WOOD RESEARCH
58 (1): 2013
33-42

REMOVAL OF LIGNIN FROM CELL MICROREGIONS IN SWEET BAMBOO (*DENDROCALAMUS BRANDISII*) CATALYZED BY CO(SALEN). EFFECT OF COMPOSITIONS IN CATALYTIC SYSTEM

Xue-Fei Zhou, Jian-Xin Qin
Kunming University of Science and Technology
Kunming, China

Key Laboratory of Solid Waste Treatment
and Resource Recycle of Ministry of Education
Southwest University of Science and Technology
Mianyang, China

Guangxi Key Laboratory of Chemistry and Engineering of Forest Products
Guangxi University of Nationalities
Nanning, China

Guangxi Key Laboratory of Biorefinery
Guangxi Academy of Sciences
Nanning, China

(Received January 2012)

ABSTRACT

So far, apart from already known delignification of bamboo, the information available on degradation of lignin by Co(salen) catalysis cannot form the basis for understanding delignification because of differences in lignin distribution on the view of topochemistry. The present work was undertaken to elucidate topochemically the bamboo degradation by a Co(salen) catalytic system by means of scanning electron microscopy-energy dispersive X-ray analysis (SEM-EDXA) technique, and particularly focused on the relation of delignification within the cell walls of sweet bamboo (*Dendrocalamus brandisii*) to the factors that affect this catalytic oxidation such as sodium hydroxide, hydrogen peroxide, pyridine and Co(salen). Small

blocks of sweet bamboo were examined after treatment by the catalytic system. Following the catalytic oxidation, the catalytic system reacted more rapidly with cell corner (CC) lignin, while reaction with lignin in the secondary wall (S) layers appeared to be slowed by mass transfer limitations, and the residual lignin was uniformly distributed. Some compositions in catalytic system have played significant role that the delignification was increased in all microregions of bamboo cell fiber such as cell corner (CC), compound middle lamella (CML), secondary wall (S) after addition of sodium hydroxide, hydrogen peroxide, pyridine and the catalyst Co(salen) respectively. Sodium hydroxide, hydrogen peroxide, pyridine and Co(salen) produced uniform residual lignin distributions in the CC, CML and S layers.

KEYWORDS: Sweet bamboo (*Dendrocalamus brandisii*), Co(salen), secondary wall, cell corner, compound middle lamella, catalytic oxidation, delignification, SEM-EDXA.

INTRODUCTION

China is one of the key native homes and sources of modern distribution for bamboos in the world; there are more than 500 bamboo species covering over 4 million hectares. Bamboo is one of the important raw materials for industry and handicraft in south China (Liu et al. 2006, Gratani et al. 2008). Nowadays, it is a decrease in wood resources, and the new long fiber resources for pulp and paper production is pointed to non-wood plant fiber such as bamboo. Sweet bamboo is one of the bamboo species which can be more utilized in China.

Sweet bamboo is a raw material of long fiber and thin cell wall for pulp production. Shi et al. reported the approximate fiber dimensions of sweet bamboo (*Dendrocalamus brandisii*) growing in Funing County of Yunnan Province of China, such as length 2.65 mm, fiber width 15.26 μm, lumen width 14.88 μm and cell wall thickness 2.80 μm and the important chemical compositions were holocellulose 72.96 %, lignin 27.08 %, and ash content 1.12 % (Shi et al. 2009). These data predict that the sweet bamboo can be used to produce pulp with high strength like as softwood fiber.

Despite the importance of bamboo as an alternative biomass to woody plants, comprehensive research on the nature of physical and chemical characteristics of bamboo is lacking (Cao and Wu 2008, Choudhury et al. 2010), except anatomical studies (Rugolo de Agrasar and Rodriguez 2003, Zou et al. 2009, Xu et al. 2007). In the present work, *Dendrocalamus brandisii* was examined as a potential substrate for the catalytic oxidation by Co(salen) catalyst.

