WOOD RESEARCH
58 (2): 2013
165-172

VARIATION IN CELLULOSE PROPERTIES IN THE COMMON PINE (*PINUS SYLVESTRIS* L.) WOOD DURING WHITE- AND BROWN-ROT DECAY INDUCED BY *CONIOPHORA PUTEANA* AND *TRAMETES VERSICOLOR* FUNGI

Piotr Witomski, Andrzej Radomski, Janusz Zawadzki
Warsaw University of Life Sciences – Sggw, Faculty of Wood Technology
Department of Wood Science and Wood Protection
Warsaw, Poland

Witold Tomaszewski
Warsaw University of Technology, Faculty of Chemistry
Warsaw, Poland

(Received November 2011)

ABSTRACT

The wood of common pine (*Pinus sylvestris* L.) was subjected to a controlled decay by two test fungi species that induced brown-rot *Coniophora puteana* (Schum.: Fr.) P. Karst. and white-rot (*Trametes versicolor* L.: Fr.) Pilát. wood decay. The effect of the fungi on cellulose chemical properties was examined. Cellulose depolimerization occurs from the start of fungal growth in the wood. During the white-rot decay the cellulose depolimerization rate is slower, as compared with the brown-rot decay. The low-molecular-weight carbohydrate content stays at a constant level, which is indicative of a uniform cellulose depolimerization process and the use of decomposition products by the fungi. In the brown-rot decay the cellulose depolimerization process is much faster, α-cellulose is produced in lesser amount, while the cellulose depolymerization products formed are low-molecular-weight carbohydrates.

KEYWORDS: Common pine, white-rot decay, brown-rot decay, cellulose, molecular mass, size-exclusion chromatography.

INTRODUCTION

White-rot decay of wood takes place largely in the forest on live and dead trees, on timber yards and forest depots, wooden structures (primarily of deciduous wood), less frequently in buildings (Grzywacz 1997, Schwarze et al. 2000, Mańka 2005). The white-rot fungi produce cellulolytic, hemicellulolytic and lignolytic enzymes. As a result, both cellulose with hemicelluloses are decomposed, as is lignin (Adaskaveg and Gilbertson 1986, Dill and Krapelin 1986, Adaskaveg et al. 1990, Eriksson et al. 1990, Adaskaveg et al. 1995).

In the initial growth stage the fungi use up the low-molecular-weight substances stored, which are accumulated in wood. Substances that undergo decomposition afterwards are hemicelluloses and celluloses of low-degree of polymerization (Krutul 1994, 1998, Krutul and Krasnodębska 1986). The compounds undergo rapid decomposition on account of their simple structure, a clearly lower degree of polymerization, as compared with α-cellulose and because of their location outside cellulose micelles.

Discrepancy of the results obtained from analyses of wood or trees decomposed in nature by white-rot fungi are indicative of a multitude of factors involved here that affect decomposition rate and pattern. Depending on the environmental conditions the same fungus species may selectively decompose lignin or simultaneously both lignin and cellulose (Adaskaveg and Gilbertson 1986, Dill and Kraepelin 1986, Adaskaveg et al. 1990, 1995). In view of a slow cellulose decomposition in the white-rot decay, the resultant variation in wood properties occurs at a slow rate.

Characteristic of the brown-rot decay is a very rapid holocellulose decomposition. Affected by decomposition process are both hemicelluloses and cellulose, the long chains of which undergo cleavage into the increasingly shorter chains to result eventually in a complete decomposition into monosaccharides utilized as nutrients. Thus the wood becomes deprived of its cellulosic skeleton and, in consequence, it gradually loses its strength and cohesion. In the last stage of the process the wood crumbles into powder.

General observation shows that carbohydrate decomposition leads to a spectacular fall in wood strength. The rate of change observed may be diverse and depends on the fungus/wood kind combination. Variation in the cellulose content observed clearly indicates a decomposition of this component to be involved (Ważny et al. 1963, Rogaliński and Kubiak 1967, Winandy and Morrell 1993), although some scientists believe only the low-molecular weight cellulose chains in the amorphous areas to undergo decomposition.

