

Miha Humar¹, Marjeta Šentjerc², Marko Petrič¹

EPR spin trapping – A new technique for observation and characterisation of free radicals during photodegradation of wood

*EPR spinske stupice – nova tehnika za
zabilježbu i razvrstavanje slobodnih
radikala tijekom svjetlosne razgradnje
drva*

Original scientific paper Izvorni znanstveni rad

Received – primljeno: 17. 06. 2003. Accepted – Prihvaćeno: 16. 07. 2003.

*UDK: 634*814; 634*813*

ABSTRACT Absorption of UV light by wood or its components promotes the formation of free radicals. These radicals induce the depolymerization of lignin and cellulose. We found Electron Paramagnetic Resonance (EPR) spin-trapping technique was a useful method to observe and characterize free radicals formed during photodegradation of wood or its components. During photodegradation of wood, using a spin trap DEPMPO three types of radicals were identified: hydroxy and carbon-centered as well as hydroperoxide species. We calculated proportions between free radical-DEPMPO adducts after UV-irradiation of wood, cellulose and brown rotted wood (as a model for lignin). As copper is known as a photo-stabilizing agent, we also investigated photodegradation of copper treated wood. Proportions between DEPMPO-radical adduct in UV-irradiated treated and untreated wood, or its components, significantly differed.

Key words: EPR spin trapping, DEPMPO, photodegradation of spruce wood, copper

¹ University of Ljubljana, Biotechnical Faculty, Department of Wood Science & Tehnology, Rožna dolina, Cesta VIII/34, SI - 1000 Ljubljana, Slovenia

² Institute Jozef Stefan, Jamova 39, SI - 1000 Ljubljana, Slovenia

SAŽETAK Upijanje ultraljubičastog (UV) svjetla u drvo ili njegove sastojke doprinosi stvaranju slobodnih radikala. Ovi radikali započinju depolimerizaciju lignina i celuloze. Pronašli smo da je hvatanje spina elektronskom paramagnetičkom rezonancijom učinkovita metoda za zabilježbu i razvrstavanje slobodnih radikala tijekom svjetlosne razgradnje drva ili njegovih sastojaka. Za vrijeme svjetlosne razgradnje drva, rabili smo spinsku stupicu DEPMPO da bismo ustanovili tri vrste novonastalih radikala: hidroksi i ugljično-centričnih vrsta, kao i hidroperoksidnih radikala. Izračunali smo omjere među DEPMPO aduktima slobodnih radikala nakon UV zračenja cjelovitog drva, celuloze i drva razgrađenog smeđom truleži, koje je poslužilo kao model za lignin. Kako je bakar isto poznat kao tvar koja usporuje svjetlosnu razgradnju, također smo ispitivali i svjetlosnu razgradnju drva zaštićenog bakrenim solima. Omjer između DEPMPO adukata radikala kod UV-osvijetljenog drva koje je bilo prirodno ili onog tretiranog, ili pak kod sastojaka drva, bitno se razlikovao.

Cljučne riječi: EPR spinska stupica, DEPMPO, svjetlosna razgradnja drva, bakar

INTRODUCTION

Wood is a naturally occurring polymer composite, composed of cellulose, hemicelluloses, lignin and extractives. Unprotected wood is susceptible to weathering and photooxidative degradation (Hon 1991). The most important reason for photooxidative degradation is a relatively narrow band of the electromagnetic spectrum of sunlight, i.e., UV radiation (Fengel and Wegener 1989, Hon 1992). Photodegradation of wood reduces performance and longevity of finishes on wood, and if surface coats are applied to surface of weathered wood, they fail too early (Evans *et al.* 1996, Schmid *et al.* 2000). Absorption of UV light by wood or its components promotes formation of free radicals. Free radicals induce depolymerization of lignin and cellulose, and the presence of oxygen facilitates the formation of radical species like hydroxy (phenoxy), carbon-centered and hydroperoxide radicals (Hon 1991, Grelier *et al.* 2000).

