WOOD RESEARCH
52 (1): 2007
1-12

PRE-TREATMENT OF SPRUCE CHIPS BY FUNGI WITH AIM TO IMPROVE THE PULP PROPERTIES

Ladislav Reinprecht, Rastislav Solár, Anton Geffert, František Kačík
Faculty of Wood Sciences and Technology, Technical University in Zvolen, Slovakia

ABSTRACT

Spruce chips applied for a pulp production are in the first place stored, usually 1 or 2 months. In this time their molecular structure can be changed due to atmospheric and bio-deterioration processes, and some of the occurring changes may have a positive effect on the pulp quality. To estimate some principles of the biotic effects in a natural pile, in the presented work, spruce chips have been subjected to model degradation processes caused by selected white-rot fungi: *Phanerochaete chrysosporium, Heterobasidion annosum, Onnia circinata* or *Climacocystis borealis,* and the brown-rot fungus *Fomitopsis pinicola*, under laboratory conditions during 3 or 6 weeks. The aim of our experiment was to find advisable storage conditions of chips to achieve satisfactory acid sulphite pulp quality. 3-week degradation of chips by *Phanerochaete chrysosporium* did not influence the yield and strength of pulps markedly, and brought a benefit of reduced Kappa number, reduced contents of lipophilic compounds and diminished the amount of rejects.

KEY WORDS: Spruce, chips, storing, wood-destroying fungi, chemical structure of wood, pulp

INTRODUCTION

Biotechnology has a wide potential for gradual introduction in the pulp and paper industries. Its application may improve the economy of wood processing to paper providing benefits in mechanical and chemical pulping, in bleaching steps, conversion of organic compounds in spent liquors, and in waste water cleaning, as well.

Laboratory and pilot plant application of white-rot fungi for the pre-treatment of raw materials confirmed marked energy savings in production of mechanical pulps (MP, TMP, CHMP) or in refining of chemical pulps (Setliff et al. 1990, Akhtar et al. 1992, Messner and Srebotnik 1994). The energy savings in mechanical disintegration of fungally pre-treated raw materials and refining of chemical pulps can be attributed to so called "bio-pulping effect" resulting from the swollen, softened and partly delignified cell walls of a substrate after biodegradation. In this respect, the application of lignin-selective fungi (e.g. strains of *Ceriporiopsis subvermispora*), and short time of pre-treatment connected with a minimal weight loss of the raw materials, seems to be more suitable (Messner and Srebotnik 1994, Reinprecht and Solár 1998).

The combination of bio-pulping with sulphite and sulphate processing of wood improved mechanical properties and diminished Kappa numbers of pulps (Oriarian et al. 1990, Messner et al. 1993, Messner and Srebotnik 1994, Wolfaard et al. 1996), however often at the expense of their optical properties (Oriarian et al. 1990, 1991). According to our experience, the diminished Kappa numbers of pulps from hardwoods pre-treated by white rot fungi and cooked by alkaline processes (kraft, NSSC) result more probably from their partial biotic delignification than from the increased rate of abiotic delignification (Solár et al. 2001a). However, the pre-treatment of some softwoods and hardwoods by ascomycete *Ophiostoma piliferum* (not degrading lignin) increased markedly the efficiency of both kraft and sulphite delignifications and improved the pulps properties (Wall et. al. 1996). In this case also the diminished consumption of cook chemicals was reported due to their better penetration into extractive-less wood. Pre-treatment of hard woods by white-rot fungi followed by cooking in acidic media (sulphite and organosolv processes) resulted in Kappa reduction and often in an increase in the rate of wood delignification in its first phase (Ferraz et al. 1996, Solár et al. 1998, 2001a). The increased rate of delignification renders the possibility of reduction of pulping time or pulping temperature.

The released ultrastructure of wood degraded by white-rot fungi, its increased porosity and permeability may undoubtedly improve the wood penetration with cook media, and thus accelerate its chemical delignification (Ferraz et al. 1996, Solár et al. 2001b).

