, S.; Johansson, E. (1990). The kinetics of lignin reactions during oxygen bleaching. Part 3. The reactivity of 4-n-propylguaiacol and 4,4′-di-n-propyl-6,6′-biguaiacol, *Holzforschung*, 44(4), 291-296

[10] Ljunggren, S. (1986). Kinetics aspects of some lignin reactions in oxygen bleaching. *J. Pulp Pap. Sci.,* 12 (2), J54-J57

[11] Johansson, E.; Ljunggren, S., (1994). The kinetics of lignin reaction during oxygen bleaching, Part 4. The reactivities of different lignin model compounds and the influence of metal ions on the rate of degradation, *J. Wood Chem. Technol.*, 507-525

[12] Adler, E.; Lindgren, B. O.; Saeden, U. (1952). The *β*-guaiacyl ether of *α*-veratrylglycerol as a lignin model. *Svensk Papperstidn.*, 55(7), 245-254.

[13] Ibrahim, W.; Lundquist, K. (1994). Synthesis of *erythro* and *threo* forms of lignin models of the arylglycerol *β*-guaiacyl ether type. *Acta Chem. Scand.*, 48(2), 149-151.

[14] Li, S.; Lundquist, K.; Soubbotin, N. (1994). Separation of diastereomers of lignin model compounds of the 1,3-diol type as borate complexes by ion-exchange chromatography. *Holzforschung*, 48(6), 509-511.

[15] Li, S.; Lundquist, K.; Nagnus, P. (1995). Synthesis of guaiacylglycerol *β*-guaiacyl ether. *Acta Chem. Scand.*, *49*(8), 623-624.

[16] Akiyama, T.; Sugimoto, T.; Matsumoto, Y.; Meshitsuka, G. (2002). *Erythro/threo* ratio of *β-O*-4 structures as an important structural characteristic of lignin. Part 1: Improvement of ozonation method for the quantitative analysis of lignin side-chain structure. *J. Wood Sci.*, 48(3), 210-215.

[17] Ohmura, S.; Yokoyama, T.; Matsumoto, Y. (2009). Progress of oxidation of non-phenolic lignin moiety in an oxygen bleaching process via the conversion of non-phenolic into phenolic lignin moiety. *J. Wood Sci.*, 58(3), 243-250

[18] Yokoyama, T.; Matsumoto Y.; Yasumoto, M.; Meshitsuka, G. (1996). The role of peroxide species in carbohydrate degradation during oxygen bleaching. Part II: Effect of oxygen pressure on the degradation of lignin and carbohydrate model compounds and on the reaction selectivity. *J. Pulp Pap. Sci.*, 55(5), J151-J154.

[19] Yokoyama, T.; Matsumoto, Y.; Meshitsuka, G. (1999). Reaction selectivity of active oxygen species in alkaline oxygen bleaching. *J. Wood Chem. Technol.*, 19(3), 187-202.

[20] Yokoyama, T.; Matsumoto Y.; Meshitsuka, G. (2005). Characterization of active oxygen species under alkaline oxygen bleaching conditions. *Holzforschung*, 59(3), 269-275.

[21] Konishi, F.; Yokoyama, T.; Matsumoto Y. (2009). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions. Part 1: Paper on the anomeric position. *Holzforschung*, 63(1), 52-60.

[22] Yokoyama, T.; Nakagawa, A.; Konishi, F.; Matsumoto, Y. (2011). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions III: Effects of the origin of active oxygen species. *J. Wood*

Sci., 57(6), 512-519.

[23] Nakagawa, A; Yokoyama, T.; Matsumoto Y. (2012). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions. Part 2: Study on the C-2 position. *J. Wood Chem. Technol.*, 32(1), 10-22.

[24] Nakagawa, A.; Yokoyama, T.; Matsumoto, Y. (2012). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions IV: Appearance of kinetic isotope effect in the reaction between methyl glucoside and deuterated methyl glucoside. *J. Wood Sci.*, *58*(6), 563-569.

[25] Yokoyama, T.; Nakagawa, A.; Matsumoto, Y. (2015). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions. Part 5: Comprehensive results on experiments using deuterium labeled methyl glucosides. *J. Wood Chem. Technol.*, 35 (6), 450-463

[26] Gierer, J.; Yang, E.; Reitberger, T. (1992). The reactions of hydroxyl radicals with aromatic rings in lignins, studied with creosol and 4-methylveratrol. *Holzforschung*, 46(6), 495-504.

[27] Gierer, J.; Yang, E.; Reitberger, T. (1994). On the significance of the superoxide radical ($O_2{}^{\bullet-}$/$HO_2{}^{\bullet}$) in oxidative delignification, studied with 4-*t*-butylsyringol and 4-*t*-butylguaiacol. *Holzforschung*, 48(5), 405-414.

[28] Gierer, J.; Yang, E.; Reitberger, T. (1996). The reactions of chromophores of the stilbene type with hydroxyl radical ($HO^{\bullet}$) and superoxide radical ($O_2{}^{\bullet-}$/$HO_2{}^{\bullet}$) part 1: The cleavage of the conjugated double bond. *Holzforschung*, 50(4), 342-352.22.

[29] Gierer, J.; Yang, E.; Reitberger, T. (1996). The reactions of chromophores of the stilbene type with hydroxyl radical (HO$\bullet$) and superoxide radical (O$_2{}^{\bar{\bullet}}$/HO$_2\bullet$) part 2: Reactions other than cleavage of the conjugated double bond. *Holzforschung*, 50(4), 353-359.

[30] Sugimoto, T.; Morishita, T.; Matsumoto, Y.; Meshitsuka, G. (2000). Effect of oxygen pressure on the oxidation of syringyl alcohol initiated by manganese (III) acetate. *Holzforschung*, 54(3), 262-268.

[31] Criss, D. L.; Elder, T.; Fisher, T. H.; Schultz, T. P. (2002). Effect of the α-and γ-hydroxyls on the alkaline hydrolysis rate of nonphenolic β-O-4 lignin diastereomers. *Holzforschung*, 56(1), 67-72.

Chapter 3

Stereo-preferential degradation of the *erythro* and *threo* isomers of *β-O*-4 type lignin model compounds under hydrogen peroxide bleaching conditions

3.1 Introduction

Concerning of environmental issues, the chlorine dioxide-based elemental chlorine free (ECF) as well as oxygen-based totally chlorine free (TCF) bleaching sequences received significant attention. The environmentally benign chemical bleaching reagents including ozone (O_3), oxygen (O_2) as well as hydrogen peroxide (H_2O_2) are being commercially used. Under oxygen bleaching treatment, the several oxygen species including superoxide anion radical ($O_2^-\bullet$), hydroperoxyl anion (HO_2^-), hydroxyl radical ($HO\bullet$) as well as peroxyl radical ($R\text{-}O\text{-}O\bullet$) generated *in situ* by the reaction between co-phenolic compound and molecular oxygen. These radical found to plays an important role in the delignification processes under oxygen alkaline treatment.

In decades, hydrogen peroxide has been used as bleaching agent for mechanical pulp where the hydrogen peroxide can eliminate chromophores from the lignin structure for brightening effect. By the way, in case of kraft pulp, the delignification is needed since not all chromophores can be eliminated. Under hydrogen peroxide treatment, the main species generated in the reaction are hydroxyl radicals ($HO\bullet$) and oxyl anion radical ($O^-\bullet$) which formed via the decomposition of hydrogen peroxide and metal-catalyzed decomposition mechanism. These species showed to be involved in the degradation of lignin.

Many studies have been observed the reactivity of delignification under both oxygen and hydrogen peroxide treatment. Otherwise, only few are deeply investigate the effect of the reactivity of the oxygen species on the lignin structure under those two treatments. Due to the complexity of oxygen species profile formed under oxygen treatment, hydrogen peroxide treatment condition was chosen in this study.

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3.2 Objectives of this work

Under hydrogen peroxide treatment, the degradation of compound *1*, *2*, and *3* was occur in the reaction with active oxygen species (AOS) generated by the decomposition of hydrogen peroxide in the system. The objectives of this work are shown in the following text.

1. To investigate the effect of the structural of model compound on the stereo-preferential degradation under hydrogen peroxide treatment. In this study, three different non-phenolic *β*-O-4 type lignin model compounds were used.

2. To observed the effect of pH on the stereo-preferential degradation. In this study, the initial pH of the reaction solution were vary from 13.3 to 9.5 where the pH variation was divided into two groups, relatively high initial pH conditions (initial pH 13.3, 12.8, or 11.9) and relatively low initial pH conditions (initial pH 11.5, 11.0, 10.5, or 9.5).

3. To investigate the stereo-preferential degradation of the non-phenolic *β*-O-4 type lignin model compounds under Fenton's reagent system.

3.3 Materials and methods

3.3.1 Materials

All purchased chemicals including semiconductor grades sodium hydroxide (NaOH), Ferric Chloride ($FeCl_3$), ferrous chloride ($FeCl_2$) as well as 30% Hydrogen peroxide solution (H_2O_2) were used without further purification. The Ultra purified water was used in this experiment. Under this study under the hydrogen peroxide treatment, three different lignin model compounds were used including 2-(2-methoxyphenoxy)-1-(3,4-dimethoxyphenyl)propane-1,3-diol (*1*), 2-(4-hydroxyme-

thyl-2-methoxy-phenoxy)-1-(3, 4-dimethoxyphenyl) propane-1,3-diol (*2*), and 2-(4-me-thoxymethyl-2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl)propane-1,3-diol (*3*). The synthesis method of these non-phenolic *β*-O-4 type lignin model compound were followed the steps as reported by Adler et al. [1] The detailed of the model compound synthesis was shown in chapter 2. The anion exchange chromatography was used in order to separate the two isomer (*E* and *T*) by converting them into boron complexes. [2-4] The purification was confirmed using the ozonation method by converting them into Erythronic or Threonic acid, as the degradation product from *E* or *T* isomer respectively. [5] The ^{1}H and ^{13}C NMR were used as the method to confirm the purification of the compound obtained where the data were shown in chapter 2.

3.3.2 Methods

Alkaline hydrogen peroxide treatment

Sodium hydroxide solution (various concentrations), 0.36 mmol/L of Ferric chloride ($FeCl_3$), and 1.0 mmol/L of lignin model compound (*1E*, *1T*, *2E*, *2T*, *3E*, or *3T*) was contained in the reaction solution. After preparation, the reaction solution then transferred into the Teflon vessel and heated to 95ºC. The 30% hydrogen peroxide solution was added in order to initiate the reaction. During the reaction, the 30% hydrogen peroxide solution was added into the vessel for ten times with constant interval of 10 minutes where the total amount added was approximately 1.8 mmol. A portion of the reaction solution was withdrawn at prescribed reaction times for further quantification of the residual lignin model compound and pH measurement. Each kind of the reaction was repeated three time to confirm the reproducibility. By the way, the reaction of compound *3* was runs only once in some reactions conditions.

Fenton's reagent treatment

A reaction solution contain 0.36 mmol/L of ferrous chloride ($FeCl_2$) and lignin model compound (*1E* or *1T*) was prepared without addition of alkali. To initiate the reaction, 1% of hydrogen peroxide solution was added into the vessel and stepwisely added every 10 minutes with total amount of hydrogen peroxide added 60 μmol. The reaction was run three times to confirm the reproducibility. By the way, under one time addition condition, compound *1E* was also treated with 200 μl of the 1% hydrogen peroxide solution which initially added at once at the beginning of the reaction was not repeated the reaction.

Quantification of residual lignin model compound

The withdrawn reaction solution was immediately cooled in a cold-water bath, and then neutralized with acetic acid. To the mixture was added an internal standard compound, 4-chlorophenol, as a methanol solution. After filtration, the resulting mixture was injected into HPLC (LC-10A, Shimadzu Co., Kyoto, Japan) equipped with an SPD-M10A detector (280 nm, Shimadzu Co.) to quantify the employed lignin model compound on the basis of a calibration line prepared for each of compound *1E*, *1T*, *2E*, *2T*, *3E*, or *3T*.

The conditions of HPLC were as follows. Column: Luna 5u C18 (2) 100A (150 mm x 4.6 mm, Phenomenex Inc., Torrance, CA, USA); oven temperature: 40℃; flow rate: 1.0 mL/min; solvent system for the analysis of compound *1E*, *1T*, or *3T* in the reaction: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 44/56 for 27.5 min and maintained for 10 min, gradient to 15/85 immediately and maintained for 5 min, total time 50 min; solvent system for the analysis of compound *2E* or *2T*: gradient CH_3OH/H_2O (v/v) from 15/85 to 30/70 for 35 min and maintained for 15 min, gradient to 15/85 immediately and maintained for 5 min, total time 55 min; solvent system for the analysis of compound *3E*: gradient CH_3OH/H_2O (v/v) from 15/85 to 35/65 for 35 min and maintained for 5 min, gradient to 15/85 immediately and maintained for 10 min, total time 50 min.

3.4 Results and Discussion

3.4.1 Reaction system description

In this study, each of the isomer of compound *1*, *2*, and *3* was individually treated under hydrogen peroxide treatment where the stereo-preferential degradation was observed. As the reference, the pair of the *E* or *T* isomer of each model compound was also treated under the same condition as individual addition conditions. An iodometric titration was used to confirm that there was no oxidation ability at the end of the reaction (reaction time 120 minutes) in all runs. The ferric ion was added as the chloride salt in all runs to overshadow the effect of other metals possibly present in the reaction system, to enhance the generation of radical species as well as to stimulate a hydrogen peroxide bleaching process. During the reaction, almost all the ferric ions as precipitated and aggregated as an oxide and hydroxides under the employed conditions with relatively high alkalinity (pH > 9-10).