Co(salen) is cobalt N,N'-bis (salicylaldehyde) ethylenediamine and it has the ability to reversibly bind oxygen discovered by Tsumaki in 1938. Many cobalt(II) dioxygen carriers have been discovered since then (Simándi 2003). Some of them have properties which make them good candidates for industrial and/or medicinal applications. On the other hand, there is an increasing interest in the oxidation of lignin (Badamali et al. 2009, Haikarainen 2005) or bleaching of paper pulp (Jia and Zhou 2008, 2009) using cobalt complexes as catalysts which is a hotly pursued approach for the development of environmentally benign and ecologically sustainable chemical processes in pulp and paper industry. We report the effect of compositions in catalytic system on the removal of lignin from bamboo cell microregions (S, CC, CML) by catalytic oxidation using the Co(salen) complex as the catalyst.

Studies on the degradation characteristics of bamboos by Co(salen) will contribute to widening of the scope for application of these fast-growing grasses into pulp and paper industry.

However, studies on the degradation of bamboo by Co(salen) are limited and particularly the topochemistry in the process of catalytic oxidation, although the topochemistries on bamboo have been studied (Lybeer and Koch 2005a, b, Kim et al. 2008). Lignin is a major component of the cell wall of fibers, parenchyma cells, and vessels in woody bamboo tissue and is responsible for many mechanical properties. Therefore, the present work topochemically investigated the delignification within the cell walls of sweet bamboo fibers by the Co(salen) catalytic oxidation using SEM-EDXA technique with a particular focus on the effects of compositions in catalytic system.

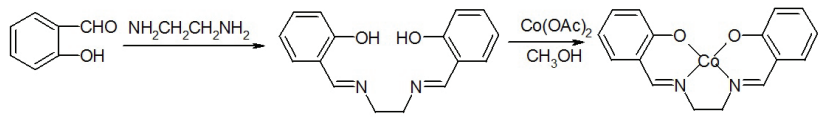
Determination of lignin distribution may be of particular importance in the introduction of new chemistries in pulp bleaching. The oxidants such as hydrogen peroxide and molecular oxygen might be expected to react quickly enough to result in non-uniform lignin distribution if mass transfer within the fiber becomes limiting especially when Co(salen) as a catalyst is used in reaction system (Argyropoulos 2001). Excessive delignification of one region of the fiber can leave the cellulose open to attack by nonselective radicals generated in bleaching stages. Knowledge of the effect of process parameters on the lignin distribution in the fibers could, therefore, be helpful in improving an established bleaching process.

MATERIAL AND METHODS

Preparation of Co(salen)

Schiff bases were prepared by the reported condensation procedures (Bozell et al. 1995). The stoichiometric amount of salicylaldehyde (2.44 g) dissolved in methanol (25 mL) was added dropwise to ethylene diamine solution (0.6 g in 25 mL methanol). The solution was refluxed for 2 h and bright yellow crystals of N, N′-ethylenebis(salicylideneamine) [salen] were obtained. The yellow crystals were separated by filtration, washed, and dried in vacuum. They were then recrystallized from methanol to obtain salen.

A mixture of methanol (50 mL) and water (20 mL) was degassed by bubbling nitrogen gas through it. Salen (1 g) was dissolved in methanol (50 mL). Cobalt acetate (0.929 g) was dissolved in 20 mL of degassed water and added dropwise into the solution of salen in methanol. The reaction mixture was refluxed at 60°C for 2 h in an inert atmosphere. It was then cooled, filtered and washed with water. The reddish brown crystals of Co(salen) were vacuum-dried. See scheme:



Bamboo samples and oxidation reaction

Bamboo culms of the species *Dendrocalamus brandisii* (48 months old) were sampled in the Funing County in Yunnan Province (China). The bamboo has been dried for more than one year in ambient condition. Such dried bamboo is widely used in practical applications. Blocks of about 1-2 cm along the grain were cut from the middle parts of the bamboo culms. Small blocks (1 × 1 × 5 mm) were cut from the sampled materials.

The oxidation reactions were carried out in 100 mL capacity three-necked glass reactor fitted with a cooling condenser. For a typical oxidation, bamboo blocks (2 g), pyridine (0.15 mL), hydrogen peroxide (0.1 mL 30 %), sodium hydroxide (6 mL 10.0 $g.L^{-1}$), and distilled

water (30.0 mL) were charged to the reactor. To this mixture, Co(salen) was added with pyridine : Co(salen) molar ratio 1:1, and the contents were stirred for 5 h at constant temperature 90°C.

