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# Fungal Biodegradation of Lignin Graft Copolymers from Ethene Monomers

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# FUNGAL BIODEGRADATION OF LIGNIN GRAFT COPOLYMERS FROM ETHENE MONOMERS

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## ABSTRACT

White rot *Basidiomycetes* were able to biodegrade styrene (1-phenylethene) or methyl methacrylate (4-methyl-2-oxy-3-oxopent-4-ene) graft copolymers of lignin containing different proportions of lignin and polystyrene [poly(1-phenylethylene)] or polymethyl methacrylate [poly(1-methyl-1-(1-oxo-2-oxypropyl)ethylene)]. The biodegradation tests were run on lignin/styrene copolymerization products which contained 10.3, 32.2, and 50.4 wt% lignin while biodegradation tests were run on lignin/methyl methacrylate copolymerization products which contained 11 to 18 wt% lignin. The styrene polymer samples were incubated with white rot *Pleurotus ostreatus, Phanerochaete chrysosporium, Trametes versicolor*, and brown rot *Gloeophyllum trabeum*. The methyl methacrylate polymer samples were incubated with white rot *Pleurotus ostreatus, Trametes versicolor*, and *Phlebia radiata*. White rot fungi degraded the



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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 and polymethyl methacrylate sheets were not degradable in these tests. Degradation was verified by weight loss, quantitative ultraviolet spectrophotometric analysis of both lignin and styrene residue, and scanning electron microscopy of the plastic surface for both incubated or control samples. Brown rot fungus did not affect any of these plastics.

## INTRODUCTION

Plastics contribute a significant part by weight or by volume of the waste in municipal landfills, and this plastic fraction is projected to increase. Since plastics became an integral part of contemporary life, opposition to landfilling plastics has grown because most synthetic polymers are resistant to biodegradation. The annual consumption of thermoplastic polystyrene has risen to $1.3 \times 10^6$ t in Western Europe [1] and to $2.5 \times 10^6$ t in the United States [2]. The annual consumption of polymethyl methacrylate has risen to $320 \times 10^3$ t in the United States [2]. Both materials are extremely recalcitrant to bioconversion, however. In addition, opposition to incinerating plastics exists because of the potential of hazardous emissions. On the other hand, blending polymers or grafting some components on 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 have been reported to introduce some naturally occurring polymers of microbial or plant origin, such as starch [3], cellulose [4], and poly(hydroxybutric acid) [5], into a synthetic polymer structure. 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 $50 \times 10^6$ t of lignin are released annually by the pulping industry. This immense amount of biomass is utilized far below its potential value. However, the polyaromatic nature of lignin may represent an enormous supply of chemicals for the production of an engineered material which can replace expensive petrochemicals with renewable raw material of comparatively low cost [6]. The increase in lignin utilization value might be achieved by copolymerization of lignin with synthetic monomers [7]. There are numerous works claiming copolymerization of lignin and some alkyl compounds [8]. Recently, a method has been developed for free radically grafting styrene onto lignin [9–13].

Polystyrene and polymethyl methacrylate are extremely resistant to biodegradation, yet some modification of polystyrene derivatives by soil microflora was reported [14]. Although the recalcitrant nature of lignin impedes its easy conversion, under the right environmental factors biological systems can transform lignin to varying extents [15, 16]. The ultimate transformation of lignin in nature, its

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complete oxidation to $CO_2$, takes place primarily by the white rot *Basidiomycetes*. White rot *Basidiomycetes* are well known for their ability to degrade lignin [16]. Brown rot fungi, in contrast, leave the lignin essentially undegraded. However, there is extensive evidence that incubation with brown rot fungi changed the structure of lignin so that it was increasingly susceptible to biodegradation by other groups of microorganisms [17]. In this paper we report how copolymerization of lignin with styrene or methyl methacrylate monomer increases susceptibility of the resulting grafted lignin product, and particularly its polymeric sidechain, to fungal degradation.

## MATERIALS AND METHODS

### Lignin, Lignin–Polystyrene and Lignin–Polymethyl Methacrylate Complex, and Polystyrene and Polymethyl Methacrylate Homopolymer

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The tested lignopolystyrene (LPS) and polymethyl methacrylate polymerizates were synthesized at the Department of Chemistry of the University of Detroit Mercy. The polymers were synthesized by solution polymerization using dimethylsulfoxide as solvent. Throughout this paper, all percents are calculated on a weight per weight basis and all temperatures are in degrees Centrigrade.

Prepare sample A by placing pure styrene or methyl methacrylate in a conical flask and bubble it with nitrogen ($N_2$) for 10 minutes. Prepare sample B by placing lignin, calcium chloride, and dimethylsulfoxide in a conical flask, stir until dissolved, and bubble the solution with $N_2$ for 10 minutes. Samples A and B are stirred while being purged with $N_2$. Add a 30 wt% aqueous solution of $H_2O_2$ to sample B, and bubble it with $N_2$ for 20 minutes. Add solution A to solution B. After 5 minutes of stirring and bubbling $N_2$ through the reaction mixture, the flask is stoppered and placed in a 30°C bath and stirred at 4 Hz for 48 hours. All reactions are terminated by opening the reaction vessel. This terminated slurry can then be added to 10 times its volume of acidified water (pH 2) and the polymer recovered by filtration. Extensive studies of this copolymerization technique, the properties of the products, and proof of copolymerization have been published separately [9, 11].