Under hydrogen peroxide bleaching conditions, the H_2O_2 (pKa≈11.6) or HO^- (hydroperoxide anion, conjugate base of H_2O_2) decomposed followed by bimolecular and/or unimolecular mechanisms depending on the reaction conditions. The bimolecular decomposition of the H_2O_2 or HO^- is directly form without the generation of radical species. On the other hands, several radical species including $HO\bullet$ (hydroxyl radical, pKa≈11.9), $O\overline{\bullet}$ (oxyl anion radical), $O_2\overline{\bullet}$ (superoxide anion radical), and/or $HO_2\bullet$ (hydroperoxyl radical) are generated from unimolecular mechanism. Moreover, the presence of both Fe^{3+} and Fe^{2+} catalyze the decomposition mechanism. By the way, under the conditions employed in this study, the $HO_2\bullet$ (hydroperoxyl radical) is readily

dissociated to $O_2\overset{-}{\bullet}$ (superoxide anion radical) which has low oxidation ability to oxidize the model compound. As the result, the $HO\bullet$ (hydroxyl radical), $O\overset{-}{\bullet}$ (oxyl anion radical) are the most responsible for the degradation of the compound *1*, *2*, and *3* among generated radical species. In addition, the chain-type reaction might be propagated due to the reaction between the radical center in compound *1*, *2*, and *3* and O_2. Otherwise, this chain-type reaction do not efficiently propagate and consequently degrade the compound as minor mode because the reaction was run without the pressurized O_2. As the conclusion, the stereo-preferential degradation of compound *1*, *2*, and *3* should correspond mainly to the difference in the reactivity between $HO\bullet$ and $O\overset{-}{\bullet}$.

3.4.2 Results

Degradation of compound *1*, *2*, or *3* at relatively high initial pH condition

As shown in Fig. 3-1 to 3-9, it shows the degradation of the compound *1*, *2*, and 3 both *E* and *T* isomer at an initial pH of 13.3, 128, or 11.9 with the final pH of 13.3, 12.5 and 11.5, respectively. The decreasing of the pH can mainly be attributed the formation of acidic reaction products. At pH 13.3, the stereo-preference was *T* over *E* isomer in the degradation of compound *1* and *3*. By the way, the degradation of compound *2* showed no clear stereo-preference. In contrast, the stereo-preference of *E* over *T* isomer was observed in the degradation of any compound at an initial pH of 12.8 and 11.9 except the degradation of compound *3* at initial pH 11.9 which showed no stereo-preferential degradation. The results suggested that the degree of the stereo-preference was dependent on the structure of model compound employed. Moreover, the degree of the degradation of any compound became less with the decrease of the initial pH.

Degradation of compound *1* at relatively low initial pH condition

The stereo-preferential degradation of compound *1E* or *1T* was shown in Figure 3-10 to 3-13 with an initial pH of 11.5, 11.0, 10.5, or 9.5 and the final pH was 7.5, 6.0, 4.0, or 3.5, respectively. There was no clear stereo-preference observed between *E* and *T* isomers at any pH under these conditions. The degradation became clearly greater with the decrease of the initial pH.

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Degradation of compound *1* in Fenton's reagent

The degradation of compound *1E* or *1T* under Fenton's reagent is illustrated in Figure 3-14. Under the condition employed, the initial and final pH were 5.5 and 3.1, respectively. According to the observed result, there was no clear stereo-preference between *E* and *T* isomers, which is in accordance with the result in previous report. [6] The amount of H_2O_2 used was 1/30 of that in the common reaction, because compound *1E* or *1T* already disappeared at a reaction time of 10 min after using the same amount of H_2O_2 as in the common reaction. This results can attributed to the difference in the H_2O_2 decomposition mechanism between Fenton's and common reaction systems. Moreover, based on the results obtained, the generated $HO \bullet$ and $HO_2 \bullet$ quite efficiently oxidized the compound rather than H_2O_2. Because the compound *1E* was remained after the reaction using the same amount of H_2O_2 initially added at once (the '×' marks in Figure 3-14), it is at least certain that the oxidation power of H_2O_2 is efficiently utilized to oxidize the compound in the stepwisely reaction.

FIGURE 3-1. Change in the yield of compound *1E* or *1T* when each compound was individually treated at the initial pH of 13.3. Each compound was run three times.

FIGURE 3-2. Change in the yield of compound *1E* or *1T* when each compound was individually treated at the initial pH of 12.8. Each compound was run three times.

FIGURE 3-3.   Change in the yield of compound *1E* or *1T* when each compound was individually treated at the initial pH of 11.9. Each compound was run three times.

FIGURE 3-4.   Change in the yield of compound *2E* or *2T* when each compound was individually treated at the initial pH of 13.3. Each compound was run three times.

FIGURE 3-5.   Change in the yield of compound *2E* or *2T* when each compound was individually treated at the initial pH of 12.8. Each compound was run three times.

FIGURE 3-6.   Change in the yield of compound *2E* or *2T* when each compound was individually treated at the initial pH of 11.9. Each compound was run three times.

FIGURE 3-7. Change in the yield of compound *3E* or *3T* when each compound was individually treated at the initial pH of 13.3. Each compound was run three times.

