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BIOMECHANICAL PULPING OF ASPEN CHIPS BY *PHANEROCHAETE CHRYSOSPORIUM*: FUNGAL GROWTH PATTERN AND EFFECTS ON WOOD CELL WALLS

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ABSTRACT

Evaluation of the potential of biopulping requires a better understanding of its physical and chemical basis. Here we investigate the fungal treatment used on wood chips prior to mechanical pulping in a bench-scale biomechanical pulping process currently under study. Scanning electron microscopy was used to observe fungal growth and the degradation of nutrient-supplemented aspen chips after a 3-week treatment with the white-rot basidiomycete *Phanerochaete chrysosporium* strain BKM-F-1767. The fungus grew well both across the chip surfaces and throughout the wood cells. The fungus penetrated the chips through the lumina of wood vessel and fiber cells as well as through natural wood cell pits and fungal bore holes. Partial degradation of the cell lumen walls by secreted fungal enzymes was evident. Erosion troughs and localized wall fragmentation or thinning were clearly visible as was a generalized swelling and relaxing of the normally rigid wood cell wall structure.

Keywords: *Phanerochaete chrysosporium* Burds., white-rot fungi, basidiomycetes, biopulping, aspen wood, wood degradation, fungal bore holes, erosion troughs, cell-wall degradation.

INTRODUCTION

There is current industrial interest in evaluating the potential of biological pulping processes that use white-rot fungi or their isolated enzymes (Bar-Lev et al. 1982; Eriksson and Kirk 1985; Eriksson and Vallander 1982). The fungal species selected for study have generally been from among those fast-growing

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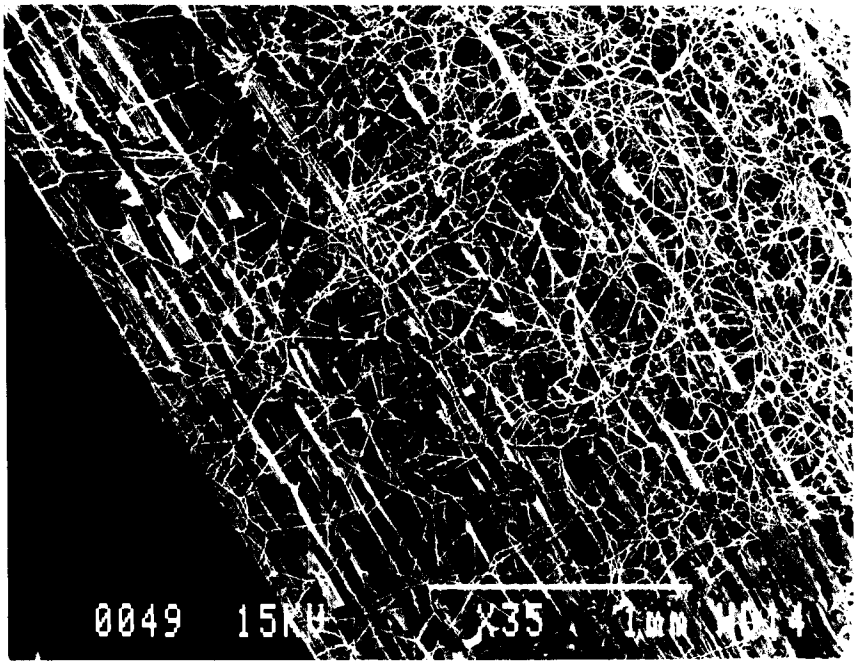

FIG. 1. Web-like hyphal network on the surface of a nutrient-supplemented aspen wood chip during a 3-week treatment by *Phanerochaete chrysosporium* strain BKM-F-1767. (× 35)

basidiomycetes capable of selective wood delignification (Blanchette et al. 1985; Eriksson and Kirk 1985). The most highly studied species is *Phanerochaete chrysosporium* (Eriksson and Kirk 1985; Kirk 1988; Otjen et al. 1987).

