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THERMOGRAVIMETRIC ANALYSIS OF FLAX SHIVES DEGRADED BY *PLEUROTUS OSTREATUS* AND *CERACEOMYCES SUBLAEVIS*

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ABSTRACT

The polysaccharide-degrading and laccase enzymes released during the controlled degradation of flax shives by the fungal colonists *Ceraceomyces sublaevis* and *Pleurotus ostreatus* were assayed. Thermogravimetric analysis was used to identify the major components of the degraded flax shives. Changes in the peak patterns of derivative thermogravimetry were only observed 8 days after the start of fungal incubations, coinciding with an increased content of degrading enzymes. Two major peaks of thermogravimetric decomposition were observed; one at 280 °C, believed to represent holocellulose, and one at 450 °C representing lignin. The two fungal colonists degraded different components of the flax shive. *P. ostreatus* released higher levels of pectic, xylanase and cellulase enzymes into the substrate and, as a result, degraded the holocellulose present in shive more efficiently than lignin. *C. sublaevis* released higher levels of laccase in the substrate and, subsequently, degraded lignin more efficiently. The difference in the enzyme-release patterns of the two fungi was correlated with the changes in thermogravimetric weight loss of the different components of shive.

INTRODUCTION

Wood pyrolysis and combustion degradation patterns and the use of thermal analysis for understanding the decomposition of wood by fungi has been reviewed by Browne [1], Beall and Eickner [2] and recently by Nguyen et al. [3]. The profiles of weight loss with increasing temperature during the pyrolysis of wood and paper (thermogravimetric profiles) have been shown to be composites of the profiles of the individual components: cellulose, hemicellulose and lignin [4,5]. The woody product of *Linum usitatissimum L.* that remains after the commercially useful flax fibres have been removed is defined as the shives. They have very little value [6] and consist primarily (90–98%) of three polymeric materials: cellulose, hemicellulose and lignin [7]. Ruminants are unable to digest the cellulose component because of the large content of lignin (24.5%) [8]. Basidiomycetes of the genus *Pleurotus* are able to oxidise lignin-bearing materials without prior chemical or biological treatment [6,9] and another, *Ceraceomyces sublaevis*, causes spoilage of flax stems during storage [10].

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Thermogravimetric analysis has been used to determine the relative degrees of deterioration of wood degraded by fungi [11]. Cowling [12] has reported differences in the degradation of wood by *Polyporus versicolor* (white rot) and *Poria monticola* (brown rot). However, changes in the thermogravimetric profiles of the degraded wood have not yet been related to the presence of different fungal polysaccharide-degrading enzymes released during colonization; this is the aim of the present study.

MATERIALS AND METHODS

Fungi and substrate

The two basidiomycetes used in this study were *Ceraceomyces sublaevis* (Bres.) Julich and *Pleurotus ostreatus* (Jacq. ex Fr.). Flax shives (10 kg) remaining after the desiccation of stand-retted flax stems [13] were washed in deionized water to remove any superficial impurities and dried at 70 °C. The dry shives (5 g) were wetted with 15 ml of deionized water in flasks and the flasks were sterilized for 30 min at 15 lbf in^{-2} before being inoculated with three plugs 2 mm in diameter cut from the growing edge of a ten-day-old culture plated on agar (MA, Oxoid) and incubated at 20 °C in near darkness. Flasks from each treatment were harvested every four days for measurement of weight loss, analysis of the components of the degraded shive for the presence of polysaccharide-degrading enzymes, and for thermogravimetric profiling.

