Bull. Environ. Contam. Toxicol. (1999) 63:335-342

Bulletin of Environmental Contamination and Toxicology

Biodegradation of Polyvinylchloride (PVC) by White Rot Fungi

Z. Klrbas,[1] N. Keskin,[1] A. Güner[2]

[1] Hacettepe University, Faculty of Science, Department of Biology, 06532 Beytepe, Turkey
[2] Hacettepe University, Faculty of Science, Department of Chemistry, 06532 Beytepe, Turkey

Received: 29 March 1999/Accepted: 22 June 1999

Amounts of recalcitrant plastics in the environment and the time required for their total mineralization to CO_2 have recently been fully understood. There is a growing interest in biodegradability of the plastics in landfills (Lee et al., 1991). A major area of environmental concern is the disposal of the solid wastes. Solid wastes which beside plastics include packaging materials, polyurethane, polystrenes, and other polymeric substances are rapidly fulling up available landfill sites. The existence of organisms able to metabolize xenobiotics is of considerable evolutionary interest in the past 50 years (Madigan et al., 1997).

Recent studies in the laboratory have shown that under nutrient (nitrogen, carbon or sulfur) limiting conditions, the white-rot fungi, the members of Basidiomycotina are able to degrade a wide variety of structurally diverse environmentally persistent organopollutans and xenobiotics to carbon dioxide. The ability to degrade such a diverse group of compounds has been shown to be dependent on a nonspesific and nonstereo-selective lignin degrading system. Lignin is a complex heteropolymer and is possibly the most-difficult-to degrade naturally occuring organic compound. The lignin degrading system consists of a group of peroxidases commonly known as ligninases, which catalyze the initial oxidative depolymerization of lignin polymer (Aust and Bumpus, 1987; Bumpus and Aust, 1987; Bumpus et al., 1988; Shah et al., 1992). It is considerably time-saving to use the organisms which have ligninases because the initial oxidation of chemicals is often the most difficult step in biodegradation.

It was the purpose of this study to determine if the white rot fungi had the ability to degrade recalcitrant polyvinyl chloride.

MATERIALS AND METHODS

The Pleurotus species were obtained from Dr. I.F Zadrazil (Weidranweg, 4 3300 Braunschweigh, Federal Republic of Germany), *Phanerochaete chrysosporium ME 446* was taken from Dr. T.K. Kirk (U.S. Department of Agriculture Forest Products Lab. Madison, Wisconsin 53 705 U.S.A.) and *Poliporus versicolor* was provided from Inonti University, Department of Biology/TURKEY. The fungi were cultured at 30°C on Patoto Dekstroz Agar (Difco) slants. Subcultures were made routinely every 20-30 days.

PVC having low molecular weight was used for the studies. The polymer supplied from Aldrich Chemical Company Inc. PVC was transformed into films before adding in the liquid culture media. As solvent, tetrahydrofuran (THF) (99% Merck) was used. Weightiness and thickness of the films were determined before transforming into the media. Spectroscopical analyses were made with FTIR (Fourier Transform Infrared Spectroscopy) and UV-VIS (Visible Ultra Violet Spectroscopy).

Correspondence to: N. Keskin

Before beginning to study, optimum conditions were aimed to be determined for reproduction of funfi. Liquid medium employed for the determination of optimum conditions was prepared, making some changes which was recommended by Forney and Reddy (1979) and Kirk (1981). Content of the medium was determined to constitute (g/L); 0.2 KH_2PO_4; 0.5 $MgSO_4$; $7H_20$; 0.1 $CaCI_2$; 0.5 NH_4NO_3; 0.1 yeast extract (Difco); 10 glucose (Merck) and distilled water. The pH was adjusted to 4.5 by 0.1*M* NaOH and 0.1*N* HCl.

Another liquid medium containing veratryl alcohol (0.4 m*M*/ ml) (Sigma Co) which was advised by Asther and Corrieu (1987) was modificated and then used for the determination of PVC experiment. The medium was preparet to include (g / L); 0.2 KH_2PO_4; 0.02 $CaCI_2$; 0.05 $MgSO_4$; $7H_20$; 10 mL glycerol; 0.5 NH_4NO_3; 0.2 yeast extract (Difco) 0.5 glucose (Merck); 0.01 mL Tween 80 (Sigma Co.) and stock mineral solvent. The medium was buffered to pH 4.5 by 0.1 *M* Na 2,2 dimethyl succinate as recommended by Tien and Kirk (1988). Stock mineral solution was prepared as (g / L); 1.4 $ZnSO_4.7H_20$; 1.0 $Fe(SO_4)_27H_20$; 0.1 $CoCl_2.6H_2O$; 0.1 $CuSO_4.H_2O$; 0.5 NaCl; 1.0 $MnCl_2.4H_20$. Veratryl alcohol and stock mineral solution was added into the media to quantify 1mL / L after sterilisation of with millipore filtration (0.45 µ diam). Cultures were grown in 250 ml Erlenmeyer flasks containing 50 mL media.