Some strains of fungi were also tested in removing of fats, terpenoids and other interfering compounds prior to pulping of the conifers (Fischer et al. 1996, Koehler et al. 1996, Wall et al. 1996, Martinez-Iñigo et al. 1999). The diminished contents of lipophilic extractives eases the process of pulp sheet formation and improves properties of the product. In this respect, white-rot fungi, e.g. *Ceriporiopsis subvermispora, Phanerochaete chrysosporium, Trametes versicolor,* and also some brown-rot, soft-rot and sapstain fungi were effective.

Enzymes – lignin peroxidase, manganese peroxidase, laccase, etc. used in pre-bleaching step caused a deep removal of residual lignin from pulps, thus diminishing the consumption of bleaching chemicals in the process (Egan 1985, Viikari et al. 1987). A weak point of isolated enzymes is their sensitivity to environment and often need of the addition of mediators (Call and Mücke 1996). Some biomimetic systems based on metaloporfyrines (Cui 1990, Nagakava et al. 1989) and semisynthetic oxalate/manganese/peroxidase systems are more effective delignification agents as natural peroxidases (Shimada 1996). Recently various complex moieties with oxidative properties are tested for pulps and wood delignifications. Complexes based on copper, hydroperoxides and heterocyclic amines showed extremely high delignification efficiency. The systems, similar to the latter one, are supposed to be active in the initial stages of white-rot by lignin-selective strains of fungi (Messner et al. 2003).

In natural piles, at non-sterile conditions, the chips are often attacked by various wood-destroying fungi and moulds. For example, in big piles of spruce chips in the pulp factory Biocel Paskov – Czech Republic the following fungi *Heterobasidion annosum, Onnia circinata (syn. Onnia leporina, Inonotus leporinus), Stereum sanguinolentum, Armillaria ostoyae, Trichoderma cf. viride, Phellinus pini var. abietis (syn. Phellinus chrysoloma, P. abietis), Gloeophyllum odoratum (syn. Anisomyces odoratus, Osmoporus odoratus, Trametes odorata), Climacocystis borealis (syn. Leptoporus borealis)* and *Fomitopsis pinicola (syn. Fomes marginatus)* have been repeatedly identified (Reinprecht and Solár 2002).

The aim of this contribution was to estimate, in a model experiment, the influence of time of storing the spruce chips in the presence of selected fungi on their degradation and on the quality of pulp produced by acid sulphite process in the presence of magnesium ions.

MATERIAL AND METHODS

Spruce chips

Fresh commercial sawmill chips of spruce wood (*Picea abies* Karst. L.) were obtained from the pulp factory Biocel Paskov. Their dimensions varied within the range 15-25 mm (length) and 3-10 mm (width). Chips, before their intentional decay, were divided into 80 comparable charges, each of approximately 15-18 g, than sterilised in dry kiln at 103 °C during 6 hours, and weighted in the oven dry state (m_0).

Intentional decay of spruce chips in laboratory conditions

Individual charges of spruce chips were moisturised with sterilised distilled water to a moisture content of 40-50 %, and inserted into Kolle's flasks for an intentional decay by white-rot fungi *Heterobasidion annosum*, *Onnia circinata, Climacocystis borealis, Phanerochaete chrysosporium* (K-3), or with the brown-rot fungus *Fomitopsis pinicola.* Chips (16 charges for each fungus) were placed directly onto the mycelium of fungus grown in flasks on the malt-agar cultivating soil. Biodegradations lasted 3 or 6 weeks at the temperature of 30 ± 1 °C, with the aim to model natural storage conditions. After decay processes the charges of chips were taken out from the flasks, deprived of mycelia, pre-dried 2 days under laboratory conditions and than dried in a dry kiln at 103 °C to the oven dry state (m_1).

Evaluation of the intentional decay processes in spruce chips

The rot of spruce chips has been evaluated by the following criteria:

- mass loss $\Delta m = /m_0 - m_1/ : m_0$,
- acetone extract (8 hours),
- HPLC analyses of triglycerides in acetone extract (Kačík and Solár 1999),
- benzene-ethanol extract (2:1, 8 hours),
- cellulose content determined by the modified method of Kűrschner and Höffer - (Kačík and Solár 1999),
- lignin content (by Tappi standard, T-13m method).