Enzyme extraction and assay

Samples of shives (5 g) were homogenized with 0.3 M KCl (150 ml) for 4 min and the extracts clarified and dialysed in cool tap-water overnight. The activities of the pectinase, cellulase and xylanase enzymes in the extracts were assessed by measuring the release of reducing groups from their respective substrates: sodium polypectate (5 g l^{-1}), carboxymethyl cellulose (1 g l^{-1}) and xylan (1 g l^{-1}). Substrate solutions (2 ml) were mixed with 1 ml of enzyme extract, and incubated at 30 °C for 1 h and the concentrations of the reducing groups determined colourimetrically with 3,5-dinitrosalicylic acid [14]. Laccase activity was assayed by incubating the substrate, 2 ml of 2,6-dimethoxy phenol with 1 ml of enzyme extract at 30 °C. The reaction was then stopped with 0.5 ml dimethoxy sulphoxide and the absorbance at 468 nm was measured [15].

Analysis of the degraded shive

Reductions in shive weights were measured after drying the flasks containing the degraded shives to constant weight at 70 °C. Samples of the

degraded and undegraded shives were repeatedly chlorinated and extracted with monoethanolamine and ethanol six times to remove lignin and the residual lignins [16,17], the removal of lignin being visually monitored by the colour formed on addition of the amine. Amounts of holocellulose and the degree of their polymerization were measured with cupriethylenediamine solutions [18]. The major carbohydrate constituents of the cellulose and holocellulose fractions were determined by the methods of Doree [19] and of Norris and Preece [20] respectively. Lignin was determined by extraction with sulphuric acid [16] and pectin by extraction with ammonium oxalate [19].

NaOH extraction

This test was carried out to determine the effect of removing hemicellulose from the flax shives by extracting with NaOH (40 g l^{-1}) for 5 h at 20 °C and to observe the peak decomposition for cellulose and lignin without the masking effect of hemicellulose.

Thermogravimetric system

Thermal weight-loss profiles of the shive were analysed using 3–5 mg samples, milled to pass a 40 mesh sieve. These were suspended into a furnace in a platinum crucible from a Stanton–Redcroft microbalance (TG, 750) connected to a temperature programmer, atmospheric controlling system and computer-controlled recorder. The flow rate of air through the system was 10 ml min^{-1} and temperature gradients were 20 °C min^{-1} from room temperature to the endpoint temperature. The crucible was cleaned at the end of each run by firing in air at about 600 °C. Thermogravimetry (TG) and derivative thermogravimetry (DTG) of the various treatments were analysed. Three runs were done for each treatment.

RESULTS

Weight loss and enzyme activity following incubation with fungi

There was a greater loss in shive dry weight after 24 days of degradation by *P. ostreatus* than by *C. sublaevis* (Table 1). When shives were degraded with *P. ostreatus*, maximum activities of polygalacturonase (PG), pectin-lyase (PL), and xylanase were detected after 16 days from the start of incubation; thereafter the activities decreased slowly. Laccase activity was maximal after 20 days of incubation and cellulase activity remained low throughout (Fig. 1(a)).