Sample preparation and SEM-EDXA

Small blocks and those obtained from the oxidation reaction were subjected to washing and immersion with distilled water at room temperature for one week, and then solvent extraction with benzene/alcohol (2/1) for 24 h. The extracted blocks were dehydrated in a graded series of alcohol (30, 50, 70, 90, 95, 100 % CH_3CH_2OH) and followed by displacement in anhydrous chloroform (CH_3Cl_3) for the following bromination. The bromination was carried out according to the published methods (Saka et al. 1982). After bromination, the blocks were washed and extracted with chloroform in Soxhlet extractor to remove unreacted bromine (Br_2). The chloroform present in blocks was removed with anhydrous acetone (CH_3COCH_3) by displacement. The small air-dried blocks were measured for elemental analysis of bromine within the S, CC and CML of fiber with a L30ESEMTMP SEM-EDXA operating at 20 kV, 1×10^{-9} A. Bromine count measurements (average number in each wall layer) were made for each sample. The percentage of bromine atom can be used to estimate the lignin concentration in different cell morphological areas as bromine bound with lignin is very stable under electron beam bomdardment (Saka and Thomas 1982).

RESULTS AND DISCUSSION

Effect of catalytic reaction

The lignin distribution varies in the different fiber cell microregions of *Dendrocalamus brandisii* (Untreated sample, Fig. 1). The lignin concentration was distributed as CC > CML > S based on the BrWt % counts shown in Fig. 1. The CC contained higher concentration of lignin, which contrasted with the finding by Kim et al. Kim et al. found that *Phyllostachys pubescens* bamboo exhibited higher lignin concentration in CML (Kim et al. 2008).

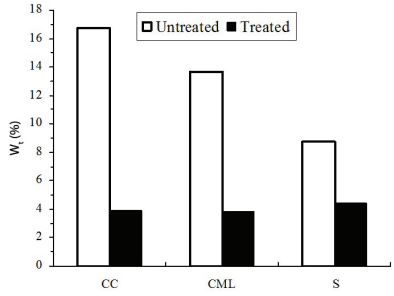


Fig. 1: Br counts for lignin in different cell morphological microregions of untreated and treated bamboo samples.

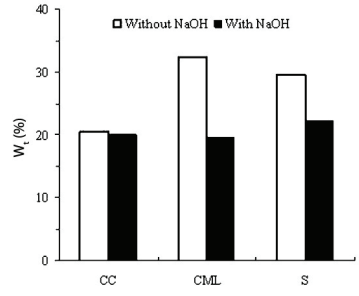


Fig. 2: Effect of sodium hydroxide addition on the removal of lignin present in cell microregions during the Co(salen) catalytic oxidation.

The topochemical analyses employed in the present work demonstrated that lignin was considerably removed during the Co(salen) catalytic oxidation. On the other hand, there were also marked differences in the process of delignification, as shown in Fig. 1, the lignin in S layers

of bamboo fiber cell had resistance against the attack by Co(salen) catalytic system as compared to CC and CML. Degradation of the lignin in S layers was mainly confined to the inner part where concentration of lignin is the lowest according to Fig. 1 as obtained by SEM-EDXA. We suggest that the higher resistance to secondary wall of fiber to Co(salen) catalytic degradation is related to the composition, concentration and spatial distribution of lignin in this cell wall layer. The secondary wall lignin in bamboo fiber was dominated by G- and S units, not by H- units (Kim et al. 2008, Higuchi 1987). It is conventionally assumed, especially for G units, that the G-type lignin is more resistant to chemical and biological treatments (including oxidation and demethylation) than the S- or H-type lignin (Filley 2002, Faix et al. 1985).

Effect of sodium hydroxide addition

It was necessary to study the effect of NaOH on the degradation of lignin in bamboo fiber cell, since the oxidation of alkyl group of phenolics proceeds only after the conversion of -OH group to its sodium phenolate salt (Kshirsagar et al. 2009). As can be seen from Fig. 2, we have observed that in this reaction the role of NaOH is significant. The extent of delignification is increased by NaOH addition.

In enzyme-catalyzed reactions the initial step of the catalytic cycle is general base-catalyzed proton abstraction of the substrate (Winkler et al. 2008, Campbell and Farrell 2009) and this could occur in our system as well. The NaOH addition is also likely to increase the amount of active cobalt-oxygen species in the solution (Kervinen et al. 2003). It should be noted that the addition of NaOH had less effect on the delignification in CC of bamboo cell fiber (Fig. 2).

