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Characteristics of Wood Meals by Laccase Delignification*1

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ABSTRACT

On nitrobenzene oxidation of aspen, spruce, and knauf wood meals gave rise to vanilline, syrigaldehyde, p-hydroxybenzoaldehyde, vanillic acid, and other minor oxidation products. The phenolic aldehydes (p-hydroxybenzaldehyde, vanilline, and syringaldehyde) are derived from oxidative degradation of the corresponding 4-hydroxyphenylpropane units and their ethers. The lignin content of knauf wood meals was different as the concentration of NaOH solution and cooking temperature. The lignin contents of aspen, spruce, and knauf wood meals were decreased as laccase treatment. The laccase caused C-oxidation, demethylation, cleavage in phenolic groups and C-C cleavage in syrigyl structures.

Keywords : wood meals, nitrobenzene oxidation, vanilline, syringaldehyde, laccase delignification

1. INTRODUCTION

Wood is comprised primarily of roughly spindle-shaped cells. The thickened walls are composites of the three structural polymers, with contiguous cells held together by the lignin. The basic morphology of wood cell walls is determined by the cellulose, which makes up approximately 45% of the weight of wood. It is linear polymer of anhydrocellobiose units linked by β-1,4-glycosidic bonds. Van der Waal forces and hydrogen bonding interactions between and within cellulose molecules, however, make natural cellulose structually complex; the individual cellulose molecules are arrayed in bundels known as microfibrils, each of which contains approximately 40 individual cellulose molecules.[1,2] Hemicelluloses make up 25 to 30% of the weigtht of wood. Like cellulose, their backbones are linear β-1,4-linked monosaccharide polymers, but the hemicellose molecules are much shorter than cellulose molecules. Hemicelluloses have noncrystalline or only poorly crystalline, so that exist more as a gel than as orderly oriented fibers.[3] Lignin has an entirely different structure from cellulose and hemicellulose. It is a branched polymer of substituted phenylpropane units joined by C-C and C-O-C linkages. Lignin is achiral despite the presence of numerous asymmetric carbons a result of its formation via the polymerization of free radical precusors.[4]

Most of all, there are major enzymes acting directly or indirectly on lignin; ligni peroxidase (LiP), manganese peroxidase (MnP), and laccase. There is an evidence that all three

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enzymes can act with low molecular weight mediators to bring about lignin oxidation.[5-10] Especially, laccases are blue copper oxidases that catalyze the 1-electron oxidation of phenolics, aromatic amines, and other electron-rich substrates. It oxidize the phenolic units, in lignin to phenoxy radicals, which can lead to aryl-Cα cleavage.[11] Laccase catalyzes four consecutive 1-electron oxidants, then transfers the electrons to molecular oxygen, reducing it to water, and returning the enzyme to its native state. Most white-rot fungi produce laccase.[12-14] Laccase can oxidize nonphenolic lignin-related in the presence of certain auxiliary substrates; ABTS (2, 2 - azinobis - 3 - ethylthiazoline - 6 - sulfonate) is one such substrate.[15]

At using wood chips of pulping and bleaching, it is useful knauf chips besides general softwood and hardwood chips.

The goal of this experiment is compared degree of delignification with 3 different wood meals.

2. EXPERIMENTALS

2.1. Materials

Laccase was isolated from *Trametes versicolor*.[16] Aspen, spruce and knauf wood meals were from State University of New York at College of Environmental Science & Forestry, U.S.A.