This polymerization method was used to create lignin/styrene copolymerization products which contained 10.3 (LPS10), 32.2 (LPS32), and 50.4 (LPS50) wt% lignin, respectively. It was used to create a lignin/methyl methacrylate copolymerization product which contained 11.2 wt% lignin. The copolymerizates 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 0.15 mm thick by 5 to 7 cm diameter circular plastic film. The films had a smooth, hydrophobic surface and, when used as a coating on birch wood, had a contact angle 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 as received from Westvaco Corporation, North Charleston, SC, USA. It was washed and reprecipitated before use in biodegradation studies. Polystyrene homopolymer, material RIPO, was used as received from Amoco Chemical Company, P.O. Box 400, Naperville, IL 60566, USA. The polymethyl methacrylate homopolymer was 150,000 molecular weight plastic pellets from J. T. Baker. The 2.5 mm diameter by 2.5 mm

long cylindrical pellets were tested directly for biodegradation and were compression molded into 0.25 mm thick by 7 cm diameter circular films for testing. All compression moldings were done at 150°C and 192 kPa pressure for 1 minute.

## Organisms and Cultivation

The microorganisms used in this work were *Basidiomycetes*: white rot *Phanerochaete chrysosporium* Burdsall, *Trametes versicolor* I (L. ex Fr.) Quelet (ATCC 11235), *Pleurotus ostreatus* v. florida (F6) (Jaquin ex Fr.), Kummer, and *Phlebia radiata*. The activities of the white rot fungi were compared with brown rot fungus *Gloeophyllum trabeum* (Pers. ex Fr.), Murrill. White rot fungi were from the culture collection of the Institute of Forstbotanic of The Universitat Göttingen, 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%. The tested fungi were cultivated on solid 2.5% agar medium. The media contained the same concentrations of mineral salts, glucose, and a reduced content of nitrogen, as specified by Kirk et al. [18] and Kern [19].

The plates inoculum consisted either of a piece of straw from the maintaining culture or of 2 × 5 mm plugs from a 7-day-old plate culture. The plates were placed at 25°C in a thermostated chamber at 100% humidity for 68 days. Plates were sealed with a parafilm tape. Every 7 days the plates were aseptically opened to exchange the air. Powdered analyzed material (10 mg) was placed in triplicate 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 minutes 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.

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## Evaluation of Polymer Degradation

Biodegradation was followed by weight loss, by decrease of the lignin and polystyrene components from the biodegraded complex, and by scanning electron microscopy of the decayed polymer. Tested powdered copolymerizates were intimately bound with the growing fungi; thus direct measurements of the loss of polymer weight was impossible. To evaluate loss of the tested copolymerizate, the nitrogen content of the aliquot of the dry collected material from the triplicates of inoculated plates and uninoculated control was measured. The amount of the 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 age and medium. The computed fungal biomass was subtracted from the recovered material in this way.

The nitrogen in the polymerizates from inoculated plates and uninoculated controls was determined by elemental analysis after combustion of the dry sample in a quartz combustion reactor at 1020°C. 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 quantity of separate components, lignin and polystyrene, in the treated copolymer was analyzed by UV spectroscopy using multicomponent analysis methods. Known mixtures of pure components were used to calibrate the spectrophotometer. Then mixtures of unknown composition were analyzed. Dry lignopolystyrene complexes from inoculated plates and uninoculated controls were solubilized in a dioxane–ethanol–dichloroethane (7:3:5 by volume) mixture. The chosen solvent showed almost zero absorbance at wavelengths of 236 to 250 nm and no absorbance above this range. The spectrophotometer measurements of the absorbance of the solubilized polymerizate and the calculations of the concentrations of the lignin and polystyrene components were performed on a Hewlett-Packard 8451A Diode Array Spectrophotometer and its software package, Multicomponent Analysis.

For lignin–polymethyl methacrylate polymerizate sheets, the fungal mat that had grown throughout the plastics was carefully washed out with a stream of distilled water. The plastic sheets were vacuum dried at 45°C and then weighed for the calculation of weight loss during incubation.

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### Scanning Electron Microscopy (SEM)

The pieces of pressed lignin–styrene copolymer (approximately 0.4 $cm^2$) from both the uninoculated control plates and from the plates with fungi were withdrawn after 68 days of incubation in a 25°C bath at 100% humidity. Pieces of pressed lignin–methyl methacrylate copolymer from both the uninoculated control plates and from the plates with fungi were withdrawn after 60 days of incubation. Withdrawn copolymerizates were then mounted on SEM stubs, sputter coated with gold to a thickness of about 10 nm, and observed and photographed using a Phillips SEM 515.

## RESULTS AND DISCUSSION

### Verification of Quantitative Ultraviolet Spectrophotometric Analysis

The amount of polymer identified by mixed solvent extraction and ultraviolet spectrophotometry of control, uninoculated plates after 68 days of incubation is shown in Table 1. The data show close to quantitative recovery for polymers containing 50 or more weight percent lignin but some decrease in polymer recovered with increase in styrene content.

### Synthesis of Polymerizates

Data for a spectrum of reactions run to optimize yield and create samples of different molecular weights and compositions are given in Table 2. In these reactions the monomer used was 1-phenylethene [100-42-5]. All of these reactions were stirred at a rate of about 4 Hz throughout the synthesis.

