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Fungal Biodegradation of Lignopolystyrene Graft Copolymers

OLEG MILSTEIN,[1] ROLF GERSONDE,[1] ALOYS HUTTERMANN,[1] MENG-JIU CHEN,[2] AND JOHN J. MEISTER[2]*

Forstbotanisches Institut der Universität Göttingen, 3400-Göttingen, Germany,[1] and Department of Chemistry, University of Detroit Mercy, P.O. Box 19900, Detroit, Michigan 48219-3599[2]

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White rot basidiomycetes were able to biodegrade styrene (1-phenylethene) graft copolymers of lignin containing different proportions of lignin and polystyrene [poly(1-phenylethylene)]. The biodegradation tests were run on lignin-styrene copolymerization products which contained 10.3, 32.2, and 50.4% (wt/wt) lignin. The polymer samples were incubated with the white rot fungi *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, and *Trametes versicolor* and the brown rot fungus *Gloeophyllum trabeum*. White rot fungi degraded the plastic samples at a rate which increased with increasing lignin content in the copolymer sample. Both polystyrene and lignin components of the copolymer were readily degraded. Polystyrene pellets were not degradable in these tests. Degradation was verified for both incubated and control samples by weight loss, quantitative UV spectrophotometric analysis of both lignin and styrene residues, scanning electron microscopy of the plastic surface, and the presence of enzymes active in degradation during incubation. Brown rot fungus did not affect any of the plastics. White rot fungi produced and secreted oxidative enzymes associated with lignin degradation in liquid media during incubation with lignin-polystyrene copolymer.

Plastics are a significant part by weight and volume of the waste in municipal landfills, and this plastic fraction of waste is projected to increase. Since plastics became an integral part of contemporary life, opposition to depositing plastics in landfills has grown, because most synthetic polymers are resistant to biodegradation. The annual consumption of thermoplastic polystyrene has risen to 1.3×10^6 tons (1 ton = ca. 907 kg) in Western Europe (31) and 2.5×10^6 tons in the United States (40). The material is extremely resistant to bioconversion. In addition, opposition to incinerating plastics exists because of the potential of hazardous emissions. On the other hand, blending polymers or grafting some components onto the main polymer backbone may bring a significant alteration of the properties of the initial components. One may enhance the degradability of plastics by linking selected, readily degradable substituents into the polymer chemical structure.

Several attempts to introduce some naturally occurring polymers of microbial or plant origin, such as starch (24), cellulose (4), and poly(hydroxybutric acid) (5), into a synthetic polymer structure have been reported. The resulting products have shown appreciable biodegradability of the naturally occurring fraction of the plastic mixture. Lignin is the second most abundant biopolymer after cellulose but has not previously been used in these degradable plastics. Lignin occurs in the cell walls of all woody plants. It is a polymer with several attractive structural features and a variety of reactive functional groups. It is the biggest natural source of polyaromatics. About 50×10^6 tons of lignin are released annually by the pulping industry. This immense amount of biomass is utilized far below its potential value. Because of its polyaromatic nature, lignin may represent an enormous supply of chemicals available for replacing expensive petrochemicals with renewable raw material of comparatively low cost in the production of engineered materials (7). The increase in lignin utilization value might be achieved by copolymerization of lignin with synthetic monomers (8). Numerous works claim copolymerization of lignin and some alkyl compounds (23). Recently, a method for grafting styrene onto lignin by using free radicals has been developed (25–28, 30).

Polystyrene is extremely resistant to biodegradation, yet some modification of polystyrene derivatives by soil microflora has been reported (33). Although the recalcitrant nature of lignin impedes its easy conversion, under the right environmental conditions biological systems can transform lignin to various extents (10, 19). The ultimate transformation of lignin in nature, its complete oxidation to CO_2, is achieved primarily by the white rot basidiomycetes, which are well known for their abilities to degrade lignin (19). Brown rot fungi, in contrast, leave lignin essentially undegraded. However, there is extensive evidence that incubation with brown rot fungi changes the structure of lignin so that it is increasingly susceptible to biodegradation by other groups of microorganisms (18). In this paper, we report how copolymerization of lignin and styrene monomer increases susceptibility of the resulting lignin-polystyrene product, and particularly its polystyrene moiety, to fungal degradation.

MATERIALS AND METHODS

LPS complex and lignin and polystyrene homopolymers. The tested lignopolystyrene polymers (LPS) were synthesized in the Department of Chemistry of the University of Detroit Mercy. The polymers were synthesized by solution polymerization with dimethyl sulfoxide as solvent.