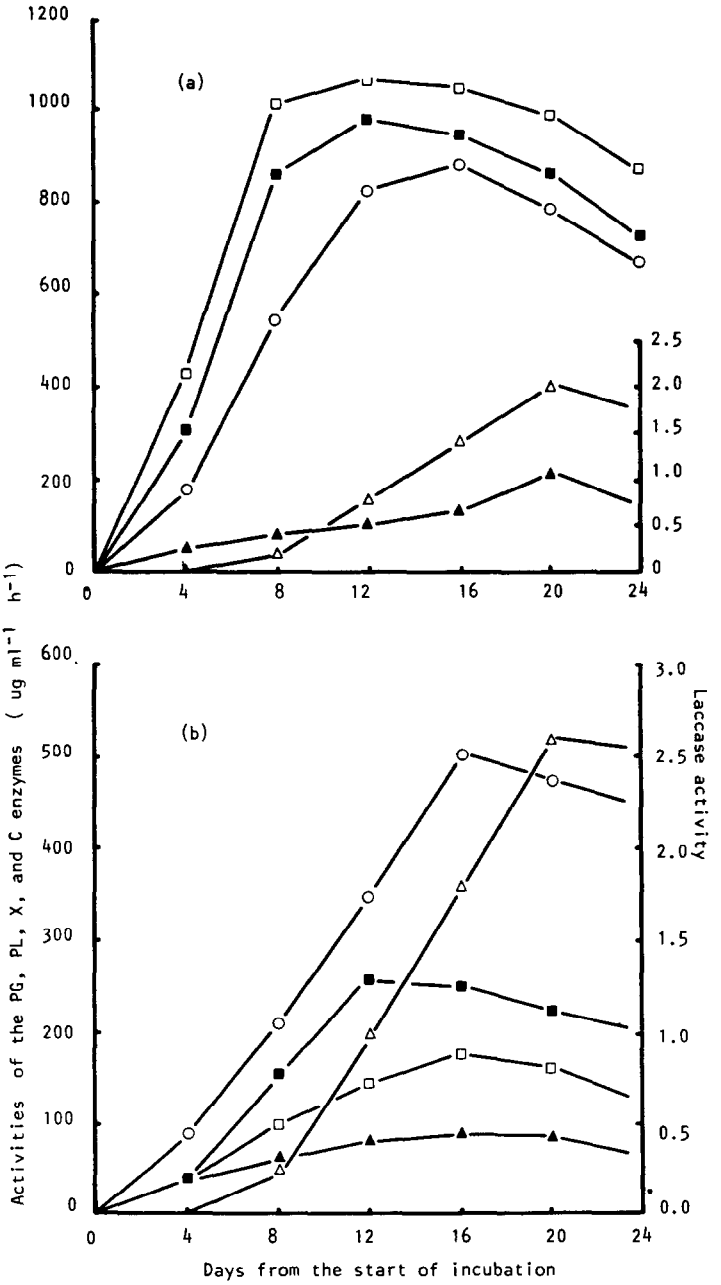

Fig. 1. Activities of polygalacturonase (PG, □), pectin-lyase (PL, ■), xylanase, (X, ○), cellulase (C, ▲) and laccase (L, △) enzymes produced by (a) *P. ostreatus* and (b) *C. sublaevis* when incubated with flax shives. PG and PL activities are expressed as $\mu g\ ml^{-1}\ h^{-1}$ galacturonic acid equivalent; X and C activities are expressed as $\mu g\ ml^{-1}\ h^{-1}$ glucose equivalent. An increase in absorption (468 nm) of 0.01 min^{-1} at 30 °C was considered equivalent to a unit of laccase activity.

TABLE 1

Comparison of weight losses, yields and degrees of polymerization (DP) of the holocellulose in undegraded shives and in those degraded with *C. sublaevis* or *P. ostreatus*

Treatment	Weight loss (%)	Yield (%)	DP
Undegraded	0.0	70.4	756
C. sublaevis	11.1	64.4	645
P. ostreatus	25.1	59.2	630
S.E. of means	0.34	0.45	

The activities of the PG, PL, xylanase and cellulase enzymes were much lower in *C. sublaevis* than in *P. ostreatus* and the activity of laccase was much greater. The activity of xylanase peaked after 16 days from the start of incubation with *C. sublaevis*, the PL activity after 12 days and the laccase activity after 20 days (Fig. 1(b)). Much less holocellulose was present in degraded shives after incubation with fungi than in undegraded samples and the holocellulose remaining was less polymerized (Table 1).

