Effects of lipases on birch extractives

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ABSTRACT: Fresh birch and birch sulfate pulp were treated with commercial lipases in order to study the hydrolysis of lipophilic esters which may be harmful in papermaking. Enzymatic treatments were performed on extractive-containing water suspensions obtained from birch powder, on fresh birch powder and on birch sulfate pulp. Changes in the amounts of different esterified lipid compounds in the materials were analyzed by capillary gas chromatography and mass spectrometry. The lipid composition of the suspension was similar to that commonly found in the extractives isolated from birch with neutral organic solvents. The fatty acid esters constituted the major lipid group in the suspension. About 80% of these esters were hydrolyzed to the corresponding free acids by the lipases. Other types of esters were hydrolyzed to a lower extent. Extractives in birch powder were less accessible to the lipase treatment than those in the suspension. The amounts of fatty acid esters were decreased by about 50%. Fatty acids and terpenoids were the major compound groups of birch sulfate pulp. The lipases were capable of hydrolyzing almost all the esterified lipid groups in the pulp.

KEYWORDS: Betula, bibliographies, ester groups, extractives, hydrolysis, pitch.

Lipophilic wood extractives which are soluble in neutral organic solvents may cause problems in papermaking processes. Depositable extractives of pulps adhere to different parts of the paper machine or may appear as dirt spots in the paper. This causes interruptions in paper production and decreases paper product quality. In addition, extractives are thought to increase the yellowing, i.e., brightness reversion, of the pulp and paper products, to cause odor problems and to increase the necessity for chlorination of the pulp *(1–4)*.

The amount and composition of lipophilic extractives vary between wood species. The lipophilic extractives in hardwood consist mainly of fatty acids esterified with glycerol, fatty alcohols, sterols, and terpene alcohols. In addition, there are small amounts of free fatty acids, free alcohols, and hydrocarbons. High proportions of saturated and dienoic fatty acids are found among the acids. The resin acids and other diterpenoids typical of pine and spruce are completely absent in birch. By contrast, triterpenoids are present in birch in a great variety. Birch contains more neutral extractives than pine *(5–7)*.

Pitch problems are dependent on the type of pulp processing, chemical or mechanical. Pitch is liberated from the pulp fibers at various processing stages and particularly when there is a change of pH and/or temperature *(8)*. Sulfate cooking under alkaline conditions does not lead to a complete removal of extractives. As the extractives contain many highly reactive chemical compounds, it is obvious that they may undergo considerable changes during bleaching. In chlorine bleaching, the double bonds of the pitch triglycerides are chlorinated, and the substances formed may affect the formation of pitch deposits. The behavior of extractives during bleaching has not been studied in detail, presumably due, at least partly, to the analytical problems involved *(2, 9, 10)*. When mechanical pulp is used, triglycer-

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I. Properties of the enzymes

Enzyme preparation	*Lipase activity*	*Specific lipase activity, nkat/mg protein*	*Esterase activity*	*Specific esterase activity, nkat/mg protein*	*Protein content*
Candida (powder)	670 nkat/mg	6700	10 nkat/mg	100	100 mg/g
Aspergillus sp. (liquid)	280,000 nkat/mL	28000	11000 nkat/mL	1100	10 mg/mL

ides, fatty acids, and resin acids have been shown to be the most troublesome extractives in softwood *(11, 12)*.

Current methods for reduction of pitch problems include the use of wood species low in extractives, the seasoning of wood chips thus causing degradation of unsaturated compounds, triglycerides, and fatty acid esters, and the addition of alum or talc to the pulp *(13)*. Recently new biotechnical methods, such as enzymatic and fungal treatments, have also been developed for softwood. The effects of enzymatic treatments on the reduction of pitch problems in birch-based pulp and paper processing technology have not previously been reported.