It is well known, that treatment of wood with copper, or copper and chromium containing formulations, can reduce photodegradation of wood surface (Feist and Williams 1991, Liu *et al.* 1994, Schmidt *et al.* 2000). Schmidt and co-workers (2000) suggested that copper(II) traps free radicals and therefore photostabilise wood. Electrons are possibly transferred from organic radical to copper, and so the concentration of free radicals in wood is reduced. However, the exact mechanisms of electron trapping are not resolved yet.

The Electron Paramagnetic Resonance (EPR) spin trapping method appears as one of the most appropriate for assessing free radical formation in biological systems. It has been successfully applied in numerous

biological systems. Nitrones have emerged as the most popular spin traps and recently the cyclic 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline *N*-oxide (DEPMPO) has received the most attention. It yields distinct and characteristic, spin adducts with O₂ and HO• radicals (Frejaille *et al.* 1995) and it is much more stable than the most frequently used spin trap DMPO. Reaction of hydroxy, carbon-centered or hydroperoxide radicals with DEPMPO produces very persistent DEPMPO-radical spin adducts. The reaction was shown to be stereo specific and roughly twice as rapid as the reaction of different radicals with DMPO (Frejaille *et al.* 1995). The lifetime of the mentioned spin trap adducts was around 45 minutes, which is significantly more than the lifetime of naturally-occurring radicals in UV-irradiated wood. Electron paramagnetic resonance is accepted and well-known method for detection but has not been used to characterize photo-induced free radicals in wood (Hon and Chang 1982, Fengel and Wegener 1989). Therefore, up to our best knowledge, our measurements are for the first time reported method for *in situ* observation and characterization of free radicals during photodegradation of wood by spin trapping technique.

MATERIAL AND METHODS

Norway spruce (*Picea abies* Karst) wood, and its components cellulose and lignin, were investigated during this experiment. Very fine wood sawdust (Mesh 150) untreated, or treated with copper(II) containing solutions, brown rotted wood (as a model for lignin) or Na-cellulose were used. BRW was obtained from untreated wood exposed to brown rott fungi (*Coniophora puteana*

Substrate	Radical type forming adducts with DEPMPO		
	HO	HOO	Carbon-centered
	Relative concentration [%]		
Wood	35.2	6.5	58.3
BRW	24.3	37.8	37.9
Cellulose	48.5	15.7	35.8
Wood + Cu(II) octanoate + ethanolamine	50.3	1.8	47.9
Wood + Cu(II) sulphate	92.8	2.7	4.5

ZIM L009 or *Gloeophyllum trabeum* ZIM L017 (Raspor *et al.* 1995)) for 16 weeks. *C. puteana* decomposed 49 % and *G. trabeum* 57 % of the initial mass of the wood. Afterwards, both BRW were mixed together and mashed into sawdust. According to the standard EN 113 (ECS 1989) spruce wood samples (1.5 x 2.5 x 5.0 cm) were vacuum impregnated with two copper based aqueous solutions; copper(II) sulphate or with copper(II) octanoate with ethanolamine ($C_{EA} = 20\%$). Concentration of copper was 0.75 %, being equal in both preparations. We let those samples to dry for 16 weeks in a dry and dark place. Afterwards, we ground them into a fine sawdust (Mesh 150). This sawdust was stored in a dark and dry place for 16 weeks to ensure that free radicals formed during the process of grounding were terminated.

For electron paramagnetic resonance (EPR) measurements, approximately 0.01 g of the sawdust was mixed with 30 μ l of aqueous solution of 0.1M DEPMPO and put into small tubes. The suspension of sawdust and DEPMPO solution was then exposed to UV irradiation ($\lambda = 280$ nm) for 1 hour. Afterwards, we put the suspension into a small glass capillary and inserted it into resonator. Then the EPR measurements started. Measurements were performed at room temperature on Bruker ESP-300 X-band spectrometer. (Microwave Frequency 9.62 GHz, Microwave Power 20 mW, Modulation Frequency 100 kHz, Center Field 341.5 mT, Field width 14.0 mT, Modulation Amplitude 0.01 mT, 4 scans) For the simulation of EPR spectra, the computer program WinSIM (Duling 1996) was used.