One type of biopulping process, termed "biomechanical pulping," involves the fungal treatment of chips prior to mechanical refining (Eriksson and Kirk 1985; Myers et al. 1988). The potential benefits of biomechanical pulping include energy savings during mechanical fiberization and refining of chips (Eriksson and Kirk 1985), improvement of paper sheet strength properties (Myers et al. 1988), and reduction of undesirable wastes (Eaton et al. 1980; Eriksson and Kirk 1985).

Increased understanding of the effects of fungi on wood under conditions likely to be used in a biopulping process is needed to most effectively evaluate the potential of biomechanical pulping. The information currently available generally concerns non-nutrient-supplemented wood blocks treated for extensive periods of time (e.g., months: Cowling 1961; Otjen et al. 1987).

Here we investigate the degradation caused by a promising strain of *P. chrysosporium* on nutrient-supplemented aspen chips of a size standard to the pulp industry. The conditions used model a high-yield fungal treatment used in a bench-scale biomechanical pulping process currently under study. The treatment gives both energy savings (Leatham et al. unpublished results) and improved sheet strength properties (Myers et al. 1988). We report the pattern of fungal growth on the chips as well as the degradative action caused by the enzymes secreted.

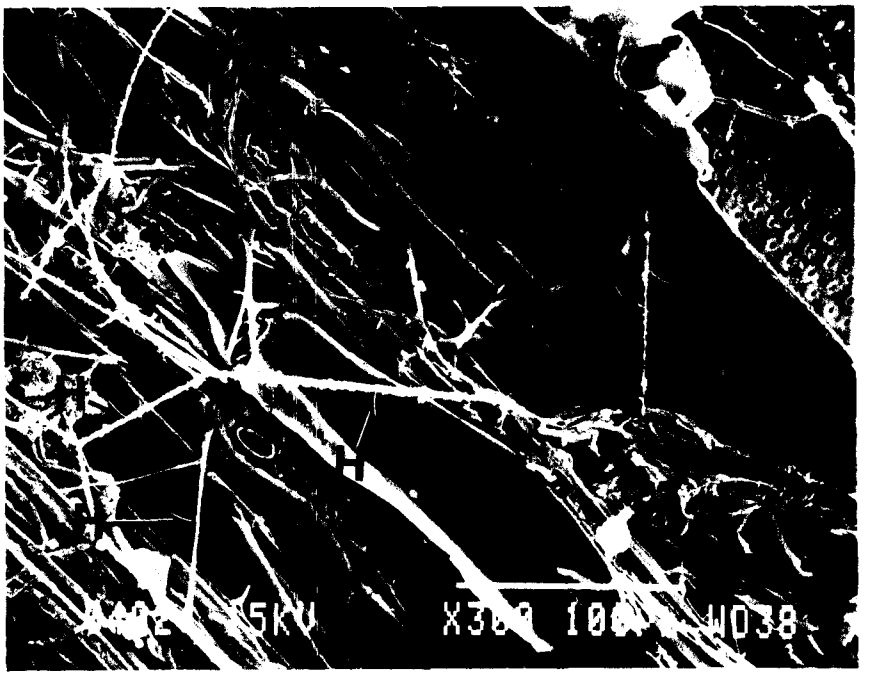

FIG. 2. Hyphae (H) bridging vessel or fiber cells on wood chip surfaces with little contact with the wood. (× 300)

MATERIALS AND METHODS

Fungal treatment of wood chips

Phanerochaete chrysosporium strain BKM-F-1767 was grown on wood chips supplemented with a chemically defined liquid medium (Myers et al. 1988). Approximately 60 g (oven-dry basis) of 19-mm aspen (*Populus tremuloides* Michx.) chips at 60% final moisture content supplemented with a low-nitrogen, glucose-containing liquid medium [including 2.5% glucose, 0.025% nitrogen, minerals and vitamins] were steam-sterilized (121 C) and then inoculated (Myers et al. 1988). Incubation was in cotton-stoppered 500-ml Erlenmeyer flasks under an air atmosphere, maintained at 39 ± 1 C and 70% relative humidity (RH). Fungal activity was stopped after a 3-week incubation by placing the colonized chips in a 70% ethanol solution for 15 min.