Pulps preparation

Sound and intentionally biodegraded spruce chips were submitted to acid sulphite (Magnefite) pulping. Laboratory cooks were carried out under the same conditions in 6 stainless Hägglund autoclaves (V = 750 ml). Autoclaves were immersed into an oil bath equipped with a programmed mode of heating and a drive gearing mechanism. Conditions of pulping were as follows:

- total SO_2 in the liquor 7.0 %
- wood to liquor ratio 1 : 3.8
- time to reach the temperature of 105 °C 30 min
- heating at the temperature of 105 °C 60 min
- time to reach the pulping temperature of 140 °C 45 min
- pulping at the temperature of 140 °C 170 min

Characteristics of the pulps

- screened yield and amount of rejects (gravimetric determination)
- Kappa number (according to the standard ISO 302)
- brightness (expressed in % of the value for MgO as a standard at λ = 457 nm)
- breaking length (according to the standard STN 50 0340)
- tear index (according to the standard ISO 5270)

Sheets of pulps were prepared from unbleached pulps refined to approximately 30 °(SR), following the standard ISO 5269-2.

RESULTS AND DISCUSSION

Intentional decay of spruce chips was accompanied by the mass losses depending on the duration of fungal attack and fungus used (Tab. 1). The short-term rot of chips led only to their moderate weight loss. Prolonged time of biodegradation was accompanied by more apparent weight loss of a substrate. The highest drop in the weight of chips (Δm ≅ 14-15 %) caused their 6-week degradation by *H. annosum* and *F. pinicola*. The least active in this regard was the white-rot fungus *C. borealis* causing only 3 % sample weight reduction.

Tab. 1: Mass losses of spruce chips (Δm) resulting from their model decay by selected fungi

Chips attacked by the fungus:	3 weeks		6 weeks	
	Δm (%)	v (%)	Δm (%)	v (%)
Phanerochaete chrysosporium	1.69	17.9	6.45	24.3
Heterobasidion annosum	2.27	21.2	13.85	23.2
Onnia circinata	1.34	22.0	4.34	29.8
Climacocystis borealis	N		2.96	41.0
Fomitopsis pinicola	N		14.97	13.4

n - (number of charges) = 8, v - coefficient of variation , N - one or more charges were infected with moulds

The amounts of extractives in fresh and by fungi attacked spruce chips varied due to fungus applied and medium used for the extraction (Tab. 2). Bio-attack increased the contents of benzene-ethanol and acetone extracts in the spruce chips, with exception of benzene-ethanol extract determined in chips degraded with *P. chrysosporium*. Increased contents of extractives in the degraded spruce chips may be explained by formation of non-volatile low molecular weight degradation products of wood during its rotting.

Tab. 2: Benzene-ethanol and acetone extracts from the intentionally decayed spruce chips

Chips attacked by the fungus:	Benzene-ethanol extract (%)		Acetone extract (%)	
	after 3 weeks	after 6 weeks	after 3 weeks	after 6 weeks
Phanerochaete chrysosporium	0.50	1.16	0.84	0.98
Heterobasidion annosum	2.44	2.15	1.38	1.17
Onnia circinata	2.13	2.21	1.03	1.43
Climacocystis borealis	-	1.64	-	1.02
Fomitopsis pinicola	-	3.97	-	0.79

(Each result is average of 2 analyses)

Note: Extracts from the fresh chips: Benzene-ethanol = 1.26 %, Acetone = 0.43 %

From the viewpoint of paper production more significant than contents of semi-polar and polar extractives is the content of lipophilic compounds in the examined series of chips. From this reason the high resolution gas chromatography (HRGC) of acetone extracts was performed. The obtained data are presented in Tab. 3. The 6-week model decay of spruce chips by selected fungi resulted in much deeper removal of lipophilic compounds. The drop in the contents of fatty acids ranged from 43 to 69 %, and reduction in contents of sterol-esters and triglycerides ranged from 66 to 90 %. The least efficient in this respect were white-rot fungus *O. circinata* and brown-rot fungus *F. pinicola*. Reduction of lipophilic compounds in chips due to their natural long-term storing in a pile was observed in a practice, as well. The combined biotic and abiotic degradation of lipophilic compounds in medium-term storing of the chips of conifers is beneficial from the viewpoint of pulp sheets preparation (Reinprecht and Solár 2002).