Effect of hydrogen peroxide addition

By adding H_2O_2 as the source of oxygen to the reaction mixture, a higher delignification appears in bamboo cell fiber than without adding hydrogen peroxide, as shown in Fig. 3. This can be interpreted involving high-valent metal oxo intermediates formed by heterolytic cleavage of the O-O bond where H_2O_2 is the oxidant (Chellamani and Alhaji 2009).

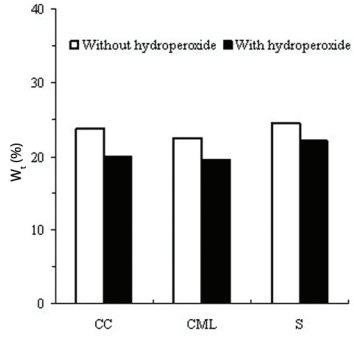


Fig. 3: Effect of hydrogen peroxide addition on the removal of lignin present in cell microregions during the Co(salen) catalytic oxidation.

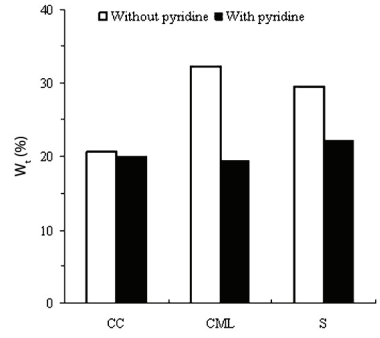


Fig. 4: Effect of pyridine addition on the removal of lignin present in cell microregions during the Co(salen) catalytic oxidation.

Effect of pyridine addition

In order to determine the dependence of the catalytic oxidation reaction on the axial ligand, the oxidation was carried out with axial ligand: Co(salen) molar ratio 1:1 using pyridine as the

axial ligand because tetradentate Schiff base catalysts like Co(salen) are known to bind oxygen most effectively when a so-called axial ligand (AL)- also binds to the cobalt producing the corresponding penta-coordinate (Rybak-Akimova et al. 1997, Smith et al. 2010, Sasaki et al. 1989). The results are plotted in Fig. 4.

Significant effect of pyridine on the delignification in CC, CML and S layers of bamboo cell fiber was found so the lignin concentration, as BrWt % showed in Fig. 4, decreased when oxidation reactions were carried out. Previous mechanistic studies of the oxidation of phenolic substrates have provided qualitative support for the significance of the axial ligand in this catalytic cycle (Rajagopalan et al. 2008).

Effect of Co(salen) addition

The catalyst-like nature of the complex is evident from the fact that the same changes are observed at addition of Co(salen) (Fig. 5) as reported for the catalase-like activity of Co(salen) complexes, in which mixing the solutions of complex and H_2O_2 produces a reactive species which is responsible for the catalase-like activity as well as for the oxidation of substrate (Chellamani and Alhaji 2009, Chavez and Mascharak 2000).

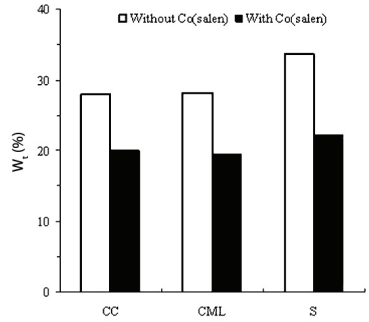


Fig. 5: Effect of Co(salen) addition on the removal of lignin present in cell microregions during the Co(salen) catalytic oxidation.

CONCLUSIONS

During the Co(salen) catalytic oxidation of small bamboo blocks the lignin concentration in various microregions of cell fiber decreases in the sequence: CC >CML > S. The effect of compositions in catalytic system on the removal of lignin in CC, CML, S layers of cell fiber was studied in the catalytic oxidation. Compositions such as NaOH, H_2O_2, pyridine and Co(salen) show a distinct positive effect on the topodelignification for the CC, CML, S layers in sweet bamboo cell fiber.