Sample preparation and scanning electron microscopy (SEM)

Fungus-treated chips were dehydrated in a series of ethanol solutions of 85%, 95%, and 100% for 5 min each. They were then critical-point dried, mounted onto metal stubs, and coated with a 200 Å layer of gold. Chip interiors were studied on samples that had been split in the radial plane with a clean sharp razor blade prior to gold coating. Specimens were examined in a JEOL 840[2] scanning

[2] The use of trade and company names is for the benefit of the reader and use does not constitute an official endorsement of approval of any service or product by the USDA to the exculsion of others that may be suitable.

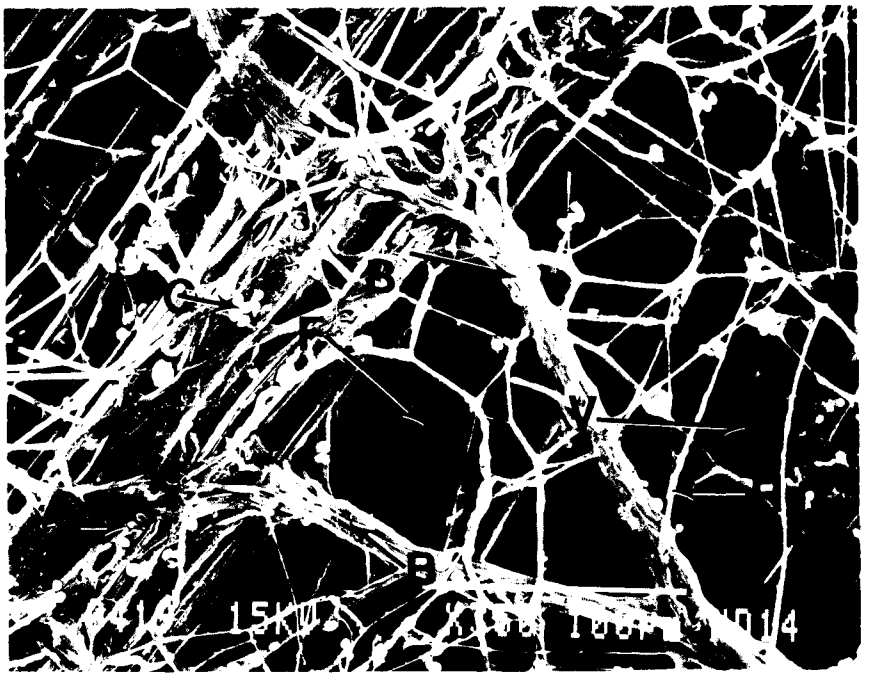

FIG. 3. Fungal conidia (C), hyphae (H), and hyphal bundles (B) observed on wood chip surfaces near wood vessel (V) and fiber (F) cells. (× 350)

electron microscope using an accelerating voltage of 20 kV. Several points should be noted. The occasional collapsed hyphae observed in some micrographs apparently resulted from sample dehydration. Drying isolated hyphae in ethanol in the absence of critical-point drying did not prevent their collapse. Collapsed hyphae were not included in diameter measurement. The preparation method may have introduced additional artifacts. For example, during sample preparation some hyphae may have been displaced from their *in vivo* location.

OBSERVATIONS

Fungal growth on and inside wood chips

After the 3-week growth period, vegetative hyphae of *P. chrysosporium* had formed a strong web-like network over the chip exterior surfaces (Fig. 1). In agreement with the earlier observations of Eriksson et al. (1980a), the hyphal network rapidly colonized the chip surfaces by traversing above the chip surface. Supported by only minute areas of wood contact (Fig. 2), hyphae were able to span directly across several vessel or fiber cells or lumina at a time. These exterior hyphae generally displayed a variety of diameters, between 1 and 3 μm. However, some hyphae were constricted into thread-like configurations.