By adding 10 mL sterile distilled water into fungi stock cultivate which were produced on the PDA slants during 7d at 30°C, transformed into mycelium suspensions and added into the liquid media. Fungi were reproduced during 7d in the incubator which had 30°C and 150 rpm. Fungi biomasses which were obtained after homogenisation in the sterile virtis homogeny indicator, added into liquid media provided that it would be 5 ml homogenate / 50 mL liquid medium. Polymer films contributed into media containing fungi were processed by disinfection application which was advised by Lee et al (1991). Fungi were incubated at 30°C and 150 rpm. PVC films were taken from liquid media at intervals of 15 d. for the purpose of washing scrap of fungi on the films, films were cleaned with 70% ethyl alcohol -distilled water- 70% alcohol and they became dried at 50°C. The films with a temperature balanced by elapse of 24 hours, were measured for determination of their weights using precision scale and their thickness was, measured by sensitive micrometer. Spectroscopic analyses were carried out with UV-VIS and FTIR. While films were being added into the media, they were processed with disinfection on the basis of same methods.

The media mentioned above, containing fungi and PVC as only carbon source were cultivated, and their degree of utilising PVC was tried to be determined. For this purpose films were taken at intervals of 15 d and their thickness and weightiness were determined. After spectroscopic analyses carried out with UV-VIS and FTIR, they were added into the media upon being treated with chemical disinfections process.

Mycelium suspensions were filtrated from Whatman No.1 filters, which were kept at 70°C for 24 hours and retained for 1 d in desiccators, Dried papers containing mycelium mass were left at 50°C for 24 hours. After that their weights were measured keeping with dessicator for balancing the temperature. Reproduction value was expressed in mg dry mycelium / mL liquid medium.

For the purpose of examining the impact of pure oxygen over biodegradation, as suggested by Faison and Kirk (1983), Tien and Kirk (1988), pure oxygen, starting from day 3 of incubation was gently flushed to the liquid media containing fungi and PVC films, with 3 d intervals, providing 5.04 L /min rate during the experiment (a total of 30 days).

Viscosity measurements of the original and biodegraded PVC samples were made by using an Ubbelonde capillary viscosimeter in tetrahydrofuran (THF) at 25°C. Temperatures were controlled within ±0.02°C and flow times were measured with an

occuracy of ±0.1 s. The concentration of PVC in THF solutions was changed in the range of 0.2 - 0.1 gdL^{-1}. The effect of the shear rate was ignored because the molecular weight of the polymer was not high. The concentration dependence of the viscosity dilute polymer solutions is described by well known Huggins equation; $\eta_{sp}/_{c} = [\eta] + k_H[\eta]^2 c$. Here, $\eta_{sp/c}$ is the reduced specific viscosity, [η] is the intrinsic viscosity, and k_H is the Huggings constant or Huggins slope coefficient. The intrinsic viscosity is a characteristic function for a single molecule in solution. It depends on molar mass, structure and conformation of the polymer molecules, on the solvent power and temperature. The dimension of [η] is a measure of the effective hydrodynamic volume of the polymer in solution. In this study, the intrinsic viscosities were evaluated as avarage values of the intercepts of plots of $\eta_{sp/c}$ as polymer concentrations (correlation coefficients are 0.98 ± 0.01).

RESULTS AND DISCUSSION

Before proceeding the studies regarding biodegradation of polyvinyl chloride with white rot fungi, it is intended to determine the conditions where fungi are active and the species to display maximum activity in such circumstances and to compare the relationship between reproduction activity and biodegradation. It is important because once the lignolytic activity has been triggered, the rate at which the biodegredation occurs is also a function of ambient pH, temperature and oxygen concentration (Leszkiewicz and Rinner 1988).