During the growth of *Gloeophyllum trabeum* in pine wood Curling et al. (2001, 2002a, b) followed chemical variations, as well as their associated changes in physical properties of wood. In the incipient stage (0 to 5 % of weight loss) the authors observed detachment of side substituents from main hemicellulose chains, and decomposition of shorter chains in hemicellulose molecules, viz. in galactan and araban. During the weight loss process in the range of 5 to 20 % not only the hemicellulose main chain was decomposing but also were all the hemicellulose-ranked components. Galactan and araban depolymerization continued, while mannan and xylan decomposition starts. According to Curling et al. (2001) cellulose decomposition was not to take place until an advanced decomposition stage, at a weight loss above 20 %. A study by Clausen and Kartal (2003) also demonstrated substantial changes in hemicellulose content at an 18 % weight loss of white pine wood caused by *Postia placenta* fungus. Weight losses of individual polysaccharides reached, respectively: 50 (araban), 34 (ramnan), 34 (xylan), 22 (galactan), and 9 % (mannan). According to the authors the glucan content stayed on the same level even during the subsequent decomposition with a weight loss that reached 26 %, which could suggest no cellulose decomposition. Nevertheless, it should be noted that the mentioned authors focused their

attention exclusively on hemicellulose analysis, ignoring the variation in cellulose itself. Hence, their views may not reflect a full picture of the effect of decomposition of wood components on wood properties.

Contradictory to this belief would be the results of a study of Flournoy et al. (1991) on the degree of polymerization of America sweet-gum (*Liquidambar styraciflua*) wood decayed by *Postia placenta* fungus, which shows a rapid fall in degree of polymerization from the initial 1200 to 900 at a weight loss as low as 5 %, 750 at a weight loss of 10 %, 500 at a weight loss of 20 %, down to a level of ca. 200 at 37 % of weight loss. As seen, the issue requires a more detailed study.

MATERIAL AND METHODS

Common pine (*Pinus sylvestris* L.) wood samples were subjected to a controlled decomposition after a modified PN-EN-113 2000 Standard procedure. The biological material used was a test white-rot fungus *Trametes versicolor* (L.: Fr.) Pilát and *Coniophora puteana* (Schum.: Fr.) P. Karst. Despite that *T. versicolor* is known to occur largely on wood of deciduous species, Cartwrigth and Findlay (1951) report occasional disease cases on conifers. In preliminary tests the ability of this fungus species for pine wood decomposition was examined.

The culture was grown on a maltose-agar medium. Samples for the study were common pine (*Pinus sylvestris* L.) wood taken from the sapwood zone. The number of the samples in each recurrent decomposition time equaled four. The wood samples were exposed to the decaying influence of the fungus for a period of one week to 12 months, and removing the successive four samples after the following scheme: for the first month - every week, for the second month - every two weeks, and for the successive months – every four weeks. This procedure allowed to obtain a series of samples with increasingly longer fungus-exposure time, thus, with a rising weight loss.

The decomposed wood samples prepared in this way were subjected to chemical analyses. In the advanced decomposition stage the wood exhibited a very low cohesion and mechanical strength. For fear of an excessive disintegration that might disturb repeatability of the results in the event of the analyses of too fine chip fractions, the wood was not comminuted in a conventional way, i.e. by mechanical cutting, grinding and screening. Shavings ca. 0.3 mm thick were cut with a plane from moistened wood blocks. The shavings were then carefully disintegrated manually into smaller fragments. On selecting suitable sample fractions for analyses (ca. 2 x 1 x 0.3 mm in size) wood (shavings) moisture content was determined by drying in a dryer to a constant weight.

Such samples were extracted with a mixture of chloroform/ethanol (93/7 % vol.) with the purpose of depriving wood of the extractives (Antczak et al. 2006). The content of cellulose was determined according to Kürschner-Hoffer method whereas α-cellulose was quantified using procedure described by Krutul (2002). The samples of cellulose separated by Kürschner-Hoffer method were dissolved and analyzed in accordance to the procedure applied by Dupont and Mortha (2004). 15 mg of cellulose was soaked in 3 ml of distilled water for 12 hours. The liquid was then decanted and the cellulose was treated twice with 2.5 ml of methanol for 45 minutes. For the final rinse a similar procedure was repeated with 2.5 ml of dimethylacetamide (DMAc) but the sample was treated with the solvent initially 45 minutes and then 12 hours. The refined cellulose was dissolved in 3 ml 8 % (w/v) LiCl/DMAc. The solution was diluted to obtain concentration 1 % (w/v) LiCl/DMAc and filtered with 0.45 μm PTFE disposable filters.