RESULTS AND DISCUSSION

EPR spin trapping technique was found to be a very useful method to observe and characterize free radicals formed during photodegradation of wood or its components. The comparison of experimental EPR spectra with the simulated ones has shown

that three forms of radical adducts were formed during photodegradation of untreated wood in suspension with DEPMPO (Table 1, Figure 1). They were identified as hydroxy and carbon-centered and hydroperoxide radical adducts according to their EPR parameters (Frejaville *et al.* 1995). It is exactly DEPMPO, which enabled identification of such short-lived radicals. By direct EPR measurements, not using the spin trap, we would not be able to identify various radical species. The occurrence of these radicals during photodegradation of wood is well known and documented (Hon 1991, Sundell *et al.* 2000, Grelier *et al.* 2000). However, the proportions and ratios between them have not been discussed yet. In our experiment, proportions between these radical adduct were calculated by computer simulation of EPR spectra (Figure 1A). During photodegradation of wood, EPR signal of carbon-centered radical adducts was the most intensive, and more than 58 % of EPR signal was assigned to this radical adduct. Second the most abundant radical adduct was the hydroxy radical-DEPMPO adduct (35 %), while hydroperoxide radical adduct species were the least abundant (6 %) (Table 1). Our observations and cited literature indicate that carbon-centered radicals are more stable than the hydroxy ones (Fengel and Wegener 1989, Hon 1991, Grelier *et al.* 2000). The statement of Grelier and coworkers (2000) also suggested, that less stable R-OH \cdot radicals were changed into more stable and therefore more abundant carbon-centered radicals. In wood that was not exposed to UV light, we could not detect any free radicals. This may be explained by the well-known fact, that wood, stored in dark and dry places almost does not contain any intrinsic free radicals (Fengel and Wegener 1989).

Relative concentrations of free radical adducts in photo-degraded BRW (Brown Rotted Wood) and cellulose differ from those in photo-degraded wood. The major difference between BRW and wood was observed in the ratio between carbon-centered radical

Table 1.

Portions of DEPMPO free radical adducts in wood or its components, after one-hour irradiation with UV light, as calculated from the best fit of computer simulated to the experimental EPR spectra. Udjeli DEPMPO adukata slobodnih radikala u drvu i njegovim sastojcima, nakon jednosatnog ozračivanja UV svjetlošću, izračunati prema najboljem poklapanju eksperimentalnih s kompjuterski simuliranim EPR spektrima.

Figure 1.

EPR spectra of DEPMPO-radical adducts in wood after 1^h irradiation with UV light ($\lambda = 280$ nm) (A): the experimental spectrum – a thin gray line, and the simulated EPR spectrum – a thick black line. The best fit was obtained as a superimposition of the spectral components of different DEPMPO-adducts, shown in B. EPR spinski spektri DEPMPO – radikal adukata u drvu nakon jednosatnog zračenja UV svjetlom ($\lambda = 280$ nm) (A): eksperimentalni spektar je tanka sival inija, a simulirani EPR spektar je debela crna linija. Najbolje poklapanje je postignuto nadograđivanjem spektralnih komponenti različitih DEPMPO adukata, prikazanih u B.