Tab. 3: The amount of fatty acids, esters of sterols and triglycerides in intentionally decayed spruce chips (expressed in mg / 100 g of the oven dry chips)

Chips attacked by the fungus:	Fatty acids (mg/100g)		Esters of sterols and triglycerides (mg/100g)	
	after 3 weeks	after 6 weeks	after 3 weeks	after 6 weeks
Phanerochaete chrysosporium	104.4	55.2	2.8	2.7
Heterobasidion annosum	134.8	55.5	13.0	4.5
Onnia circinata	123.2	102.2	8.2	6.5
Climacocystis borealis	-	63.5	-	2.0
Fomitopsis pinicola	-	99.1	-	2.1

(Each result is average of 2 analyses)

Note: Extracts from the fresh chips: Fatty acids = 178.2 mg / 100 g, Esters of sterols and triglycerides = 19.4 mg / 100 g

Intentional decay of spruce chips led to partial degradation and removal of all basic wood components. From practical point of view the amounts of lignin and cellulose removed by fungal action are interesting (Tab. 4). As seen from the table, all model biodegradations of chips by white-rot fungi removed preferentially some portions of lignin, however they were only small, and mainly parallel degradation of lignin and cellulose have been observed. This fact can be explained by prevailing erosive and suppressed delignification activity of all white-rot fungi tested. From the point of view of cellulose degradation the least dangerous were the white-rot fungi *C. boreal*is and *P. chrysosporium*. On the other hand, the brown-rot fungus *F. pinicola* caused an expressive drop in cellulose content and corresponding relative increase of lignin content in chips. However, also the white-rot fungus *H. annosum* removed 17.5 % of cellulose, and it was very aggressive in this respect (Tab. 1 and 4 – see calculation: 100 - {47.8 x [(100 – 13.85) : 100] : [49.9 : 100]}= 17.48).

For pulping, the series of fresh and 6-weeks (or 3-weeks by *P. chrysosporium*, as well) intentionally biodegraded spruce chips were used (Tab. 5, Fig. 1). Model biodegradations of sawmill chips reduced the yields of pulps more or less apparently. This is in a good agreement with the data concerning the total mass and cellulose removal by the applied fungi. As expected, the most expressive loss of the pulp yield occurred in pulping the chips degraded by the brown-rot fungus *F. pinicola*. The data concerning the contents of rejects point out at positive influence of chips biodegradations by white-rot fungi on this value. The reduction in the amount of rejects may result from improved permeability of the biodegraded raw material. Though a drop in the yield of

pulp is undesired from the viewpoint of the process economy, the targeted degradation of chips by fungi at their storing may be counterbalanced by markedly diminished content of residual lignin in the pulp, expressed by their lower Kappa number.

Tab. 4: Lignin and cellulose in the biodegraded spruce chips

Chips attacked by the fungus:	Lignin (%)		Cellulose (%)	
	after 3 weeks	after 6 weeks	after 3 weeks	after 6 weeks
Phanerochaete chrysosporium	26.8	27.0	49.8	48.6
Heterobasidion annosum	26.7	28.4	48.1	47.8
Onnia circinata	26.7	27.0	47.6	48.4
Climacocystis borealis	-	26.2	-	48.8
Fomitopsis pinicola	-	31.7	-	43.1

(Each result is average of 2 analyses)

Note: Basic components in the fresh chips: Lignin = 27.2 %, Cellulose = 49.9 %

Tab. 5: Basic characteristics of unbleached sulphite pulps from spruce chips

Chips attacked by the fungus:	Yield (%)	Yield * (%)	Rejects (%)	Kappa number
Fresh chips – control	48.9	48.9	0.250	25.8
P. chrysosporium - 3 weeks	47.9	47.1	0.024	23.0
P. chrysosporium - 6 weeks	48.9	45.7	0.060	20.3
H. annosum - 6 weeks	39.4	33.9	0.115	18.1
O. circinata - 6 weeks	48.1	46.0	0.206	22.1
C. borealis - 6 weeks	48.3	46.9	0.135	21.9
F. pinicola - 6 weeks	34.0	28.9	0.494	22.7