Sample A was prepared by placing pure styrene in a conical flask and bubbling it with nitrogen (N_2) for 10 min. Sample B was prepared by placing lignin, calcium chloride, and dimethyl sulfoxide in a conical flask, stirring the solution until the additions were dissolved, and bubbling the solution with N_2 for 10 min. Samples A and B were stirred while being purged with N_2. A 30% (wt/vol) aqueous solution of H_2O_2 was added to sample B and bubbled with N_2 for 20 min. Solution A was added to solution B. After 5 min of stirring and bubbling N_2 through the reaction mixture, the flask was stoppered, placed in a 30°C bath, and stirred at 4

* Corresponding author.

TABLE 1. Recovery of copolymers incubated on uninoculated plates[a]

Incubated material	Recovered polymer (% [wt/wt] of initial)	Solubilized polymer (% [wt/wt] of recovered)	Polymer determined spectrophotometrically (% [wt/wt] of recovered)
Lignin	84 ± 2.1	99 ± 4.8	103 ± 1.2
LPS50	96 ± 4.2	96 ± 5.3	94 ± 0.6
LPS32	90 ± 4.6	93 ± 3.9	89 ± 0.9
LPS10	89 ± 5.1	90 ± 4.5	86 ± 0.8
Polystyrene	89 ± 5.1	90 ± 4.5	86 ± 0.8

[a] Data are averages of values for 10 uninoculated plates. A total of 10 ml of tested material was incubated for 68 days on agar plates. The tested copolymer was solubilized in 4 ml of a dioxane-ethanol-dichloroethane (7:3:5) mixture, and the contents of lignin and polystyrene were determined spectrophotometrically.

Hz for 48 h. All reactions were terminated by opening the reaction vessel. This terminated slurry was then added to 10 times its volume of acidified water (pH 2), and the polymer was recovered by filtration. Extensive studies of this copolymerization technique, the properties of the products, and proof of copolymerization have been published separately (26, 27).

This polymerization method was used to create LPS which contained 10.3% (LPS10), 32.2% (LPS32), and 50.4% (LPS50) (wt/wt) lignin. The copolymers tested for biodegradation were supplied in two aggregate forms: as a fine powder of numerically more than 100 mesh and as a compression-molded sheet of circular plastic film 0.15 mm thick with a 5- to 7-cm diameter. The films had smooth, hydrophobic surfaces and, when formed on birch wood, contact angles with water of 110 to 120°. The lignin (kraft pine lignin; Indulin AT) was derived as a by-product of kraft pulping of soft wood. It was supplied and used in copolymerization reactions as received from Westvaco Corp., North Charleston, S.C. It was washed and reprecipitated before use in biodegradation studies. Polystyrene homopolymer, material RIPO, was used as received from Amoco Chemical Co., Naperville, Ill. The cylindrical pellets (2.5 mm in diameter by 2.5 mm long) were tested directly for biodegradation and were compression molded into circular films 0.25 mm thick by 7 cm in diameter for testing. All compression moldings were done at 150°C and 192 kPa of pressure for 1 min.

Organisms and cultivation. The microorganisms used in this work were basidomycetes: white rot fungi *Phanerochaete chrysosporium* Burdsall, *Trametes versicolor* I (L. ex Fr.) Quelet (ATCC 11235), and *Pleurotus ostreatus* var. florida (F6) (Jaquin ex Fr.), Kummer. The activities of the white rot fungi were compared with that of brown rot fungus *Gloeophyllum trabeum* (Pers. ex Fr.), Murrill. White rot fungi were from the culture collection of the Forstbotanisches Institut, Göttingen University, 3400-Göttingen, Germany. *G. trabeum* was generously provided from the collection of Bundesanstalt für Materialforschung und-prüfung, Berlin, Germany.

The cultures were maintained either on slants with 2.5% malt agar or in conical 500-ml flasks with sterile, chopped, moistened wheat straw with a moisture content of about 60% (wt/wt). The tested fungi were cultivated either on solid 2.5% agar medium for the study of copolymer biodegradation or in liquid medium for the comparative study of the patterns of the analyzed laccase and peroxidases. The two media contained the same concentrations of mineral salts and glucose and a reduced content of nitrogen as specified by Kirk et al. (20) and Kern (16).

The liquid medium (30 ml in 500-ml conical flasks) was placed in both control and test flasks, and test flasks also received 50 mg of lignin, polystyrene, or the LPS complex. Mycelia grown for 9 days in the liquid medium without any added polymer and then homogenized with Ultra-Thorax were used as inoculum. The flasks were inoculated in triplicate with equal 4-ml volumes of homogenized mycelia. Inoculated flasks were incubated in the dark as standing cultures for 3 weeks at 25°C. The plate inoculum consisted either of a piece of straw from the maintaining culture or of plugs (2 by 5 mm) from a 7-day-old plate culture. The plates were placed at 25°C in a thermostatted chamber at 100% humidity for 68 days. Plates were sealed with Parafilm tape. Every 7 days, the plates were aseptically opened to exchange the air. Three 10-mg volumes of powdered analyzed material were placed on a piece of sterile dialysis membrane to facilitate recovery of the incubated material. Pressed plastic films measuring 0.4 cm^2 were placed directly on the surface of the solid medium near the inoculum. Tested polymers were disinfected by treatment for 15 min with 70% ethanol and dried under aseptic conditions before introduction into the incubation media. No significant UV absorbance was found in the disinfection liquid after it was drained from the test films.