Also evident on the chip surfaces were tightly organized bundles of 4 to 7 hyphae from which many of the network hyphae originated (Fig. 3). Each bundle was approximately 7 to 13 μm in diameter. In addition, many elliptically shaped conidia (asexual spores for vegetative propagation) typical for *P. chrysosporium* were present (Fig. 3).

Wood cell-wall degradation was quite extensive on some chip surfaces. Hyphae

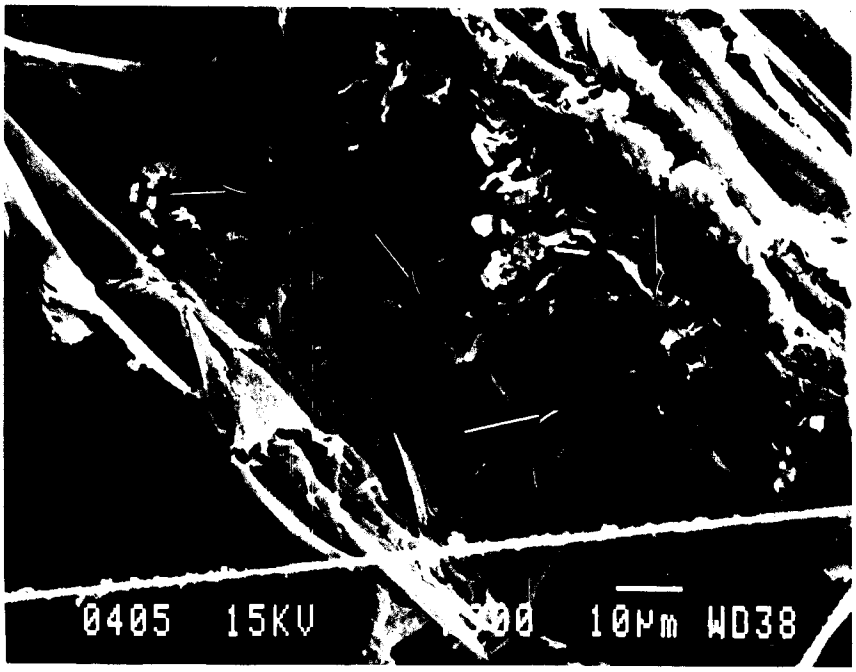

FIG. 4. Fragmented portions of a vessel cell wall broken up by the action of enzymes secreted by fungal hyphae (H). (× 900)

appeared more abundant in areas where large portions of the cell wall were fragmented and separated from the main body of the cell (Fig. 4). Many hyphae projected from the network into the wood cell lumina. Hyphae were observed lying along the longitudinal axes of the wood cells and penetrating through the outer walls into the lumina of adjacent vessels or fibers. Fungal bore holes like those reported by Eriksson et al. (1980a, b) gave the hyphae lateral movement through the wood cell walls (Fig. 5).

Fungus action on the interior cell walls of wood chips

When viewed from the radial plane, hyphae were frequently observed that had penetrated into the inside of the chips, colonizing vessel and fiber lumens. The interior hyphae were generally larger in diameter than the exterior hyphae, with many being as wide as 6 μm. The tightly organized hyphal bundles noted on the surface of the chips were not found within the chips.

Hyphae were most prevalent in vessels, less prevalent in fibers, and least prevalent in ray cells. Hyphae preferentially grew parallel to the longitudinal axes of vessel and fibers. However, hyphae within these cells spread in other directions as well. In the walls, hyphae spread transversely through naturally occurring openings (pits) and also through fungal bore holes that were approximately 1 to 7 μm in diameter.

Although chips colonized for 3 weeks by *P. chrysosporium* showed little evidence of the separation of the wood fiber cells previously reported in studies using longer incubations (Otjen et al. 1987; Ruel et al. 1981), widespread degradation was nevertheless readily evident. Localized wood cell-wall thinning and fragmentation

FIG. 5. A bore hole (B) produced by a fungal hypha (H) at the chip surface. (× 1,000)

(see discussion) as well as bore holes used by the hyphae to gain entry into new cells (Fig. 6) were clearly visible.