Yield * - calculated from the original (non bio-attacked) chips

Tab. 6: Optical and mechanical characteristics of unbleached sulphite pulps from spruce chips

Chips attacked by the fungus:	Number of revolutions	Freeness (^oSR)	Brightness (% MgO)	Density of sheets ($g.cm^{-2}$)	Breaking length (km)	Tear index ($mN.m^2g^{-1}$)
Fresh chips – control	4000	29	56.3	65.2	7.9	10.35
P. chrysosporium - 3 weeks	3400	32	52.0	65.3	7.1	9.80
P. chrysosporium - 6 weeks	3000	30	55.5	64.0	7.2	8.47
H. annosum - 6 weeks	2450	46	56.4	56.8	5.9	7.04
O. circinata - 6 weeks	2600	28	49.2	58.8	6.3	6.60
C. borealis - 6 weeks	2500	30	49.6	61.4	7.2	7.69
F. pinicola - 6 weeks	1700	30	47.8	61.9	6.1	6.46

Selected physical-mechanical properties of the examined pulps are presented in Tab. 6. The targeted decay of chips reduced the number of revolutions in refining to given degree (^oSR). A drop in the number of revolutions is due to loss of pulp firmness resulting from partial delignification and polysaccharides decomposition of the raw material by fungi. This observation confirmed the potential savings in energy in the pulp refining step. In this respect the extreme results were achieved in refining the pulps from chips degraded by *H. annosum* and *F. pinicola*. These fungi have

consequently caused the deepest decay of spruce wood, as expressed by the amounts of removed total mass, lignin and cellulose (Tab. 1 and 4).

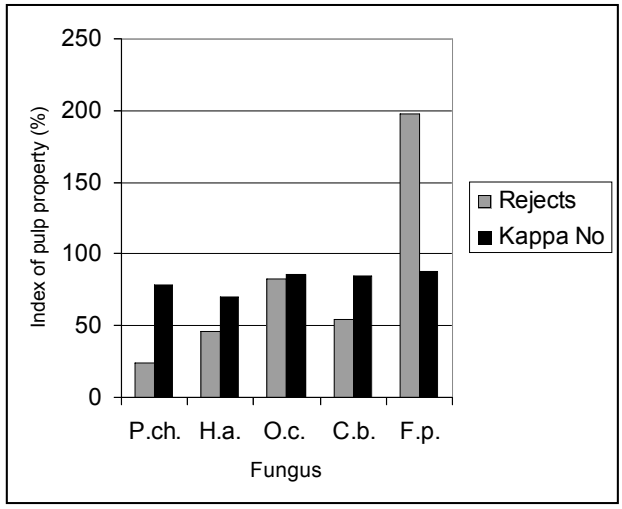

Fig. 1: Rejects and Kappa number of pulps prepared from the bio-attacked spruce chips (6-weeks attack by the fungi P. chrysosporium, H. annosum, O. circinata, C. borealis or F. pinicola) in a comparison to those prepared from fresh chips

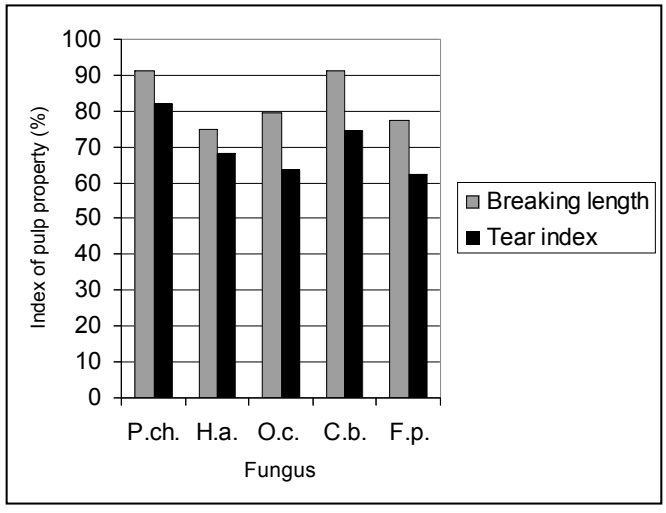

Fig. 2: Breaking strength and tear index of pulps prepared from the bio-attacked spruce chips (6-weeks attack by the fungi P. chrysosporium, H. annosum, O. circinata, C. borealis or F. pinicola) in a comparison to those prepared from fresh chips