2.2. Alkaline Nitrobenzene Oxidation

Samples of wood meals (0.5～2 g) equivalent to 30～100 mg of lignin placed in stainless steel autoclaves, were mixed with nitrobenzene (2.3 mL) and 2 M NaOH (40 mL). The autoclaves, after being sealed tightly with teflon gaskets and screw caps, were heated in an oil bath at 170°C for 2.5 h. The reaction mixture, after cooling to ambient temperature with cold water, was extracted twice with chloroform (10 mL×2) to remove any excess nitrobenzene and its reduction products. After adding an internal standard solution (1 mL) of 2,6-dimethoxyphenol (0.5% in dioxane), the mixture was acidified with 4 M hydrochloric acid and then extracted three times with ethyl ether (15 mL×3). The combined ether extract after drying over Na_2SO_4 was evaporated in vacuum. The resulting products after silation with BSTFA were then analyzed by GC. GC analysis were conducted on a Hewlett-Packard 5880 model equipped with a flame ionization detector and a fused silica capillary column (50 m×0.25 mm) of a 007- series bonded phase using nitrogen as a carrier gas. Temperatures at the injection and detector ports were 250 and 280°C, respectively. The temperature profile for product analysis was set initially at 160°C for 10 min followed by a gradual increase at a rate of 5°C/min to 270°C and then held there isothermally for 30 min.

2.3. Non-pressurized Oxygen-laccase Treatment

A wood meal was suspended in pH 4.5 sodium acetate buffer solution. The wood meal slurry was introduced into a 2 ℓ reactor in laccase activity 60 IU/g pulp, 2% HBT mediator and 1% consistency under room temperature with a continuous flow of O_2 through the system for 3 days. The blank sample without laccase was conducted in the same conditions.

2.4. Alkaline Extraction

Non-pressurized oxygen-laccase treated wood meals were then washed with distilled water. A portion of treated wood meal was then extracted with dilute sodium hydroxide solution using a

2.5% alkali charge and O_2 flow at 10% consistency and 80°C for 1 h.

2.5. Wood Meal Analysis

The treated wood meal was analyzed for lignin contents following the corresponding Tappi standard procedures. Phenolic hydroxyl analysis was conducted by sodium periodate oxidation method.

3. RESULTS and DISCUSSIONS

3.1. The Characteristics of Aspen, Spruce, and Knauf Wood Meals by Nitrobenzene Oxidation

As according as listed in Table 1, spruce wood meals gave rise to vanilline as the major product in a yield range of 25% based on the klason lignin. In addition, Insignificant amounts of p-hydroxybenzoaldehyde, vanillic acid, and other minor oxidation products were obtained. In contrast, aspen wood meals produced vanilline (11%) and syringaldehyde (35%) as the major products on nitrobenzene oxidation. In case of knauf wood meals, vanilline (15, 3%) and syringaldehyde (34, 14%) were the major products as like aspen wood.

On nitrobenzene oxidation, phenolic aldehydes (p-hydroxybenzaldehyde, vanilline, and syringaldehyde) are derived from oxidative degradation of the corresponding 4-hydroxyphenylpropane units and thier ethers, in particular the corresponding 4-O-alkylated and α-O-4 and β-O-4 lignin substructures, under imposed reaction conditions. Moreover, aromatic acids related to phenolic aldehydes are produced only in negligible amounts from lignins. It is well established that benzaldehyde and 4-O-

Table 1. The characteristics of wood meals

	Aspen wood	Spruce wood	Core of knauf	Bark of knauf
Moisture content(%)	6.76	7.31	6.24	6.85
Lignin content(%)	19.06	28.51	11.90	16.91
Klason lignin	18.42	27.82	11.20	16.17
Acid soluble	0.64	0.69	0.70	0.74
Phenolic OH ($mmol_{CH_3OH}$/g lignin)	0.43	0.69	0.31	0.39
Nitrobenzene oxidation*(%)				
Vanilline	11.19	25.13	15.99	3.01
Vanillic acid	0.78	2.14	1.00	0.25
Syringic acid	2.16	-**	1.59	0.55
Syringaldehyde	34.99	-	34.12	13.86
Hydroxybenzoaldehyde	0.38	0.40	0.86	
Hydroxybenzoic acid	2.83	-	-	
Alcohol-benzene extractives(%)	2.47	non-checked	2.36	2.73