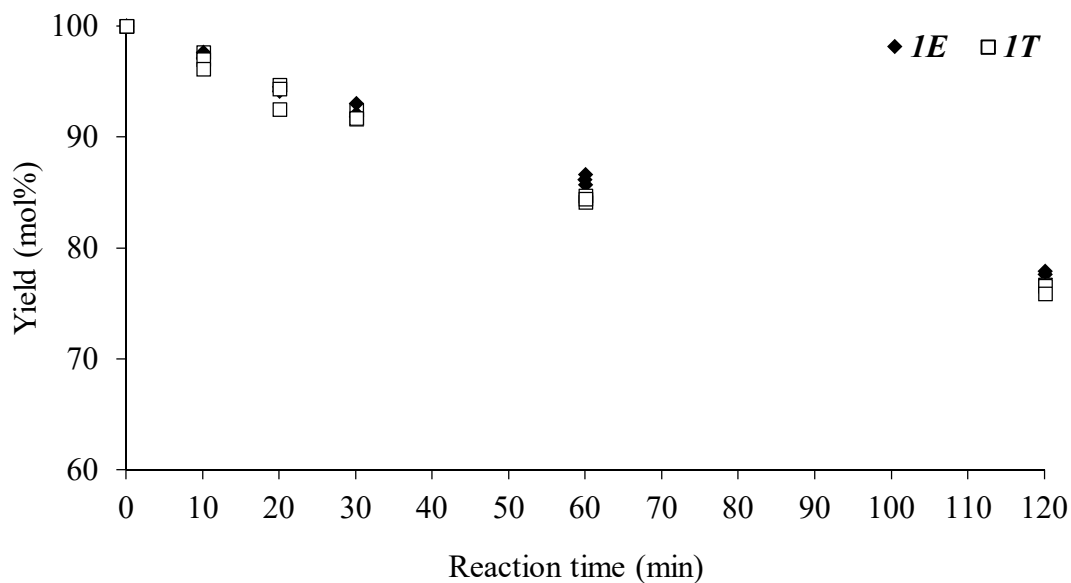**FIGURE 3-8.** Change in the yield of compound *3E* or *3T* when each compound was individually treated at the initial pH of 12.8. Each compound was run only once.
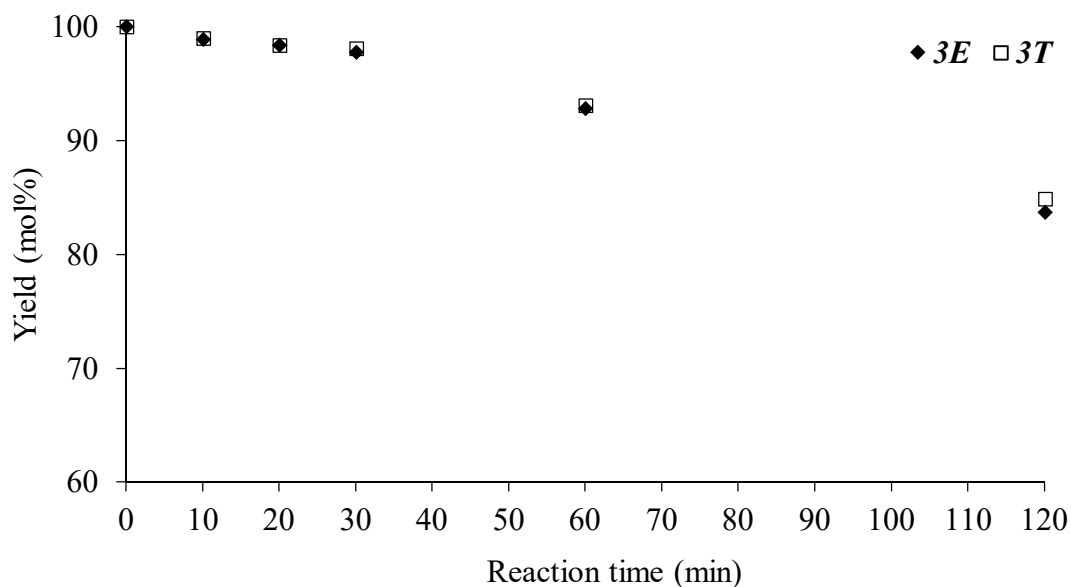
Figure 3-9. Change in the yield of compound *3E* or *3T* when each compound was individually treated at the initial pH of 11.9. Each compound was run only once.
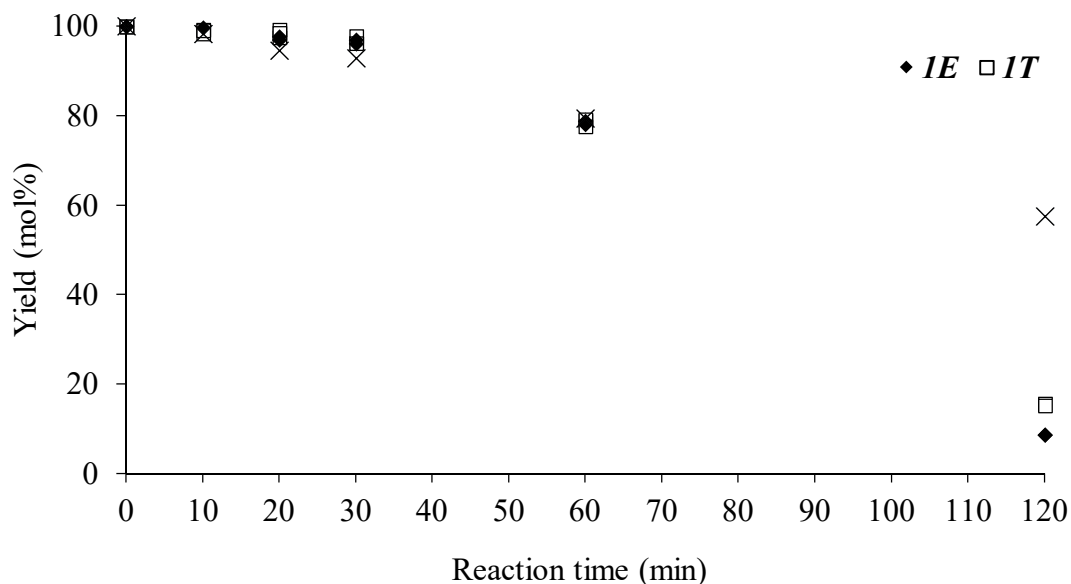
Figure 3-10. Change in the yield of compound *1E* or *1T* when each compound was individually treated at the initial p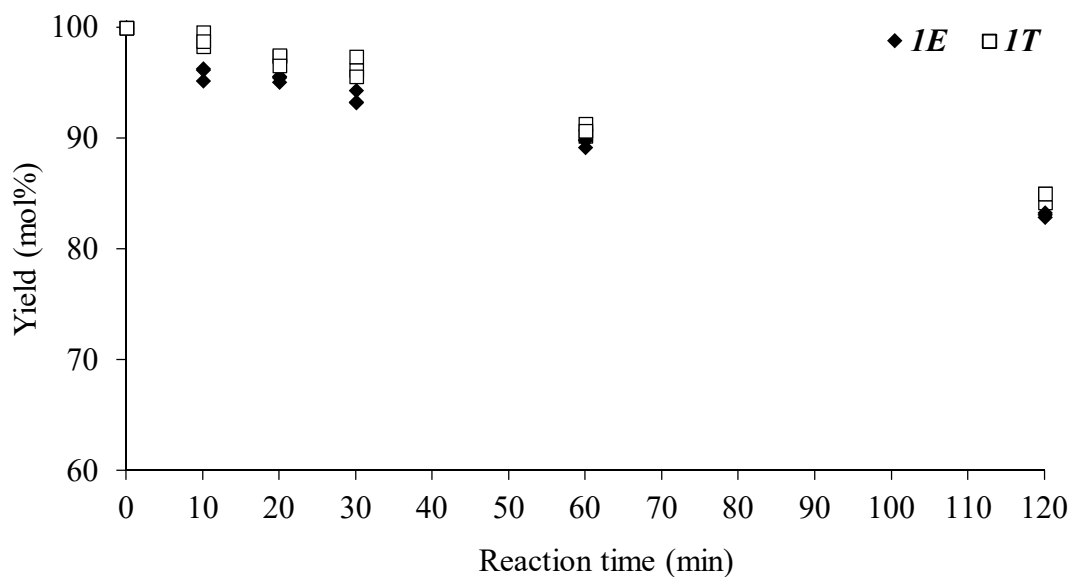H of 11.5. Each compound was run three times. The 'x' marks show the yield of compound *1E* when treated with a low initial concentration of compound *1E* (0.2 mmol/L). The reaction was run only once.
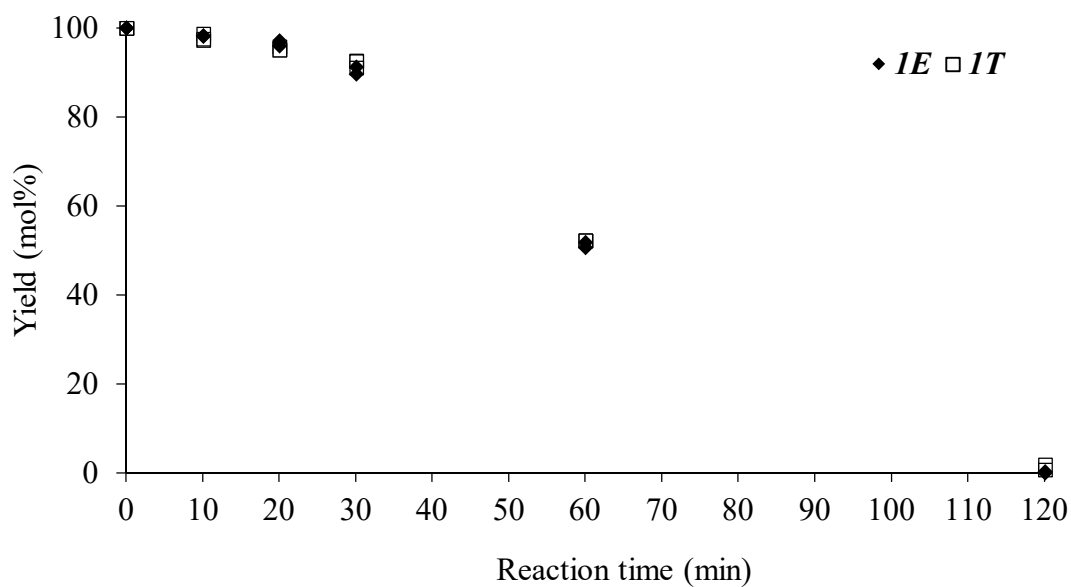
Figure 3-11. Change in the yield of compound *1E* or *1T* when each compound was individually treated at the initial pH of 11.0. Each compound was run three times.
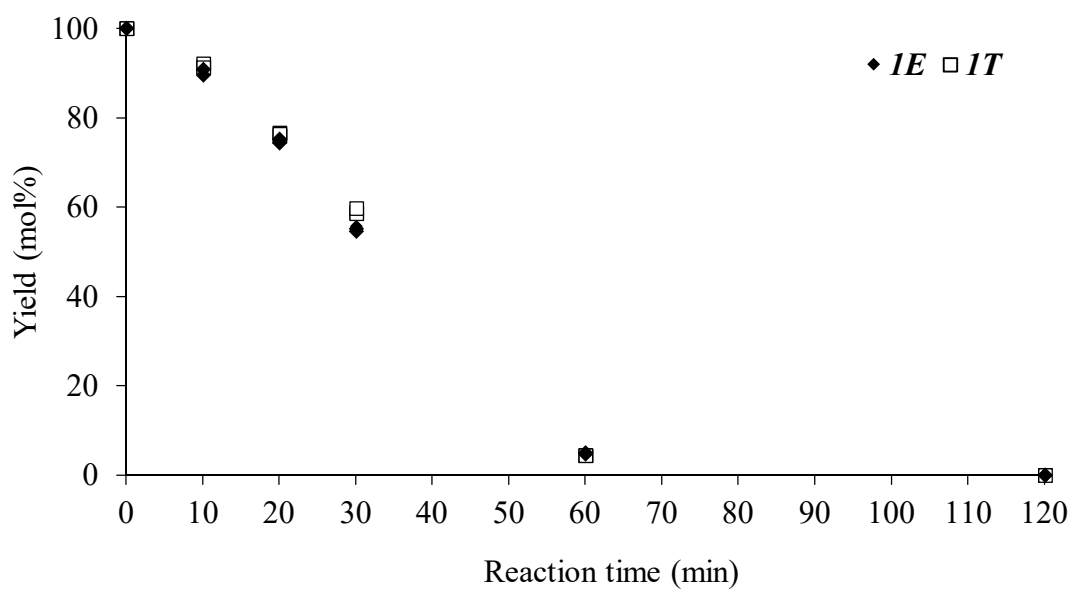
Figure 3-12. Change in the yield of compound *1E* or *1T* when each compound was individually treated at the initial pH of 10.5. Each compound was run three times.
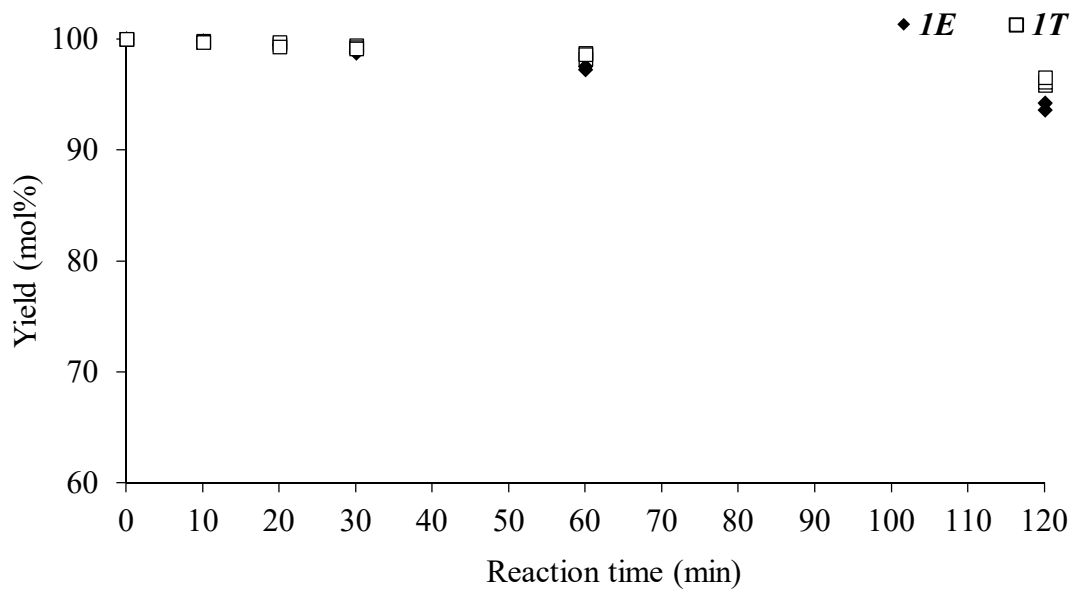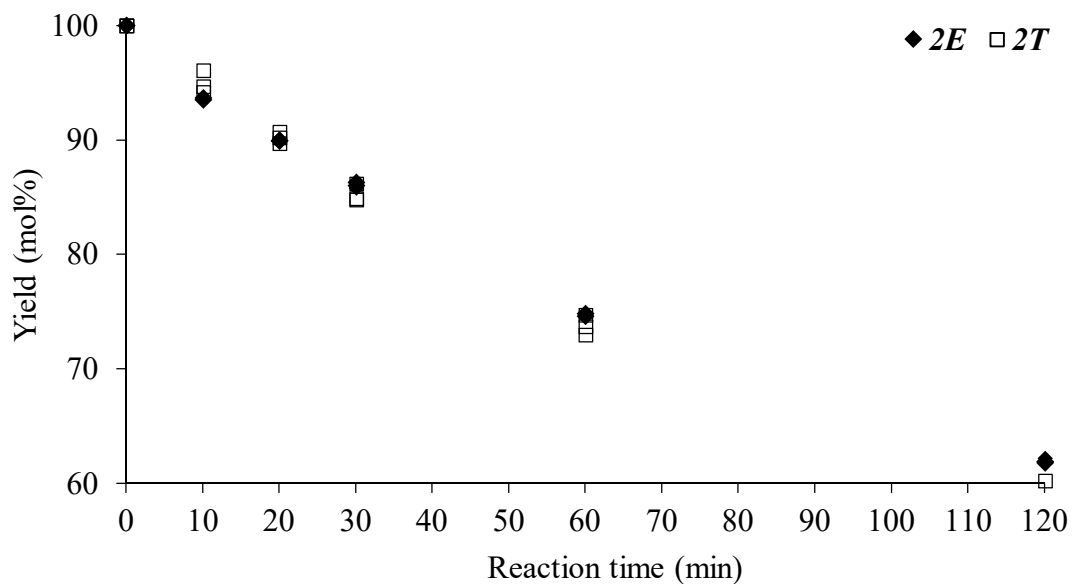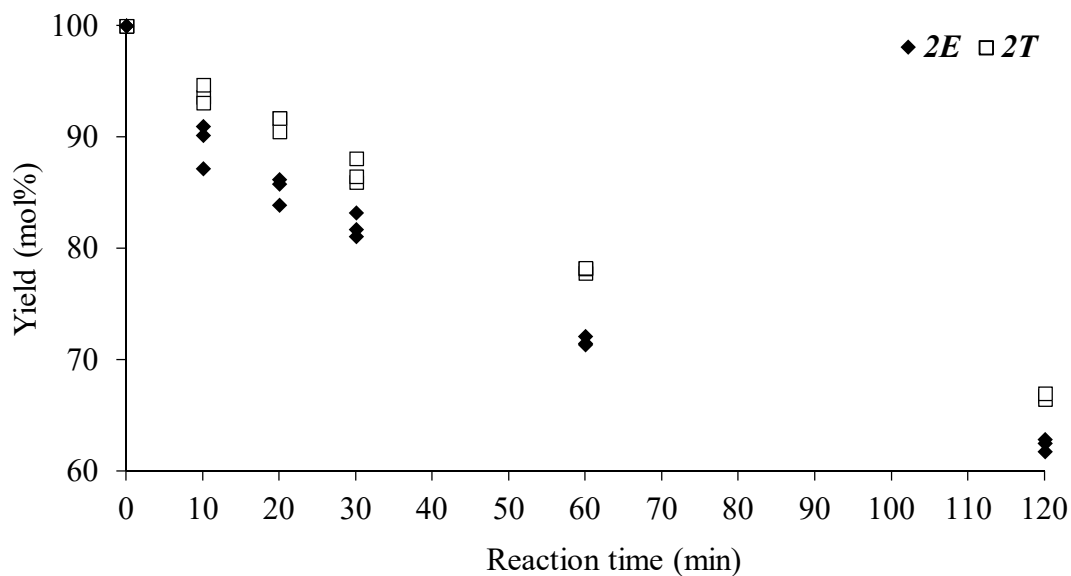
100
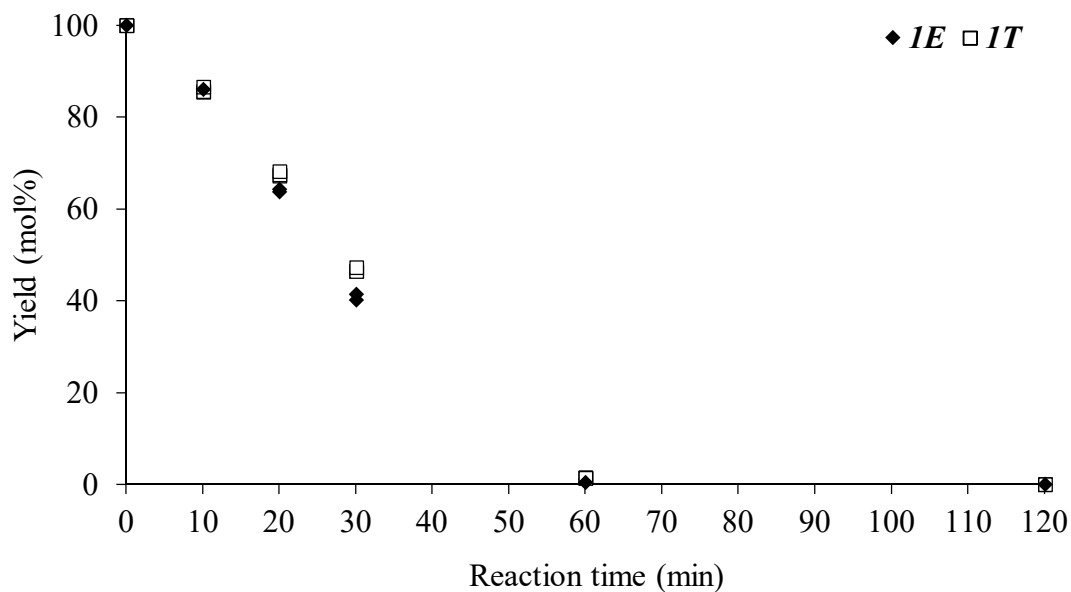
Figure 3-13. Change in the yield of compound *1E* or *1T* when each compound was individually treated at the initial pH of 9.5. Each compound was run three times.