Thermogravimetric analysis

Chemical analysis of the flax shives indicated that the main components were cellulose (46.0%), hemicellulose (26.2%) and lignin (23.1%). As the temperature was progressively increased during the thermogravimetric analysis, moisture was eliminated from the sample. With further increase in temperature, maximum pyrolysis of pectins occurred at 234°C, of hemicelluloses at 265°C, of cellulose at 362°C and of lignin at 472°C. Holocellulose, which contains both hemicellulose and cellulose, peaked at 271°C (Table 2). During the first 6 days of incubation, the thermogravimetric (TG) weight-loss curve and derivative thermogravimetric (DTG) profiles for both fungal-colonized and uncolonized shives were the same. Changes only became obvious after 8 days of incubation (Figs. 2, 3 and 4) and this coincided with an increase in the enzyme activity of the colonized shives. The pyrolytic losses in weight at thermogravimetric temperatures, representing the major structural components, became smaller as the fungal degradation continued. After 16 days from the start of incubation with *C. sublaevis* and *P. ostreatus*, the TG weight losses for holocellulose (cellulose and hemicellulose) at about 276°C were 48% and 36% of the total weight, respectively, compared with a weight loss of 54% for uncolonized, undegraded shives (Figs. 2, 3 and 4). At the end of the incubation period of 24 days, the weight losses for holocellulose at DTG peaks of about 275°C were 46% and 32% respectively for shives degraded by *C. sublaevis* and *P. ostreatus* (Figs. 3 and 4).

The undegraded shives which had been extracted with 4% NaOH at ambient temperature for 5 h showed a shift in the DTG peak to 321°C and the weight loss of the DTG peak was 50% (Fig. 5). The DTG peak for

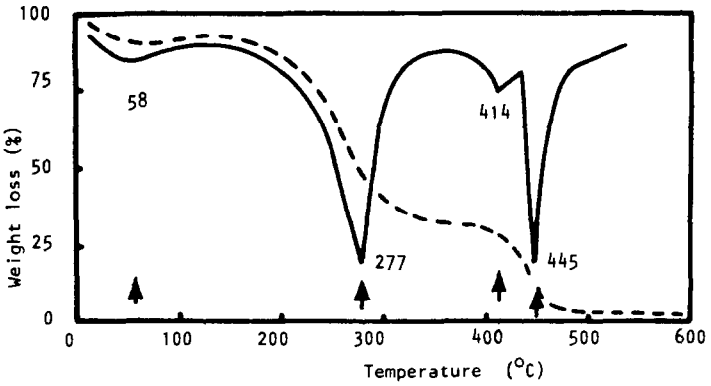

Fig. 2. The thermal analysis of undegraded flax shives showing thermogravimetric (TG) weight loss (———) and derivative thermogravimetry (DTG) profiles (— — —) of the components.

holocellulose at about 300 °C was followed by a minor peak at 414 °C and the weight loss of the undegraded shives was around 14%. After fungal colonization for 24 days, the minor DTG peak at 414 °C was not detected in shives degraded by *P. ostreatus* and only trace activity was detected in shives degraded by *C. sublaevis*. This was followed by another DTG peak at about 450 °C representing the pyrolysis of the lignin fraction present in the undegraded shives. The TG weight loss at this DTG peak was around 19% (Fig. 2). After fungal degradation for 16 days, the DTG peak at 414 °C was present as a shoulder on the lignin decomposition peak of about 450 °C. Again, after fungal degradation, the DTG peak for lignin had shifted to a slightly higher temperature of 458 °C and 11% and 16% TG weight losses were recorded for shives degraded by *C. sublaevis* and *P. ostreatus* respectively (Figs. 3 and 4).

At higher temperatures (800 °C) further decomposition was not detected in all the samples tested. However, nearly 7% and 18% of residual solid material of shives degraded by *C. sublaevis* and *P. ostreatus* respectively were detected at the end of the TG analysis. During TG analysis of the control samples, residual solid materials were not detected (Figs. 2, 3 and 4). Holocellulose of the undegraded flax shives was also analysed and the thermal decomposition peak was at 271 °C (Table 2).