*: % of klason lignin **: Trace amount

alkylated vanilline, such as veratraldehyde, readingly undergo the Canizzaro reaction under the conditions of nitrobenzene oxidation to produce benzoic acid and benzyl alcohol, and 4-O-alkylated vanillic acid and 4-O-alkylated vanillyl alcohol, respectively. Collectively, these findings indicate that Canizzaro reaction does not play an important roll during nitrobenzene oxidation of lignins. It follows that base-catalyzed cleavage of α- and β-aryl ether bonds producing the corresponding phenolate anions precedes the oxidative cleavage of C_α-C_β bonds. Because of thier stability toward alkaline hydrolysis under the test conditions, aryl-O-4 linked lignin units are resistant to nitrobenzene oxidation.[17]

3.2. The Characteristics of Knauf Wood Meals by NaOH Cooking

As according as listed in Table 2, the lignin content of knauf wood meals was different as the concentration of NaOH solution and cooking temperature. The lignin content was decreased as high concentration of NaOH solution and high cooking temperature, but phenolic hydroxyl group was inverse. As more treatments resulted in making new phenoxy radicals.

3.3. The Effect of Non-pressurized Oxygen-laccase Two Stage Treatment on Lignin Removal

The result of the two stage treatment of laccase to aspen and spruce wood meals summarized in Table 3. The lignin contents were decreased with further treatment, especially, the alkaline extraction of wood meals after first laccase treatment was more effective than non-alkaline extraction at the second stage laccase treatment. In case of phenolic hydroxyl group, initial phase was accompanied by significant reduction, where subsequent alkaline has little influence on this functional group.

Table 2. The characteristics of NaOH cooking knauf wood meals

	Yield(%)	Lignin content(%)		Phenolic OH*
		Klason lignin	Acid soluble	
Core of knauf[1]	69.7	15.99	11.01	0.59
Bark of knauf	64.4	0.12	0.30	0.42
Core of knauf[2]	60.7	15.05	10.01	1.04
Bark of knauf	57.7	0.14	0.13	0.85
Core of knauf[3]	55.0	13.16	9.43	1.17
Bark of knauf	51.8	0.07	0.08	0.92
Core of knauf[4]	53.1	11.41	7.47	1.56
Bark of knauf	50.5	0.06	0.08	0.98

*: $mmol_{CH3OH}$/g lignin

1: 0.5 N NaOH (20 mL/g wood meal), 100°C, 1 hr.
2: 1 N NaOH (20 mL/g wood meal), 100°C, 1 hr.
3: 1 N NaOH (20 mL/g wood meal), 130°C, 1 hr.
4: 1 N NaOH (20 mL/g wood meal), 150°C, 1 hr.

Table 3. The effect of non-pressurized oxygen- laccase two stage treatment on the aspen and spruce wood meals on lignin removal in different alkaline treatments

Treatment	AL	ALE	ALEL	ALELE	ALELEo	ALL	ALLE	ALLEo
Yield(%)	92.0	90.5	89.4	82.6	82.0	90.2	83.4	83.0
Lignin content (%)	16.07	14.74	13.86	12.95	12.24	15.51	14.83	13.76
	0.08	0.09	0.09	0.08	0.08	0.08	0.08	0.08
Reduction(%)	15.3	22.2	26.8	31.6	35.4	18.2	21.8	27.4
Phenolic OH*	0.24	0.21	0.20	0.17	0.13	0.23	0.21	0.20
Treatment	SL	SLE	SLEL	SLELE	SLELEo	SLL	SLLE	SLLEo
Yield(%)	92.2	91.3	90.4	87.9	85.4	86.8	83.1	82.5
Lignin content (%)	26.00	24.96	23.95	22.14	21.45	25.47	23.96	22.12
	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.01
Reduction(%)	8.7	12.4	16.0	22.3	24.7	10.6	15.9	22.4
Phenolic OH*	0.32	0.30	0.30	0.28	0.24	0.29	0.29	0.27

*: $mmol_{CH3OH}/g$ lignin
A: aspen wood meal S: spruce wood meal L: non-pressurized oxygen-laccase treated
E: NaOH extracted Eo: NaOH extracted + O_2 flow

3.4. The Characteristics of Non-pressurized Oxygen-laccase Treated of Knauf Wood Meals

It has significant reduction of lignin contents as laccase treatment on Table 4. But, the phenolic hydroxyl group was little decreased.