Though in practice, the long-term storing of cut chips may sometimes lead to increased brightness of the pulp (Reinprecht and Solár 2002), the brightness of the pulps from rotten sawmill chips was almost in all cases reduced to a different degree, according to time and fungus used. An acceptable drop in the brightness of pulp caused *P. chrysosporium*, while the degradation by *H. annosum* even increased this property negligibly (Tab. 6).

It is a known fact, that long-term storing of chips in a pile leads to a moderate drop in the density of resulting pulp sheets (Reinprecht and Solár 2002). The targeted fungal degradations, except from those performed by *P. chrysosporium* that influenced the density of sheets negligibly, led to generally deeper reduction of this value (Tab. 6).

Fungal degradation of spruce chips resulted in a drop in breaking length and tear index of the pulp. The least influence on these mechanical properties of pulps showed the biodegradation of chips by *P. chrysosporium* (Tab. 6, Fig. 2). Diminished values of tear index of the pulps from degraded raw materials point out at a shortening of cellulose fibres in the process of biodegradation.

The problem of prospective introduction of biotic or bio-mimetic agents into the practice is very broad and deserves much more space than provided in this contribution. There are still many questions to be answered especially those concerning the applicability of selected fungi and properties of pre-treated raw material, the uniformity of pre-treatment and sometimes occurring shift in the fungus character, asepsis as well as the mode of cultivation.

CONCLUSIONS

The obtained experimental data concerning the alterations of spruce wood chips resulting from their intentional degradation by fungi and the final pulp properties allow us to derive the following conclusions:

- mass loss of the chips depended on the fungus applied (the highest drop in weight caused white-rot fungus *H. annosum* and brown-rot fungus *F. pinicola*) and the time of degradation (an increase from 3 to 6 weeks resulted in 3-6 times deeper drop in weight),
- the model bio-attacks of chips, with exception of application of *P. chrysosporium*, increased the contents of benzene-ethanol and acetone extracts in chips,
- biodegradations led to a marked 43 to 90 % reduction in the contents of lipophilic compounds in chips which is important from viewpoint of pulp sheets formation,
- erosive character of all used white-rot fungi was expressed by minimal changes of lignin and cellulose contents in the bio-attacked chips; lignin rapidly increased and cellulose rapidly decreased only by the influence of brown-rot fungus *F. pinicola*,
- yield of pulps from rotten chips was diminished proportionally to weight loss of cellulose in chips (highest drop in the pulp yield caused *H. annosum* and *F. pinicola*),
- biodegradations of spruce chips by the applied white-rot fungi resulted in marked reduction of rejects in the pulps, and in apparent drop of their Kappa number,
- refining of pulps from the intentionally rotten chips to a freeness of approx. 30 ° SR proceeded with a diminished number of revolutions (by 15 - 58 %, depending on the time of degradation and fungus used),
- 3- or 6-week model degradation of chips by *P. chrysosporium* did not influence the yield and physical properties of pulps markedly, however it brought a benefit of reduced Kappa number, diminished contents of lipophilic compounds in wood and the amounts of rejects in pulps expressively.

- white-rot fungus *P. chrysosporium* is known to resist to sepsis, provides satisfactory delignification of wooden substrates (as can be seen from this work, not only of hardwoods), and from this reason it can be well applicable for chip pre-treatments in a practice.

ACKNOWLEDGEMENTS

The authors wish to express their thanks to the factory Biocel Paskov in Czech Republic for sponsoring the research in this area, and are also obliged to collaborators for their skilful assistance. We also thank to the Grant Agency of the Slovak Republic (Grant No. 1/1360/04 and Grand No. 1/1359/04) for financial support of this work.