The Nippon Paper Co. has employed the enzymatic pitch control technology in the papermaking process for several years. An *Aspergillus* lipase (Resinase), produced in industrial scale by Novo Nordisk A/S, is marketed for this purpose. By removing the triglycerides from softwood mechanical pulp using this lipase a significant reduction in pitch problems has been obtained. The enzymatic method has been used successfully in at least two paper mills in Japan in the treatment of mechanical pulp of red pine *(14, 15)*. Fischer and Messner *(16)* also demonstrated that the triglycerides in unbleached softwood sulfite pulp are a major source of the formation of sticky substances. The adhesiveness of the pitch and the amount of triglycerides were considerably reduced in these studies by a lipase treatment.

A fungal pretreatment has been used successfully to reduce the pitch content of thermomechanical pulps of Southern yellow pine. The nonpathogenic fungus, *Ophiostoma piliferum* with the trade name Cartapip, marketed by Sandoz, reduces the pitch content by cell metabolism. Treatment of wood chips for two weeks with the fungus decreased both the total resin acid and total fatty acid content of the wood by approximately 40%. The esterified fatty acids were almost completely hydrolyzed to the corresponding free acids during the microbial treatment *(8, 11, 12, 17)*.

In this work, the use of *Candida cylindracea* lipase in the hydrolysis of different lipophilic esters present in extractives of fresh birch and birch sulfate pulp was studied as a method of reducing the pitch problems in pulp and papermaking processes. A comparison of *Candida* and *Aspergillus* lipases in the treatment of sulfate pulp was also performed.

Materials and methods

Materials

Fresh debarked birchwood and never-dried unbleached birch sulfate pulp with kappa number of 24.4, obtained from Metsä-Serla Group, Ltd, Äänekoski mills, were used as raw materials. Wood chips were air-dried to 5% moisture content and ground with a hammer mill to fine sawdust with a particle size less than 0.5 mm. Lipase Type B from *Candida cylindracea* was purchased from Biocatalysts LTD (United Kingdom) and *Aspergillus* sp lipase (Resinase A) from NOVO Enzyme Process Division (Denmark).

Preparation of the extractive-containing substrate from birch

The fresh birch sawdust was suspended in water (2% dry weight suspension) for 30 min at 50°C. The conditions were optimized in a series of treatments in which different sawdust concentrations (10–50 g/L) and different reaction times (0.5–3 h) were used. After the treatments, the suspensions were centrifuged (1350 rpm for 12 min). The supernatant containing colloidal and dissolved substances (extractive-containing substrate) was used for enzymatic treatments.

Enzymatic treatment of the extractive-containing substrate

To 200 mL of the extractive-containing substrate of birch (original pH 5.3) buffered with potassium dihydrogen phosphate (50 m*M*) and

sodium hydroxide to pH 5.8, 40 mg lipase from *Candida* was added. The suspension was incubated in a shaking water bath (135 rpm) for 24 h at 40°C. The reference suspension was incubated without the enzyme.

Enzymatic treatment of birch powder

Birch sawdust (5.2 g) was suspended in 95 mL of 50 m*M* sodium acetate buffer, pH 5.8. To this solution 100 mg of *Candida* lipase was added. The suspension was incubated in a shaking water bath (135 rpm) for 24 h at 40°C. The reference suspension was incubated without addition of enzyme.