The analyses of average molecular mass of cellulose were carried out by size-exclusion chromatography (SEC). The SEC analyses were performed on Shimadzu liquid chromatograph equipped with GRAM-10.000 column and precolumn (Polymer Standard Service). The mobile

chase was 0.5 % LiCl/DMAc previously degassed in ultrasonic bath and percolated through nutsche filter (0.2 μm PTFE). The chromatographic analyses were carried out at 80°C and flow rate of 2 ml.min^{-1}. The injection volume was 200 μl. The collected data were analyzed using PSS WinGPC scientific 2.74 software and PSS Calibration program V2.99 (both from Polymer Standard Service). All used chemicals were of analytical grade and obtained commercially from different suppliers.

RESULTS AND DISCUSSION

Changes in cellulose and α-cellulose content

Values of the changes in cellulose content and quality occurring in wood decayed by fungi are reported in Fig. 1 and 2.

Cellulose underwent slow decomposition (Fig. 1) in wood decomposed by *T. versicolor* fungus. The changes were close to a linear course and were occurring at a rate of ca. 6-7 per 10 % of wood weight loss. The cellulose content variation was described by a regression eq.: $y = 0.0064x^2 - 0.9508x + 51.585$ at a coefficient of determination $R^2 = 0.9364$. The percentage of α-cellulose in the cellulose stayed at a constant level throughout the decomposition process, independent of the degree of wood decomposition and varied at a level of ca. 65 to 70 %.

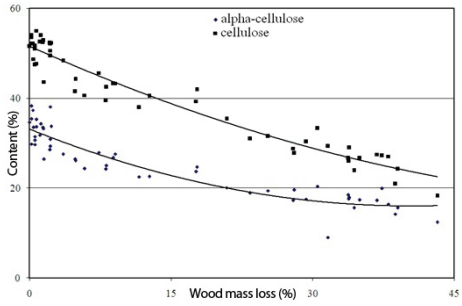

Fig. 1: The quantitative and qualitative changes of cellulose in the wood decayed by white-rot fungi.

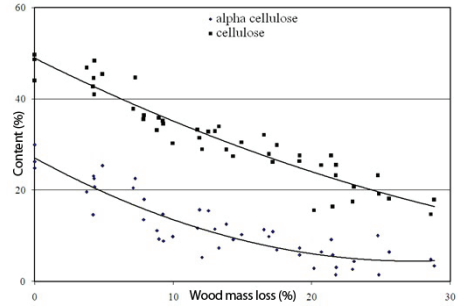

Fig. 2: The quantitative and qualitative changes of cellulose in the wood decayed by brown-rot fungi.

In the brown-rot wood decay induced by *C. puteana* fungus variation in cellulose content was of a dramatic character (Fig. 2). Already in the initial decomposition stages significant decline in cellulose content could be noted; a drop in weight by 5 % was accompanied by a drop in the content of this component by ca. 8 %, while a weight loss by 10 % gave rise to a decline in cellulose content by nearly 20 %. Towards the end of the experiment, at a 30 %-wood decomposition the cellulose content was as low as ca. 15 %. The variation was described in terms of the regression equation $y = 0.0133x^2 - 1.5117x + 48.955$, at a coefficient of determination $R^2 = 0.957$. Cellulose decomposition was accompanied by cellulose degradation. The percentage of α-cellulose in cellulose was found to vary from ca. 60 % in the incipient decomposition stages to ca. 20 % at a wood weight loss by 30 %. This relationship was described in the equation $y = 0.0304x^2 - 1.6578x + 27.12$, at a coefficient of determination $R^2 = 0.8152$.