REFERENCES

1. Akhtar, M. et al., 1992: The white-rot fungus *Ceriporiopsis subvermispora* saves electric energy and improves strength properties during biomechanical pulping of wood. In: Biotechnology in the Pulp and Paper Industry, Proceedings of the 5-th International Conference on Biotechnology, Uni. Publishers Co. Ltd., Tokyo, Pp. 3-8
2. Call, H. P., Mücke, I., 1996: The Laccase-Mediator system (LMS) - a new concept. Application of lignin degrading enzymes. Proceedings of the 6-th International Conference on Biotechnology in the Pulp and Paper Industry, Vienna, Pp. 27-32
3. Cui, F., 1990: Biotechnology in Pulp and Paper Manufacture. Boston, Buterworth-Heineman. 481 pp.
4. Egan, M., 1985: 2nd Annual Pulp and Paper Chemical Outlook Conference, Corpus Information Services. Ltd., Don Mills, Ontario. In: Trotter P.C. Biotechnology in the pulp and paper industry. A review. Part 1: Tree improvement, pulping and bleaching, an dissolving pulp applications. Tappi J., 68: 198-204
5. Ferraz, A., Mendonca, R., Cotrim, A. R., da Silva, F. T., 1996: The use of white rot fungi as a pre-treatment for organosolv delignification of *Eucalyptus grandis* wood. Proceedings of the 6-th International Conference on Biotechnology in the Pulp and Paper Industry, Vienna, Pp. 221-224
6. Fischer, K., Akhtar, M., Messner, K., Blanchette, R. A., Kirk, T. K., 1996: Pitch reduction with the white-rot fungus *Ceriporiopsis subvermispora*. Proceedings of the 6-th International Conference on Biotechnology in the Pulp and Paper Industry, Vienna, Pp. 193-198
7. Kačík, F., Solár, R., 1999: Analytická chémia dreva (Analytical chemistry of wood). Technical University in Zvolen, 368 pp.
8. Koehler, L. F., Dinus, R., Malcom, E., Rudie, A., Farrell, R., Brush, T., 1996: Enhancing softwood mechanical pulp properties through chip treatment with *Ophiostoma piliferum*. Proceedings of the 6-th International Conference on Biotechnology in the Pulp and Paper Industry, Vienna, Pp. 225-228
9. Martinez-Iñigo, M. J., Immerzeel, P., Gutierrez, A., del Río, J. C., Sierra-Alvarez, R., 1999: Biodegradability of extractives in sapwood and heartwood from Scots pine by sapstain and white-rot fungi. Holzforschung, 53: 247-252