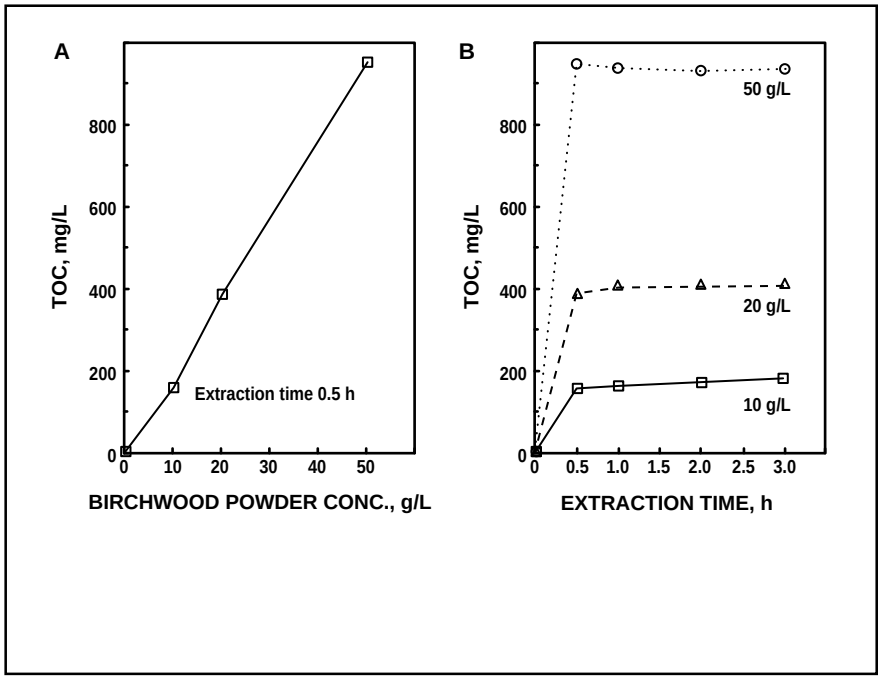
Enzymatic treatment of birch sulfate pulp

The birch sulfate pulp (75 g fresh weight, equivalent to 8.8 g dry) was suspended in 150 ml of 50 m*M* acetate buffer, pH 5.8, resulting in a consistency of 3.9%. To this suspension 150 mg of *Candida*-lipase or 0.35 ml of *Aspergillus*-lipase (Resinase) was added. The suspensions were incubated as described previously for the extractive substrate and the birch powder. After incubation the pulps were filtered. The control pulp sample was treated in the same way but without addition of enzyme.

Analytical methods

The birch sawdust and the birch sulfate pulp before and after enzymatic treatments were analyzed for lipophilic extractives. Vacuum-dried samples (1 g) were extracted with 40 mL dichloromethane (DCM) in Soxhlet extractors for 8 h and the solvent was removed by vacuum evaporation. The dry extract yields were determined. The DCM-soluble free and esterified lipid compounds were separated by gas chromatography (GC) with an HP1 (25 m x 0.32 mm, film thickness 0.17 μm) fused silica capillary column *(18)*. The compounds were identified by gas chromatography/mass spectrometry (GC/MS) *(19)*. Quantitative determinations were based on peak areas relative to heptadecanoic acid, which served as a quantitative internal standard.

1.The effect of birchwood powder concentration (A) and extraction time (B) on the TOC of the extractive-containing suspension at 50°C



The extractive substrates were analyzed for total organic carbon (TOC) and lipophilic compounds. TOC was determined using an Ionics Model 1270 analyzer. For the determination of lipophilic compounds the extractive substrate (100 mL) after adjusting the pH to 2–3 were extracted with diethylether *(20)*. The free and esterified lipids in the ethylether extracts were then analyzed by GC and identified by GC/MS as described above. The reproducibility of the determination of the amount of lipids was about ±5% *(18)*.

Lipase activity was assayed by the olive oil emulsion method at pH 7 and 37°C as described previously *(21)*. One unit of lipase activity (nkat = nanokatal) was defined as the amount of enzyme which liberated 1 mmol of fatty acid per second under the assay conditions. Esterase activity was determined using α-naphthyl acetate as substrate at pH 5.3 and 50°C *(22)*. Protein contents in the enzyme preparations were determined by the method of Lowry *et al. (23)*.