Enzymes secreted by the hyphae acted on cell lumen walls at extended distances from the hyphae. The fungus formed erosion troughs on the lumen walls (Fig. 7) similar to those previously reported for *Trametes* (syn. *Polyporus*) *versicolor* (Levy 1974). In areas of marked fungal action, the erosion troughs were much wider than the diameter of the hyphae (Fig. 8). Wall material was removed both near hyphal tips and at points of close contact with the lateral surfaces of the hyphae. Interior hyphae were often enveloped in a slime sheath (Fig. 9) similar to that reported in other fungi, which probably gave increased contact with the cell wall (Palmer et al. 1983; Procter 1941).

The fungal treatment resulted in bulk overall changes to the cell wall structure of the nutrient-supplemented aspen wood chips. As observed in cross-sections, the changes included (1) swelling, (2) softening or relaxing (partial collapse) of the rigid tube-like cells, and (3) localized areas of thinning and fragmentation (Fig. 10).

DISCUSSION

The use of scanning electron microscope improved our understanding of the sequence of events leading to the modification of wood chips during a 3-week treatment with *P. chrysosporium*. The events included chip surface colonization, surface penetration, interior colonization, and the partial degradation of the internal cell walls, which was initiated at the lumen surfaces.

The tightly organized bundles of hyphae observed on the chip surfaces have

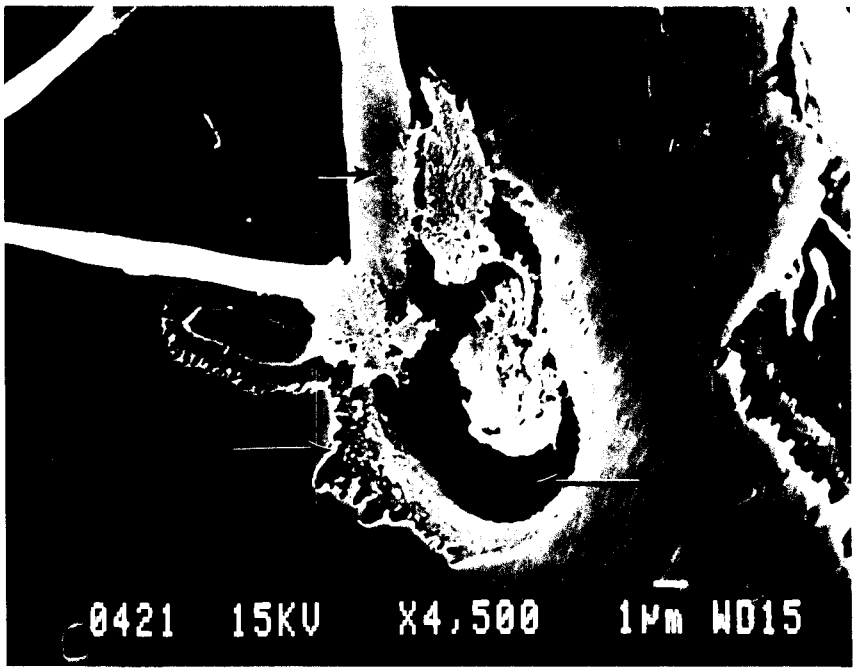

FIG. 6. A bore hole (B) through a vessel lumen wall and enzyme degradation of the wall (W) around the hole periphery caused by a fungal hypha (H). (× 4,500)