TABLE 1. Recovery of the Copolymerizates Incubated for 68 Days on Uninoculated Plates[a,b,c]

| Incubated polymeric material | Recovered polymer (wt% of initial) | Solubilized polymer (wt% of recovered) | Polymer determined spectrophotometrically (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]The data are averages of the material determined from 10 uninoculated plates.
[b]A total of 10 mL of the tested material was incubated for 68 days on the agar plates.
[c]The tested copolymerizate was solubilized in 4 mL of dioxane–ethanol–dichloroethane (7:3:5) mixture, and the content of the lignin and polystyrene was determined spectrophotometrically.

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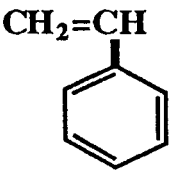

**1-Phenylethene**

The data of these reactions show that

1. Copolymer can be formed from reaction mixtures which have a weight ratio of lignin to styrene anywhere in the range of 0.05 to 5.
2. There is an optimum ratio of peroxide to chloride to lignin that produces maximum yield and styrene conversion.
3. The optimum yield occurs at a mole ratio of hydroperoxide to chloride ion of 0.783 and a mole ratio of hydroperoxide to lignin ($M_n$) of 140.
4. At these ratios, quantitative conversion of styrene to polymer occurs.
5. There is a broad range of halide ion concentrations that produce high but not maximum yield.

Proof of formation of graft copolymer was completed by mass balance of fractionated reaction product, solubility tests, wetting tests, phase partitioning tests, and FT-IR analysis. The fractionation of the graft copolymer is diagrammed in Eq. (1).

TABLE 2. Composition and Yield of Copolymerization Reactions

| Sample number | Composition, g | | | | | Yield, g/wt% |
|---|---|---|---|---|---|---|
| | Lignin | Styrene | $CaCl_2$ | $H_2O_2$, mL | Solvent | |
| 2-1 | 2.00 | 18.76 | 2.02 | 1.0 | 20.04 | 17.80/85.74 |
| 2-2 | 2.00 | 18.76 | 2.01 | 2.0 | 20.00 | 20.28/97.69 |
| 2-3 | 2.00 | 18.76 | 2.07 | 3.0 | 19.99 | 20.37/98.12 |
| 2-4 | 2.01 | 18.77 | 2.02 | 4.0 | 20.02 | 19.10/91.92 |
| 2-5 | 2.01 | 18.78 | 2.02 | 5.0 | 20.02 | 18.53/89.13 |
| 2-6 | 3.03 | 18.78 | 2.00 | 2.0 | 20.00 | 19.14/87.76 |
| 2-7 | 2.00 | 18.76 | 1.01 | 2.0 | 20.10 | 18.84/90.75 |
| 2-8 | 2.01 | 18.79 | 1.52 | 2.0 | 20.01 | 18.77/90.24 |
| 2-9 | 2.00 | 18.79 | 2.01 | 2.0 | 20.05 | 18.81/90.48 |
| 2-10 | 2.01 | 18.76 | 2.52 | 2.0 | 20.07 | 18.98/91.38 |
| 2-11 | 2.01 | 4.69 | 2.04 | 2.0 | 20.01 | 5.68/84.78 |
| 2-12 | 2.01 | 9.39 | 2.02 | 2.0 | 20.00 | 10.42/91.40 |
| 2-13 | 2.01 | 14.07 | 2.03 | 2.0 | 20.10 | 14.95/92.79 |
| 2-14 | 2.01 | 18.76 | 2.03 | 2.0 | 20.01 | 19.52/93.98 |
| 2-15 | 2.02 | 23.45 | 2.04 | 2.0 | 20.07 | 23.76/93.29 |
| 2-16 | 2.00 | 18.76 | 2.00 | 2.06 g | 20.00 | 19.24/92.68 |
| 2-17 | 2.01 | 18.78 | 2.00 | 2.02 g | 20.00 | 19.63/94.42 |
| 2-18 | 2.00 | 18.79 | 2.00 | 2.07 g | 20.00 | 19.19/92.30 |
| 2-19 | 8.00 | 28.15 | 8.00 | 8.0 | 40.02 | 33.16/91.73 |
| 2-20 | 8.04 | 18.76 | 8.00 | 8.0 | 40.03 | 24.14/90.07 |
| 2-21 | 8.01 | 9.39 | 8.00 | 8.0 | 40.10 | 15.45/88.79 |
| 2-22 | 8.01 | 18.74 | 6.00 | 8.0 | 40.04 | 24.59/91.93 |
| 2-23 | 8.01 | 18.76 | 6.00 | 8.0 | 40.02 | 24.36/90.99 |
| 2-24 | 8.04 | 18.75 | 6.00 | 8.0 | 40.05 | 15.96/59.57 |
| 2-25 | 8.00 | 9.38 | 2.08 | 8.0 | 40.08 | 14.56/83.77 |
| 2-26 | 8.03 | 9.38 | 4.04 | 8.0 | 40.09 | 14.98/86.04 |
| 2-27 | 8.01 | 9.38 | 6.28 | 8.0 | 40.00 | 15.89/91.37 |
| 2-28 | 8.00 | 9.38 | 6.00 | 4.0 | 40.0 | 14.86/85.50 |
| 2-29 | 8.01 | 9.39 | 6.00 | 6.0 | 40.0 | 15.13/86.95 |
| 2-30 | 8.01 | 9.39 | 6.00 | 10.0 | 40.0 | 15.86/91.18 |
| 2-31 | 8.01 | 9.39 | 2.00 | 2.0 | 40.0 | 13.30/76.44 |
| 2-32 | 8.03 | 9.38 | 4.03 | 4.0 | 40.0 | 15.41/88.51 |
| 2-33 | 8.07 | 9.38 | 6.03 | 6.0 | 40.0 | 15.60/89.40 |
| 2-34 | 8.02 | 18.76 | 6.02 | 8.0 | 40.0 | 24.93/90.14 |
| 2-35 | 0.00 | 9.39 | 6.02 | 8.0 | 40.0 | 0.00/ 0.00 |