Optimum conditions for fungal activity and growth were determined before the biodegradation experiments. For all the white rot fungi the best growth was observed at in pH 4.5 and 150 rpm. Species of Pleurotus, *Poliporus versicolor* and *Phanerochaete chrysosporium ME 446* showed maximal growth rates at 30, 35 and 40°C respectively. Then the species having growth activity were determined from the species mentioned above. Species showing growth were also investigated for their biodegradative abilities, hence a comparative analyses of biomass-biodegradation relationship could be afforded. After setting optimum conditions for white rot fungi, the fungi have been left to incubation in conditions assigned to them in liquid media containing 1% glucose as the carbon producing source. Following the incubation, the active species among the fungi in terms of reproduction have been determined respectively as follows, *Poliporus versicolor, Pleurotus sajor caju, Phanerochaete chrysosporium, ME 446, Pleurotus ostreatus, Pleurotus sapidus, Pleurotus eryngii, Pleurotus florida* (Figure 1).

For the purpose of examining the impact of pure oxygen on biodegradation of PVC in the liquid media, pure oxygen starting from day 3 of incubation has been released during the entire time of experiment. By this way, the impact of oxygen over biodegradation could be compered in normal conditions (without extra oxygen) as well as in conditions in which pure oxygen was distributed evenly. Depending upon lignin enzyme system, it has been observed that desegregation in oxygen containing circumstances of many compound featuring polychloric and polyaromatic structure begins with the end of day 3, maximises at days 18 and 20 and continues until day 30 in gradually diminishing extends (Faison and Kirk, 1983). For that reason days 15 and 30 were set to be the most appropriate days for taking films out from the liquid media. But, since no apparent difference was found out in polymer films in day 15, it was concluded accordingly that the values displayed in the beginning and day 30 should be based in evaluation of the results.

Polyvinyl chloride composes of C-C, C-H, C-Cl structures. Therefore our study aims to reveal the change in the spots where these bonds locate. In order to determine the quantity of biodegradation, FTIR signified as a sensitive method, was used out of many spectroscopic methods available. The decrease in percentage of the films exhibited in day 30 was also determined in utilisation of UV-ViS.

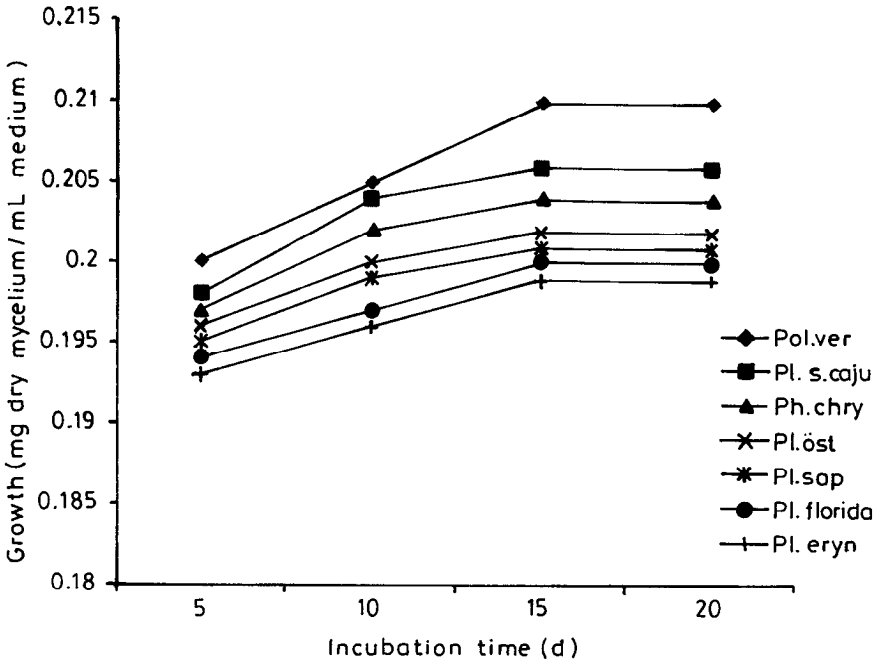

Figure 1. Growth of the fungi at the optimum conditions in the liquid medium containing only glucose as the carbon source.

