Biodegradability of Lignin–Polypropylene Composite Films

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ABSTRACT. Biological degradation of composite lignin-polypropylene films containing 4 % organocell lignin was confirmed by treatment with lignin-degrading enzymes produced by the white-rot fungus Phanerochete chrysosporium. The kinetics of P. chrysosporium culture in the presence of lignin-containing and lignin-free polypropylene films show that the fungus produced lignin-degrading enzymes into the liquid medium during incubation with the lignin-polypropylene film. The degree of biodegradation of both types of film was followed by monitoring their mechanical properties. Correlation was found between the decrease of elongation at break and the amount of released lignin fragments into the extracellular fluid in the course of microbial treatment. The incorporation of lignin into polyolefins represents a new way of using wastes from pulp and paper industry to reduce the environmental impact factor of waste plastics.

From the viewpoint of environmental protection the plastic wastes, especially those from packaging materials, represent a potential waste problem. Various approaches were examined to develop partially or completely biodegradable plastics. Generally, the biodegradable plastics could be obtained by introducing organic compounds consumable by microorganisms. This makes the films fragile and thereby more readily degradable.

Regarding the detrimental effect of starch on the mechanical properties of the resulting films (Huang et al. 1990) some attempts were made to apply the lignin component of biomass in the production of biodegradable plastics (Lee et al. 1991; Milstein et al. 1992).

Lignin is one of the main wood components. It is a commercially available nontoxic amorphous phenylpropane biopolymer which can be obtained as a by-product in pulp production. In nature it is degraded by a number of microbial species (Kirk et al. 1987), mainly by white-rot fungi. The best known and the most studied of these is Phanerochete chrysosporium. When cultured under lignonolytic conditions, P. chrysosporium secretes two extracellular heme peroxidases, viz. lignin peroxidase (ligninase) (Kirk et al. 1987) and manganese (II) peroxidase (Kuwahara et al. 1984) which, along with an H2O2-generating system, are the major components of its lignin degradative system (Tien et al. 1987).

Polypropylene films containing 0.4–10 % (W/W) lignin with acceptable mechanical strength were obtained in the absence of commercial stabilizers (Košíková et al. 1993a).

The objective of the present paper was to investigate the biodegradability of the lignin-polypropylene composites using P. chrysosporium.

MATERIALS AND METHODS

Microorganism. The white-rot fungus Phanerochete chrysosporium, strain ME-446 (ATCC-34543) DSM, Germany, was maintained at 25 °C on 2 % (W/V) malt-agar slants. The inoculum was prepared by washing the spores from the slant into a sterile 0.1 % solution of Tween 80. The resulting suspension was filtered through sterile muslin to remove fragments of hyphae and the spore concentration was adjusted to the final value of 5 × 10⁷/mL. The fungus was incubated at 37 °C without agitation in shallow liquid culture, viz. 100 mL fluid in a 1-L Erlenmeyer flask. The basal medium was prepared according to Kirk et al. (1978). Flasks were flushed with pure oxygen on the day of inoculation and after 3 d. Cultures were sampled at time intervals for dry mass and extracellular fluid analyses.

Preparation of the composite lignin–polypropylene films. The polymer blends were obtained by mixing isotactic polypropylene powder (Tatren, Slovnaft, Bratislava) with powdered lignin samples (4 %) in a roll mill for 1 h and then granulated in a homogenizer (Fortuna, Slovakia) at 200 °C. The composite lignin–polypropylene films (40–60 µm thick) were prepared from the granulates by extrusion in the absence of commercial stabilizers. The lignin co-product of methanol based on organosol-
Vented pulping of spruce wood was obtained from a pilot-plant run by the Organocell GmbH for Pulp and Environmental Engineering, München (Germany).

**Biodegradation of lignin–polypropylene composite films.** Experiments were conducted in the same cultivation condition as described above but a 1 g film sample was added to 100 mL of the cultivation medium prior to sterilization. The films were cut to strips 15 × 90 mm in size to measure the mechanical properties. Release of lignin from the film into the extracellular fluid was monitored by assaying the absorbance at the wavelength maximum of lignin during the incubation period. At the same time, the strips were taken and their mechanical properties measured. Biodegradation experiments with only the extracellular fluid were done in a similar manner. The fungal mycelium and extracellular fluid were separated by centrifugation (10000 g, 10 min) and the extracellular fluid in a tartrate buffer at pH 3 was used as a crude enzyme preparation. To each flask 0.6 % H₂O₂ and NaN₃ were added, the latter to prevent bacterial contamination. The film tested and sampling were the same as in experiments with the fungus.

**Analytical methods.** Cultures were suction-filtered, washed several times with water (the film tested was thoroughly rinsed with distilled water) and dried at 105 °C overnight for dry mass analysis. Filtrates from the culture flasks were centrifuged (10000 g, 10 min) and subjected to the following analyses. Absorption spectra were recorded on a UV/VIS Specord M40 (Carl-Zeis Jena, Germany) at room temperature using cuvettes of 10-mm light path. Ligninase activity was determined by measuring the initial rate of veratraldehyde production from veratryl alcohol (Tien et al. 1984). Manganese peroxidase was assayed in culture fluids by following the initial rate of oxidation of the phenol red in the presence of Mn(II) at room temperature (Kuwahara et al. 1984). Glucose in the culture medium was determined with 3,5-dinitrosalicylic acid (Miller et al. 1959). Extracellular protein was measured by the Lowry method with bovine serum albumin as standard. The elongation data were obtained with an Instron tester on specimens cut in accordance with the Czechoslovak norm no. 640 604. Measurement of each sample was repeated six times.

**RESULTS AND DISCUSSION**

**Degradation of lignin–polypropylene composite films.** Fungal growth in the presence of films is illustrated in Fig. 1. The presence of both films in the medium mildly inhibits fungal growth but lignin added (at a concentration corresponding to the amount of lignin in the film) to the culture liquid slightly increased the growth rate.

![Fig. 1](image-url)