Results and discussion

Properties of the lipases

Two commercial enzymes, produced by *Candida cylindracea* and *Aspergillus* sp. (Resinase A), were used in this work. The activities of the enzymes were assayed using two different methods using olive oil and α-naphthyl acetate as substrates. The *Candida* lipase was obtained in a dried form and the activity was measured from a suspension of 10 mg solids/mL phosphate buffer. The *Aspergillus* preparation was a liquid with a 4-fold higher specific lipase activity and an 11-fold higher esterase activity than that of the *Candida* enzyme **(Table I)**. Both lipases were most active at neutral pH. The temperature optimae of the *Candida* and *Aspergillus* lipases on olive oil as substrate were 45°C and 40°C, respectively (results not shown).

The lipase from *C. cylindracea* yeast has previously been used suc-

II. Effect of *Candida* lipase treatment on the amounts of free and esterified lipid compounds present in the isolated substrate of fresh birch.

Compound group/ component	Untreated substrate, mg/L			Lipase-treated substrate, mg/L		
	Free	Esterified	Total	Free	Esterified	Total
Fatty acids	2.86	9.23	12.09	9.48	2.12	11.60
saturated C12-C_{26}	1.36	1.64	3.00	2.63	0.51	3.14
unsaturated C_{18}	1.50	7.59	9.09	6.85	1.61	8.46
Fatty alcohols C_{16}-C_{28}	0.41	0.07	0.48	0.26	0.09	0.35
Hydrocarbons C_{23}-C_{29}	0.10	0	0.10	0.10	0	0.10
Sterols	0.13	0.58	0.71	0.15	0.45	0.60
Terpenes and terpenoids	0.43	3.46	3.89	0.89	2.78	3.67
betulaprenols	0	2.50	2.50	0.26	2.10	2.36
triterpenoids	0	0.96	0.96	0.26	0.68	0.94
squalene	0.43	0	0.43	0.37	0	0.37
Total	3.93	13.34	17.27	10.88	5.44	16.32

cessfully for several biotechnical applications *(24, 25)*. It has a broad substrate specificity, hydrolyzing virtually any ester bonds not only in triglycerides but also in various natural and synthetic esters. Several esterases, e.g., wax esterase, have also been identified in the commercial *C. cylindracea* preparation *(26)*. Broad substrate specificity makes this lipase suitable for degradation of troublesome esters in the extractives of birch. The lipase from *Aspergillus* is especially developed for the hydrolysis of triglyceride constituents of wood resins.

Preparation of the extractive-containing substrate from fresh birch powder

In order to make the extractives more accessible to the lipase treatment, they were isolated from fresh birch by extraction with water. The conditions of the treatment were optimized by monitoring the release of organic compounds. The TOC-content of the extractive-containing substrate, obtained after 30 min with different sawdust concentrations at 50°C, correlated directly with the wood powder concentration **(Fig.1A)**. During the first half hour, all compounds contributing to the TOC-value were dissolved (Fig. 1B). On the basis of these results, a method using a 20 g/L suspension of birchwood powder and a reaction time of 30 min at 50°C was chosen. The TOC-value of this substrate was 400 mg/L, corresponding to 2% of the original wood dry weight. The lipid concentration in the suspension was 17 mg/L, corresponding to 0.1% w/w of dry wood.

Composition of the extractive-containing substrate of birch

The extractive-containing substrate of birch contained the same lipid compound groups as generally observed in the extracts isolated from birch with neutral organic solvents. However, their amounts were lower in this preparation. The lipid compounds consisted of fatty acids, fatty alcohols, hydrocarbons, sterols, and terpenoids. About 80% of the lipids were in esterified form **(Table II)**.

The main group of the lipids consisted of fatty acids comprising about 70% w/w of the lipids. The fatty acids occurred mainly (76%) in esterified form. About 95% of the fatty acids consisted of shorter-chain (C12–C18) fatty acids, unsaturated C18-acid (linoleic acid) and saturated C16-acid (palmitic acid) predominating. The amounts of other straight-chain lipids were low.