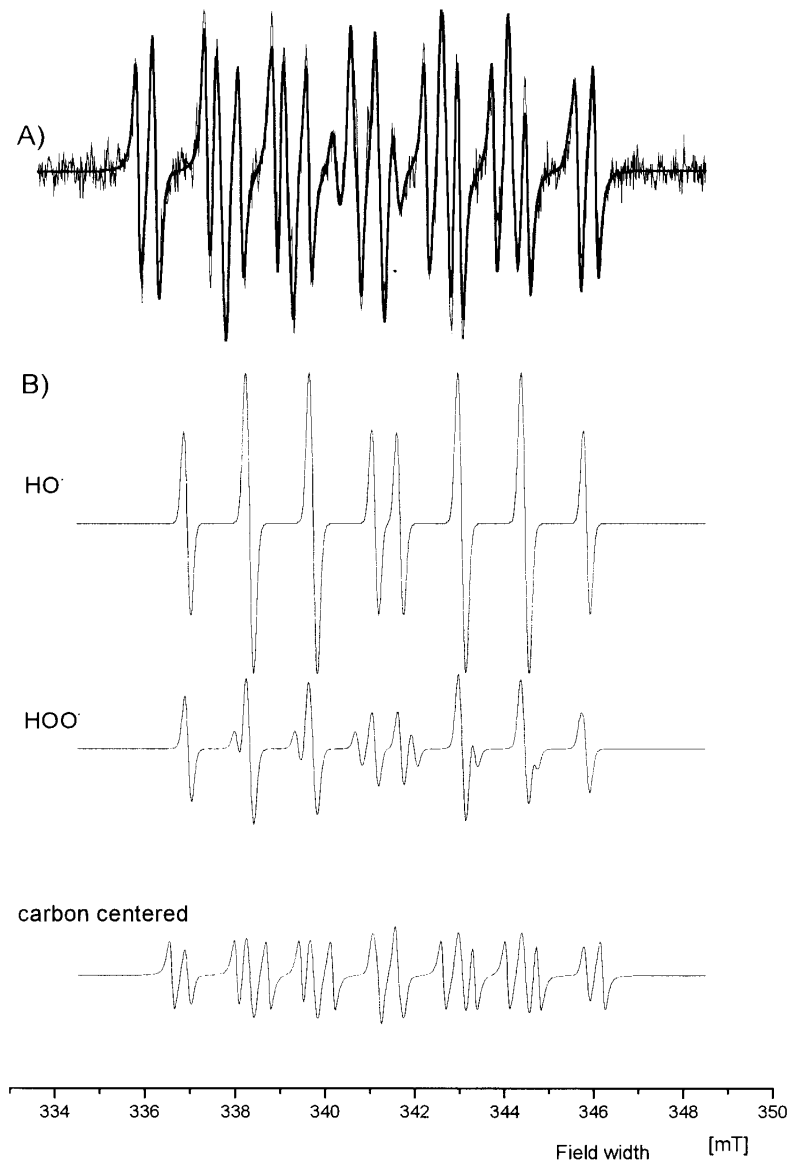
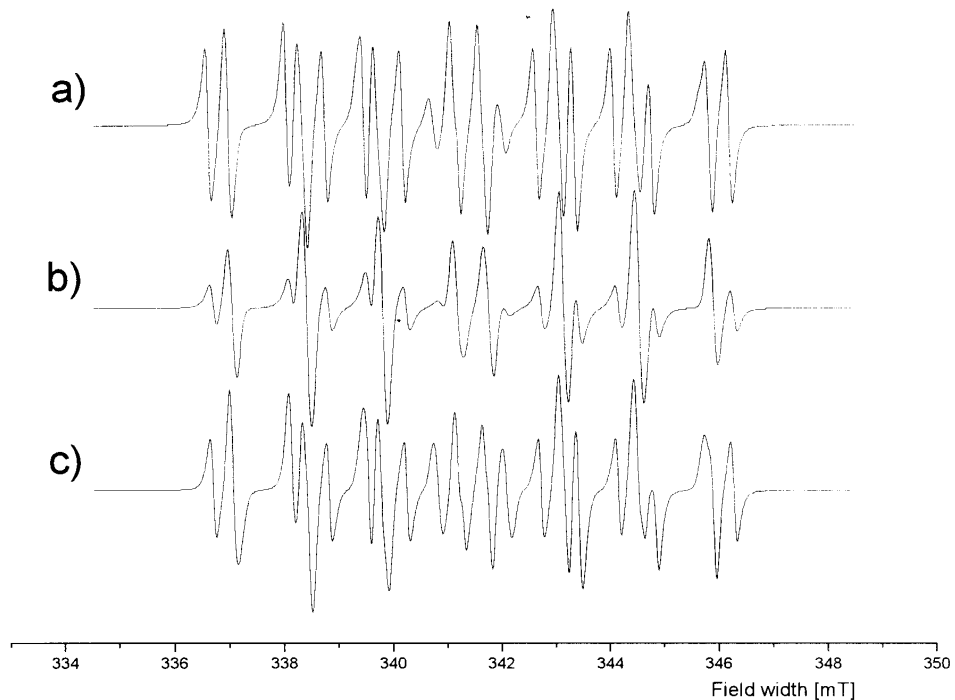