The study carried out to reveal the impact of white rot fungus over PVC films in the liquid media comprised of comparison between beginning and day 30 spectroscopes of the media equipped with and without pure oxygen. The change in C-H, C-Cl bonds as made clear with the calculations are provided in Table 1 and 2. Again, due to the very weak nature of C-C bonds in the constitution of polymer and hard recognition of them, they are excluded from our study. As given in Table 1, the decrease values in % in C-H quantities that polymer films contain in the liquid media with pure oxygen has remarkably increased in comparison to the pure oxygen free media. Here, the effect of oxygen on C-H bond is given in increase expressed in %. For instance, it is whereas determined that *Poliporus versicolor* forms a decrease of 82.15% on C-H bonds in normal conditions, such values is found out as 90.85% in pure oxygen containing media. The fact once more proves that the effect of oxygen on lignolytic enzymes is much notable, as observed by the quantities of decrease in percentage in C-H bonds for the oxygen containing media respectively indicate 65.51%, 65.09%, 74.47% and 90.25% for *Pl. ostreatus, Pl. sapidus, Pl. eryngii, florida* which characterises a weaker feature in terms of reproduction than *Poliporus versicolor, Pleurotus sajor caju* and *Ph. chrysosporium.* The opinion here is that oxidative effect of oxygen takes the role. Many studies surveying effect of pure oxygen over biological desegregation are in support our thought (Kirk et al., 1983; Faison and Kirk, 1983, 1985; Bumpus et al., 1988; Leszkiewicz and Rinner, 1988; Roch et al., 1989).

The explanation for the observation of the increase in C-Cl bonds composed in PVC films in liquid media containing white rot fungi at oxygen free media, is made to lie on the coupling of chlorine atoms in the content of medium in lieu of hydrogen atoms broke away as a result of biodegradation. Besides the values of increase in C-Cl bonds in PVC films at pure oxygen containing media is less as per oxygen free liquid media. The

substitution of oxygen with hydrogen atoms broke away from C-H bonds and subsequent formation of carbonyl atoms is expressed to reason such decrease. As given in Table 3, it is confirmed by the higher increase in pure oxygen containing media at carbonyl groups than the increase taken place in normal conditions.

Table 1. Quantities of decrease in percentage (%) in C-H bonds in normal and pure oxygen-containing conditions, for the PVC films.

Fungi	Normal conditions	O_2 containing
Pol. versicolor	82.15	90.85
Pl. sajor-caju	58.11	85.57
Ph. chrysosporium	53.10	77.82
Pl. ostreatus	55.01	65.51
Pl. sapidus	49.37	65.09
Pl. eryngii	53.73	74.47
Pl. florida	37.80	90.25
Control	Not Applicable	

Table 2. Quantities of increase in percentage (%) in C-Cl bonds in normal and pure oxygen-containing conditions, for the PVC films.

Fungi	Normal conditions	O_2 containing
Pol. versicolor	167.33	116.73
Pl. sajor-caju	125.30	111.34
Ph. chrysosporium	119.06	103.10
Pl. ostreatus	128.70	114.96
Pl. sapidus	110.72	110.28
Pl. eryngii	125.86	121.02
Pl. florida	131.36	111.20
Control	Not Applicable	

Table 3. Quantities of increase in percentage (%) in C=0 bonds in normal and pure oxygen-containing conditions, for the PVC films.

Fungi	Normal conditions	O_2 containing
Pol. versicolor	167.66	234.50
Pl. sajor-caju	113.02	300.91
Ph. chrysosporium	131.18	176.38
Pl. ostreatus	124.18	128.53
Pl. sapidus	115.16	286.48
Pl. eryngii	151.23	202.19
Pl. florida	147.41	157.54
Control	Not Applicable	

No expectation should be raised and is rational as for the quantities of decrease in percentage values in C-H bonds and increase in C-Cl and C=O groups is related to each other, beyond foregoing remarks. Because, the chlorine and oxygen atoms supposed to substitute hydrogen atoms will be entering into cell to form C-Cl, C=O groups in a different manner depending upon the fungus type and thus it is thought that oxygen atoms in turn will have different effect over the films contained in the liquid media. For instance, whereas the increase quantity of *Poliporus versicolor* in C-Cl bonds in oxygen containing conditions is 116.73% which displays the most reproductive activity, it has been 111.20% in *Pl. florida* with minimum activity. Our thought is further supported by the occasion that the increase in C=O groups in oxygen containing media is 234.50% in *Poliporus versicolor* 300.91% in *Pl. sajor caju* and 176.98% in *Ph. chrysosporium.*

Before the PVC films, in liquid media, given with pure oxygen or left in normal conditions are incubated with fungi, UV-ViS based spectroscopic analysis has been performed. The change in percentage rate between very initial values and the values obtained in week 4 has been accounted (Table 4). As we may see here, quantities of decrease in percentage in PVC films retained in pure oxygen released media are higher than the films left in normal conditions. At the same time, it is also observed that the fungus determined to be active in terms of reproduction have much more effect on PVC films. The formerly carried out studies arrive at parallel reference that the pure oxygen has an effect over white rot fungi (Leskiewicz and Rinner, 1988; Faison and Kirk, 1983, 1985).