The terpenoid group comprised about 23% w/w of the lipids in the extractive substrate. The terpenoids occurred mainly (about 90%) as esters. The main terpenoids (65%) were betulaprenols, which are alcohols consisting of 6–9 isoprene units. The dominant triterpenoids were methylenecycloartanol and citrostadienol. An acyclic squalene, which is the precursor of the cyclic triterpenes, was also found in small amounts. The amount of sterols was rather low, ß-sitosterol being dominant.

III. Effect of *Candida* lipase treatment on the amounts of free and esterified lipid compounds in birch sawdust.

Compound group	Untreated birch sawdust, mg/g dw			Lipase-treated birch sawdust,mg/g dw		
	Free	Esterified	Total	Free	Esterified	Total
Fatty acids	0.85	3.05	3.90	2.94	1.64	4.58
saturated C12-C_{28}	0.42	1.84	2.26	1.62	0.65	2.27
unsaturated C_{18}	0.43	1.21	1.64	1.32	0.99	2.31
Fatty alcohols C_{16}-C_{24}	0.27	0.18	0.45	0.17	0.14	0.31
Hydrocarbons C_{23}-C_{31}	0.15	0	0.15	0.17	0	0.17
Sterols	0.17	0.30	0.47	0.18	0.32	0.50
Terpenes and terpenoids	0.19	0.51	0.70	0.15	0.55	0.70
betulaprenols	0.01	0.13	0.14	0.01	0.17	0.18
triterpenoids	0.17	0.38	0.55	0.13	0.38	0.51
squalene	0.01	0	0.01	0.01	0	0.01
Total	1.63	4.04	5.67	3.61	2.65	6.26

Enzymatic treatment of the extractive-containing substrate of birch

The buffered substrate was treated with *Candida* lipase using a dosage corresponding to about 7000 nkat/g original birch sawdust for 24 h at 40°C. The reference substrate was incubated under the same conditions but without enzyme. In the enzymatic treatment of the substrate, the amount of esterified lipids was considerably decreased (Table II). About 60% w/w of the total amount of esterified compounds was hydrolyzed by the lipase. This decrease of esterified compounds was most significant in the fatty acids; almost 80% of the esterified fatty acids were hydrolyzed to the corresponding free acids. The amounts of esterified sterols and terpenoids were reduced only to a lesser extent, about 20%.

Composition of the extractives from fresh birch

The DCM extraction of birch sawdust dissolved 2.5% of the birch dry weight. About 40% of the extract consisted of compounds eluted by GC. The amount of identified lipid compounds was 0.6% w/w of dry birch **(Table III)**. About 70% of the compounds were esterified. The main group of the extract was fatty acids comprising about 70% w/w of the lipids as in the case of the isolated substrate. They occurred mainly (80%) in esterified form. The proportion of unsaturated fatty acids was lower (42%) than in the isolated substrate (75%). The other common group, comprising 12% of the lipid compounds, consisted of terpenoids, 80% of these being triterpenoids. The proportion of terpenoids was lower in the DCM extract than in the isolated substrate, whereas the relative amounts of other groups were somewhat higher in the DCM extract. The lipid composition of the DCM-extract of birch was in agreement with that commonly found in birch *(5–7)*.

Enzymatic treatment of fresh birch

The fresh birch was treated with *Candida* lipase using a dosage of 13000 nkat/g sawdust. A control birch sample was treated in the same way but without addition of enzyme. After the *Candida* lipase treatment the DCM extract yield of the birch was decreased to 2.1% of the birch dry weight. The total amount of esterified compounds was decreased by 34% . The decrease was most significant in the fatty acids. About 50% of the esterified fatty acids and even 65% of the saturated fatty acids were hydrolyzed. The esters of fatty alcohols were also hydrolyzed to some extent (Table III). The amounts of esterified sterols and terpenoids remained the same. Esters in fresh birch were less extensively hydrolyzed by the lipase treatment than those in the isolated substrate, apparently due to the less accessible location of extractives in the wood powder.