ACKNOWLEDGMENT

We acknowledge the support given by the National Natural Science Foundation of P. R. China (No. 21166011; 20766002), Key Laboratory of Solid Waste Treatment and Resource Recycle of Ministry of Education of P. R. China (No. 11zxgk07), Guangxi Key Laboratory of

Chemistry and Engineering of Forest Products of P. R. China (No. GXFC11-03), Guangxi Key Laboratory of Biorefinery of P. R. China (No. GXBF11-05), and by the Foundation of Research Center for Analysis and Measurement of Kunming University of Science and Technology of P. R. China.

REFERENCES

1. Argyropoulos, D.S., 2001: Oxidative delignification chemistry fundamentals and catalysis. Oxford University Press.
2. Badamali, S.K., Luque, R., Clark, J.H., Breeden, S.W., 2009: Microwave assisted oxidation of a lignin model phenolic monomer using Co(salen)/SBA-15. Catalysis Communications 10(6): 1010-1013.
3. Bozell, J.J., Hames, B.R., Dimmel, D.R., 1995: Cobalt-schiff base complex catalyzed oxidation of para-substituted phenolics. Preparation of benzoquinones Journal of Organic Chemistry 60(8): 2398–2404.
4. Campbell, M.K., Farrell, S.O., 2009: Biochemistry. Thomson Higher Education, Belmont.
5. Cao, Y., Wu, Y.Q., 2008: Evaluation of statistical strength of bamboo fiber and mechanical properties of fiber reinforced green composites. Journal of Central South University of Technology 15: 564-567.
6. Chavez, F.A., Mascharak, P.K., 2000: Co(III)–alkylperoxo complexes: Syntheses, structure–reactivity correlations, and use in the oxidation of hydrocarbons. Acc. Chemical Research 33(8): 539-545.
7. Chellamani, A., Alhaji, N.M.I., 2009: Kinetics and mechanism of (salen)Mn^{III} –catalysed hydrogen peroxide oxidation of diphenyl sulphides. Indian Journal of Chemistry 48A(3): 346-351.
8. Choudhury, D., Sahu, J.K., Sharma, G.D., 2010: Biochemistry of bitterness in bamboo shoots. Assam University Journal of Science and Technology: Physical Sciences and Technology 6(2): 105-111.
9. Faix, O., Mozuch, M.D., Kirk, T.K., 1985: Degradation of gymnosperm (guaiacyl) vs angiosperm (syringyl/guaiacyl) lignins by *Phanerochaete chrysosporium*. Holzforschung 39(4): 203-208.
10. Filley, T.R., 2002: Lignin demethylation and polysaccharide decomposition in spruce sapwood degraded by brown-rot fungi. Organic Geochemistry 33(2): 111–124.
11. Gratani, L., Crescente, M.F., Varone, L., Fabrini, G., Digiulio, E., 2008: Growth pattern and photosynthetic activity of different bamboo species growing in the Botanical Garden of Rome. Flora 203(1): 77-84.
12. Haikarainen, A., 2005: Metal-salen catalysts in the oxidation of lignin model compounds. Ph.D. Dissertation, University of Helsinki, Finland.
13. Higuchi, T., 1987: Chemistry and biochemistry of bamboo. Bamboo Journal 4: 132-145.
14. Jia, Y.D., Zhou, X.F., 2008: Bamboo pulp totally chlorine free bleaching with co-salen biomimetic pretreatment. China Pulp & Paper 27(1): 4-8.
15. Jia, Y.D., Zhou, X.F., 2009: Structural changes of bamboo pulp residual lignin caused by Co-salen biomimetic catalytic oxidation. Journal of Beijing Forestry University 31(4): 130-134.