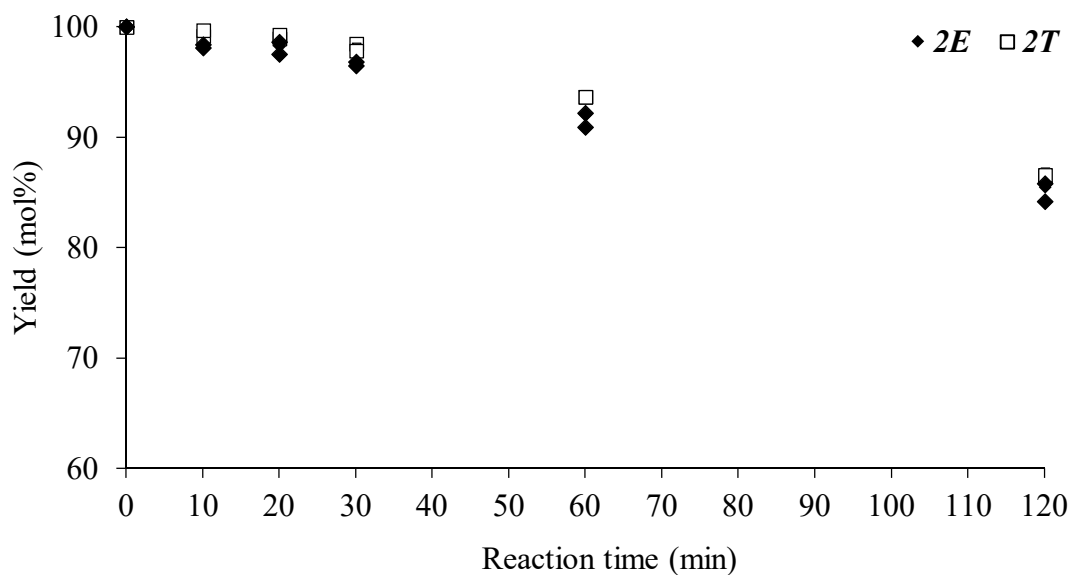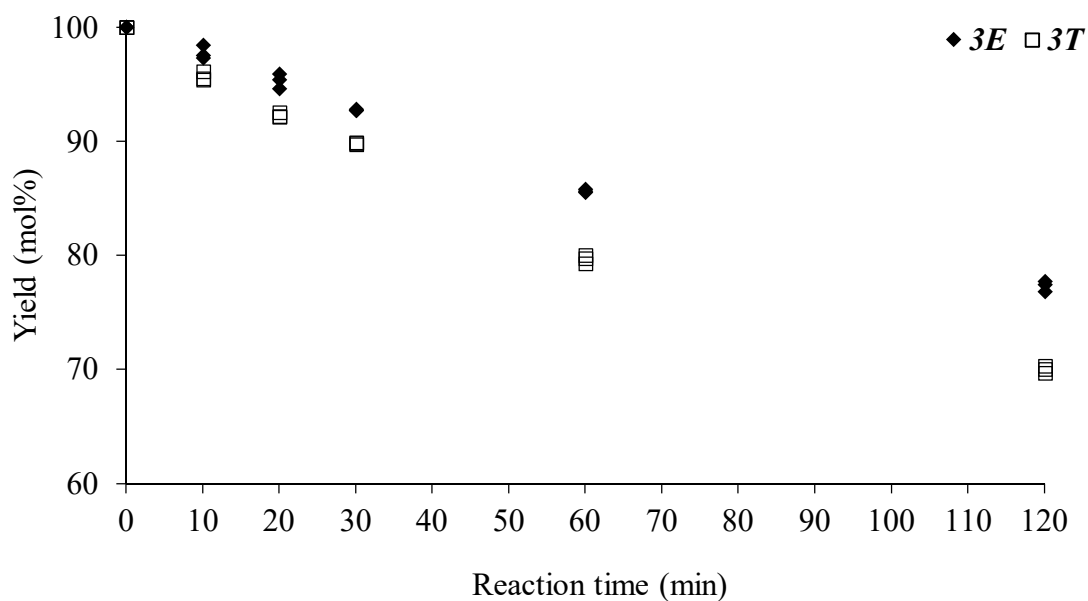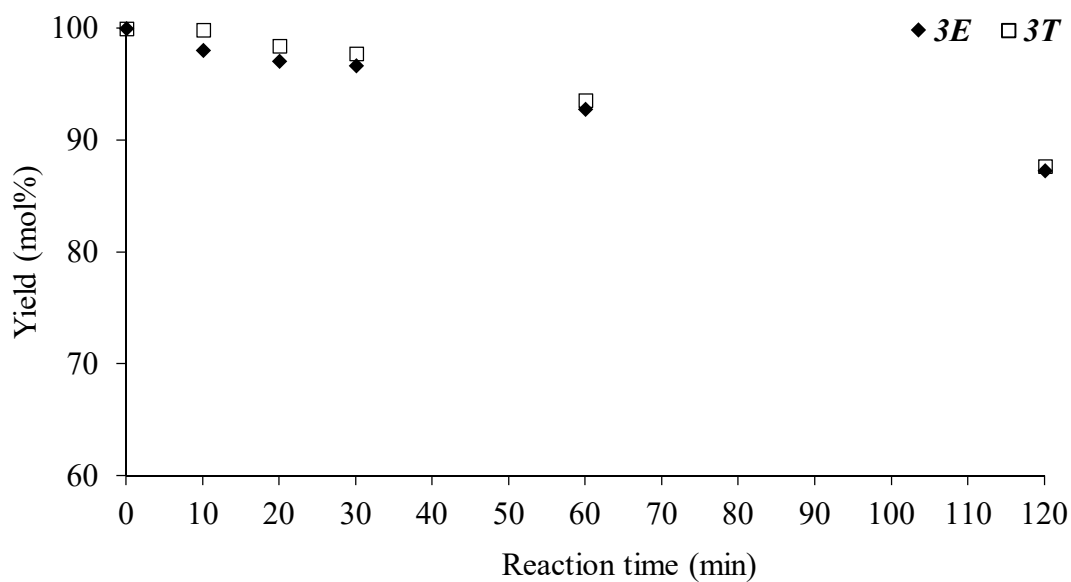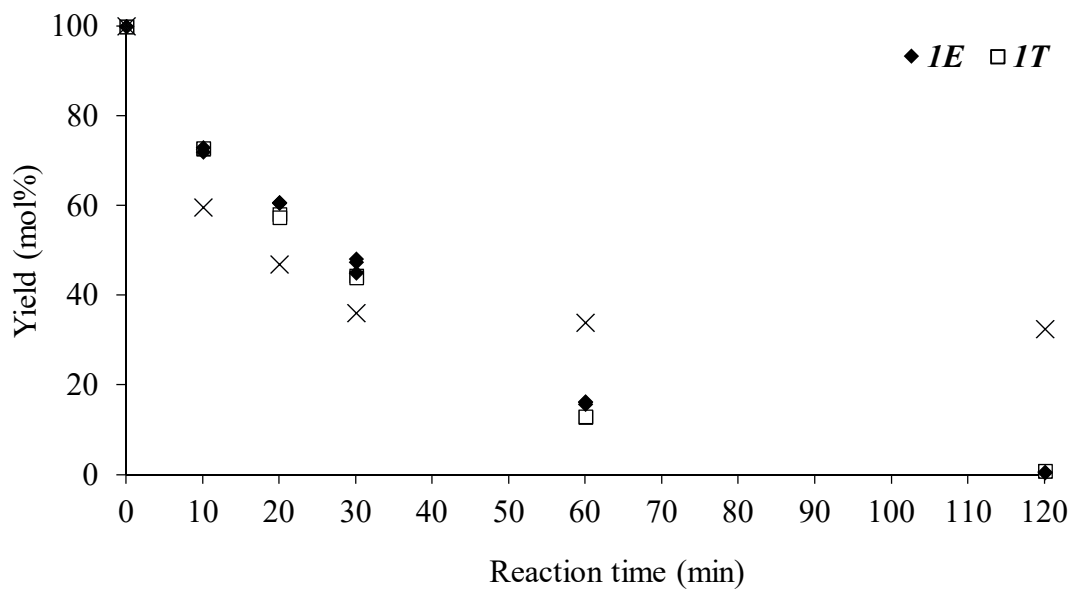**Figure 3-14.** Change in the yield of compound *1E* or *1T* when each compound was individually treated in Fenton's reagent. Each compound was run three times. The '×' marks show the yield of compound *1E* when treated adding H_2O_2 initially all at once. The reaction was run only once in this case.

TABLE 3-1: List of the initial pH, final pH, and stereo-preference observed in this study

Compound	Initial pH	Final pH	Stereo-preference
1	13.3	13.3	*T*
	12.8	12.5	*E*
	11.9	11.5	*E*
	11.5	7.5	no preference
	11.0	6.0	no preference
	10.5	4.0	no preference
	9.5	3.5	no preference
	5.5	3.1	no preference
2	13.3	13.3	no preference
	12.8	12.5	*E*
	11.9	11.5	*E*
3	13.3	13.3	*T*
	12.8	12.5	*E*
	11.9	11.5	no preference

*The grey line indicates Fenton's reagent system

3.4.3 Discussion

There are two suggestions from the previous studies can be used to discuss the observed stereo-preference.

Suggestion 1: Under high initial pH conditions (initial pH over than 12), the most responsible species for the degradation of the compound is negatively charged $O\overline{\bullet}$ which preferably to attack at the side-chain of the compound. [7-10]

Suggestion 2: Under low initial pH conditions (initial pH lower than 12), the neutral $HO\bullet$ considered to play an important role in the degradation instead of $O\overline{\bullet}$. Moreover, the $HO\bullet$ attack both side-chain and aromatic nucleus of the compound employed. [7-8]

Degradation of compound *1*, *2*, or *3* at relatively high initial pH conditions

The stereo-preferences results under high initial pH conditions can rationally be explained where the α–hydroxy group of *E* isomer of compound *1* and *3* fairly dissociates at the condition with initial pH of 13.3 while it is not the case for *T* isomer. The repulsive force working between negatively charged $O\overline{\bullet}$ and the negatively charged α-alkoxide at the side-chain of compound which further suppresses the degradation resulting in the stereo-preferential degradation of *T* isomer for compound *1* and *3*. In case of compound *2*, the dissociation mainly occurs at benzyl hydroxy group due to higher acidity of benzyl hydroxy group compare with the α–hydroxy group in the side-chain resulting in no clear

stereo-preference was observed. Under initial pH of 12.8 or 11.9, the α–hydroxy group of any compound does not greatly dissociate resulting in the stereo-preference of E over T isomer of any compound except compound 3 at an initial pH of 11.9 showed no clear stereo-preference. The results obtained under high initial pH conditions can suggested that the intrinsic stereo-preference of $O\cdot^-$ should slightly be the E isomer. Under initial pH 11.9 condition, where the responsible species for the degradation is not only $O\cdot^-$ but also $HO\cdot$, the degree of the stereo-preference shown was small. This result suggested that the $HO\cdot$ has less stereo-preferentially compared with $O\cdot^-$ which accordance to our suggestion 2 that $HO\cdot$ attack both side-chain and aromatic nucleus of the compound employed.

Moreover, the degradation became less with the decreasing of the initial pH under these high initial pH conditions. Two possible explanation for the results obtained are as follow.

(1) $O\cdot^-$ and $HO\cdot$ preferred to attack on H_2O_2 or HO_2^- rather than on the model compound at lower pH. Under initial pH 13.3 condition, the main mode in the decomposition of HO_2^- is by the attack of $O\cdot^-$ on HO_2^- which also suppressed by the electrostatic repulsive force between those two negatively charged. By the way, this repulsive forced became less with decreasing of pH due to their protonation to be conjugate acids. Moreover, it should be noted that the decomposition of H_2O_2 should decrease the degradation of the lignin model compounds.

(2) The bimolecular mechanism contributes more to the decomposition of H_2O_2 (and HO_2^-) at lower pH. The bimolecular decomposition of H_2O_2 without the formation of the radicals occurs at the maximum degree at the pH of about 11.6 (pKa of H_2O_2). In additions, the degree of degradation was in the order of compound $2>3>1$ at any

initial pH. The results suggested that the presence of benzyl hydroxymethyl and methoxymethyl in compound *2* or *3* enhances the reaction with $O^{\bar{•}}$ or $HO•$.

Degradation of compound *1* at relatively low initial pH and Fenton's reagent conditions

Under low initial pH conditions, the results showed that almost no clear stereo-preference was observed between two isomers at any pH. The results suggested that the $HO•$, as the most responsible species for the degradation of lignin model compound under these low initial pH conditions, has almost no stereo-preference or a small preference toward *E* isomer. This small or no stereo-preference of $HO•$ can be explained by its reactivity that preferably to attacks the lignin model compound both at side-chain and aromatic nuclei.

The degradation of the compound became greater with decreasing of initial pH under these conditions. It should be noted that the degradation is significantly accelerated at a reaction time of about 40-50 min or 30 min at the initial pH of 11.5 or 11.0, respectively, but is rapid from the beginning at an initial pH of 10.5 or 9.5, where the most responsible species should constantly be $HO•$ at any initial pH. In the reaction at an initial pH of 11.5, the pH was 9.9 or 9.2 at a reaction time of 30 or 60 min, respectively, and the precipitates and aggregates of Fe disappeared until a reaction time of about 60 min under this reaction condition. The results suggested that the unimolecular decomposition of H_2O_2 contributes more with decreasing of pH and predominates as the main mode when the pH becomes lower than 9.5-10.0. This predominates of the unimolecular mechanism should be due to the dissolution of the precipitates and aggregates and consequent increase of free Fe^{3+} (and Fe^{2+}) in the solution. Additional, the yield of compound *1E* was

higher than that in the common reaction when 0.2 mol/L of compound was run (1/5 of common reaction) at an initial pH of 11.5. (the '×' marks in Figure 3-10)) Moreover, the final pH is 9.5 which higher than 7.5 observed in the common reaction. The results suggests the small amount of acidic product produced from small amount of employed compound resulting in the small decrease of pH which delays the change in the H_2O_2 decomposition mechanism from bimolecular to unimolecular.