Enzymatic treatment of birch sulfate pulp

The DCM extract yield of the dried sulfate pulp was 0.7% w/w. A DCM extract yield of 0.9% w/w in birch kraft pulp has previously been reported by Laamanen *(9)*. The amount of extractives in the pulp was much lower than in the fresh birch. In the production of birch kraft pulp, most of the lipophilic extrac-

IV. Amounts of lipid compounds in the birch sulfate pulp and degree of hydrolysis of esters by *Candida* and *Aspergillus* lipases.

Compound group	*Untreated sulfate pulp, mg/g dw*			*Hydrolysis of esters, %*	
	Free	*Esterified*	*Total*	*Candida*	*Aspergillus*
Fatty acids	0.58	0.50	1.08		
saturated	0.26	0.25	0.51	50	67
unsaturated	0.32	0.25	0.57		
Fatty alcohols	0.06	0.03	0.09	95	98
Hydrocarbons	0.03	0	0.03		
Sterols	0.24	0.12	0.36	45	62
Terpenes and terpenoids	0.56	0.54	1.10	0	18
betulaprenols	0.19	0.24	0.43	0	20
triterpenoids	0.27	0.30	0.57	0	16
squalene	0.10		0.10		
Total	1.47	1.19	2.66	28	43

tives present in the wood are dissolved in the alkaline pulping liquor. The amount of lipids was 0.3% w/w of the dry pulp. The esterified compounds in the pulp comprised 45%, which was much lower than in the birch **(Table IV)**. Fatty acids and terpenoids were the major lipid compound groups, each comprising about 40% w/w of the lipids. The proportions of terpenoids and sterols were higher and that of fatty acids lower compared with the birch. Hydrophobic sterol and terpenoid esters remain unsaponified to a large extent during sulfate pulping and therefore remain in the pulp slurry as a result of incomplete washing *(7)*.

The birch sulfate pulp was treated with the *Candida* and *Aspergillus* lipases using a dosage of about 11000 nkat/g pulp. Of the total amount of esterified lipids about 30% was hydrolyzed by the *Candida* lipase and about 40% by the *Aspergillus* lipase. The lipases were capable of hydrolyzing almost all the esterified compound groups of the lipids, although the *Aspergillus* lipase was somewhat more efficient. The esters of saturated fatty acids, fatty alcohols and sterols were rather effectively hydrolyzed by both lipases. The *Candida* lipase was incapable of degrading the esters of betulaprenols and triterpenoids, whereas the *Aspergillus* enzyme hydrolyzed them to some extent.

Triglycerides of unbleached softwood sulfite pulp have been shown to be extensively hydrolyzed (86%) by the *Aspergillus* lipase (Resinase) treatment *(16)*. In these experiments, the enzyme dosage was higher (17500 nkat/g pulp) but the reaction time shorter (2 h) than that used in our work.

Conclusions

Different types of birch-derived extractive-containing substrates were studied. A representative sample of lipophilic compounds was isolated from fresh birch by water extraction and used as a model mixture for hydrolysis experiments. The lipid composition of the isolated substrate was rather similar to that commonly found in birch when isolated with neutral organic solvents. However, the amount of lipids in the DCM extract was five-fold as compared with that in the isolated substrate. The *Candida* lipase hydrolyzed 80% of the esterified fatty acids, the main lipid group of the extractive substrate. Other groups could also be hydrolyzed by the lipase, but to a lower extent.

Esters in fresh birch sawdust were less extensively hydrolyzed by the *Candida* lipase treatment than those in the extractive substrate, apparently due to the less accessible location of extractives in the wood powder. The amount of esterified fatty acids was reduced by about 50%. It is possible that the remainder of the fatty acid esters might have been degraded by increasing the enzyme dosage, which however was not optimized in this study. Furthermore, all other lipid groups were hydrolyzed to some extent, with the exception of sterols and terpenoids.