Figure 2.

Simulated EPR spectra of DEPMPO free radical adducts after 1 hour irradiation of wood with UV light in wood (a), cellulose (b) and brown rotted wood (c). Simulirani EPR spektri DEPMPO adukata slobodnih radikala nakon jednosatnog ozračivanja drva UV svjetlošću za drvo (a), celulozu (b) i drvo razgrađeno smodom truleži (c).



adducts and hydroperoxide radical adducts. While the relative concentration of carbon-centered radical adducts in wood was higher than in BRW (58 % and 38 % respectively), the relative concentration of hydroperoxide radical adducts increased from approximately 6 % in wood to 38 % in BRW (Table 1, Figure 2). The proportion of hydroxy radical adducts in cellulose had a value of 49 %, while the relative portions of hydroperoxide radical adducts was about 16% and the relative portion of carbon centered radical adducts remained almost the same as in BRW (Table 1, Figure 2). We presume that the reason for these differences originates from completely different photodegradation mechanisms of cellulose and BRW (Hon 1991). Grelier and co-workers (2000) proposed that, in a lignin system, during exposure to UV and in the presence of oxygen, R-OH[•] radicals are formed first. These radicals are rather unstable (Sundell *et al.* 2000) and are subsequently changed into carbon-centered radicals on the third or fifth position of benzene ring (Fengel and Wegener 1989, Grelier *et al.* 2000). Finally, from these radicals quinone is formed, (Leary 1968) and the surface of photo degraded wood changes color. It is believed, that, during the process of photodegradation of cellulose, free radicals are important intermediates as well. When cellulose is subjected to UV light, the glycosidic linkages are cleaved (Hon 1991). Therefore, the formation of free radicals is located at the C-1 and C-4 position (Hon, 1976). In general, hydroxy radicals generated in cellulose, are more stable as com-

pared to carbon-centered radicals because the carbon-centered radicals readily undergo secondary termination reactions (Hon 1991). Thus, this could explained the observed higher relative portion of hydroxy radical adducts in cellulose in our experiment and relatively lower concentration of carbon centered radical adducts compared to wood or lignin.

It is well known that the presence of copper can protect wood surface to some extent against photodegradation (Feist and Williams 1991, Liu *et al.* 1994). From our results (Figure 3, Table 1) it can be seen that, in the presence of copper(II) sulphate, mainly hydroxy radical adducts were formed (93 %), and only 3 % of spectra were assigned to hydroperoxide radical adduct species and 5 % to carbon-centered radical adducts. Similar, but less significant reaction occurred also in wood, treated with Copper(II) octanoate with ethanolamine (Table 1, Figure 3). Thus formation of carbon-centered radical adducts was somehow prevented. Copper(II) maybe trapped free electrons during fragmenting of hydroxy radicals into carbon-centered radicals. The other possibility is that copper could provide resistance against UV-degradation by blocking free phenol groups, which are the reactive sites for photochemical reactions (Ross and Feist 1993, Grelier 2000) Copper(II) sulphate is more likely to adsorb on these groups than copper(II) octanoate with ethanolamine (Petrič *et al.* 2000), and consequently the observed proportions between radical adducts were different.

