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Fungal bio-treatment of spruce wood with *Trametes versicolor* for pitch control: Influence on extractive contents, pulping process parameters, paper quality and effluent toxicity

Teris A. van Beek [a,*], Bram Kuster [a], Frank W. Claassen [a], Taisto Tienvieri [b], Frédérique Bertaud [c], Gilles Lenon [c], Michel Petit-Conil [c], Reyes Sierra-Alvarez [d]

[a] *Laboratory of Organic Chemistry, Natural Products Chemistry Group, Wageningen University, Dreijenplein 8, 6703 HB Wageningen, The Netherlands*
[b] *UPM Kymmene Corporation, Teollissuustie 1, P.O. Box 51, FIN-37601 Valkeakoski, Finland*
[c] *Centre Technique du Papier, Domaine Universitaire, BP. 251, 38044 Grenoble Cedex 9, France*
[d] *Department of Chemical and Environmental Engineering, University of Arizona, P.O. Box 210011, Tucson, AZ 85721-0011, United States*

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Abstract

Lipophilic low molar-mass constituents in wood chips for the paper industry result in low quality pulp, pitch deposition, and effluent toxicity. New biotechnological solutions such as fungal pre-treatment of wood chips can reduce pitch problems. This laboratory-scale study focuses on the potential and limitations of a fungal bio-treatment of Norway spruce chips with the white-rot fungus *Trametes versicolor*. Different fungal treatment conditions were compared. A 4-week fungal treatment reduced the concentration of resin acids and triglycerides by 40% and 100%, respectively, but neither lowered the energy requirements of the TMP process nor significantly affected the morphological fiber characteristics and the physical pulp properties. The pre-treatment led to slightly poorer optical properties. The *Trametes versicolor* fungal treatment contributed to a less toxic effluent and improved the biodegradability. A treatment of 2–3 weeks appears optimal.

Keywords: Lipophilic wood extractives; Biodegradation; Norway spruce; White-rot fungus; Effluent toxicity; Pitch

1. Introduction

Lipophilic wood constituents such as resin acids, free fatty acids, sterols, triglycerides and sterol esters occur in low concentrations in wood (Fengel and Wegener, 1989). In spite of being present at a concentration of only 1–3%, their presence leads to serious technical and environmental problems in pulp and paper mills (Allen, 1980; Hillis and Sumimoto, 1989). The deposition of extractives (pitch) is responsible for technical problems with paper machines and a lower paper quality (Beatson et al., 1999; del Rio et al., 1999). Certain constituents such as resin acids cause increased aquatic toxicity of paper industry effluents. This is especially true for the thermomechanical pulping (TMP) process (Munkittrick and Sandstrom, 2003; Walden and Howard, 1977).

Pitch problems in pulping processes can be diminished by efficient debarking and seasoning logs and wood chips and by adding pitch control agents (Allen et al., 1991). In the past decade as an alternative method, wood chips have been treated with different wood-inhabiting fungi with the aim of degrading lipophilic wood constituents (Farrell et al., 1993; Gutierrez et al., 2001; Messner et al., 2003). Biopulping is a biotechnological process involving the treatment of wood chips with white-rot fungi prior to pulping.

* Corresponding author. Tel.: +31 317 482376; fax: +31 317 484914.
E-mail address: teris.vanbeek@wur.nl (T.A. van Beek).

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This process can lead to energy savings during the refining of wood pulp, improvement of paper quality, and waste reduction (Akhtar et al., 2000; Mansfield and Esteghlalian, 2003; Messner, 1998). Earlier it was shown that the white-rot fungi *Bjerkandera* sp. strain BOS55 and *Trametes versicolor* (strain LaVec 94–6) were able to eliminate 95% of the triglycerides and 58–87% of other lipophilic extractive classes relative to an abiotic control from Scots pine sapwood by a 2 week solid-state fermentation (Martínez-Iñigo et al., 1999; Dorado et al., 2000). This elimination was accompanied by a 7- to 17-fold reduction in toxicity in the Microtox bioassay. This appears promising, however, effects on the pulping process, effluent composition and toxicity, and paper quality are largely unknown. This research evaluates the effects of a fungal pre-treatment of industrially used spruce chips on a laboratory scale on pulp and paper quality, TMP process parameters, chemical degradation of non-polar constituents, and effluent toxicity to pinpoint advantages and potential problems before a pilot-scale trial.