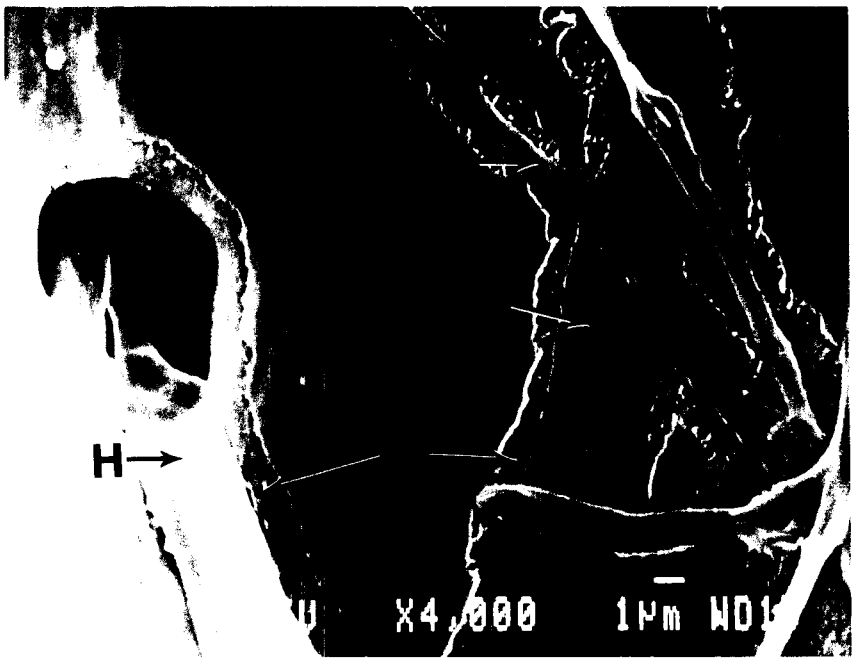

FIG. 7. Erosion troughs (E) produced by enzymes secreted by (collapsed) fungal hyphae (H) lying in the centers of the troughs. (× 4,000)

FIG. 8. Enzyme erosion troughs (E) wider than the hyphae that produced them. One trough containing the (collapsed) hypha (H) that produced the trough (in trough center) measured approximately 4 μm. (× 1,900)

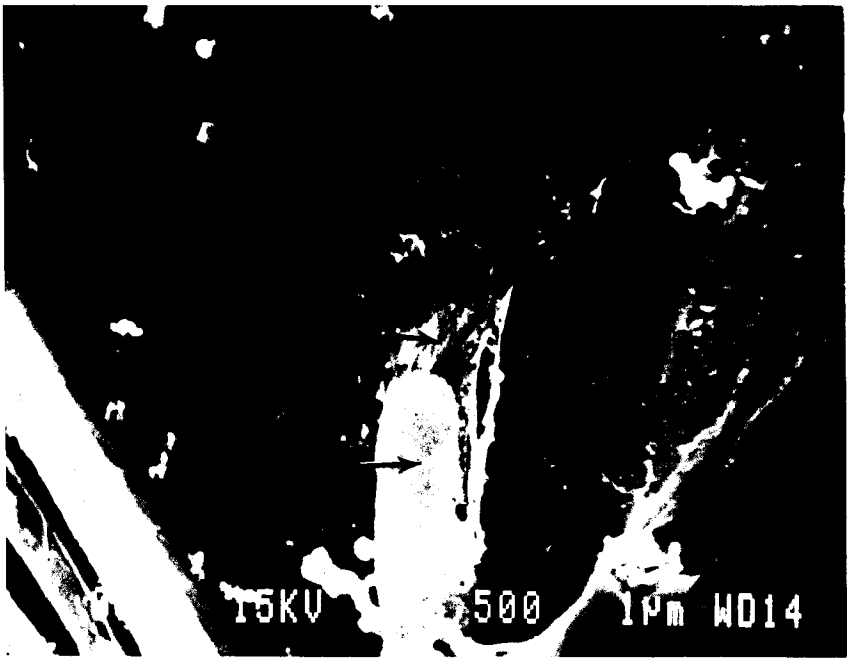

FIG. 9. Secreted slime sheath (S) enveloping a hyphal tip (H) that retracted during sample preparation. (× 300)