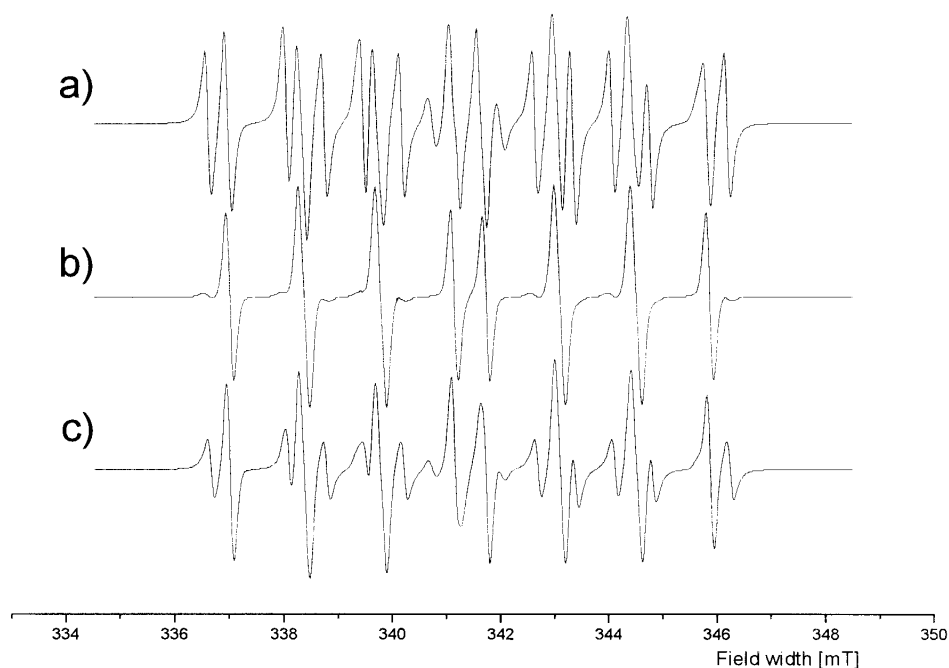


Figure 3.

Simulated EPR spectra of DEPMPPO free radical adducts after 1 hour irradiation with UV light in untreated wood (a), wood, treated with the aqueous solution of copper(II) sulphate (b) or in wood, treated with the aqueous solution of copper(II) octanoate with ethanolamine ($C_{Cu} = 0.75\%$). Simulirani EPR spektri DEPMPPO adukata slobodnih radikala nakon jednosatnog ozračivanja UV svjetlošću za netretirano drvo (a), za drvo tretirano vodenom otopinom bakrovog (II) sulfata (b), te za drvo tretirano vodenom otopinom bakrovog (II) otkanoata s etanol aminom ($C_{Cu} = 0.75\%$).

CONCLUSIONS

The electron paramagnetic resonance (EPR) spin trapping method seems to be suitable technique for observation and characterization of free radicals involved in the photodegradation of wood or its components.

We identified three forms of DEP-MPO-radical adducts as a result of UV irradiation of wood or its components; hydroxy and carbon-centered radical adducts as well as hydroperoxide radical adducts. Proportions between these radical adducts in UV irradiated wood, cellulose and lignin significantly differ. This confirmed the already reported different mechanism of photo-degradation of cellulose.

It seems that treatment of wood with copper(II) may prevent forming of carbon-centered and hydroperoxide radicals, while we observed significant proportion of hydroxy radical adducts in copper(II) treated wood. We presume that copper(II) may trap free radicals during fragmentation of hydroxy radicals into carbon-centered radicals.