16. Kervinen, K., Allmendinger, M., Leskelä, M., Repo, T., Rieger, B., 2003: In situ ATR-IR spectroscopy: A powerful tool to elucidate the catalytic oxidation of veratryl alcohol in aqueous media. Physical Chemistry Chemical Physics 5(20): 4450–4454.
17. Kim, J.S., Lee, K.H., Cho, C.H., Koch, G., Kim, Y.S., 2008: Micromorphological characteristics and lignin distribution in bamboo (*Phyllostachys pubescens*) degraded by the white rot fungus *Lentinus edodes*. Holzforschung 62(4): 481-487.
18. Kshirsagar, V.S., Garade, A.C., Mane, R.B., Patil, K.R., Yamaguchi, A., Shirai, M., Rode, C.V., 2009: Characterization of clay intercalated cobalt-salen catalysts for the oxidation of p-cresol. Applied Catalysis A: General 370(1/2): 16–23.
19. Liu, Q.F., Zhang, X.D., Zhou, J.X., Shu, H.L., 2006: Advances in research on the cold endurance of bamboo in China. World Forestry Research 19(5): 59-62 (in Chinese).
20. Lybeer, B., Koch, G., 2005a: A topochemical and semiquantitative study of the lignification during ageing of bamboo culms (*Phyllostachys viridiglaucescens*). IAWA Journal 26(1): 99–109.
21. Lybeer, B., Koch, G., 2005b: Lignin distribution in the tropical bamboo species *Gigantochloa levis*. IAWA Journal 26(4): 443-456.
22. Rajagopalan, B., Cai, H., Busch, D.H., Subramaniam, B., 2008: The catalytic efficacy of Co(salen)(AL) in O_2 oxidation reactions in CO_2-expanded solvent media: Axial ligand dependence and substrate selectivity. Catalysis Letters 123(1/2): 46–50.
23. Rugolo de Agrasar, Z., Rodriguez, M.F., 2003: Culm anatomy of native woody bamboos in Argentina and neighboring areas: Cross section. Bamboo Science & Culture, Journal of the American Bamboo Society 17(1): 28-43.
24. Rybak-Akimova, E.V., Otto, W., Deardorf, P., Roesner, R., Busch, D.H., 1997: Kinetics and equilibria of dioxygen binding to a vacant site in cobalt(ii) complexes with pentadentate ligands. Inorganic Chemistry 36(13): 2746-2753.
25. Saka, S., Whiting, P., Fukazawa, K., Goring, D.A.I., 1982: Comparative studies on lignin distribution by UV microscopy and bromination combined with EDXA. Wood Science and Technology 16(4): 269-277.
26. Saka, S., Thomas, R.J., 1982: Evaluation of the quantitative assay of lignin distribution by SEM-EDXA technique. Wood Science and Technology 16(1): 1-18.
27. Sasaki, I., Pujol, D., Gaudemer, A., Thuery, P., Claudel, R., Zarembowitch, J., 1989: Geometry and spin state effects on the catalytic efficiency of cobalt(II) complexes toward the oxidation of 2,6-di-t-butylphenol by dioxygen. New Journal of Chemistry 13(12): 843-848.
28. Shi, Z.J., Hui, C.M., Zhang, J.Y., Wu, C.H., Quin, Y.J., 2009: Research on chemical composition and fiber morphology of *Dendrocalamus brandisii*. Biomass Chemical Engineering 43(4): 21-24.
29. Simándi, L.I., 2003: Advances in catalytic activation of dioxygen by metal complexes, Kluwer Academic Publishers, Dordrecht.
30. Smith, K.M., McNeil, W.S., Abd-El-Aziz, A.S., 2010: Organometallic-mediated radical polymerization: Developing well-defined complexes for reversible transition metal-alkyl bond homolysis. Macromolecular Chemistry and Physics 211(1): 10–16.
31. Winkler, A., Lyskowski, A., Riedl, S., Puhl, M., Kutchan, T.M., Macheroux, P., Gruber, K., 2008: A concerted mechanism for berberine bridge enzyme. Nature Chemical Biology 4(12): 739-741.
32. Xu, M., Ren, H.Q., Guo, W., Li, X., 2007: Advance of researches on bamboo fiber and its cell ultrastructure. Nonwood Forest Research 25(4): 82-89.

33. Zou, L.H., Jin, H., Lu, W.Y., Li, X.D., 2009: Nanoscale structural and mechanical characterization of the cell wall of bamboo fibers. Materials Science and Engineering C 29(4): 1375–1379.

Xue-Fei Zhou, Jian-Xin Qin
Kunming University of Science and Technology
P.O. Box A302-12, Building No. 5
Xinying Yuan, No. 50,
Huancheng East Road
Kunming 650051
Yunnan Province
China
Corresponding author: lgdx602@tom.com

Key Laboratory of Solid Waste Treatment
and Resource Recycle of Ministry of Education
Southwest University of Science and Technology
Mianyang, China

Guangxi Key Laboratory of Chemistry and Engineering of Forest Products
Guangxi University of Nationalities
Nanning, China

Guangxi Key Laboratory of Biorefinery
Guangxi Academy of Sciences
Nanning, China