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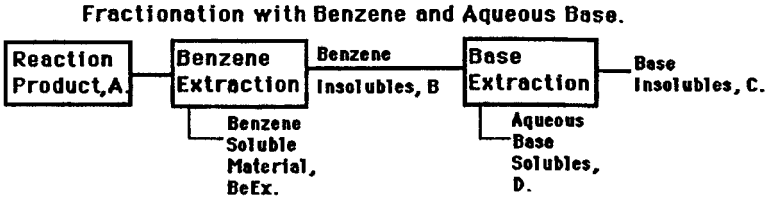


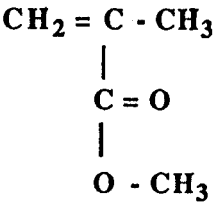

Equation 1. Flow diagram of copolymer fractionation process.

The reaction product, A, was extracted with benzene for 48 hours. The benzene-soluble material was recovered by evaporating the benzene, and the solid was labeled fraction BeEx. The solid not dissolved in benzene was labeled fraction B and was slurried with 0.5 M sodium hydroxide for at least 16 hours. This solution was filtered, and the filtrate was dialyzed against water for 3 to 5 days using dialysis tubing. The solid filtered from the base was washed with 2 M hydrochloric acid, washed with distilled water, dried, and labeled fraction C. The dilute, dialyzed solution was then dried or freeze dried to recover base-soluble fraction D.

When a mixture of lignin and polystyrene homopolymer is treated this way, two pure samples are produced. BeEx is pure styrene homopolymer and D, the fraction soluble in aqueous base, is pure lignin. For a copolymerization reaction product, none of the fractions are pure. Fraction BeEx, the benzene-soluble part of the product, contains polystyrene homopolymer and the graft copolymer that has long polystyrene chains on it. Fraction C contains graft copolymer with medium-sized polystyrene chains on it. Fraction D is unreacted lignin and graft copolymer with tiny polystyrene chains on it.

This polymerization method was used to create copolymerization products for exposure to microorganisms which contained 10.30 (LPS10), 32.17 (LPS32), and 50.41 (LPS50) weight percent of lignin, respectively. These are samples 2-14 (LPS10), 2-34 (LPS32), and 2-27 (LPS50).

Data for synthesis and fractionation of lignin-polymethyl methacrylate polymerizates are given in Table 3. In these reactions the monomer used was 4-methyl-2-oxy-3-oxopent-4-ene [80-62-6].



**4-Methyl-2-Oxy-3-Oxopent-4-ene**

The fractionation of the reaction product was done in a Soxhlet apparatus. The reaction product, product A, was extracted with benzene for 48 hours. The benzene-soluble material, fraction C, was recovered by evaporating the benzene. The solid

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not dissolved in benzene was labeled fraction B. Samples 3-1A, B, and C are the plastics incubated with white rot fungi to determine degradability in the lignin-polymethyl methacrylate products.

### SEM Visualization of Copolymerizates Overgrown with Fungi

Four or 5 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 by the overgrown mycelial mats of *P. chrysosporium* and *T. versicolor*. Growth of *P. ostreatus* over the tested LPS was less intensive than the other two white rot fungi. All of the applied white rot fungi and the brown rot *G. trabeum*, after 2 weeks of cultivation, completely overgrew the tested lignin powder. However, the brown rot *G. trabeum*, even after 3 weeks of cultivation, 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 encompassment of the particles of the tested LPS by white rot mycelia was visualized clearly in the SEM, as shown in Fig. 1. Moreover, mycelia of the white rot fungi had produced mucilage material outside the hyphae. This material engulfed particles of the degraded LPS, thus enhancing close contact between the fungi and the surface of the polymer complex, as shown in Fig. 1.

The adhesion of microorganisms to surfaces of various compositions is a decisive step in microbially-induced corrosion [20]. Presumably the active colonizers of polymer are able to adhere due to their ability to produce exocellular polymers composed primarily of nonionic and anionic polysaccharides. It was reported that

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TABLE 3. Poly(Lignin-*graft*-(1-methyl-1-(1-oxo-2-oxypropyl)ethylene)) Copolymers Produced with Two Different Chloride Ion to Hydroperoxide Mole Ratios

| Sample number | 3-1 | 3-2 |
|---|---|---|
| Lignin (g) | 1.50 | 1.50 |
| Monomer[a] (g) | 12.38 | 12.38 |
| 30% $H_2O_2$ (mL) | 1.50 | 1.50 |
| DMSO (g) | 16.02 | 16.02 |
| R[b] | 0.86 | 3.88 |
| Yield: Product A (%) | 94.2 | 88.2 |
| Lignin content (%): | 11.2 | |
| Yield: Fraction B (%) | 70.4 | 70.4 |
| Lignin content (%): | 18 | |
| Yield: Fraction C (%) | 29.6 | 29.6 |
| $T_g$ (Product A, °C) | 108.0 | 111.5 |

[a]4-Methyl-2-oxy-3-oxopent-4-ene.

[b]Mole ratio of chloride ion to hydrogen peroxide in the reaction mixture.