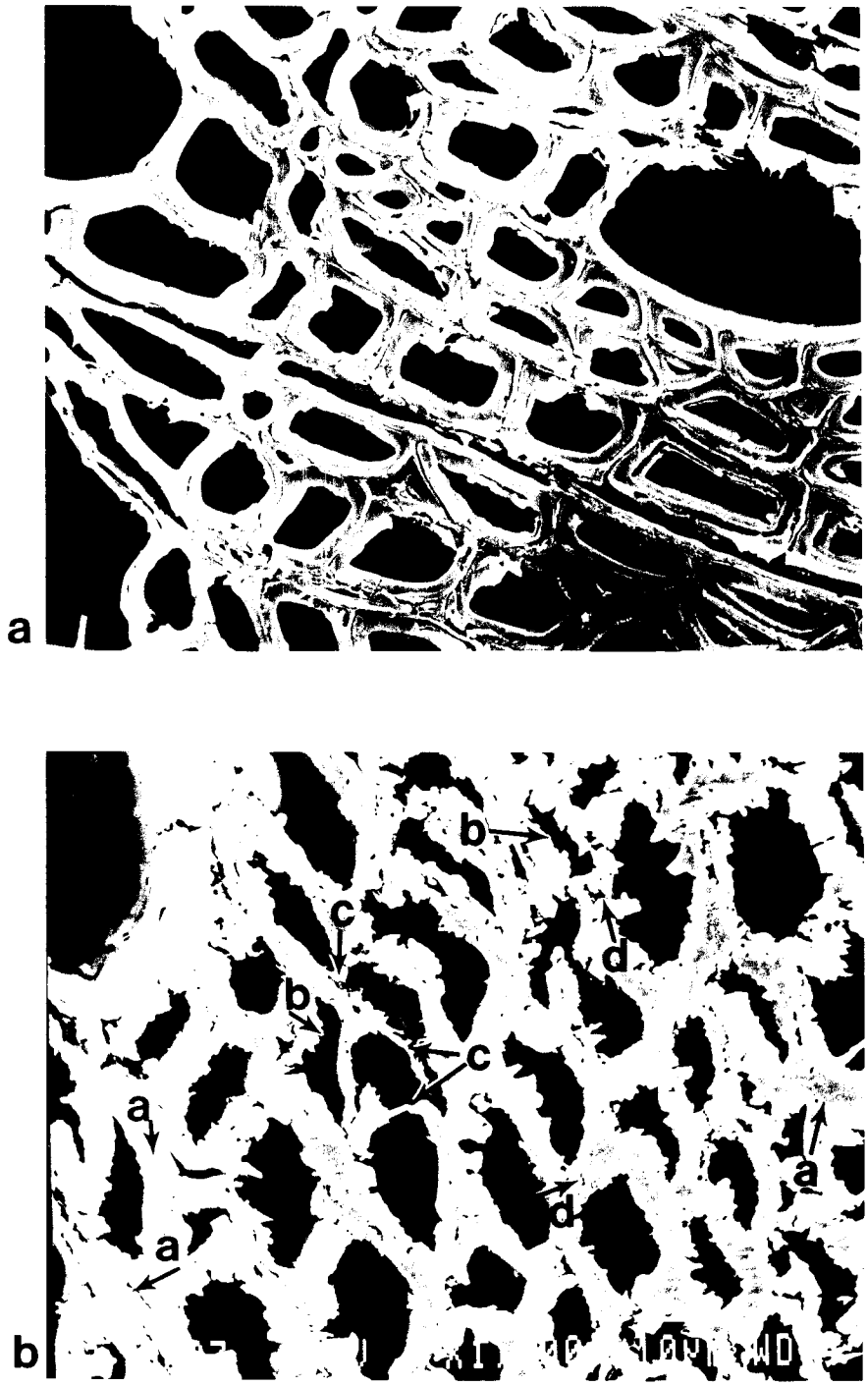

FIG. 10. The normally rigid wood cell wall structure within an aspen chip (A) was modified by the model 3-week fungal treatment used in the bench-scale biomechanical pulping process. (B) Modifications included (1) cell wall swelling (a), (2) enzymatic softening or relaxing resulting in the partial collapse of the tubelike-cell structure (b), and (3) localized areas of wall thinning (c) or fragmentation (d). (× 1,000)