Table 4. Quantities of decrease in percentage (%) in polymer films in normal and pure oxygen-containing conditions, as revealed in UV-ViS results accounted according to the original.

Fungi	Decrease in percentage (%)	Decrease in percentage (%)
Pol. versicolor	19.32	53.70
Pl. sajor-caju	17.84	37.20
Ph. chrysosporium	16.52	32.90
Pl. ostreatus	13.55	27.60
Pl. sapidus	13.17	21.80
Pl. eryngii	11.92	13.20
Pl. florida	10.10	11.10
Control	Not Applicable	

Table 5. The change in thinness and weight of the polymer films in normal and pure oxygen-containing conditions.

Fungi	Weight of the films (mg)			
	Normal conditions		O_2 containing	
	Time		Time	
	0. day	30. days	0. day	30. days
Pol. versicolor	0.0307	0.030	0.0363	0.0361
P. sajor-caju	0.0318	0.0317	0.0342	0.0340
Ph. chrysosporium	0.0335	0.0335	0.0357	0.0356
Pl. ostreatus	0.0365	0.0365	0.0398	0.0398
Pl. sapidus	0.0326	0.0326	0.0401	0.0401
Pl. eryngii	0.0286	0.0286	0.0412	0.0412
Pl. florida	0.0298	0.0298	0.0287	0.0287

The films introduced to liquid media after being measured in respect of thinness and weight have been taken out from respective media in days 15 and 30 and subjected to repeated measurements for thinness and weight to understand any possible loss of weight and thinness. But, since no change is observed in day 15 in PVC films, it is resolved that the results obtained in day 30 be expressed. As provided in the Table 5 no change has taken place in the weight of some films in oxygen containing and free media, as well as control group, as compared to the initial measurements. However, the UV-ViS and FTIR based spectroscopic analyses show actual changes in PVC films (Table 1, 2, 3, 4). Then the data concerning weight loss remains improper and insufficient in biodegradation studies to attain desired results. Therefore it is of the convection that such studies should be absolutely backed by more than one methods. Such conviction is also stated in other relevant studies (Benedict et al., 1983).

The viscosity measurements of the original and biodegraded samples were given in Table 6. As shown here, the intirinsic viscosity (η) of the original films is 0.61. This value

decreased at the biodegraded films. The fungi which have the most biodegradative activities decreased the intrinsic viscosity. The films treated with *Poliporus versicolor, Pl. sajor caju, Ph. chrysosporium* gave the intrinsic viscosities 0.30, 0.41, 0.43 respectively. In addition *Pl. ostreatus, Pl. sapidus, Pl. eryngii, Pl. florida* gave the intrinsic viscosities 0.50, 0.50, 0.51, 0.53.

Table 6. Viscosity measurements of the original and biodegraded PVC samples.

Fungi	[η]	r^2
PVC-0 (original)	0.61	0.99
PVC-1 (*Poliporus versicolor*)	0.39	0.98
PVC-2 (*Pl. sajor caju*)	0.41	0.98
P-3 (*Ph. chrysosporium*)	0.43	0.98
PVC-4 (*Pl. ostreatus*)	0.50	0.97
PVC-5 (*Pl. sapidus*)	0.50	0.97
PVC-6 (*Pl. eryngii*)	0.51	0.95
PVC-7 (*Pl. florida*)	0.53	0.95

Many studies on biodegradation have been focused on polymers containing filling matter such as amyl, poly - β - hydroxy butyrate, cellulose (Brand1 et al., 1990; Lee et al., 1991; Seppala et al., 1991; Osawa et al., 1994). These filling matters introduced into the structure of polymers have an accelerating effect towards biodegradation by microorganisms of polymers in outside environment (Gould et al., 1990; Barak et al., 1991; Goheen and Wool, 1991). Flat chained polymers are being preferred bearing low molecule weight for the studies focusing on biodegradation of pure polymers. The actual studies remark the less the molecule weight and chain folding degree of the polymer, the much easy biodegradation will occur (Albertsson et al., 1987; Klemchuck, 1989).

There are many studies about thermal and photo degradation of PVC (Braun, 1975; Decker, 1984; Owen, 1976; Seppala et al., 1991). But there is not any about the biodegradation of PVC. Finally, our study proves that PVC having low moleculer weight can be exposed to biodegradation at the optimum conditions containing oxygen by use of the white rot fungi.

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