10. Messner, K. et al. 1993: Bio-sulfite pulping: Current state of research. In: FEMS Symposium; Lignin Biodegradation and Transformation. Book of Proceedings, Forbitec editions, Lisboa. Pp. 197-200
11. Messner, K., Srebotnik, E., 1994: Biopulping: An overview of developments in an environmentally safe paper-making technology. FEMS Microbiology Reviews, 13, Ed. Elsevier. Pp. 351-364
12. Messner, K., Fackler, K., Lamaipis, P., Gindl, W., Srebotnik, E., Watanabe, T., 2003: Overview of White-rot Research: Where We are Today. Deterioration and preservation - Advances in our changing world. ACS Symposium, Series, Vol. 845, 16 pp.
13. Nagakava, M., Shimada, M., Higuchi, T., 1989: The proceedings of the 34-th Lignin Symposium, Nagoya, 109 pp.
14. Oriarian, T. P., Labosky, J. P., Blankenhorn, P. R., 1990: Kraft pulping and papermaking properties of by *Phanerochaete chrysosporium* degraded aspen. Tappi J., 73: 147-152
15. Oriarian, T. P., Labosky, J. P., Blankenhorn, P. R., 1991: Kraft pulping and papermaking properties of by *Phanerochaete chrysosporium* degraded red oak. Wood Fiber. Sci., 23: 316-327
16. Reinprecht, L., Solár, R., 1998: Využiteľnosť enzymatického systému húb pri výrobe buničín. (Applicability of the enzymatic system of fungi in the pulp industry). In: Vybrané procesy pri chemickom spracovaní dreva, 2nd International symposium, Zvolen, Pp. 177-184
17. Reinprecht, L., Solár, R., 2002: Experimentálne zistenie typu húb na štiepkach, ich vplyvu na straty drevnej suroviny i kvalitu buničiny, a možnosti ochrany štiepok voči týmto hubám. (Experimental analyses of fungi in the piles of chips, their effect on the wood mass losses and pulp quality, and the possibilities of chips protection against fungi). Research report of the Technical University Zvolen in Slovakia – for the pulp factory Biocel Paskov in Czech Republic, Zvolen, 42 pp.
18. Setliff, E. C. et al., 1990: Biomechanical pulping with white-rot fungi. Tappi J., 73: 141-147
19. Shimada, M., 1996: Recent advances in microbial, enzymatic and biomimetic lignin degradation research. Proceedings of the 6-th International Conference on Biotechnology in the Pulp and Paper Industry, Vienna, Pp. 17-23
20. Solár, R., Gajdoš, E., Reinprecht, L., 1998: Uncatalyzed and catalyzed organosolv delignification of sound and pre-treated beech wood by *Trametes versicolor.* Part 2. - Delignification of pre-treated chips. Drevársky výskum, 43, (3-4): 29-37
21. Solár, R., Kurjatko, S., Reinprecht, L., 2001a: Influence of hornbeam wood pretreatment by white-rot fungus *Phanerochaete chrysosporium* on the course of organosolv, kraft and neutral sulphite delignifications. Part. 1 - Pulping and the selected properties of pulps. Wood Research, 46, (3): 11-20
22. Solár, R., Kurjatko, S., Liptáková, E., Mamoň, M., Vacek. V., 2001b: Influence of hornbeam wood pretreatment by white-rot fungus *Phanerochaete chrysosporium* on the course of organosolv, kraft and neutral sulphite delignifications. Part. 2: Selected properties of the biodegraded material. Wood Research, 46, (4): 9-21
23. Standards: ISO (302, 5270, 5269-2) and STN (50 0340)
24. Viikari, L. et al., 1987: Proceedings of the 3rd International Symposium on Biotechnology in the Pulp and Paper Industry. STFI, Stockholm, Pp. 67-72

25. Wall, M. B., Stafford, G., Noel, Y., Fritz, A., Iverson, S., Farrell, R. L., 1996: Treatment with *Ophiostoma piliferum* improves chemical pulping efficiency. Proceedings of the 6-th International Conference on Biotechnology in the Pulp and Paper Industry, Vienna, Pp. 205 – 210
26. Wolfaard, J. F., Bosman, J. L., Jacobs, A., Male, J. R., Rabie, C. J., 1996: Bio-kraft pulping of softwood. Proceedings of the 6-th International Conference on Biotechnology in the Pulp and Paper Industry, Vienna, Pp. 211-216

Prof. Ing. Ladislav Reinprecht, PhD.
Faculty of Wood Sciences and Technology
Technical University in Zvolen
T. G. Masaryka 24
960 53 Zvolen
Slovak Republic
E-mail: reinpret@vsld.tuzvo.sk

Prof. Ing. Rastislav Solár, PhD.
Faculty of Wood Sciences and Technology
Technical University in Zvolen
T. G. Masaryka 24
960 53 Zvolen
Slovak Republic
E-mail: rsolar@vsld.tuzvo.sk

Assoc. Prof. Ing. Anton Geffert, PhD.
Faculty of Wood Sciences and Technology
Technical University in Zvolen
T. G. Masaryka 24
960 53 Zvolen
Slovak Republic
E-mail: geffert@vsld.tuzvo.sk

Prof. RNDr. František Kačík, PhD.
Faculty of Wood Sciences and Technology
Technical University in Zvolen
T. G. Masaryka 24
960 53 Zvolen
Slovak Republic
E-mail: kacik@vsld.tuzvo.sk