DISCUSSION

Ligno-celluloses are complex and heterogeneous at both macroscopic and molecular levels [22]. During thermogravimetric analysis, physical and chemical changes are detected as changes in sample weight [3]. This approach has been used in the current study to examine changes occurring in flax shives

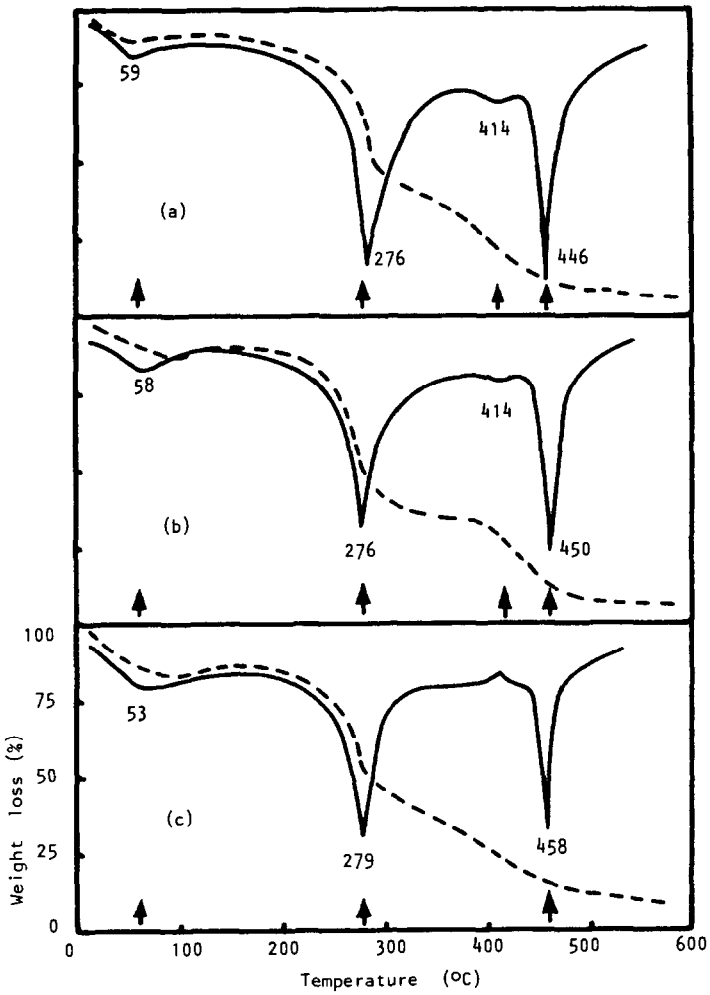

Fig. 3. The thermal analysis of flax shives degraded by *C. sublaevis* after 8 (a), 16 (b) and 24 (c) days of incubation, showing TG weight loss (———) and DTG profiles (— — —) of the components.

during fungal degradation. When wood is pyrolysed at a low heating rate (5 °C min^{-1}), hemicelluloses decompose to predominantly volatile products (CO, CO_2 and condensable vapours) between 200 and 280 °C. Cellulose, which has already undergone some chemical transformation at lower temperatures, decomposes to volatile products at an increasing rate between 280 and 500 °C, the decomposition occurring at ca. 320 °C. The volatile products of lignin, which has already undergone some change in functional groups, starts to volatilize at 420 °C [22]. At this stage, the carbon content of the residual material rapidly increases. The main constituents of flax shives found in this study, i.e. cellulose, hemicellulose and lignin, are similar to those found by Norman and Jenkins [8] and Sharma [6]. In the analysis of