The amount of extractives in the birch sulfate pulp was much lower than in the birch because most of the lipophilic compounds are dissolved in the alkaline pulping liquor. Almost all the lipid groups in the pulp were hydrolyzed in the enzymatic treatments with *Candida* and *As-*

pergillus (Resinase) lipases. However, the *Aspergillus* lipase was more efficient. As in the case of the birch, the *Candida* lipase was incapable of hydrolyzing the terpenoid esters in the pulp.

Treatment of fresh birch and birch sulfate pulp with *Candida* lipase, in addition to the *Aspergillus* preparation originally developed for the hydrolysis of triglyceride constituents of wood resin, should provide a useful method for reducing pitch problems in the papermaking process using birch.

Literature cited

1. Allen, L. H., *Tappi J.* 63(2): 81(1980).
2. Allen, L. H., *Pulp & Paper Canada* 76(5): T139(1975).
3. Jay, K., *Paper* 203(10): 31(1985).
4. Sjöström, J., "Detrimental Substances in Pulp and Paper Production," Ph.D. thesis, Åbo Akademi University, Åbo, Finland, l990.
5. Fengel, D. and Wegener, G.,"Wood, Chemistry, Ultrastructure, Reactions," Walter de Gruyter, Berlin-New York, 1984.
6. Holmbom, B., "Constituents of Tall Oil. A Study of Tall Oil Processes and Products," Ph.D. thesis, Åbo Akademi University, Åbo, Finland, l978.
7. Paasonen, P., "On the Non-volatile Eethylether Extractives of Birch Wood and theChanges in Composition Effected by Aging and Sulfate Pulping," Ph.D. thesis, University of Helsinki, Finland, l966.
8. Brush, T. S., Farrell, R.L. and Ho,C., *Tappi Journal* 77(1):155 (1994)
9. Laamanen, L., *Paperi ja Puu* 11: 615(1984).
10. Dreisbach, D. D., and Michalopoulos, D. L., *Tappi J.* 72(6): 129(1989).
11. Wendler, P. A., Brush, T. S., and Farrell, R. L., *1991 International Symposium on Wood and Pulping Chemistry Proceedings,* Melbourne, APPITA, Vol. I, p. 501.
12. Wendler, P. A., Brush, T. S., Iverson, S., *et al., Kemia-Kemi* 19: 262(1992).
13. Hassler, T., *Tappi J.* 71: 195(1988).
14. Fujita, Y., Awaji, H., Taneda, M., *et al., Tappi J.* 75: 117(1992).
15. Fujita,Y., Matsukura, M., *et al., Tappi J.* 74(4):177(1992).
16. Fischer, K., and Messner, K., *Tappi J.* 75: 130(1992).
17. Blanchette, R. A., Burnes, T. A., Farrell, R. L., *et al., Tappi J.* 75: 102(1992).
18. Fagernäs, L., Ekman, R., and Sipilä, K., *Fuel Processing Technology* 33: 137(1993).
19. Alen, R., Fagernäs, L., and Kuoppala, E., *Fuel Processing Technology* 28: 175(1991).
20. Ekman, R., and Holmbom, B., *Nordic Pulp and Paper Research J.* 16(1989).
21. Mustranta A., *Microbiol. Biotechnol.* 38: 61(1992).
22. Poutanen, K., and Sundberg, M., *Appl. Microbiol. Biotechnol.* 28: 419(1988).
23. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., *et al., J. Biol. Chem.* 193: 265(1951).
24. Chambou, B., and Klibanov A. M., *Biotechnol. Bioeng.* 26: 1449(1984).
25. Langrand, G., Baratti, J., Buono, G., *et al., Tetrahedron Lett.* 27: 29(1986).
26. Brahimi-Horn, M-C., Guglielmino, M. L., and Sparrow, L. G., *J. Biotechnol.* 22: 299(1989).

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