Evaluation of polymer degradation. Biodegradation was monitored by measurement of weight loss, in particular by decrease of the lignin and polystyrene components from the biodegraded complex, and by scanning electron microscopy (SEM) of the decayed polymer. Tested powdered copolymers were intimately bound with the growing fungi; thus, direct measurements of the loss of polymer weight were impossible. To evaluate loss of the tested copolymer, the nitrogen contents of the aliquots of dry collected material from the triplicate samples on inoculated plates and the uninoculated control were measured. The amount of nitrogen determined was extrapolated to the amount of fungal biomass by applying the same nitrogen/biomass ratio as was found in the pure fungus from the cultures of identical ages and media. The computed fungal biomass was subtracted from the amount of recovered material.

The nitrogen in the polymers from inoculated plates and uninoculated controls was determined by elemental analysis after combustion of the dry sample at 1,020°C in a quartz combustion reactor. Individual components, particularly nitrogen, were separated and eluted on chromatographic column PQS and detected and measured with the help of a thermal conductivity detector in the elemental analyzer (EA 1108; Carlo Erba Instruments). The quantities of separate components (lignin and polystyrene) in the treated copolymer were analyzed by UV spectroscopy by using multicomponent analysis methods. Known mixtures of pure components were used to calibrate the spectrophotometer before mixtures with unknown compositions were analyzed. Dry LPS complexes from inoculated plates and uninoculated controls were solubilized in a dioxane-ethanol-dichloroethane (7:3:5, by volume) mixture. The chosen solvent showed an A_{236} to A_{250} of almost zero and no absorbance above this range. Spectrophotometer measurements of the absorbance of the solubilized polymer and calculations of the concentrations of the lignin and polystyrene components were performed on a Hewlett-Packard 8451A Diode Array Spectophotometer with its software package, Multicomponent Analysis.