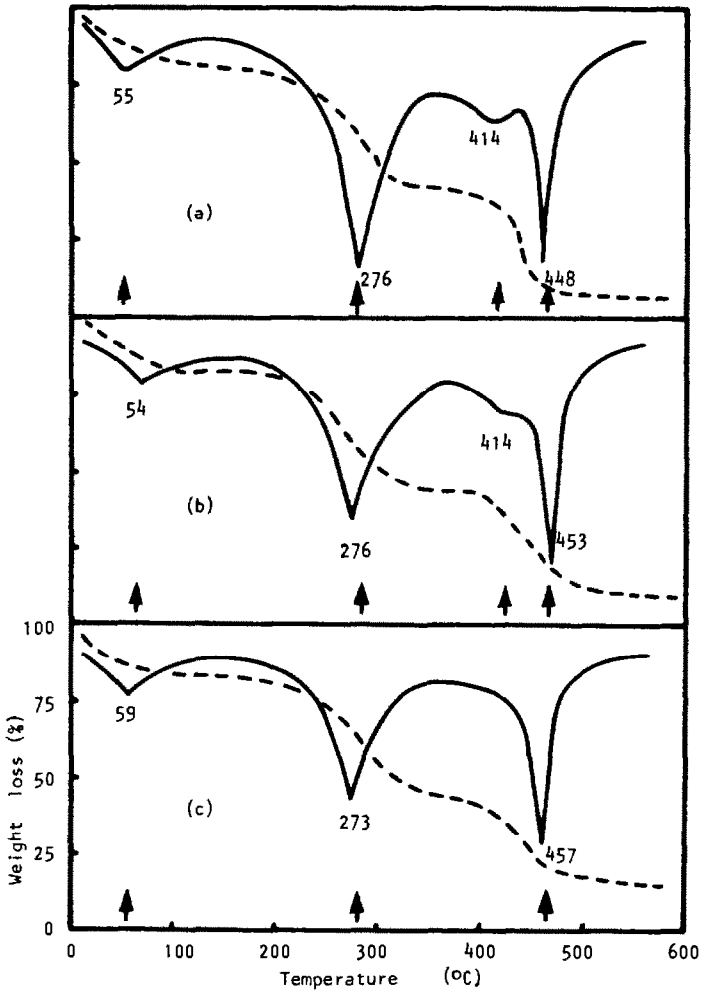

Fig. 4. The thermal analysis of flax shives degraded by *P. ostreatus* after 8 (a), 16 (b), 24 (c) days of incubation, showing TG weight loss (———) and DTG profiles (— — —) of the components.

flax, there were no separate decomposition peaks for hemicellulose and cellulose, only a single peak at about 280–300 °C. This could have been because a higher rate of heating (20 °C min^{-1}) was used under the experimental conditions. An individual peak for cellulose was only detected after hemicellulose had been removed with NaOH from the shive. The pyrolysis of cellulose and hemicellulose at about 300 °C was followed by a smaller peak at 414 °C which may represent the thermal degradation of residual hemicellulose present in close association with lignin.

The two fungi produced different quantities of degrading enzymes and the differences in enzyme activities correlated with the weight losses of the various shive components, the amounts of holocellulose and the extent of its

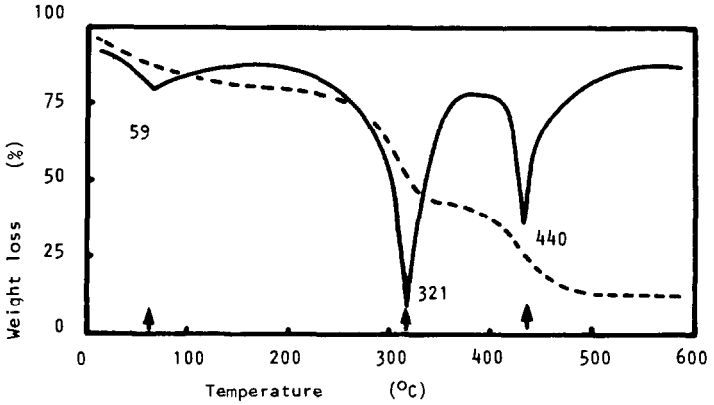

Fig. 5. The thermal analysis of flax shives extracted with NaOH to remove hemicellulose, showing TG weight loss (———) and the DTG profiles (— — —) of cellulose and lignin.

polymerization [6]. *C. sublaevis* selectively degraded the flax xylan and lignin without significantly degrading the cellulose, while *P. ostreatus* degraded most of the polysaccharides but not the lignin. The thermogravimetric profiles of wood depend not only on the cellulose content but also on the changes in lignin and hemicellulose [23–25]. Profiles of thermal decomposition changed when shives were extracted with NaOH and the shift of the DTG peak from 279 °C to 321 °C may reflect the partial removal of hemicelluloses. The height of the DTG peak for lignin in the NaOH-treated shive was smaller and the peak temperature (440 °C) was slightly lower than that for untreated shives because NaOH treatment partly removes lignin [19].