3.5 Conclusion

According to the results obtained, we can suggest that the intrinsic stereo-preference of $O^{-}\bullet$ generated by the decomposition of hydrogen peroxide at high alkalinities is slightly toward the **E** over the **T** isomer. By the way, the intrinsic stereo-preference appears differently when the electrostatic repulsion exists between the $O^{-}\bullet$ and side-chain with the dissociated α-hydroxyl group. The HO$\bullet$ shows a further smaller stereo-preference when it is similarly generated from the decomposition of hydrogen peroxide at alkalinities lower than those for the generation of $O^{-}\bullet$. These stereo-preferences can mainly be attributed to the difference in reactivity between $O^{-}\bullet$ and HO$\bullet$. Because $O^{-}\bullet$ is known to preferably attack the side-chain while HO$\bullet$ reacts with the aromatic nucleus as well as the side-chain, the structural difference between the *erythro* and *threo* isomers should affect the reaction of $O^{-}\bullet$ more than that of HO$\bullet$. The change of the H_2O_2 decomposition mechanism was observed where the bimolecular mechanism, as the most contributable under high initial pH condition of any compound, had been switched into unimolecular mechanisms which considered to be the most viable under lower initial pH conditions.

3.6 Stereo-preferential degradation of the *erythro* and *threo* isomers of *β-O*-4 type lignin model compounds under sodium treatment (as reference)

3.6.1 Introduction

Pulp bleaching stage, where the residual non-carbohydrate constituents as well as lignin are removed from the pulp, is one of chemical processing in the pulp manufacture to increase brightness, improve cleanliness as well as reducing pitch content. Several bleaching reagents has been used in this step to remove residue lignin remained after cooking process including oxygen, ozone, as well as chlorine. According to the environmental reasons, the chlorine bleaching has been used as a combined stage together with another environmental friendly reagent. Several chlorine-containing bleaching methods and their reactants are shown in Table 3-2.

TABLE 3-2: Chlorine-contain bleaching methods, reactants, and chlorine species generated in the reaction system.

Bleaching method	Reactant
Chlorination	Molecular Cl_2
Hypochlorite bleaching	Hypochlorous acid (HClO)
Chlorine dioxide bleaching	Chlorine dioxide (ClO_2)
Chlorite bleaching	Sodium chlorite ($NaClO_2$)

Chlorination and Hypochlorite bleaching

There are three chlorine species including molecular chlorine (Cl_2), hypochlorous acid (HClO) as well as hypochlorite (ClO^-) presented in the reaction system depending on the pH of the system. Under chlorination stage, Molecular chlorine (Cl_2) considered to plays an important role in the system where pH below 2. The main purpose of chlorination stage is not to improve the brightness but to make the residual lignin soluble in both alkali and water. On the other hand, in the hypochlorite bleaching stage, the main purpose is to decrease the amount of residual lignin presence in pulp which normally conducts at pH 8-9. The hypochlorous acid (HOCl) (pKa 7.53) considered to be the active species in the reaction pH between 4-7.5. The hypochlorite (ClO^-), anion of hypochlorous acid (HClO), suggested to be the dominant species when the pH value over than 8.5.

Chlorine dioxide bleaching (Chlorite bleaching)

The chlorine dioxide bleaching systems are more complicated than the hypochlorite bleaching system due to the interconversion of oxidizing species as well as formation of hypochlorous acid (HOCl) during the oxidation. For the chlorine dioxide bleaching, the pH is a significant factor. Molecular chlorine (Cl_2) plays an important role in delignification process under low pH (pH< 3) condition. At higher pH, the chlorite ion (ClO_2^-) and Hypochlorous acid (HOCl) suggested to be the primary reduction products of chlorine dioxide (ClO_2). [11-13] These two can further produced various chlorine active species including chloride ion (Cl^-) as well as chlorate ion (ClO_3^-). According to the previous studies, 6-chlorovanillin, fumaric acid as wekk as monochloroacetic acid was obtained as the products from the chlorite holocellulose preparation of spruce wood

sample. These obtained 6-chlorovanillin and monochloroacetic acid suggested the cleavage at the side-chain. On the other hands, the formation of fumaric acid indicates attacks on the aromatic rings. [14-15] It can be suggested that chlorine dioxide chlorine dioxide (ClO_2) or chlorites (ClO_2^-) may attack both on the side-chain as well as on the aromatic nuclei of the lignin compound. Several studies has been researched on the lignin reactions under chlorine dioxide or chlorite bleaching system. The aromatic rings of the lignin model compound was oxidized resulting in the formation of quinone and muconic acid. [16-23] Two single electron transfer suggested to be the reaction mode for the reaction between chlorine dioxide and etherification of lignin. [24] The studies on the reaction mechanism between lignin and chlorine dioxide has been performed in many researches using non-phenolic lignin model compound. [25-28] All the results suggested that the delignification proceeds via reaction pathways as shown in Figure 3-15 By the way, the data on the stereo-preferential degradation of non-phenolic lignin model compound is still lacking and need to be investigated as the references. Sugimoto et al. has been delignified the wood meal samples using $NaClO_2$ treatment following with ozonation analysis to examine the proportion between erythro (*E*) and threo (*T*) isomers in the lignin sample. The results showed that there were no significantly different in degradation degree between those two isomers. By the way, under employed reaction conditions, this stereo-preferential degradation results not only from the reactivity of chlorine active species but also other topochemical factors. [5, 29] In this study, the non-phenolic *β*-O-4 type lignin model compounds were treated under $NaClO_2$ system to observe the stereo-preferential degradation between erythro (*E*) or threo isomer (*T*) as a reference for the results obtained from oxygen and hydrogen peroxide treatment.
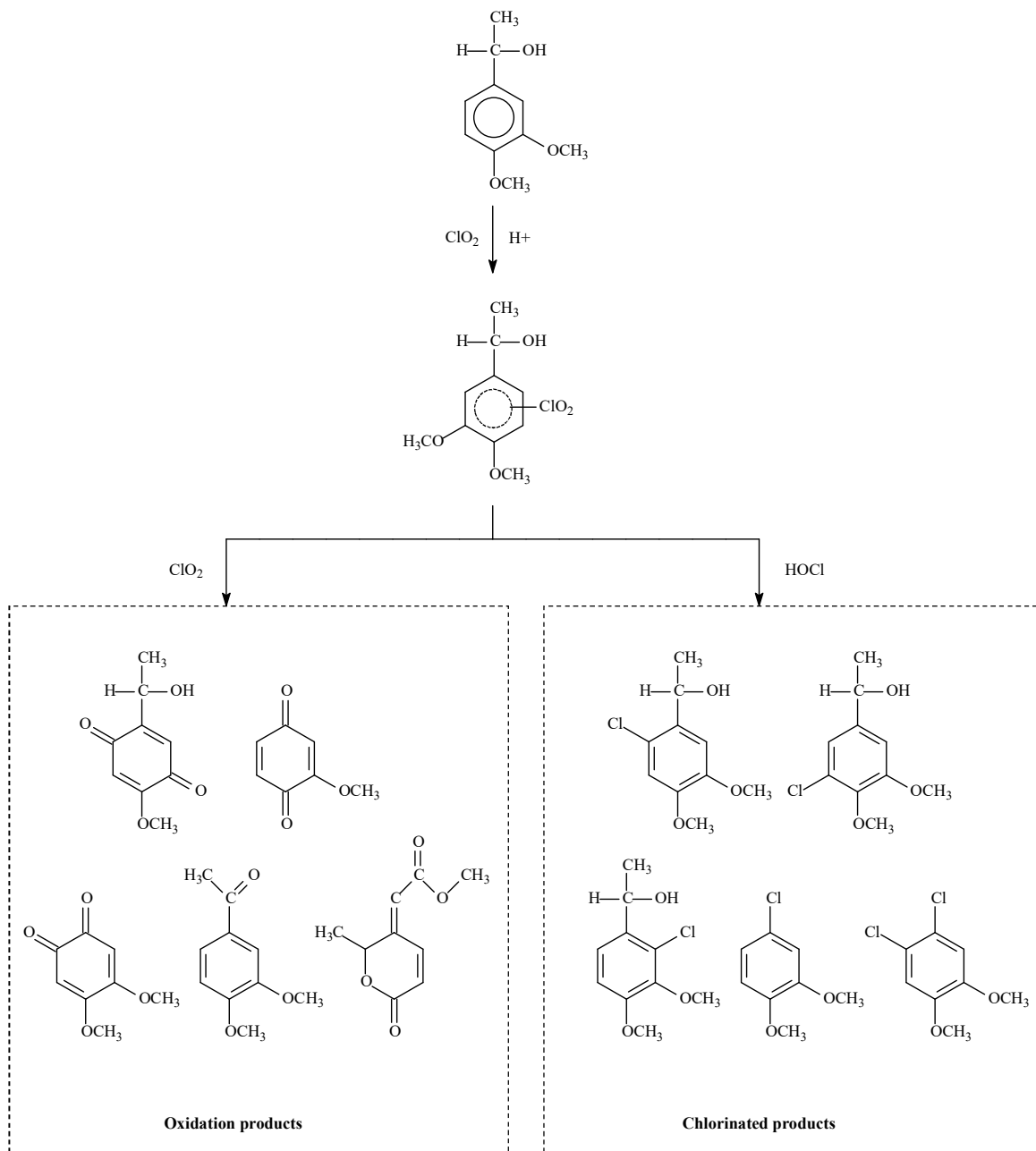
FIGURE 3-15. The reaction pathways between lignin and chlorine dioxide [28]

3.6.2 Materials and methods

3.6.2.1 Materials

All chemicals except lignin model compound were purchased and used without any purification. The lignin model compound **1**, 2-(2-methoxyphenoxy)-1-(3, 4- dimethoxyphenyl) propane-1, 3-diol,was synthesized according to the method reported in Adler et al. [1] The detailed of the model compound synthesis and ^{1}H and ^{13}C NMR results were shown in chapter 2.

3.6.2.2 Methods

NaClO$_2$ treatment

1.0 mmol/L of lignin model compound (**1E**, **1T**) was adjusted in 100ml reaction solution and 10 mmol/L of NaClO$_2$ and 200μL acetic acid were added in order to initiate the reaction. During the reaction 10 mmol/L of NaClO$_2$ and 200μL acetic acid was stepwisely added 5 times with constant interval of 10 minutes. The reaction temperature was 70ºC. A portion of the reaction solution was withdrawn at prescribed reaction times for further quantification of the residual lignin model compound and pH measurement. Each kind of the reaction was repeated three time to confirm the reproducibility.

Quantification of residual lignin model compound

The $Na_2S_2O_3$ were immediately added into the withdrawn reaction solution, following with internal standard compound, 4-chlorophenol, as a methanol solution. After filtration, the resulting mixture was injected into HPLC (LC-10A, Shimadzu Co., Kyoto, Japan) equipped with an SPD-M10A detector (280 nm, Shimadzu Co.) to quantify the employed lignin model compound on the basis of a calibration line prepared for each of compound *1E*, *1T*

The conditions of HPLC were as follows. Column: Luna 5u C18 (2) 100A (150 mm x 4.6 mm, Phenomenex Inc., Torrance, CA, USA); oven temperature: 40℃; flow rate: 1.0 mL/min; solvent system for the analysis of compound *1E*, *1T*, or *3T* in the reaction: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 44/56 for 27.5 min and maintained for 10 min, gradient to 15/85 immediately and maintained for 5 min, total time 50 min.

3.6.3 Results and Discussion

The degradation of compound *1E* or *1T* under NaClO$_2$ is illustrated in Figure 3-16. According to the observed result, there was no clear stereo-preference between *E* and *T* isomers. As mentioned in the introduction session, under chlorine dioxide treatment, the lignin model compound suggested to attack by chlorine active species generated in the reaction system resulting in the delignification process without the ionization of lignin model compound. The chlorine dioxide (ClO$_2$) and hypochlorous acid (HOCl), generated by the decomposition of chlorine dioxide (ClO$_2$), was considered as the main active species under employed conditions. The result obtained can rationally be explained that generated chlorine active species attacked both on the side-chain as well as on the aromatic nuclei of compound *1* resulting in no stereo-preference was observed.
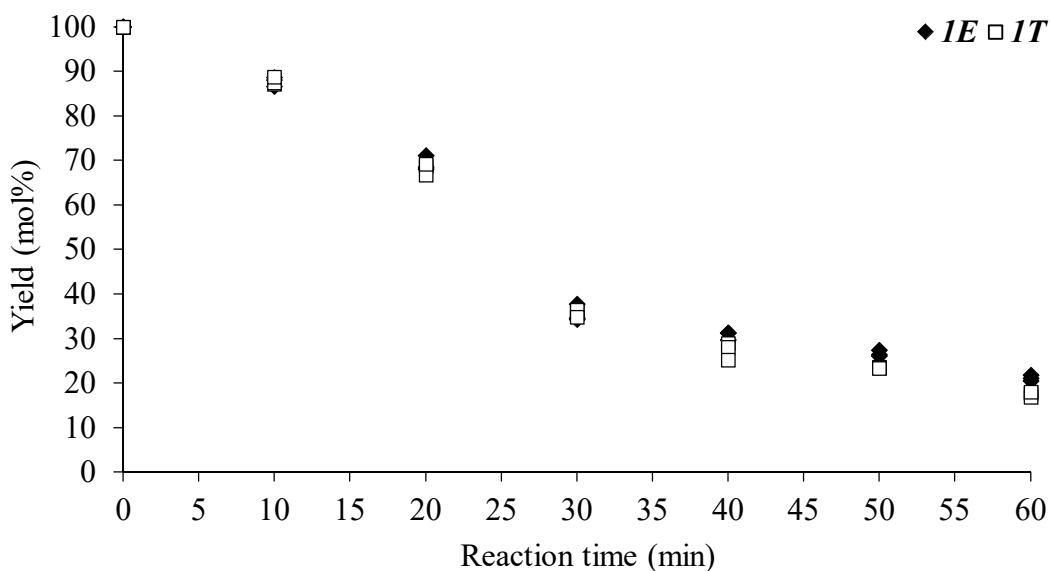
FIGURE 3-16. Change in the yield of compound *1E* or *1T* when each compound was individually treated. Each compound was run three times.