Assay of enzyme activity. Lignin peroxidase (LiP) activity

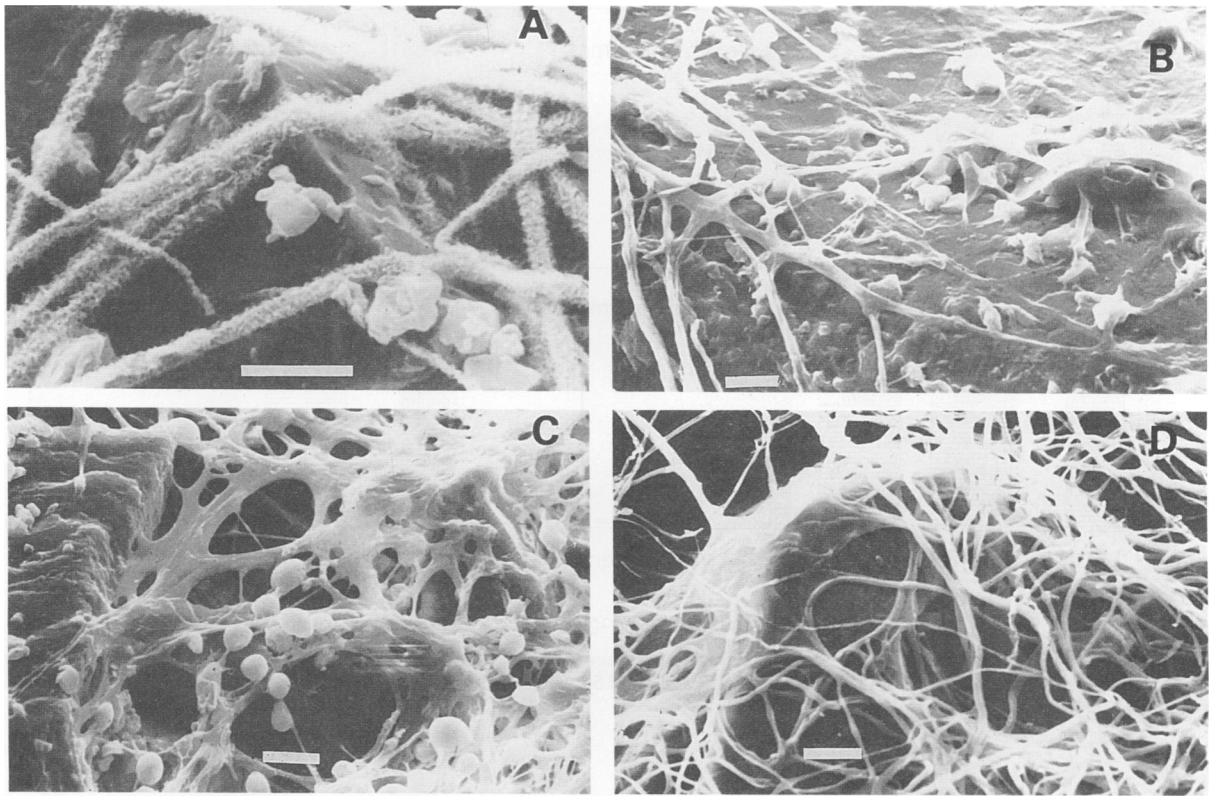


FIG. 1. SEMs of powdered LPS50 incubated for 30 days with *G. trabeum* (A), *P. ostreatus* (B), *P. chrysosporium* (C), or *T. versicolor* (D). Fungal hyphae have overgrown the polymer. The extracellular mucilage facilitates adhesion of hyphae and promotes efficient interaction with the plastic surface, as shown in B, C, and D. Bars, 10 μm.

was measured by the rate of oxidation of veratryl alcohol (42). Units of activity were equivalent to micromoles of substrate oxidized at pH 3.0 and 25°C to veratrylaldehyde [$\varepsilon_{310\ nm}$ = 9,300/(M · cm)] in 1 min as measured spectrophotometrically. The assay mixture consisted of culture medium filtered through a 0.45-μm-pore-size filter, veratryl alcohol solution (1 ml, 3 mM, in 0.33 M sodium tartrate buffer at pH 3.0), and freshly prepared 54 mM H_2O_2 (16 μl).

Laccase activity was measured by monitoring color development of syringaldazine (4-hydroxy-3,5-dimethoxybenzaldehyde azine) at 525 nm (11). The reaction was started at 25°C by the addition of 50 μl of cell-free culture medium and was monitored spectrophotometrically during the linear period for detection of the oxidation product of syringaldazine [ε_{525} nm = 65,000/(M · cm)]. The rate of the reaction was expressed in micromoles per minute per milligram of protein. The effect of peroxidase able to oxidize syringaldazine in the presence of H_2O_2 was eliminated by the addition of catalase (EC 1.11.16; 20 U [20 μl]). This addition was sufficient to remove all of the endogenous H_2O_2 from the aliquot of culture medium. Preincubation of sample with catalase was done 5 min before the laccase reaction started.

Mn(II) peroxidase (MnP) activity was measured by monitoring the oxidation of guaiacol to tetraguaiacol by monitoring A_{466}. The reaction was started at 25°C by the addition of $MnSO_4$ (25 μl, 100 mM) to 1 ml of reaction mixture composed according to the method of Aitken and Irvine (1).

SEM. The pieces of pressed copolymer (approximately 0.4 cm^2) from both the uninoculated control plates and the plates with fungi were withdrawn after 68 days of incubation in a 25°C bath at 100% humidity. Withdrawn copolymers were then mounted on SEM stubs, sputter coated with gold to a thickness of about 10 nm, and observed and photographed with a Phillips SEM model 515.

RESULTS AND DISCUSSION

Verification of quantitative UV spectrophotometric analysis. The amounts of polymer identified by mixed-solvent extraction and UV spectrophotometry of control uninoculated plates after 68 days of incubation are shown in Table 1. The data show close to quantitative recovery for polymers containing ≥50% (wt/wt) of lignin but some decrease in polymer recovered with increase in styrene content.

SEM visualization of LPS complexes overgrown with fungi. Four or five days after inoculation of plates, fungal mycelia of all of the applied white rot fungi had grown over the tested powdered LPS. The most intensive enmeshing of the LPS was observed in the overgrown mycelial mats of *P. chrysosporium* and *T. versicolor*. Growth of *P. ostreatus* over the tested LPS was less intensive than growth of the other two white rot fungi. After 2 weeks of cultivation, all of the applied white rot fungi and the brown rot fungus *G. trabeum* completely overgrew the tested lignin powder. However, even after 3 weeks of cultivation, the brown rot fungus *G. trabeum* colonized only external zones of the compact mass of the LPS powder. Growth of both white rot and brown rot fungi was sporadic in and near the applied polystyrene.

The close encompassing of particles of the tested LPS complex by white rot mycelia was visualized clearly in the