The results from the present investigation have shown that the *P. ostreatus* degraded holocellulose present in shives more efficiently than it did lignin, and that *C. sublaevis* degraded lignin more efficiently than it did holocellulose. The difference in enzyme-release patterns of the two fungi was correlated with the changes in thermogravimetric weight loss of the different components of flax shives.

Although differential thermogravimetry has been used here qualitatively, quantitative comparison can be made using peak areas as indices of thermal stability. This is useful for a rapid assessment of the degrees of fungal degradation in ligno-celluloses [11].

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REFERENCES

1 F.L. Browne, U.S.D.A. Forest Service, Forest Products Lab., Madison, WI, Report No. 2136, 1958, 69 pp.
2 F.C. Beall and H.W. Eickner, U.S.D.A. Forest Service, Forest Products Lab., Madison, WI, Res. Pap. FPL-130, 1970, 26 pp.
3 T. Nguyen, E. Zavarin and E.M. Barrall, J. Macromol. Sci. Rev. Macromol. Chem., G20 (1981) 1–65.
4 W.K. Tang, U.S.D.A. Forest Products Lab., Madison, WI, Research Paper FPL 71, 1967, 16 pp.
5 F.C. Beall, Wood Sci., 5 (1972) 102–108.
6 H.S.S. Sharma, Appl. Microbiol. Biotechnol., 25 (1987) 542–546.
7 R.J. Thomas, Wood technology: chemical aspects, Am. Chem. Soc. Symp. Ser., 43 (1977) 1–23.
8 A.G. Norman and S.H. Jenkins, Biochem. J., 28 (1929) 2160–2168.
9 T. Hiroi, K.E. Eriksson and B. Stendlund, Sven. Papperstidn., 79 (1976) 162–166.
10 A.E. Brown and H.S.S. Sharma, Trans. Br. Mycol. Soc., 86 (1986) 173–175.
11 F.C. Beall, W. Merrill, R.C. Baldwin and J.H. Wang, Wood Fibre, 8 (1976) 159–167.
12 E.B. Cowling, U.S.D.A. Forest. Tech. Bull., No. 1258, 1960, 79 pp.
13 H.S.S. Sharma, Ann. Appl. Biol., 109 (1986) 605–611.
14 G.L. Miller, Anal. Chem., 31 (1959) 426–428.
15 A. Haars and A. Huttermann, Arch. Microbiol., 134 (1983) 309–313.
16 E.B. Cowling, U.S.D.A. Forestry Service, Forest Products Lab., Madison, WI, Report No. 2177, 1960.
17 W.E. Moore and D.B. Johnson, U.S.D.A. Forest Products Lab., Madison, WI, unnumbered publication, 1967.
18 Anon, Official French Standard NF, G 06-037 (1981) 1–21.
19 C. Doree, The Methods of Cellulose Chemistry, Chapman and Hall, London, 1947, 543 pp.
20 F.W. Norris and I.A. Preece, Biochem. J., 24 (1929) 59–66.
21 B.D. Michel and A.H. Knight, J. Exp. Bot., 16 (1965) 1–15.
22 M.A. Conners and C.M. Salazar, in Proceedings of Symposium on Forest Products Research International-Achievement and the Future, 5 (1985) 12–14.
23 F.C. Beall, Wood Fibre, 1 (1969) 215–226.
24 T. Hirata and H. Abe, Mokugzai Gakkaishi, 19 (1973) 451.
25 R.D. Cardwell and P. Lunar, Adv. Chem. Ser., 164 (1976) 362–381.