Vanilline and syringaldehyde were the major products on nitrobenzene oxidation. The amounts of those were little decreased as laccase treatment.

The laccase caused C-oxidation, demethylation, cleavage in phenolic groups and C-C cleavage in syrigyl structures.

4. CONCLUSIONS

This experiment gave informaions about the characteristics of wood meals, especially, knauf wood meal, by nitrobenzene oxidation and laccase delignification. It needs to further research about knauf wood for pulping and bleaching.

REFERENCES

1. Sjostrom, E. 1993. Wood chemistry : Fundamentals and applications. Academic Press, New York, p. 293.
2. Goring, D. A. 1977. In cellulose chemistry and technology : American Chemical Society, ed. J. C. Arther, Washinton D.C., p. 273.
3. Timmel, T. E. 1967. *Wood Sci. Technol.* 1: 45.
4. Adler, E. 1977. *Wood Sci. Technol.* 11: 169.
5. Eriksson, K. E. L., R. A. Blanchette, and P. Ander. 1990. Microbial and enzymatic degradation of wood and wood components: Springer Series in Wood Science, ed. T. E. Timmell. Springer Verlog, Berlin.
6. Orth, A., M. Rzhetskaya, D. 1994. Cullen, and M. Tien. *Gene.* 148: 161.
7. Hattaka, A. 1994. *FEMS Microbiology Reviews.*

Table 4. The characteristics of non-pressurized oxygen-laccase treated core and bark of knauf wood meals

	KC	KCL	KCLE	KB	KBL	KBLE
Lignin content(%)	16.75	14.14	12.67	11.82	11.17	9.61
Klason lignin(%)	16.05	13.45	11.96	11.08	10.46	8.88
Acid soluble(%)	0.70	0.69	0.71	0.74	0.71	0.73
Phenolic OH*	0.31	0.27	0.25	0.39	0.36	0.33
Nitrobenzene oxidation**						
Vanilline	7.29	7.45	7.50	2.87	3.02	3.04
Vanillic acid	0.41	0.50	0.49	0.22	0.27	0.26
Syringic acid	0.56	0.51	0.50	0.41	0.40	0.42
Syringaldehyde	13.08	12.81	12.79	11.10	10.77	10.69
Hydroxybenzoaldehyde	-	-	-	-	-	-
Hydroxybenzoic acid	-	-	-	-	-	-

*: $mmol_{CH3OH}$/g lignin **: mole%/100 g lignin -: trace amount
KC: core of knauf wood meal KB: bark of knauf wood meal
L: non-pressurized oxygen-laccase treated E: NaOH extracted

13: 125.
8. Tuor, U., K. Winterhalter, and A. Fiechter. 1995. *J. Biotechnol.* 41: 1.
9. Gold, M., and M. Alic. 1993. *Microbiol. Rev.* 57: 605.
10. Higuchi, T. 1993. *J. Biotechnol.* 30: 1.
11. Kawai, S., T. Umezawa, and K. T. Higuchi. Arch. 1988. *Biochem. Biophys.* 262: 99.
12. Srivasan, C., T. M. D'Souza, K. Boominnathan, and C. A. reddy. 1995. *Appl. Environ. Microvial.* 61: 4274.
13. Salas, C., S. Lobos, J. Larrain, L. Salas, D. Cullen, and R. Vicuna. 1995. *Biotechnol. Appl. Biochem.* 21: 323.
14. Fukushima, Y., and T. K. Kirk. 1995. *Appl. Environ. Microbiol.* 61: 872.
15. Bourbonnais, R., and M. G. Paige. 1990. *FEBS Lett.* 267: 99.
16. Slomozynski, D., Nakas, J. P., and Tanenbaum. J. P., 1995. *Appl. Environ. Microbiol.* 63: 907.
17. Chen, C. L., 1992. In Methods in Lignin Chemistry, Springer-Verlag. ed. S. Y. Lin and C. W. Dence, p. 301.