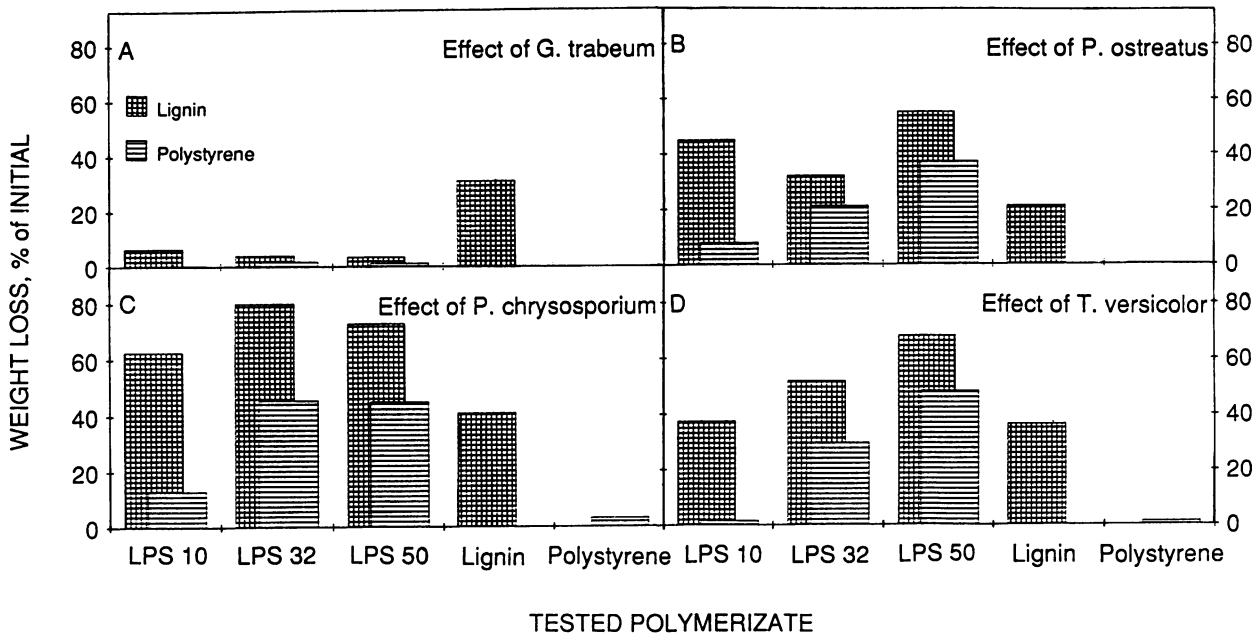


FIG. 2. Mass loss of the constituents of LPS complex graft copolymer induced by fungal metabolism during 68 days of cultivation on solid media. LPS complexes containing 10.3% (LPS10), 32.2% (LPS32), and 50.4% (LPS50) (wt/wt) lignin were incubated with *G. trabeum* (A), *P. ostreatus* (B), *P. chrysosporium* (C), and *T. versicolor* (D).

SEM (Fig. 1). Moreover, mycelia of the white rot fungi produced capsular material outside the hyphae. This material engulfed particles of the degraded LPS complex, thus enhancing close contact between the fungi and the surface of the polymer complex (Fig. 1).

The adhesion of microorganisms to surfaces of various compositions is a decisive step in microbially induced corrosion (43). Presumably, the active colonizers of polymer are able to adhere because of their abilities to produce exocellular polymers composed primarily of nonionic and anionic polysaccharides. It was reported that part of the synthesized extracellular polysaccharide of many groups of fungi constitutes a sheath covalently linked to the wall glucan and chitin (39) and playing an important role in the support and transport of depolymerizing enzymes in wood decay (34, 37). The formation of extracellular material that facilitated fungal adhesion on the surface of the LPS complex was not observed in the tested brown rot fungus *G. trabeum*, though the hyphae of the fungus were found in the vicinity of the incubated polymer particles. It appeared that in the brown rot fungus, the contact of the fungal mycelium and its interaction with the components of the incubated LPS complex were less effective than the interaction of the tested white rot fungus with polymer. The incubated lignin was also engulfed by the extracellular structures of white rot fungi, similar to what was observed by Janshekar et al. (13) during the degradation process caused by *P. chrysosporium*.

Incubation of the tested white rot fungi with LPS complex that contained an increased weight percentage of polystyrene (above 80%) caused a decrease in production of the extracellular filmlike material by the fungi (data not shown).

Mass reduction of lignin and polystyrene constituents of the LPS complex. All tested white rot fungi demonstrated an ability to decrease the weight of both constituents of LPS, no matter what ratio of the main components, polystyrene and lignin, the plastic contained (Fig. 2). These white rot basidiomycetes caused a range of weight loss of LPS copolymer that varied with the fungus with which the plastic was inoculated. The decomposing activities of *P. chrysosporium* and *T. versicolor* toward tested LPS complex exceeded the activity of *P. ostreatus* (Fig. 2B, C, and D). All tested LPS complexes have shown insignificant weight losses of their constituents after incubation with the brown rot fungus *G. trabeum* (Fig. 2A). However, *G. trabeum* was able to deplete lignin applied as a natural polymer to an extent similar to that shown by white rot fungi. Decomposition of polystyrene incubated as a homopolymer was insignificant in all tested fungi. The most efficient degradation of both constituents of LPS complex by white rot fungi was ob-