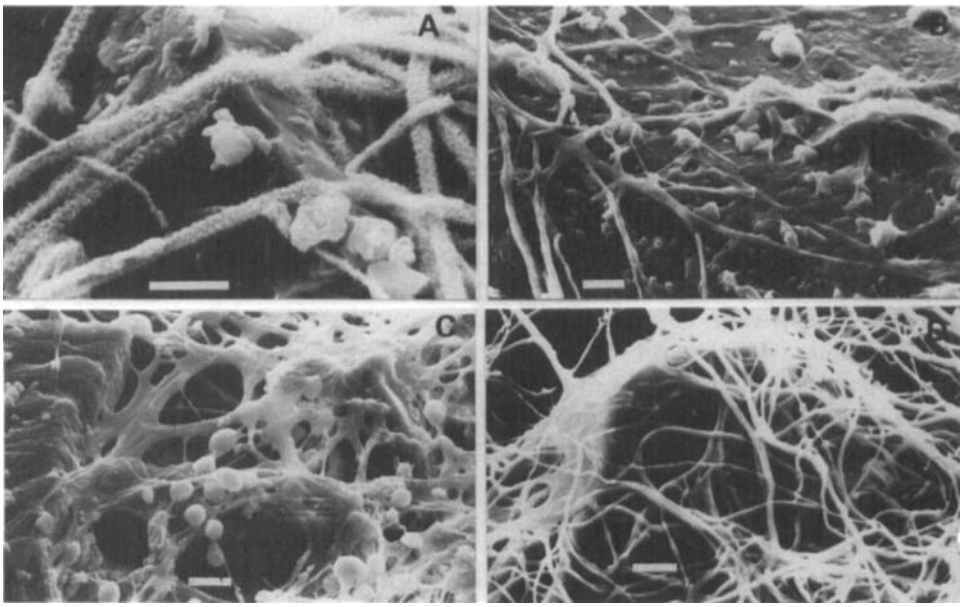


FIG. 1. Scanning electron micrographs of powdered LPS50 incubated for 30 days with *G. trabeum* (A), *P. ostreatus* (B), *P. chrysosporium* (C), and *T. versicolor* (D). Fungal hyphae have overgrown the polymerizate. The extracellular mucilage facilitates adhesion of hyphae and promotes efficient interaction to the plastic surface, as shown on B, C, and D. Bars, 10 $\mu$m.

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part of the synthesized extracellular polysaccharide of many groups of fungi constitutes a sheath covalently linked to the wall glucan and chitin [21], playing an important role in the support and transport of depolymerizing enzymes in wood decay [22, 23]. The formation of extracellular material that facilitated fungal adhesion on the surface of LPS was not observed in the tested brown rot *G. trabeum* although the hyphae of the fungus were found in the vicinity of the incubated polymer particles. It appeared that in the brown rot the contact of the fungal micelia and its interaction with the components of the incubated LPS were less effective compared with the efficiency of the interaction of the tested white rot 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. [24] during the degradation process caused by *P. chrysosporium*. Incubation of the tested white rot fungi with LPS that contained an increased weight percent of polystyrene (above 80%) caused a decrease of the production of the extracellular filmlike material by the fungi (data not shown).

All of the tested lignin–polymethyl methacrylate polymerizates were rapidly colonized by the inoculated fungi. No significant differences in the overgrowth of the three copolymer samples by fungus were observed. Polymethyl methacrylate homopolymer was not colonized by any fungus and did not support the growth of the fungi. Moreover, it slightly inhibited growth and facilitated infection by unknown microorganisms.

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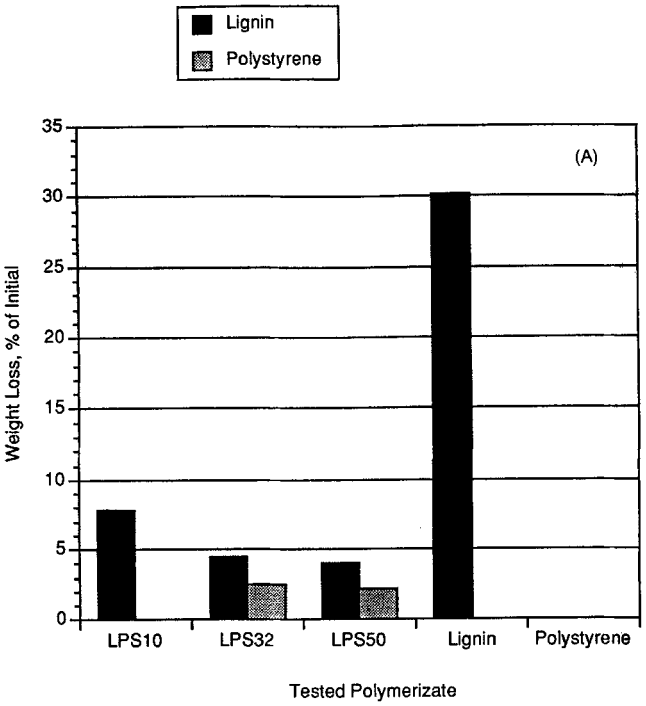
### Mass Reduction of Constituents of the Polymerizates