References

[1] Adler, E.; Lindgren, B. O.; Saeden, U. (1952). The *β*-guaiacyl ether of *α*-veratrylglycerol as a lignin model. *Svensk Papperstidn.*, 55(7), 245-254.

[2] Ibrahim, W.; Lundquist, K. (1994). Synthesis of *erythro* and *threo* forms of lignin models of the arylglycerol *β*-guaiacyl ether type. *Acta Chem. Scand.*, 48(2), 149-151.

[3] Li, S.; Lundquist, K.; Soubbotin, N. (1994). Separation of diastereomers of lignin model compounds of the 1,3-diol type as borate complexes by ion-exchange chromatography. *Holzforschung*, 48(6), 509-511.

[4] Li, S.; Lundquist, K.; Nagnus, P. (1995). Synthesis of guaiacylglycerol *β*-guaiacyl ether. *Acta Chem. Scand.*, *49*(8), 623-624.

[5] Akiyama, T.; Sugimoto, T.; Matsumoto, Y.; Meshitsuka, G. (2002). *Erythro/threo* ratio of *β-O*-4 structures as an important structural characteristic of lignin. Part 1: Improvement of ozonation method for the quantitative analysis of lignin side-chain structure. *J. Wood Sci.*, 48(3), 210-215.

[6] Bohlin, C.; Andersson, P.-O.; Lundquist, K.; Jonsson, L. J. (2007). Differences in stereo-preference in the oxidative degradation of diastereomers of the lignin model compound 1-(3,4-dimethoxyphenyl)-2-(2-methoxyphenoxy)-1,3-propanediol with enzymic and non-enzymic oxidants. J. Mol. Catal., B Enzym., *45*(1&2), 21-26.

[7] Yokoyama, T.; Matsumoto, Y.; Meshitsuka, G. (1999). Reaction selectivity of active oxygen species in alkaline oxygen bleaching. J. Wood Chem. Technol., *19*(3), 187-202.

[8] Yokoyama, T.; Matsumoto Y.; Meshitsuka, G. (2005). Characterization of active oxygen species under alkaline oxygen bleaching conditions. Holzforschung, *59*(3), 269-275.

[9] Nakagawa, A.; Yokoyama, T.; Matsumoto, Y. (2012). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions IV: Appearance of kinetic isotope effect in the reaction between methyl glucoside and deuterated methyl glucoside. J. Wood Sci., *58*(6), 563-569.

[10]   Yokoyama, T.; Nakagawa, A.; Matsumoto, Y. (2015). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions. Part 5: Comprehensive results on experiments using deuterium labeled methyl glucosides. J. Wood Chem. Technol., *35*(6), 450-463.

[11] Kolar, J.J. (1983). Chemical reactions in ClO_2 stages of pulp bleaching, *Wood Science and Technology*, 17, 117-128

[12] Patricia Gonzalez, C.Z. (2000). Effect of process modifications on AOX emissions from kraft pulp bleaching using Chilene pine and eucalyptus, *Journal of Cleaner Production*, 8, 233-241

[13] Joncourt, M.J.; Froment, P.; Lachenal, D.; Chirat, C. (2000). Reduction of AOX formation during chlorine dioxide bleaching, *TAPPI Journal*, 83, 144-148

[14]   Pearl, I.A. (1946). 6-chlorivanillin from the chlorite oxidation of lignin, *J. Am. Chem. Soc.*, 68, 916

[15] Barton, J.S. (1950). The reaction products of lignin and sodium chlorite in acid solution, *Tappi*, 33, 496-503

[16] Lindgren, B.O. (1971). Chlorine dioxide and chlorite oxidations of phenols related to lignin, *Svensk papperstidn*, 74 (3), 57-63

[17] Ni, Y.; Shen, X.H.; Van Heiningen, A.R.P. (1994). Studies on the reactions of phenolic and nonphenolic lignin model compounds with chlorine dioxide, *J. Wood*

Chem. Technol., 14 (2), 243-262

[18] Brage, C.; Eriksson, T.; Gierer, J. (1991). Reactions of chlorine dioxide with lignins in unbleached pulps. Part I. Reactions of chlorine dioxide with monomeric model compounds representing aromatic structures in residucal lignins, *Holzforschung*, 45 (1), 23-30

[19] Gunnarsson, N. P.-I.; Ljunggren, S. (1996). The kinetics of lignin reactions during chlorine dioxide bleaching Part I. Influence of pH and temperature on the reaction of 1- (4, 4- dimethoxyphenyl) ethanol with chlorine dioxide in aqueous solution*, Acta Chem. Scand.,* 50, 422-431

[20] Brage, C.; Eriksson, T.; Gierer, J. (1991). Reactions of chlorine dioxide with lignins in unbleached pulps. Part II, *Holzforchung*, 45, 47-152

[21] Mckague, A.B.; Kang, G.J.; Reeve, D.W. (1994). Reactions of lignin model dimers with chlorine dioxide, *Nordic Pulp Paper Res.*, 9 (2), 84-87

[22] Mckague, A.B.; Reeve, D.W.; Xi, F. (1995). Reaction of lignin model compounds with chlorine dioxide and sodium hydroxide, *Nordic Pulp Paper Res.*, 10 (2), 114-118

[23] Sun Yong, Z.J.-p.; Yang Gang, D. (2007). Study on the spectra of spruce lignin with chlorine dioxide oxidation, *Spectroscopy and Spectral Analysis*, 27, 1551-1554

[24] Doug, J.F.K; Svenson, R.; Chang, H.M.; Jameel, H. (2002). Effect of pH on the inorganic species involved in a chlorine dioxide reaction system, *Industrial and Engineering Chemistry Research*, 41, 5927-5933

[25] Hamzeh, Y.; Mortha, G.; Lachenal, D.; Izadyar, S. (2008). Selective degradation of lignin polymer by chlorine dioxide during chemical pulp delignification in flow through reactor*, Polymer-Plastics Technology and Engineering*, 47, 931-935

[26] Hamzeh, Y.; Mortha, G.; Lachenal, D.; Hostachy, J.C..; Calais, C.J. (2006).

Comparative studies of chlorine dioxide reactions with muconic acid derivatives and lignin model compounds, *Journal of Wood Chemistry and Technology*, 26, 153-164

[27] Reeve, B.M. (1994). Reaction of lignin model dimer sequentially with chlorine and sodium hydroxide, *Environmental Science and Technology*, 28, 573-576

[28] Nie, S.; Liu, X.; Wu, Z.; Zhan, L.; Yin, G.; Yao, S.; Song, H.; Wang, S. (2014). Kinetics study of oxidation of the lignin model compounds by chlorine dioxide, *Chem. Eng, J.*, 241, 410-417

[29] Sugimoto, T.; Akiyama, T.; Matsumoto, Y.; Meshitsuka, G. (2002). The *erythro*/*threo* ratio of *β-O*-4 structures as an important structural characteristic of lignin. Part 2: Changes in *erythro*/*threo* (E/T) ratio of *β-O*-4 structures during delignification reactions Holzforschung, *56*(4), 416-421.

Chapter 4

Formation of aldehyde and acid as the main reaction products obtained in the reaction of *β-O*-4 type lignin model compounds under oxygen and hydrogen peroxide treatments

4.1 Introduction

Regarding to high potential of wood biomass as the raw material for value added materials, chemicals, it has recently attracted great interest. In the wood cell wall, there are composed of three major components including cellulose, hemicellulose as well as lignin where the utilization of these components have been chosen as the topics in several researches. Among these three components, many researchers focusing on the utilization of lignin which expected to be a replacement of petroleum, but sufficient utilization has not yet been attained. Nowadays, lignin is mainly burned and used as the energy source in pulp and paper mills. Several studies believed that lignin can utilized to high value-added chemicals. Otherwise, the commercial high-value products from lignin are absence due to its heterogeneous structure as well as lack of efficient depolymerization and modification techniques. Among the lignin utilization processes, oxidation of lignin has become interest in many studies, for example, the alkaline nitrobenzene oxidation treatment of lignin resulting in several fine chemical including vanillin, syringaldehyde, vanillic acid), and syringic acid. By the way, this nitrobenzene oxidation method cannot be practical used due to its toxicity. [1, 2] Many other conversion methods has also been published is several studies both from actual lignin and lignin model compound as shown in Table 4-1.

TABLE 4-1: Conversion of lignin into fine chemicals

No.	Lignin substrate	Methods	Product yields	Ref
1	*Populus* wood	Steam-exploded with presence of Cu^{2+} and Fe^{3+}	12% of total yield of vanillin and syringaldehyde	[3]
2	Organosolv beech wood lignin	Alkaline solution with H_2O_2 as oxidant with presence of La/SBA-15 as catalyst under microwave irradiations (200W)	5.99-9.94% of vanillin, 7.78-15.66% of syringaldehyde, 2.42-5.19% of vanillic acid, 0-2.45% of syringic acid, 0.23-0.7% of acetovanillone, 0-0.1% of acetosyringone	[4]
2	*Pinus spp.* wood	Alkaline solution under oxygen pressure	10% of vanillin	[5]
3	Kraft lignin from *Pinus spp.*	Alkaline solution under oxygen pressure	3-8% of vanillin	[6]
4	Alkaline lignin from sugarcane bagasse	Oxygen pressure with the presence of Pd/Al_2O_3 as catalyst	4.4% of vanillin, 6.7% of syringaldehyde, and 7.3% of ρ-hydroxybenzaldehyde	[7]
5	Organosolv lignin	Oxygen as oxidant with presence of Pd/CeO_2 as catalyst	5.2% of vanillin, 0.87% of guaiacol, and 2.4% of ρ-hydroxybenzaldehyde	[8]

TABLE 4-1: Conversion of lignin into fine chemicals (continued)

No.	Lignin substrate	Methods	Product yields	Ref
6	Lignin from enzymatic hydrolysis of steam-explosion cornstalks	Alkaline solution under oxygen pressure with the presence of $LaMnO_3$ as catalyst	3.7-4.32% of vanillin, 3.6-9.33% of syringaldehyde, 0.8-2.2% of ρ-hydroxybenzaldehyde	[9]
7	Lignin from enzymatic hydrolysis of steam-explosion cornstalks	Alkaline solution under oxygen pressure with the presence of $LaCoO_3$ as catalyst	3.9-4.55% of vanillin, 2.1-9.99% of syringaldehyde, 0.9-2.23% of ρ-hydroxybenzaldehyde	[10]
8	Dimeric β-O-4 type lignin model compound	Alkaline solution under oxygen pressure with 1,10-phenanthroline as catalyst and $CuSO_4$	Approximately 50% of vanillin	[11]
9	β-O-4 model compound (softwood and hardwood)	poly(4-vinylpyridine)/MTO and polystyrene/MTO as catalysts with 35 wt% H_2O_2 aqueous solution, and CH_3COOH as solvent	9.8-25% of vanillic acid, 4.2-13.7 of ketone, and 23.4-32.5% of 2,6-dimethoxyphenol	[12]

4.2 Objectives of this work

Under oxygen and hydrogen peroxide treatment, the generated active oxygen species (AOS) considered to play an important role in degradation of lignin model compound. The objectives of this work are shown in the following text.

1. To observe the yield of degradation products obtained in the reaction of compound *1* under alkaline oxygen treatment both TMPh and Valc system.

2. To observe the yield of degradation products obtained in the reaction of compound *1* under hydrogen peroxide as well as fenton's reagent treatment both in alkaline and acidic conditions.

4.3 Materials and methods

4.3.1 Materials

All the chemicals except the lignin model compound were purchased and directly used with no further purification. In this study, only non-phenolic β-O-4 type lignin model compound **1**, 2-(2-methoxyphenoxy)-1-(3, 4-dimethoxyphenyl) propane-1, 3-diol, were used to observed the reaction products obtained under both oxygen and hydrogen peroxide treatment where the synthesize steps are according to Adler *et al*.[13] The detailed of the model compound synthesis was shown in chapter 2. Therefore, the anion exchange chromatography was used as isomer (**E** and **T**) separation method. [14-16] The purification was confirmed by ^{1}H and ^{13}C NMR method where the detailed data were shown in chapter 2.

4.3.2 Methods

Alkaline Oxygen Treatment

300 mL of a reaction solution including 0.50 mol/L sodium hydroxide (NaOH), 0.36 mmol/L ferric chloride, 10 mmol/L of co-existing phenolic compound (TMPh or Valc), and 1.0 mmol/L of lignin model compound (**1E**, **1T**) was prepared. It should be noted that only compound **1E** and **1T** was reacted under the TMPh condition. Moreover, a 0.10 mol/L sodium hydroxide (NaOH) was employed as a lower alkaline concentration conditions in some runs of compound **1** under Valc system. After the preparation, the

reaction solution then transferred into Teflon-coated stainless steel vessel (500 mL, Taiatsu Techno® Co., Tokyo, Japan), and adjusted oxygen pressure of 1.1 MPa (1.0 MPa as the gauge level). Low oxygen pressure (0.4 MPa or 0.3 MPa as the gauge level) was employed in some runs under TMPh system. The vessel then heated to 95℃ for 10 min and maintained for 360 min while stirring. The reaction time was defined as zero when the temperature reached 95℃. At prescribed reaction times, a portion of the reaction solution was withdrawn for quantification. Each kind of reaction was run three times to confirm the reproducibility.