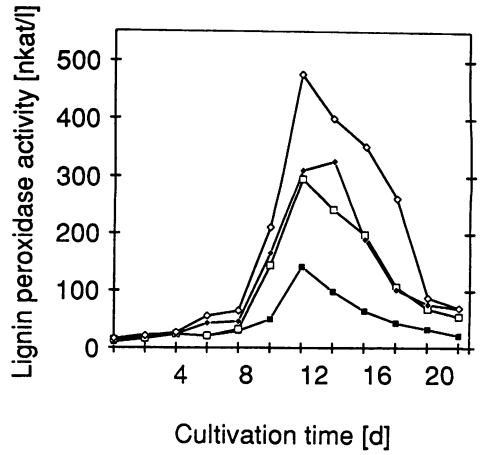


FIG. 3. Production of extracellular LiP in standing liquid cultures of *P. chrysosporium* supplemented with lignin (◇), LPS10 (□), LPS50 (◆), or nothing (■) in the basal media. d, days.

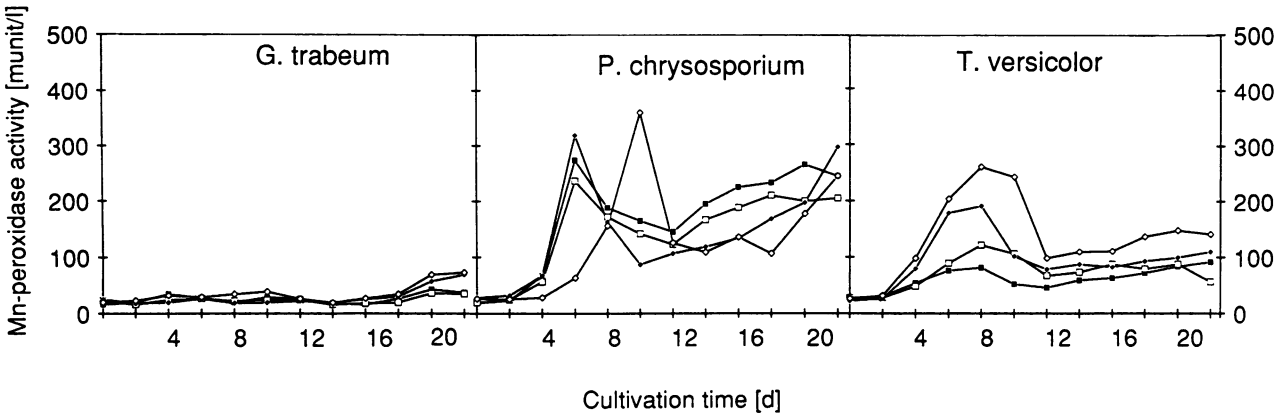


FIG. 4. Production of extracellular MnP in standing liquid cultures of *G. trabeum*, *P. chrysosporium*, and *T. versicolor* supplemented with lignin (◇), LPS10 (□), LPS50 (◆), or nothing (■) in the basal media. d, days.

served with the plastics LPS50 and LPS32, which contain 50.1 and 32.2% (wt/wt) lignin, respectively. It appeared that the level of weight loss of polystyrene component from the incubated LPS complex was correlated with concentration of lignin in the copolymer. It has to be taken into consideration that measured weight loss of the LPS complex components could be due to their mineralization as well as to their modification followed by partial solubilization in a surrounding medium. This last type of conversion might be the cause of the biodegradation of lignin homopolymer by brown rot fungus *G. trabeum*. Transformation of lignin caused by brown rot basidiomycetes increases the number of polar groups in the lignin molecule after partial demethoxylation, hydroxylation, and less mineralization of lignin (18, 19).

The tested LPS complex copolymers, particularly their lignin and polystyrene components, were degraded by white rot fungi in our experiments under conditions of solid-state fermentation. It appears that the conditions chosen for cultivation facilitate production of extracellular mucilage by the tested white rot fungi. The extracellular capsular material, in turn, improves adhesion of hyphae to the plastic surface and intensifies the oxidative potential of the fungus. Moreover, the extracellular forms of the polysaccharides are not always present in a liquid culture of *P. chrysosporium* (2). It can be assumed that white rot fungi in liquid media express their degradation potentials toward incubated plastics less than do the same fungi cultivated in the solid state.

Interestingly, a lignin-related aromatic attached via an ester linkage to a polystyrene matrix was not depleted by *P. chrysosporium* in a liquid culture in earlier studies (3). The polystyrene moiety of the copolymer incubated in this way was also not affected (3).

Patterns of oxidative enzymatic activities in liquid cultures. White rot basidiomycete fungi, particularly *P. chrysosporium*, are responsible for the decomposition of the polymeric structure of lignin. During secondary metabolism, these fungi produce and secrete into surrounding media two extracellular heme peroxidases, LiP and MnP (9, 19, 21, 41), reportedly associated with lignin degradation. However, many ligninolytic fungi do not produce detectable LiP. These white rot fungi, particularly *T. versicolor*, produce one or more laccases in addition to MnP (6, 14, 29, 32). The pattern of oxidative activity secreted into the surrounding media by white rot fungi constitutes a unique combination of these enzymes that varies among strains and with the conditions of organism cultivation.