LITERATURE

1. Duling D. 1996. EPR calculations for MS-Windows NT, 95. Version 0.96. National Institute of Environmental Health Sciences. Public software tools.
2. European Committee for Standardization. 1989. Wood preservatives; Determination of the toxic values against wood destroying basidiomycetes cultured an agar medium, EN 113. Brussels, 32.
3. Evans P.D., Thay P.D. and Schmalzl K.J. 1996. Degradation of wood surfaces during natural weathering. Effects on lignin and cellulose and on the adhesion of acrylic latex primers. *Wood Science and Technology*, 30: 411 - 422.
4. Feist, W.C., Williams, R.S. 1991: Weathering durability of chromium-treated southern pine. *Forest Products Journal*, 41(1): 8-14.
5. Fengel, D., Wegener, G. 1989: *Wood; Chemistry, Ultrastructure, Reactions*. Walter de Gruyter, Berlin, New York: pp: 345-372.
6. Frejaville, C., Karoui, H., Tuccio, B., La Moigne, F., Culcasi, M., Pietri, S., Lauricella, R., Tordo, P. 1995: 5-(Diethoxyphosphoryl)-5-methyl-1-pyrrolone *N*-Oxide: A new efficient phosphorylated nitron for the *in vitro* and *in vivo* spin trapping of oxygen-centered radicals. *Journal of Medicinal Chemistry*, 38, 258-265.
7. Grelier, S., Castellan, A., Kamdem, D.P. 2000: Photoprotection of copper-amine treated pine. *Wood and fibre science*, 32: 196-202.
8. Hon, D.N.S. 1976: Fundamental degradation processes relevant to solar irradiation of cellulose: ESR studies. *Journal of Macrobiological Science – Chemistry, A* 10: 1175-1185.
9. Hon, D.N.S. 1991: Photochemistry of wood. *In: Wood and Cellulosic Chemistry*. Eds. D.N.S. Hon, N. Shiraishi. Marcel Dekker, Inc. New York. pp. 525-555.
10. Hon, D.N.S., Chang, S.T. 1982: Participation of singlet oxygen in the photo degradation of wood surfaces. *Wood Science and Technology*, 16: 193-201.
11. Hon, D.N.S., Feist, W.C. 1992: Hydroperoxidation in photoirradiated wood surfaces. *Wood and Fibre science*, 24: 448-455.
12. Leary, G. 1968. Photochemical production of quinoid structures in wood. *Nature*, 217:672-673.
13. Liu, R., Ruddick, J.N.R., Jin, L. 1994: The influence of copper (II) chemicals on the weathering of treated wood, Part 1. ACQ treatment of wood on weathering. The International Research Group on Wood Preservation, Document IRG/WP/94-30040, 9.
14. Petrič, M., Murphy, R.J., Morris, I. 2000: Microdistribution of some copper and zinc containing waterborne and organic solvent wood preservatives in spruce wood cell walls. *Holzforschung*, 54, 23-26.
15. Raspor, P., Smole-Možina, S. Podjavoršek, J. Pohleven, F. Gogala, N. Nekrep, F.V. Rogelj, I. and Hacin. J. 1995: ZIM: zbirka industrijskih mikroorganizmov. Katalog biokultur; Biotehniška fakulteta, Katedra za biotehnologijo, Ljubljana: 98.
16. Ross, A.S., Feist, W.C. 1993: The effects of CCA-treated wood on the performance of surface finishes. *Proceedings of American Wood Preservation Association AWWA*, 1-15.
17. Schmid, S., Webster, R.D., Evans, P.D. 2000: The use of ESR spectroscopy to assess the photostabilising effects of wood preservatives. The International Research Group on Wood Preservation, Document IRG/WP 00-20186, 9.
18. Sundell, P., de Meijer, M., Miltz, H. 2000: Preventing light induced degradation of wood by acetylation: a study on lignin and lignin model compounds. *Woodcoatings. Challenges and solutions in the 21st Century*. Paper 22, 17.