Variation in cellulose molecular weight

Variation in cellulose molecular weight as a result of the growth of fungi is reported in Fig. 3, 4 and 5. Gradual disappearance of cellulose fraction of the highest molecular weight (of longest chains) took place during white-rot decay by *Trametes versicolor* fungus (Fig. 3) over the range of weight loss of 0.6 to 43.5 %. Also, due to the most prevalent cellulose fraction the peak was shifting at a rather slow pace. In undecayed wood the most prevalent was the fraction of a molecular weight of ca. 150 000, whereas in highly decayed wood (weight loss of ca. 50 %) the most represented was the fraction of a molecular weight of 35 000. Likewise, variation of the weight-average molecular weight of cellulose (cf. Fig. 5) followed a linear course at a rather slow pace and even at a highly advanced decomposition state it stayed at a high level. At the same time a rapid disappearance of the cellulose fractions of molecular weight over 500 000 was observed in "sound" wood (Sjöholma et al. 2000, Schult et al. 2002, Berggren 2003, Fengel and Wegener 2003, Rowell et al. 2005).

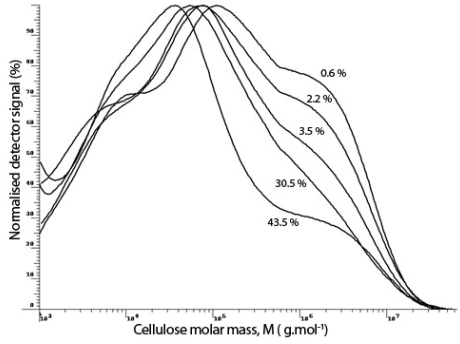

Fig. 3: Changes in the molecular-weight distribution of cellulose in the wood decayed by white-rot fungi (percentage corresponds to mass loss of wood).

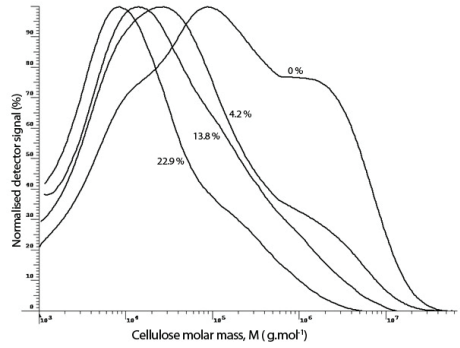

Fig. 4: Changes in the molecular-weight distribution of cellulose in the wood decayed by brown-rot fungi.

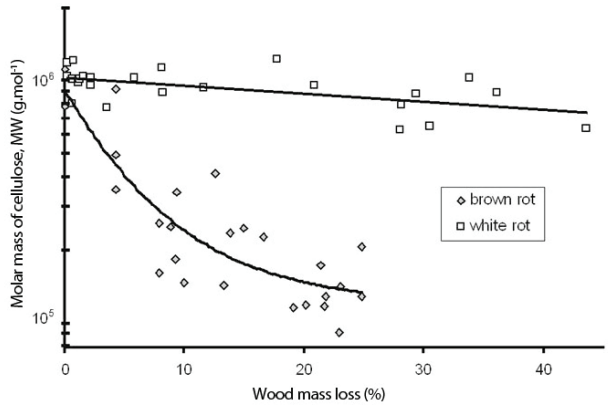

Fig. 5: Variation of weight-average molecular weight of cellulose during white- and brown-rot decay.

During the brown-rot wood decay by *Coniophora puteana* fungus (Fig. 4) as soon as the incipience of the decomposition process at a weight loss of ca. 5 % a rapid decline of the cellulose fraction of the higher molecular weight values could be observed. During subsequent decomposition at a weight loss of 20-30 % the fraction of molecular weight values of ca. 500 000 already accounted for a rather low percentage, while the most of the remaining cellulose has a degree of polymerization below 200. At the same time a rapid shift of the peak due to the most represented cellulose fraction could be observed. At a wood weight loss as low as 5 % the length of the most represented declined fivefold (the most represented fraction was the one of a molecular weight of 30 000 ("sound" wood – 150 000) and the weight-average degree of polymerization, over threefold. During subsequent wood decomposition the value changed to about 7000 at a weight loss by 25 %.