The production of LiP, MnP, and laccase enzymatic activities in the liquid cultures of *P. chrysosporium*, *T. versicolor*, and *G. trabeum* supplemented with lignin or its polymers with polystyrene is shown in Fig. 3, 4, and 5, respectively.

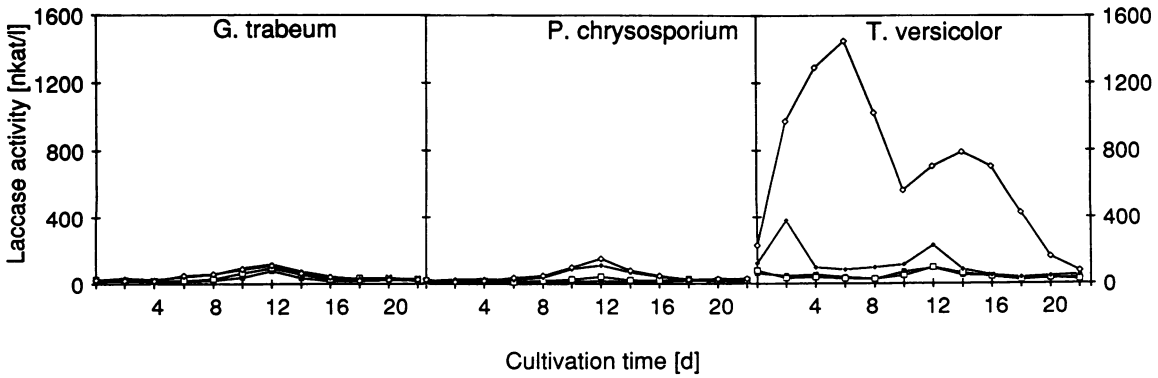
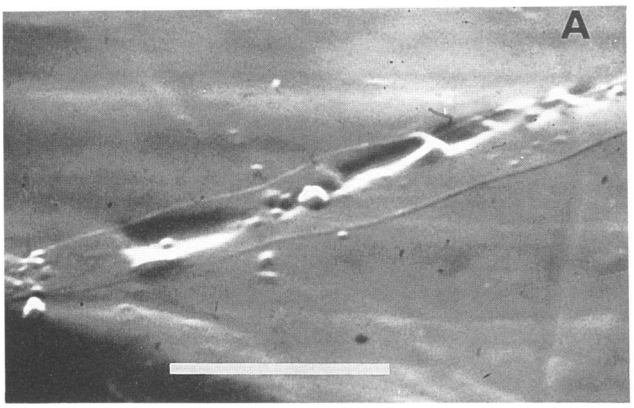
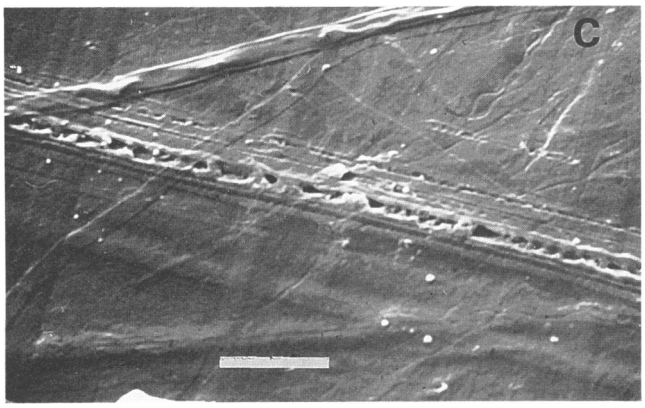


FIG. 5. Production of extracellular laccase in standing liquid cultures of *G. trabeum*, *P. chrysosporium*, and *T. versicolor* supplemented with lignin (◇), LPS10 (□), LPS50 (◆), or nothing (■) in the basal media. d, days.





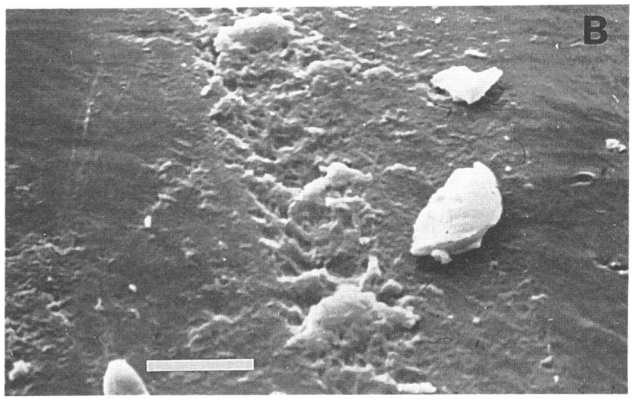


FIG. 6. SEMs of pressed LPS complex incubated for 40 days, showing different forms of surface deterioration caused by overgrown white rot fungi. Micrographs show pitting (A), striating (B), and pitting and decay (C). Bars, 10 μm.