been reported in other fungi (e.g., Thompson 1983), but not yet in *P. chrysosporium*. These hyphae bundles may function in the following ways: (1) to protect against deleterious external factors (e.g., dehydration), (2) to increase competitive advantage for the fungus by concentrating its inoculum potential in localized areas, (3) to channel nutrient and moisture resources allowing fungal outgrowth into new areas that may have fewer resources, or (4) to channel resources for the formation of fruiting structures.

It is important to realize that the anatomical arrangement of the wood cell structures may influence the entry and advancement of a fungus. Hardwoods such as aspen presumably show no major barrier to organism growth. Hardwoods consist primarily of hollow parallel vessel and fiber cells (Côté 1980). In our study, hyphae entered and advanced into the chips through vessel or fiber lumens as well as directly through the cell walls, using both natural wood pits and fungal bore holes. The number of bore holes in a cell wall appeared to be proportional to the length of time that hyphae had been in the vicinity of the cell. More bore holes were observed at the chip surface than in the interior, and interior cells containing more hyphae also contained more bore holes. It is not known whether or not the thread-like hyphae observed were penetration forms as suggested by Eriksson et al. (1980a) or how the organism preferentially chooses vessel elements for colonization.

As reported by Ruel et al. (1981) for non-nutrient-supplemented wood, the interior of nutrient-supplemented chips was attacked by *P. chrysosporium* in a manner similar to that for the chip surface. Attack was preferentially from the lumen side using secreted enzymes capable of lateral diffusion—that is, hyphae were much more frequently observed lying in erosion troughs along the lumen walls than within the cell-wall layers. The distinct erosion zones near hyphal tips suggest that the tips were a significant initial source of enzymes. During more advanced stages of decay, which characteristically showed further wall thinning and more extensive breakdown, the secreted enzymes must have penetrated, extensively degrading the innermost layers of the cell wall.

The enzyme action by *P. chrysosporium* was most extensive when hyphae directly contacted the wood cell wall. This was presumably due to either increased concentration of the enzymes or the removal of reaction end-products by the hyphae. It is likely that the diffusion of the secreted enzymes by *P. chrysosporium* is directed or aided in some way by the slime sheath, as suggested by Jutte and Sachs (1976) and Palmer et al. (1983) for other fungi. Increased slime content could explain why interior hyphae were wider in diameter than exterior hyphae.

CONCLUSIONS AND FURTHER RESEARCH

When grown for 3 weeks on nutrient-supplemented aspen wood chips under conditions used in a biomechanical pulping process, *P. chrysosporium* strain BKM-F-1767 successfully grew across all the exposed surfaces and penetrated throughout the chips. Exterior hyphae were generally narrower in diameter than interior hyphae and hyphal bundles were present. Hyphae penetrated through wood cell lumina, natural wood pits, and fungal bore holes. Inside the chips, the bulk of the degradation originated at the cell lumen surfaces. The fungus degraded the internal lumen cell walls using secreted enzymes and a slime sheath, which apparently increased wall contact. Erosion troughs, bore holes, thinning, and frag-

mentation were readily evident in the walls. The overall wood structure appeared to soften and relax in response to attack.

The chemical and thermal treatments used in chemimechanical (CMP) and chemithermo-mechanical (CTMP) pulping processes yield improvements as a result of chip softening and wood fiber swelling (Leask and Kocurek 1987). This facilitates both mechanical chip fiberization and pulp fiber refining. The increased fiber flexibility and swelling increase the interfiber bonding within paper sheets, and hence, increase sheet strength properties. Our observations suggest that the physical basis for the efficacy of the fungal treatment investigated here is likely to involve the overall enzymatic softening and swelling of the wood cell-wall fibers as well as the thinning and fragmentation of the wood cell walls in localized areas.

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