Variation of weight-average molecular weight of cellulose (cf. Fig. 5) during brown-rot decay followed an exponential course. Over the range of weight loss of 0-7 % the weight-average molecular weight became lower from 1 000 000 (DP ~ 6000) to 3 000 000 (DP ~ 1800). Observed changes were rather linear and the weight loss of cellulose chains were slower for the white-rot decay.

Hemicelluloses, cellulose and lignin undergo degradation, as follows from the study on the white-rot decay of wood. Collected data indicate that the cellulose depolymerization rate is considerably lower for white-rot decay. In the first stage, degradation involves primarily hemicelluloses, followed by cellulose, including cellulose of the highest degree of polymerization. In the brown-rot decay degradation of hemicelluloses is accompanied by degradation of cellulose. The very fact of hemicellulose depolymerization in the first stage of wood decay does not preclude simultaneous cellulose depolymerization; evidence of it is a decrease in its content as well as a lowered α-cellulose content, combined with a lowered degree of its polymerization. The results obtained coincide with the data by Flournoy et al. (1991), which suggest a decrease of the average degree of polymerization for cellulose from 1200 to 200 per a changed wood weight by 37 %.

CONCLUSIONS

Cellulose depolymerization, irrespective of the wood kind, proceeds from the onset of the fungal growth in the wood. During the white-rot decay cellulose depolymerisation proceeds at a slower rate than for the brown-rot decay. The low-molecular-weight carbohydrate content remains at a constant level, which is indicative of a simultaneous cellulose depolymerization and the use of the decomposition products by the fungus. In the brown-rot decay cellulose depolymerization proceeds violently: α-cellulose percentage declines rapidly, while the cellulose depolymerization products build up in the form of low-molecular-weight glucans. Cellulose depolymerization proceeds at a higher rate than the rate of the use of decomposition products by the fungus.

REFERENCES

1. Adaskaveg, J.E., Gilbertson, R.L., 1986: In vitro decay studies of selective delignification and simultaneous decay by the white rot fungi *Ganoderma lucidum* and *G. tsugae*. Can. J. Bot. 64(8): 1611-1619.
2. Adaskaveg, J.E., Gilbertson, R.L., Blanchette, R.A., 1990: Comparative studies of delignification caused by *Ganoderma* species. Appl. Environ. Microbiol. 56(6): 1932-1943.