LiP was found only in the culture medium of *P. chrysosporium* (Fig. 3). The rapid increase of LiP activity was observed 10 days after inoculation. LiP activity in the culture medium with or without polymer reached a maximum after 15 days and then gradually decreased (Fig. 3). Addition of either copolymer or lignin to the culture media enhanced the level of LiP activity by almost three times compared with the levels of activity in culture media from control flasks.

MnP activity was detected in the culture media of *P. chrysosporium* and *T. versicolor* and always appeared in the same period after inoculation as the LiP activity (Fig. 4). No significant MnP activity was found in the culture medium of *G. trabeum*. The MnP activity of analyzed white rot fungal media reached a maximum after 10 to 12 days. Thereafter, the enzyme level began to decrease (Fig. 4). However, in the late phase of cultivation, a second cycle of increase of MnP activity was observed in the culture media of both *P. chrysosporium* and *T. versicolor* (Fig. 4). The levels of MnP activity in the tested white rot fungi were higher in media supplemented with either lignin or its copolymers (Fig. 4).

Significant levels of laccase activity were detected only in the culture medium of *T. versicolor* (Fig. 5). Addition of lignin or LPS50 to the medium led to a rapid increase of laccase activity. The level of enzymatic activity in the medium supplemented with lignin surpassed, by a factor of almost 5, the activity level of laccase in the medium with LPS50. Under the test conditions, laccase activity in the culture medium of *T. versicolor* peaked twice during 22 days of cultivation (Fig. 5).

Although the role of fungal extracellular enzymes, particularly LiP, in lignin degradation was demonstrated (41), the few reports that support this finding are equivocal (22, 38) about the exact nature of the role of the enzyme in the degradation. On the other hand, it was reported that LiP from white rot fungus *Phlebia radiata* affects a lignin-related, nonphenolic β-*O*-4 dimer bound to a polystyrene structure (12). Furthermore, it has recently been reported that extracellular enzymes of lignocellulose-degrading *Streptomyces* spp. were capable of attacking and modifying the polyethylene portion of a degradable polyethylene (36). It was reported also that LiP and MnP preparations from a *P. chrysosporium* culture were able to oxidize the conjugated multiunsaturated structures of recalcitrant azo dyes (35).

Yet these enzymes, especially LiP, do not seem to be prerequisites for lignin degradation in vivo (38). However, LiP, MnP, and laccases can catalyze one-electron oxidation of phenolic and nonphenolic substrates, producing cation radical intermediates (17). These enzymes, particularly laccase, oxidize phenolic substrates to reactive phenoxy radicals that, in turn, can mediate the oxidation of nonphenolic substrates (15). The analyzed oxidative enzymes LiP, MnP, and laccases may modify the lignin macromolecule by introducing additional functional groups into its structure. These new functional groups render lignin and its copolymer with polystyrene more susceptible to subsequent degradation by coordinated action of the enzymatic system of the whole organism. We plan to gain further insight into the actual steps in the degradation of the copolymer by studies on ^{14}C-labeled graft polymer.

Deterioration of the plastic surface. Additional evidence of bioconversion and degradation of the copolymers was obtained by SEM of fungus-corroded surfaces of the plastics. SEM data for the LPS complex are shown in Fig. 6. SEM data for the copolymer surface after hyphae from fungus mycelium have grown over it reveal obvious traces of surface corrosion. The most common types of corrosion were striating, pitting, and occasional decay. Extensive pitting and striating were observed on the surfaces of plastics exposed to the white rot fungi, while very little deterioration of the surface of the plastic incubated with brown rot fungus *G. trabeum* or maintained on control plates could be seen.

Bioconversion and degradation of lignin-styrene graft copolymer were verified by weight loss, quantitative UV spectrophotometric analysis, and SEM and were further

supported by verification of oxidative-enzyme production during incubation. The most efficient degradation of lignin and polystyrene constituents of the copolymer by white rot fungi was observed with the plastics with the highest lignin content, indicating that the level of weight loss of polystyrene component from the incubated LPS complex was correlated with concentration of lignin in the copolymer. Polystyrene is frequently used as a packaging material, and the use of this plastic will probably increase because of growing concerns about polyvinylchloride in waste disposal streams. Currently, our society produces many commercial products of fully synthetic recalcitrant materials. Copolymerization of synthetic side chains onto naturally occurring backbones should be considered a way of producing compounds that are more easily degraded in the environment. In particular, grafting of lignin with synthetic side chains such as polystyrene will form a much more biodegradable material than synthesis of a polymer from pure, petroleum-derived hydrocarbons.

ACKNOWLEDGMENTS

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