Figures 2(A–D) show that all tested white rot fungi demonstrated an ability to decrease the weight of both constituents of LPS, no matter what the ratio of the main components, polystyrene and lignin, the plastic contained. Figures 2(A–D) are bar graphs of weight loss for each constituent of a blend of grafted, lignin-containing material and a synthetic homopolymer after 68 days of incubation with four fungi. Figure 2(A) shows the constituent loss after incubation with *G. trabeum*. Figure 2(B) shows the constituent loss after incubation with *P. ostreatus*. Figure 2(C) shows the constituent loss after incubation with *P. chrysosporium*. Figure 2(D) shows the constituent loss after incubation with *T. versicolor*. In Figures 2(A–D) the letters LPS stand for lignin polystyrene and the numbers 10, 32 and 50 designate the percent lignin in the blend. These white rot *Basidiomycetes* caused a range of weight loss of lignopolystyrene copolymerizate that varied with the fungus with which the plastic was inoculated. The decomposing activity of *P. chrysosporium* and *T. versicolor* toward tested LPS exceeded the activity of *P. ostreatus* (Figs. 2C, 2D, 2B). All tested LPS have shown insignificant weight loss of its 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 by white rot fungi was observed with the plastics LPS 50 and LPS 32 containing 50.41 and 32.17 wt% lignin, respectively. It appeared that the level of weight loss of the polystyrene component from the incubated LPS was correlated with the concentration of lignin in the copolymerizate. It has to be taken into consideration that measured weight loss of the LPS components could be due to their mineralization as well as 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 *G. trabeum*. Transformation of lignin caused by brown rot *basidiomycete* increases the amount of polar groups in the lignin molecule after partial demethoxylation, hydroxylation, and less mineralization of lignin [16, 17].

The tested LPS copolymerizates, particularly their lignin and polystyrene components, were degraded with 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 on the plastic surface and additionally leads to an increased oxidative potential for the fungus.

The lignin–polymethyl methacrylate polymerizates also showed mass losses 2 to 7 times larger than those of the sterile controls after 60 days of incubation with three white rot fungi. The data are shown in Fig. 3. The controls and polymethyl methacrylate homopolymer lost between 1 and 3 wt% of their mass during the incubation process. However, all of the lignin-containing polymerizates lost more mass than the corresponding control or the homopolymer, and polymerizate 3-A lost more mass than its lignin content when incubated with *P. ostreatus*. Of the three fungi tested on lignin–polymethyl methacrylate polymerizates, *P. ostreatus* was the most effective organism for reducing the mass of the polymer sheet. We



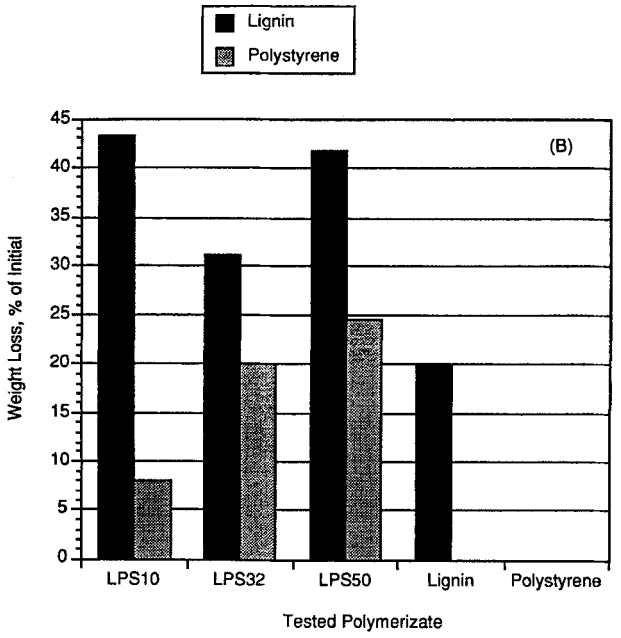




FIG. 2. Mass loss of the constituents of lignin–polystyrene graft copolymer (LPS) induced by fungal metabolism during 68 days of cultivation on solid media. LPS contained 10.3 (LPS10), 32.2 (LPS32) and 50.4 (LPS50) weight percent of lignin, respectively, and were incubated with *G. trabeum* (A), *P.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

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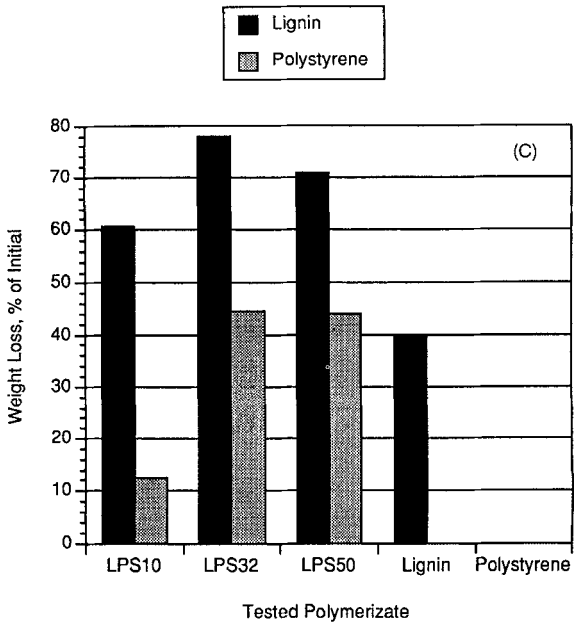