2. Methods

2.1. Microorganisms and culture conditions

Bjerkandera sp. strain BOS55 and *T. versicolor* strain LaVec94–6 (CBS 114372) were kindly supplied by Dr. J.A. Field, formerly Division of Industrial Microbiology, Department of Food Science of Wageningen University. Cultures were maintained at 4 °C on glucose and peptone yeast extract slants (per litre: 20 g of glucose, 5 g of peptone, 2 g of yeast extract and 15 g of agar). They were prepared as needed and refrigerated until used. For inoculation, strains were cultured at 27 °C for 5 days on malt extract plates (per litre: 20 g of malt extract and 15 g agar). Six millimeter agar plugs from the leading edge were used as inoculum in the experiments using wood meal.

Fungal mycelium was utilized to inoculate experiments with industrial wood chips. The mycelium was cultured in 1000 mL Erlenmeyer flasks supplied with 175 mL of a basal culture medium containing 2.2 mM N as diammonium tartrate, 56 mM glucose and BIII mineral medium (Tien and Kirk, 1988), and 20 mM 2,2-dimethylsuccinate (pH 4.5) buffer. Following sterilization, each flask was supplemented with filter-sterilized thiamine (2 mg L^{-1}) and 15 colonised agar plugs, and then incubated at 27 °C for 10–12 days. Subsequently, the culture medium was decanted. The mycelial mat was washed and re-suspended in sterile distilled water and then dispersed into slurry by gentle blending (Waring Commercial Blender©). The dry weight of the mycelium was determined by separating the mycelial mat from the culture fluid by filtration through dried and tared glass filters, rinsing with distilled water, overnight drying at 105 °C, and weighing.

2.2. Wood

After felling Norway spruce trees in Southwest Finland, the trees were left in the forest for 2 weeks and then stored for 2 weeks in the mill yard. The chip collection was a sampling over a period of 2 h, which is the time corresponding to the chipping of four truckloads. The chips were of industrial size (average chip length: 20.2 mm; average chip thickness: 4.3 mm). After the logs were debarked, they were immediately chipped. Thereafter the chips were frozen, stored at −20 °C and shipped frozen to Wageningen without delay. Before use, the frozen chips were thawed and autoclaved immediately.

2.3. Fungal treatment of industrial wood chips

Polypropylene bags ($26 \times 51 \times 10\,cm^3$) (SAC-O2©) that permitted passive aeration and a relatively constant humidity were used. The bags were filled with 3 kg (moist weight, m.w.) of chips, and autoclaved (121 °C, 30 min). The bags were allowed to cool down and then they were inoculated under aseptic conditions using mycelium slurry (0.4–1.0 g o.d. (oven dried) fungus per kg of wood) as described above. Uninfected sterile controls were also run in parallel (abiotic controls). The bags with wood chips were sealed with adhesive tape and incubated under an air atmosphere at 27 °C and 70% relative humidity for 2, 4 and 8 weeks. Experiments were conducted in triplicate. Some bags were supplied with a variable glucose addition (0%, 0.5% and 2% on a dry wood weight basis) (Table 1). Removal of total extractives, elimination of specific wood extractive constituents and total wood mass losses in fungal treatments and control samples were determined. Control and treated wood chips were subjected to mechanical pulping.

Total extractive yield was determined gravimetrically by weighing an acetone extract after evaporation of the solvent. The weight loss of the wood was determined gravimetrically. Dry weight was calculated indirectly by determining the moisture content in a sub-sample of the wood chips in order to avoid thermal modification of the wood constituents.

Table 1
Conditions of bio-treatment experiments

Samples	C0W	A2W	T2w 0G	T2w 0.5G	T2w 2G	T4w 0.5G	T8w 0.5G
Treatment	Control	Abiotic treatment	*T. versicolor*	*T. versicolor*	*T. versicolor*	*T. versicolor*	*T. versicolor*
Duration (week)	0	2	2	2	2	4	8
Glucose dose (g/100 g)	0	0	0	0.5	2	0.5	0.5