3. Adaskaveg, J.E., Gilbertson, R.L., Dunlap, M.R., 1995: Effects of incubation time and temperature on in vitro selective delignification of silver leaf oak by *Ganoderma colossum*. Appl. Environ. Microbiol. 61(1): 138-144.
4. Antczak, A., Radomski, A., Zawadzki, J., 2006: Benzene substitution in wood analysis. Annals of Warsaw Agricultural University. Forestry and Wood Technology 58: 15-19.
5. Berggren, R., 2003: Cellulose degradation in pulp fibers studied as changes in molar mass distributions. Doctoral Thesis, Stockholm, 94 pp.
6. Cartwrigth, K., Findlay, W., 1951: Decomposition and conservation of wood. (Rozkład i konserwacja drewna). PWRiL, Warsaw (in Polish).
7. Clausen, C.A., Kartal, S.N., 2003: Accelerated deterioration of brown-rot decay: Comparison of soil block test, chemical analysis, mechanical properties, and immunodetection. Forest Prod. J. 53(11-12): 90-94.
8. Curling, S., Clausen, C.A., Winandy, J.E., 2001: The effect of hemicellulose degradation on mechanical properties of wood during brown rot decay. In: The International Research Group on Wood Preservation; Section 2, Test methodology and assessment; IRG/WP 01-20219, 10 pp.
9. Curling, S., Clausen, C.A., Winandy, J.E., 2002a: Experimental method to quantify progressive stages of decay of wood by basidiomycete fungi. International Biodeterioration and Biodegradation 49: 13-19.
10. Curling, S., Clausen, C.A., Winandy, J.E., 2002b: Relationships between mechanical properties, weight loss, and chemical composition of wood during incipient brown-rot decay. Forest Prod. J. 52(7-8): 34-39.
11. Dill, I., Kraepelin, G., 1986: Palo Podrilo: Model for extensive delignification of wood by *Ganoderma applanatum*. Appl. Environ. Microbiol. 52(6): 1305-1312.
12. Dupont, A.L., Mortha, G., 2004: Comparative evaluation of size-exclusion chromatography and viscometry for the characterisation of cellulose. Journal of Chromatography A 1026(1-2): 129-141.
13. Eriksson, K.-E., Blanchette, R., Ander, P., 1990: Microbial and enzymatic degradation of wood and wood components. Springer Series in Wood Science, 407 pp.
14. Fengel, D., Wegener, G., 2003: Wood chemistry, ultrastructure, reactions. Verlag Kessel, 613 pp.
15. Flournoy, D.S., Kirk, T.K., Highley, T.L., 1991: Wood decay by brown-rot fungi: Changes in pore structure and cell wall volume. Holzforschung 45(5): 383-388.
16. Grzywacz, A., 1997: Biological specific diversity of fungi decaying wood. IVth PSMB Symposium "Building objects preservation against biological corrosion and fire". Szklarska Poręba, 13-15 november. Pp 69-77 (in Polish).
17. Krutul, D., 1998: Distribution of cellulose and lignin on the cross- and longitudinal section of Scots pine (*Pinus sylvestris* L.) stem 12th Scientific Conference of Wood Technology Faculty SGGW. Pp 149-154 (in Polish).
18. Krutul, D., 1994: Variation in cellulose content in the stem of pine wood (*Pinus silvestris* L.). Annals of Warsaw Agricultural University, Forestry and Wood Technology. No. 45: 43-49.
19. Krutul, D., 2002: Exercises of wood chemistry and chosen problems of organic chemistry. Warsaw University of Life Sciences, 175 pp (in Polish).
20. Krutul, D., Krasnodębska, B., 1986: Analysis of cellulose and latewood content in the pine stem. 2nd Scientific Conference of Wood Technology Faculty, Warsaw University of Life Sciences. Pp 85-93 (in Polish).
21. Mańka, K., 2005: Forest phytopathology. VIth ed. PWRiL. Warsaw, 368 pp (in Polish).

22. Rogaliński, K., Kubiak, M., 1967: Pinewood decomposition caused by *Merulius lacrymans* Wulf., *Poria vaporaria* (Pers) Fr. and *Gloeophyllum sepiarium* (Wulf) Karsten fungi. Sylwan 12: 11-25 (in Polish).
23. Rowell, R.M, Pettersen, R., Han, J.S., Rowell, J.S., Tshabalala, M.A., 2005: Cell Wall Chemistry. In: Wood chemistry and wood composites. (Ed. R.M. Rowell). CRC Press, Boca Raton London, New York, Washington, D.C.. Pp 35-74.
24. Schult, T., Hjerde, T., Optun, O.I., Kleppe, P.J., Moe, S., 2002: Characterization of cellulose by SEC-MALLS. Cellulose 9(2): 149–158, Kluwer Academic Publishers.
25. Schwarze, F.W.M.R., Engels, J., Matteck, C., 2000: Fungal strategies of wood decay in trees. Springer Verlag, Berlin, Heidelberg, New York, 185 pp.
26. Sjöholm, E., Gustafsson, K., Eriksson, B., Brown, W., Colmsjö, A., 2000: Aggregation of cellulose in lithium chloride/N,N-dimethylacetamide. Carbohyd. Polym. 41(2): 153–161.
27. Ważny, J., 1963: Colour reactions application for the analysis of fungi decaying wood. Folia Forestalia Polonica, series B. Timbering (Drewarstvo) 5: 63–78 (in Polish).
28. Winandy, J.E., Morrell, J.J., 1993: Relationship between incipient decay, strength, and chemical composition of Douglas-fir heartwood. Wood Fiber Sci. 25(3): 278-288.

Piotr Witomski, Andrzej Radomski, Janusz Zawadzki
Warsaw University of Life Sciences – Sggw
Faculty of Wood Technology
Department of Wood Science And Wood Protection
Warsaw, Poland
Corresponding author: janusz_zawadzki@sggw.pl

W. Tomaszewski
Warsaw University of Technology
Faculty of Chemistry
Warsaw, Poland