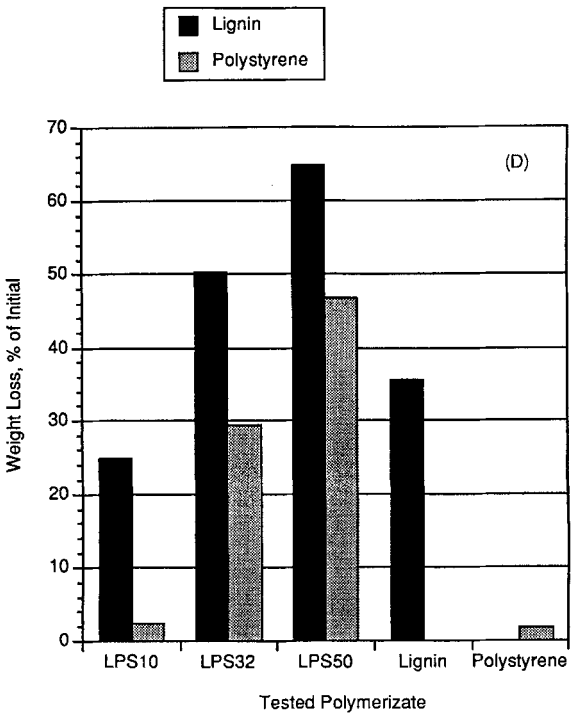


FIG. 2. Continued.

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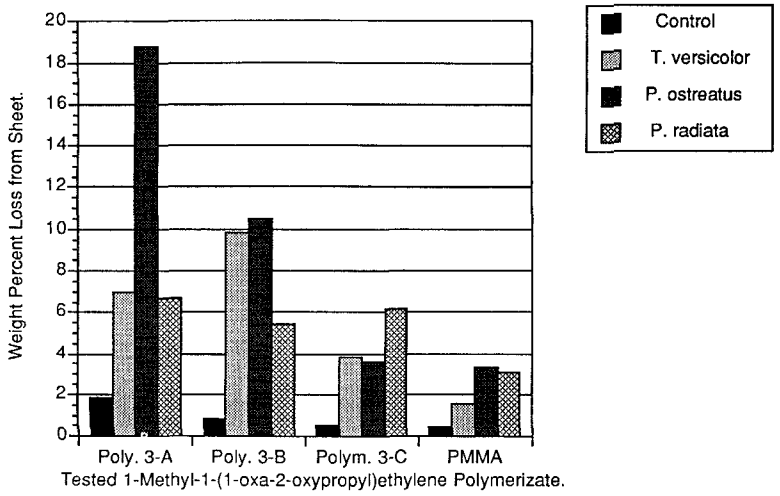


FIG. 3. Mass loss of lignin–polymethyl methacrylate reaction product induced by fungal metabolism during 60 days of cultivation on solid media. The polymerizates were incubated with white rot *P. ostreatus, T. versicolor*, and *P. radiata*.

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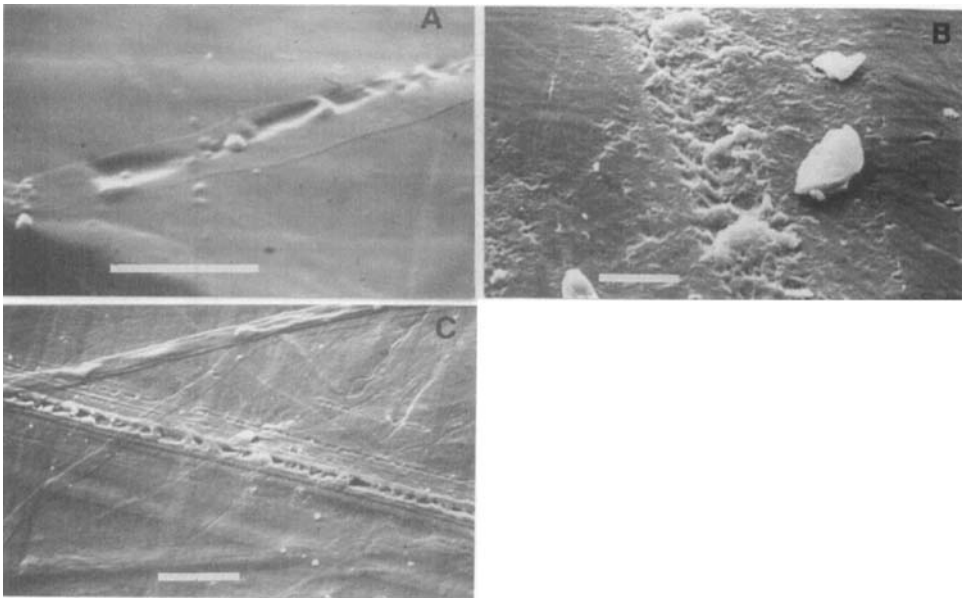


FIG. 4. Scanning electron micrographs of pressed LPS incubated for 40 days showing different forms of surface deterioration caused by overgrown white hot fungi. Pitting (A), striating (B), pitting and decay (C). Bars, 10 $\mu$m.