Alkaline hydrogen peroxide treatment

Sodium hydroxide solution (various concentrations), 0.36 mmol/L of Ferric chloride ($FeCl_3$), and 1.0 mmol/L of lignin model compound (*1E*, *1T*) was contained in the reaction solution. After preparation, the reaction solution then transferred into the Teflon vessel and heated to 95ºC. The 30% hydrogen peroxide solution was added in order to initiate the reaction. During the reaction, the 30% hydrogen peroxide solution was added into the vessel for ten times with constant interval of 10 minutes where the total amount added was approximately 1.8 mmol. A portion of the reaction solution was withdrawn at prescribed reaction times for further quantification of the residual lignin model compound and pH measurement. Each kind of the reaction was repeated three time to confirm the reproducibility.

Quantification of residual lignin model compound

The detailed quantification procedures were shown in chapter 2 and 3. The quantification was conducted using a high performance liquid chromatograph (HPLC, LC-10A, Shimadzu Co., Kyoto, Japan) equipped with a photodiode array detector (SPD-M10A, Shimadzu. The conditions of HPLC were as follows. Column: Luna 5u C18 (2) 100A (150 mm x 4.6 mm, Phenomenex Inc., Torrance, CA, USA); oven temperature: 40℃; flow rate: 1.0 mL/min; solvent system for the analysis of compound *1E* in the reaction with TMPh: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 40/60 for 27.5 min and maintained for 20 min, gradient to 15/85 immediately and maintained for 5 min, total time 60 min; solvent system for the analysis of compound *1T* in the reaction with TMPh: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 35/65 for 27.5 min and maintained for 20 min, gradient to 15/85 immediately and maintained for 5 min, total time 60 min; solvent system for the analysis of compound *1E*, *1T* and neutral degradation products in the reaction with Valc and H_2O_2 system: gradient CH_3OH/H_2O (v/v) from 15/85 to 25/75 for 7.5 min, gradient to 44/56 for 27.5 min and maintained for 10 min, gradient to 15/85 immediately and maintained for 5 min, total time 50 min; Those for the analysis of acidic degradation products were as follows: gradient CH_3OH/aqueous 1% CH_3COOH (v/v) from 15/85 to 20/80 for 45 min, gradient to 44/56 for 5 min and maintained for 15 min, gradient to 15/85 immediately and maintained for 5 min, total time 70 min.

4.4 Results and Discussion

4.4.1 Reaction system description

Oxygen treatment

As mentioned in chapter 2, several point have been indicated as followed.

1. The compound 1 is stable without the addition of oxygen under the same condition. [17]

2. Under the oxygen treatment, the compound 1 is attacked by the active oxygen species (AOS) generated in the system by the reaction between molecular oxygen and co-existing phenolic compound (2, 4, 6-trimethylphenol (TMPh) and Vanillyl alcohol (Valc) in this study). [17, 18]

3. The negatively charged oxyl anion radical ($O\bar{\bullet}$) is considered to be the major AOS generated in both Valc and TMPh system. By the way, the peroxyl radical (ROO•) is also suggested to play an important role in the TMPh system. [19, 20]

4. The chain-type reactions found to generate in the system after the disappearance of AOS.

5. Oxyl anion radical ($O\bar{\bullet}$) preferentially attacked the compound 1 at the side-chain rather than the aromatic nucleus. [17, 18, 21]

Hydrogen peroxide and Fenton's reagent treatment

As shown in chapter 3, the following points have been indicated as the general knowledge for hydrogen peroxide and fenton's reagent treatment.

1. The compound *1* is stable without the addition of H_2O_2 under the same condition. [17]

2. The compound *1* is attacked by the active oxygen species (AOS) generated in the system by the decomposition of H_2O_2 under both hydrogen peroxide as well as fenton's reagent treatment.

3. Under the pH level higher than 11.9 (pK$_a$ value of HO•), oxyl anion radical (O$\overline{\bullet}$) considered to be the most responsible species for the degradation of compound *1*. [17, 22]

4. Hydroxyl radical (HO•) play an important role in the delignification together with oxyl anion radical (O$\overline{\bullet}$) under the reaction system with the pH lower than 11.9. [22]

5. The chain-type reactions do not propagate under employed reaction conditions due to its low oxygen pressure. [22]

6. Each radical have different reactivity on the lignin model compound where the oxyl anion radical (O$\overline{\bullet}$) preferentially attacked the compound *1* at the side-chain while hydroxyl radical (HO•) attacked both side-chain and aromatic nucleus. [17, 18, 21]

4.4.2 Results and discussion

Oxygen treatment

As shown in Table 4-2, it shows the yields of compound *1* as well as the degradation products based on both the initial mole amount as well as the mole amount of disappearing of compound *1*. All the yield of reactions products discussed are based in the mole amount of disappearing of compound *1*.

➤ TMPh system

In TMPh system, Clear peak of veratraldehyde as well as several broad product peaks (not yet been identified) was observed on the HPLC chromatogram (monitored at 280 nm) for both oxygen pressure conditions at 0.4 and 1.1 MPa. Under the oxygen pressure 1.1MPa, the yield of veratraldehyde was 28% and 26% for *1E* or *1T*, respectively, before the disappearance of TMPh. By the way, the yield was changed to 30% and 19% at the end of the reaction. Under the oxygen pressure 0.4 MPa, the veratraldehyde yield was 36% or 33% before the disappearance of TMPh and became 35% or 34% at the end of reaction. The neutral peroxyl radical (ROO•), as the main species in TMPh system, generated more frequently at the oxygen pressure 1.1 than at 0.4 MPa which resulting in the yields of veratraldehyde were lower in oxygen pressure of 1.1 compared to 0.4 MPa conditions where the formation of this veratraldehyde indicate the cleavage of carbon-carbon bond at the side-chain of compound *1*. Moreover, there are no acidic products observed and almost no guaiacol was detected under this TMPh system.

- Valc system

In Valc system, three clear product peaks (veratraldehyde, vanillin, and guaiacol) were also observed from HPLC chromatogram (at 280 nm) for both reaction conditions using 0.1 and 0.5 mol/L NaOH. Under 0.5 mol/L NaOH condition, the yield of veratraldehyde was 33% or 25% for *1E* or *1T*, respectively, before Valc disappeared and became 31% or 20% at 360 min. In the case of 0.1 mol/L NaOH, the yield was 27% or 36% before the disappearance of Valc and at the end of the reaction decrease to 16% or 17%, respectively. Vanillin was also observed as another clear peak in the HPLC chromatogram. By the way, this vanillin should not originated from compound *1* but mostly from Valc. Moreover, guaiacol was also observed with the yield of 12-18% in the reaction of *1E* and *1T*. The obtained guaiacol as the reaction products can indicate the cleavage of *β-O*-4 bond of compound *1*. As the results observed, the yield of veratraldehyde obtained in TMPh and Valc system are similar but the different was observed in the yield of guaiacol. Generally, the molecular oxygen can be directly oxidized guaiacol in the employed conditions which is the reason why there was no guaiacol observed under TMPh system. By the way, the observed guaiacol in Valc system might be due to more frequent cleavage of *β-O*-4 bond under Valc system compared to TMPh system.

TABLE 4-2: Yields of residual lignin model compound and degradation products under oxygen treatment

Reaction System			Base on initial mole of compound *1*						Base on mole of disappearing of compound *1* at 360 min.			
			Compound		Veratraldehyde		Guaiacol		Veratraldehyde		Guaiacol	
			A	B	A	B	A	B	A	B	A	B
TMPh	O₂:1.1 MPa	*1E*	75	57	7	13	0*	0*	28	30	0*	0*
		1T	81	57	5	8	0*	0*	26	19	0*	0*
	O₂:0.4 MPa	*1E*	78	66	8	12	0*	0*	36	35	0*	0*
		1T	73	61	9	13	0*	0*	33	34	0*	0*
Valc	NaOH: 0.5 mol/L	*1E*	85	77	5	7	2	0	33	30	13	0
		1T	84	75	4	5	2	0	25	20	12	0
	NaOH: 0.1 mol/L	*1E*	93	88	2	2	1	0	27	16	14	0
		1T	94	88	2	2	1	0	36	17	18	0

A: at the reaction time when TMPh (45 min. under 1.1MPa and 120 min. under 0.4 MPa) or Valc (60 min.) disappeared from the reaction system.

B: At the reaction time of 360 min.

*: negligible amount

Hydrogen peroxide and Fenton's reagent treatment

As shown in Table 4-3, the yields of compound *1* as well as the degradation products based on both the initial mole amount as well as the mole amount of disappearing compound *1* under both hydrogen peroxide and fenton's reagent system. As mentioned in chapter 3, the pH condition in this study divided into two system including high pH system (initial pH approximately more than 11.9) and low pH system (initial pH approximately lower than 11.9). Under both system, the four clear peaks (compound *1*, internal standard, veratraldehyde, and guaiacol) as well as one broad peaks (veratric acid) were observed under neutral products quantification conditions. This veratric acid broad peak became shaper and be able to analyzed with HPLC analysis using acidic product quantification condition. All the yield of reactions products discussed are based in the mole amount of disappearing of compound *1*.

➢ High pH system

Under high pH system, the yield of veratraldehyde was 12-27% at the end of the reaction (120 min.). The veratric acid was observed initially at the reaction time 30 min. and increase thereafter in any pH with the yield of 49-65% at the end of the reaction. Although, the formation of veratric acid considered to form not from compound *1* but *via* veratraldehyde due to the presence of hydroperoxyl anion (HO_2^-) by the dissociation of H_2O_2. These anions are further attacks the aldehyde group of veratraldehyde and resulting in formation of veratric acid. By the way, the total yield of these veratraldehyde and veratric acid were 68-78% at the end of the reaction which can indicate that the cleavage of carbon-carbon bond at side-chain was the main reaction mode under these conditions. These results can also confirmed the reaction selectivity of oxyl anion radical ($O^-•$), as the

most responsible species under this high pH system, that it predominantly attack compound *1* at side-chain rather than aromatic nucleus. Moreover, the guaiacol was also observed where this formation indicate the cleavage of *β-O*-4 bond, the yield of guaiacol was 18-25% at the end of reactions. By the way, the result from the initial pH 11.9 cannot be discussed due to the limited amount of the degradation of compound *1*.

➢ Low pH system

Under low pH system, the yield of veratraldehyde was shown in maximum during initial phase of reaction (10-30 min.) and then decreased into 0-5% at the end of the reaction. As mentioned in reaction description, the hydroxyl radical (HO•) became the main responsible species instead of oxyl anion radical (O$\overline{\bullet}$) under this low pH system both hydrogen peroxide and fenton's reagent treatment. This hydroxyl radical (HO•) attacks both side-chain and aromatic nucleus of compound *1* resulting in the low maximum yield obtained under this low pH system. By the way, no veratric acid was observed in any pH under this system. The most reasonable explanation is that the reaction of veratraldehyde under this low pH system is slow and does not frequently afford veratric acid. The maximum yield of guaiacol (5-10%) was observed during the reaction but at the end of the reaction no guaiacol was observed. The most responsible explanation is that the liberated guaiacol has been attacked by hydroxyl radical (HO•) resulting in low guaiacol yield was obtained both in H_2O_2 and fenton's reagent treatment.

TABLE 4-3: Yields of residual lignin model compound and degradation products under hydrogen peroxide and fenton's reagent treatment

Initial pH	Final pH	Com-pound	Base on initial mole of compound *1*				Base on mole of disappearing of compound *1* at 120 min.		
			Compound	Aldehyde	Acid	Guaiacol	Aldehyde	Acid	Guaiacol
High initial pH conditions system									
13.3	13.3	*1E*	78	6	11	5	27	49	22
		1T	76	3	13	6	13	55	25
12.8	12.5	*1E*	83	2	11	3	12	62	18
		1T	85	2	10	3	13	65	20
11.9	11.5	*1E*	94	1	4	0	17	58	0
		1T	96	1	2	0	26	58	0
Low initial pH conditions system									
11.5	7.5	*1E*	9	4	0	3	4	0	3
		1T	16	4	0	3	5	0	4
11.0	6.0	*1E*	0	1	0	0	1	0	0
		1T	1	1	0	0	1	0	0
10.5	4.0	*1E*	0	0	0	0	0	0	0
		1T	0	0	0	0	0	0	0
9.5	3.5	*1E*	0	0	0	0	0	0	0
		1T	0	0	0	0	0	0	0
5.5*	3.1	*1E*	1	1	0	3	1	0	3
		1T	1	0	0	2	0	0	2

* Fenton's reagent system

4.5 Conclusion

Under oxygen treatment, the 30-35% of veratraldehyde was formed according to the disappearing of compound *1.* This formation of veratraldehyde can be explained by the reaction selectivity of main AOS, oxyl anion radical ($O^-\bullet$), which predominantly attacked and cleaved bond at the side-chain of the lignin model compound.

For H_2O_2 and fenton's reagent treatment, the very high yield of veratraldehyde and veratric obtained under high pH system compared to several previous papers. This high yields can be explained by the reactivity of oxyl anion radical ($O^-\bullet$). By the way, under lower pH system, almost no veratraldehyde and veratric acid obtained which can be explained by the fact that hydroxyl radical (HO•) as the most responsible species under this system attacks both side-chain as well as aromatic nucleus of lignin model compound.