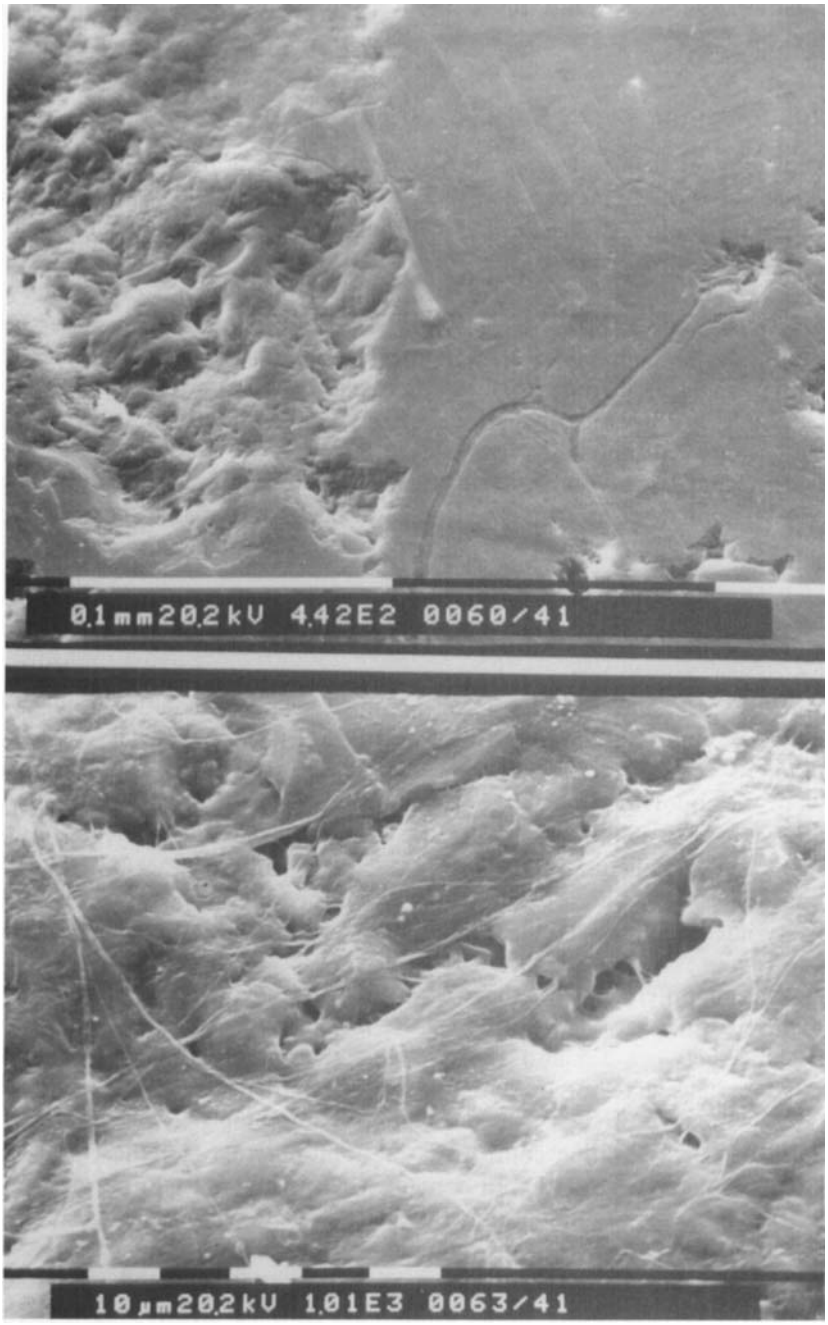


FIG. 5. Scanning electron micrographs of pressed lignin-polymethyl methacrylate graft copolymer incubated for 40 days showing different forms of surface deterioration caused by overgrowth by white rot fungi. Bars, 10 $\mu$m.

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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 fungi-corroded surfaces of the plastics. SEM data of the LPS complex are shown in Fig. 4. SEM data of the copolymer surface after hypeae from fungus mycelium have grown over it show 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 surface of plastic exposed to the white rot fungi while very little deterioration of the surface of the plastic incubated with brown rot *G. trabeum* or maintained on control plates could be seen.

The surface of lignin–polymethyl methacrylate polymerizate sheets overgrown by fungus lost its smoothness and gloss. Furthermore, the sheet of 3-A became very brittle. SEM data of these sheets are shown in Fig. 5. Again, the most common types of corrosion were striating, pitting, and occasional decay. Extensive pitting and striating were observed on the surface of plastic exposed to the white rot fungi.

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## CONCLUSIONS

Bioconversion and degradation of lignin–styrene graft copolymer was verified by weight loss, quantitative ultraviolet spectrophotometric analysis, and scanning electron microscopy. 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 the polystyrene component from the incubated LPS was correlated with the concentration of lignin in the copolymerizate. The capacity of the white rot fungi to produce weight loss in the polystyrene decreased in the order *P. chrysosporium, T. versicolor*, and *P. ostreatus*.

The lignin–polymethyl methacrylate polymerizates also showed mass losses after 60 days of incubation with three white rot fungi. The lignin-containing polymerizates lost between 3.5 and 18.8 wt% of their mass while the controls and polymethyl methacrylate homopolymer lost between 0.46 and 3.3 wt% of their mass during the incubation process. All of the lignin-containing polymerizates lost more mass than the corresponding control or the homopolymer. Further, polymerizate 3-1A lost more mass than its lignin content when incubated with *P. ostreatus*. Of the three fungi tested on lignin–polymethyl methacrylate polymerizates, *P. ostreatus* was the most effective organism for reducing the mass of the polymer sheet.

Polystyrene is frequently used as a packaging material, and the use of this plastic will probably increase because of growing concerns about polyvinyl chloride in waste disposal streams. Polymethyl methacrylate is widely used as a high impact engineering resin and a barrier plastic. Currently, our society produces many commercial products of fully synthetic, recalcitrant materials. Copolymerization of synthetic sidechains onto naturally occurring backbones should be considered as a way of producing compounds that are more easily degraded in the environment. In particular, grafting of lignin with synthetic sidechains such as polystyrene or poly-

methyl methacrylate will form a much more biodegradable material than synthesis of a polymer from pure petroleum-derived hydrocarbons.

## ACKNOWLEDGMENTS

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