References

[1] Sarkanen, K. V.; Hergert, H. L. (1971). Classification and distribution. In: Sarkanen K V, Ludwing C H (ed) Lignins, Wiley-Interscience, New York, 43-94

[2] Chen, C.-L. (1992). Nitrobenzene and cupric oxide oxidations. In: Lin S Y, Dence C W (ed) Methods in lignin chemistry, Springer-Verlag, Berlin, pp 301-321

[3] Wu, G.X.; Heitz, M.; Chornet E. (1994). Improved alkaline oxidation process for the production of aldehydes (vanillin and syringaldehyde) from steam-explosion hardwood lignin, *Ind. Eng. Chem. Res.*, 33, 718-723

[4] Gu, X.; Cheng, K.; He, M.; Shi, Y.; Li, Z. (2012). La-modified SBA-15/H_2O_2 systems for the microwave assisted oxidation of Organosolv beech wood lignin, *Maderas Cienc. Technol.*, 14, 31-41

[5] Fargues C.; Mathias A.; Rodrigues A. (1996). Kinetics of vanillin production from kraft lignin oxidation, *Ind. Eng. Chem. Res.*, 35, 28-36

[6] Araujo J. D. P.; Grande C. A.; Rodrigues A. E. (2010). Vanillin production from lignin oxidation in a batch reactor, *Chem. Eng. Res. Des.*, 88, 1024-1032

[7] Fernando G.S.; Laisse, C.A.; Nelson, M.; Cesar A.M. (2007). Experimental evaluation and continuous catalytic process for fine aldehyde production from lignin, *Chem. Eng. Sci.*, 62, 18-20

[8] Deng, W.; Zhang, H.; Wu, X.; Li, R.; Zhang, Q.; Wang, Y. (2015). Oxidative conversion of lignin and lignin model compounds catalyzed by CeO_2-supported Pd nanoparticles, *Green Chem.*, 17, 5009-5018

[9] Deng, H.; Lin, L.; Sun, Y.; Pang, C.; Zhuang, J., Ouyang, P.; Li, Z.; Liu, S. (2008). Perovskite-type oxide $LaMnO_3$: An efficient and recyclable heterogeneous catalyst

for the wet aerobic oxidation of lignin to aromatic aldehydes, *Catal. Lett.*, 126, 106-111

[10]   Deng, H.; Lin, L.; Sun, Y.; Pang, C.; Zhuang, J., Ouyang, P.; Li, J.; Liu, S. (2009). Activity and stability of Perovskite –type oxide LaCoO$_3$ catalyst in lignin catalytic wet oxidation to aromatic aldehydes process, *Energy Fuels*, 23, 19-24

[11] Azarpira, A.; Ralph J.; Lu F. (2014). Catalytic alkaline oxidation of lignin and its model compounds: A pathway to aromatic biochemical, *Bio. Energy Res.*, 7, 78-86

[12] Crestini, C.; Caponi, M.C.; Argyropoulos, D.S.; Saladino, R. (2006). Immobilized methyltrioxo rhenium (MTO)/ H$_2$O$_2$ systems for the oxidation of lignin and lignin model compounds, *Bioorg. Med. Chem.*, 14, 5292,-5302

[13] Adler, E.; Lindgren, B. O.; Saeden, U. (1952). The *β*-guaiacyl ether of *α*-veratrylglycerol as a lignin model. *Svensk Papperstidn.*, 55(7), 245-254.

[14] Ibrahim, W.; Lundquist, K. (1994). Synthesis of *erythro* and *threo* forms of lignin models of the arylglycerol *β*-guaiacyl ether type. *Acta Chem. Scand.*, 48(2), 149-151.

[15] Li, S.; Lundquist, K.; Soubbotin, N. (1994). Separation of diastereomers of lignin model compounds of the 1,3-diol type as borate complexes by ion-exchange chromatography. *Holzforschung*, 48(6), 509-511.

[16] Li, S.; Lundquist, K.; Nagnus, P. (1995). Synthesis of guaiacylglycerol *β*-guaiacyl ether. *Acta Chem. Scand.*, *49*(8), 623-624.

[17] Ohmura, S.; Yokoyama, T.; Matsumoto, Y. (2012).Progress of oxidation of non-phenolic lignin moiety in an oxygen bleaching process *via* the conversion of non-phenolic into phenolic lignin moiety, *J Wood Sci.*, 58, 243-250

[18] Yokoyama, T.; Matsumoto, Y.; Meshitsuka, G. (2005). Characterization of active oxygen species under oxygen-alkali bleaching conditions, *Holzforschung*, 59, 269-275

[19] Nakagawa, A.; Yokoyama, T.; Matsumoto, Y. (2012). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions IV: Appearance of kinetic isotope effect in the reaction between methyl glucoside and deuterated methyl glucoside. *J Wood Sci.*, 58, 563-569

[20] Yokoyama, T.; Nakagawa, A.; Matsumoto, Y. (2015). Investigation of hydrogen abstraction from methyl glucoside by active oxygen species under oxygen delignification conditions part 5: Comprehensive results on experiments using deuterium labeled methyl glucosides, *J. Wood Chem. Technol.*, 35, 450-463

[21] Posoknistakul, P.; Akiyama, T.; Yokoyama, T.; Matsumoto, Y. (2016). Stereo-preference in the degradation of the *erythro* and *threo* isomers of *β-O*-4-type lignin model compounds in oxidation process: part 1: In the reaction with active oxygen species under oxygen delignification conditions, *J. Wood Chem. Technol.*, 36, 288-303

[22] Posoknistakul, P.; Akiyama, T.; Yokoyama, T.; Matsumoto, Y. (2016). Stereo-preference in the degradation of the *erythro* and *threo* isomers of *β-O*-4-type lignin model compounds in oxidation process: part 2: In the reaction with hydroxyl and oxyl anion radicals under hydrogen peroxide bleaching conditions, J. Wood Chem. Technol., 0, 1-12

Summary

Oxygen and hydrogen peroxide treatment are considered as environmentally benign chemical bleaching methods which are received significant attention nowadays. Many studies conducted the research on the reaction mechanism of the wood components, including carbohydrate and lignin, under oxygen and hydrogen peroxide bleaching conditions. Under these treatments, the degradation of pulp components are not from the reaction with molecular oxygen itself but by the active oxygen species (AOS) generated in the reaction system. The reaction mechanism of model lignin under oxygen and hydrogen peroxide bleaching conditions has been focused in many researches. By the way, there is still lacking of detail information on the effect of difference lignin structure on the delignification reactivity. The targets of this study are to obtain the basic information on the different reactivity of the AOS on both *erythro* (***E***) and *threo* (***T***) isomer of β-O-4 type lignin model compounds under oxygen and hydrogen peroxide bleaching treatments.

Oxygen bleaching conditions

In this study, three different non-phenolic β-O-4 type lignin model compounds (compound ***1***, ***2***, and ***3***) were treated under oxygen bleaching treatments. Under employed conditions, the lignin model compound were not directly attacked by molecular oxygen but by the AOS generated in the system. These AOS were produced by the reaction between molecular oxygen and co-existing phenolic compounds (TMPh and Valc in this work). The conclusions are shown in the following text.

① The difference in the structural of *β*-O-4 type lignin model compound has an effect on the stereo-preferential degradation tendency.

> Higher acidity of the *α*–hydroxy group at the side chain of *1E* compared to *1T* resulting in the dissociation occurs at the side-chain.

> The benzyl hydroxy group in compound *2* considered to be the dissociated rather than at the side chain position due to its high acidity.

② AOS profiles produced are different depends on type of co-existing phenolic compound.

> The neutral peroxy radical in TMPh system attack directly at the side-chain of compound *1E* resulting in stereo-preferential degradation of *E* isomer.

> The presence of electro-static repulsion between negatively charged *α*–alkoxide and negatively charged oxyl anion radical ($O\overline{\bullet}$) suppressed the degradation in Valc system.

③ Another reactions factor including oxygen pressure and alkalinity of the reaction system are also effect on the stereo-preferential degradation tendency.

Hydrogen peroxide bleaching conditions

Same as oxygen treatment, the degradation of lignin model compounds occurs by attacking of generated AOS on the compound. By the way, under hydrogen peroxide treatment, the AOS generated by the decomposition of hydrogen peroxide and shown pH sensitivity as shown in following text.

> At high pH, the oxyl anion radical ($O\overline{\bullet}$) is considered to be the main radical responsible for the degradation of lignin model compound.

> The hydroxyl radical (HO•.) suggested to play an important role in delignification processes under low pH.

According to the obtained results, we can suggest that the intrinsic stereo-preference of O•⁻is slightly toward the **E** over the **T** isomer. By the way, the intrinsic stereo-preference appears differently when the electrostatic repulsion exists between the O•⁻ and side-chain with the dissociated α-hydroxyl group, while HO• shows no or small stereo-preference. These results can mainly be purported to the difference in the reactivity of both AOS where O•⁻ is known to preferably attack the side-chain while HO• reacts with the aromatic nucleus as well as the side-chain. According to the difference in reactivity between those AOS, the difference in lignin structure should effect the reaction of AOS on lignin under these employed conditions.

Degradation products

Three reaction products were observed in this work including veratraldehyde, veratric acid, and guaiacol where the proportion of each product depends on the reaction conditions. Veratraldehyde found to be the main degradation product in most cases where its formation indicates that the carbon-carbon bond cleaves between the α- and β-position in the side-chain of compound. The presence of veratric acid was confirmed to form from veratraldehyde. Moreover, the formation of guaiacol shows that the β-O-4 bond of compound certainly cleaves.

List of Publications

1. <u>Pattaraporn Posoknistakul</u>, Takuya Akiyama, Tomoya Yokoyama, and Yuji Matsumoto, Stereo-preference in the degradation of the *erythro* and *threo* isomers of *β-O*-4 type lignin model compounds in oxidation process: Part 1: In the reaction with active oxygen species under oxygen delignification conditions, *Journal of Wood Chemistry and Technology,* **2016**, 36, 288-303 DOI: 10.1080/02773813.2016.1138133

2. <u>Pattaraporn Posoknistakul</u>, Takuya Akiyama, Tomoya Yokoyama, and Yuji Matsumoto, Stereo-preference in the degradation of the *erythro* and *threo* isomers of *β-O*-4 type lignin model compounds in oxidation process: Part 2: In the reaction with hydroxyl and oxyl anion radicals under hydrogen peroxide bleaching conditions, *Journal of Wood Chemistry and Technology*, **2016** DOI: 10.1080/02773813.2016.1235588

3. <u>Pattaraporn Posoknistakul</u>, Takuya Akiyama, Tomoya Yokoyama, and Yuji Matsumoto, Predominant formation of aromatic aldehyde and acid from a dimeric *β-O*-4-type lignin model compound under hydrogen peroxide bleaching conditions with a high pH level [Submitted to Journal of Wood science]

List of Conferences

2014

1. <u>Pattaraporn Posoknistakul</u>, Satoko Shimizu, Tomoya Yokoyama, Yuji Matsumoto, Quantitative difference in the rates of the β–O-4 bond cleavage between lignin model compounds with and without γ–hydroxymethyl groups during the alkaline pulping process, The 81st Pulp and Paper Research Conference of Japan, Tokyo, Japan, June 2014 [Oral presentation]

2015

2. <u>Pattaraporn Posoknistakul</u>, Tomoya Yokoyama, Yuji Matsumoto, Effect of the structural difference of β-O-4 type lignin model compounds on the reaction with active oxygen species under oxygen bleaching conditions, International Symposium on Wood Science and Technology, Tokyo, Japan, March 2015 [Poster presentation]

3. <u>Pattaraporn Posoknistakul</u>, Tomoya Yokoyama, Yuji Matsumoto, Effect of the structural difference of β-O-4 type lignin model compounds on the reaction with active oxygen species under oxygen bleaching conditions, The 82nd Pulp and Paper Research Conference of Japan, Tokyo, Japan, June 2015 [Poster presentation]

4. <u>Pattaraporn Posoknistakul</u>, Takuya Akiyama, Tomoya Yokoyama, Yuji Matsumoto, Stereo-preferential degradation of the *erythro* and *threo* isomers of

β–O-4 type lignin model compounds under oxygen delignification conditions, International Symposium on Wood, Fiber, and Pulping Chemistry, Vienna, Austria, September 2015 [Poster presentation]

5. <u>Pattaraporn Posoknistakul</u>, Takuya Akiyama, Tomoya Yokoyama, Yuji Matsumoto, Stereo-preferential degradation of the *erythro* and *threo* isomers of *β–O*-4 type lignin model compounds under oxygen delignification conditions, The 60th Lignin Symposium, Tsukuba, Japan, November 2015 [Oral presentation]

2016

6. <u>Pattaraporn Posoknistakul</u>, Takuya Akiyama, Tomoya Yokoyama, Yuji Matsumoto, Stereo-preferential degradation of the *erythro* and *threo* isomers of *β–O*-4 type lignin model compounds under hydrogen peroxide bleaching conditions and its relation to the type of major degradation products, The 61st Lignin Symposium, Uji, Japan, October 2016 [Oral presentation]

7. <u>Pattaraporn Posoknistakul</u>, Takuya Akiyama, Tomoya Yokoyama, Yuji Matsumoto, Stereo-preference of hydroxyl and oxyl anion radicals in the reaction with the *erythro* and *threo* isomers of *β–O*-4 type lignin model compound under hydrogen peroxide bleaching conditions, 5th International Symposium on Emerging Technologies of Pulping and Papermaking & 3rd International Papermaking and Environment Conference (5th ISETPP & 3rd IPEC), Guangzhou, China, November 2016 [Oral presentation]