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Alkaline Fungal Degradation of Oxidized Polyethylene in Black Liquor: Studies on the Effect of Lignin Peroxidases and Manganese Peroxidases

Shritama Mukherjee, Patit P. Kundu
Advanced Polymer Laboratory, Department of Polymer Science and Technology, University of Calcutta, 92 APC Road, Kolkata-9, West Bengal, India
Correspondence to: P. P. Kundu (E-mail: ppk923@yahoo.com)

ABSTRACT: High-molecular-weight polyethylene is resistant to natural environmental degradation for its crystalline, hydrophobic structure. In this study, waste polyethylene bags are chemically oxidized at 80°C for 5 days by potassium dichromate solutions of various concentrations along with sulfuric acid. Absorbance peaks of carbonyl and carboxylate ions in the Fourier transform infrared spectroscopy spectra and formation of amorphous phase from crystalline one as indicated in X ray diffraction studies of oxidized polyethylenes indicate the formation of a polar hydrophilic and low-molecular-weight material after oxidation. From the scanning electron microscopy studies, it is observed that reacted polyethylene surface is disintegrated and numerous fissures are formed throughout the surface. The respective weight loss of incubated oxidized polyethylene with *Phanerochaete chrysosporium* (MTCC-787) after 15 days of incubation is 70%, respectively, in black liquor–glucose–malt extract medium. As both lignin peroxidase (LiP) and manganese peroxidase (MnP) were detected in this media, further degradation of oxidized polyethylene is carried out in four different media with varying amount of N and Mn. The weight loss is observed only in media with excess nitrogen (N) and limited manganese (Mn), the condition which enhances the presence of LiP and MnP. This indicates that these enzymes are essential for degradation of lignin as well as oxidized polyethylene. UV spectroscopic studies indicate 40% decrease in the lignin concentration. This process of fungal degradation of chemically oxidized polyethylene using black liquor is very quick compared to the other related studies, leading to the simultaneous degradation of two waste materials, polyethylene and black liquor. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40738.

KEYWORDS: biodegradation; cellulose and other wood products; degradation

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INTRODUCTION

One of the most used polymers in daily life is polyolefin due to its properties like low cost of production from cheap petrochemical feed stock, easy handling, wide range of chemical resistance, transparency in thin films, nontoxicity, and flexibility. But polyolefin is a high-molecular-weight closely packed hydrophobic hydrocarbon without any polar groups and is resistant to natural environmental degradation.[1–3] An increasing rate of production of 12% per year and difficulty in degradation naturally, polyethylene has become a main environmental pollutant and a main concern for research to facilitate its degradation.[4]

Polyethylene degradation can be achieved through chemical, thermal, or photo oxidation. Low-density polyethylene (LDPE) and linear low density polyethylene (LLDPE) films mixed with pro-oxidant have been subjected to abiotic degradation at 60°C and under sunlight for 260 days. Loss of mechanical properties and flexibility has been observed for both cases, but the level of degradation achieved in case of thermal treatment has been much quicker with less exposure time than environmentally treated polyethylene.[2] Thermal degradation has been described in another report by subjecting LDPE at 450–525°C in an inert gas chamber. Degradation product is gaseous and wax like lower molecular weight material.[5] At 600°C under nitrogen atmosphere, polyethylene degradation has been observed.[6] Other methods like photooxidation under UV, sunlight have been used for abiotic degradation of polyethylene which takes place through oxidation. Main problems regarding above mentioned abiotic degradation are productions of toxic degradation byproducts like aldehydes, ketones, and acetones, and temperature required is extensively high which is in turn costly to achieve. On another way, if microbes present in environment, that is, in soil, water, wood are used in degradation of oxidized polyethylene, it will be more environmental friendly with lesser productions of toxic byproducts. Oxidation is always the primary step which is continued further to achieve abiotic

degradation, whereas in biodegradation, oxidation is performed separately as a pretreatment. Through oxidation, carbonyl group is introduced into carbon polymer backbone of polyethylene with reduction of molecular weight.

Biodegradation of oxidized polyethylene films generally demonstrated in films infused with pro-oxidant which catalyzes oxidation process. Such kind of biodegradation has been reported with polyethylene infused with TDPA® (totally degradable plastic additives) by subjecting it into soil composting. In this case, polyethylene is thermally oxidized and subjected to soil burial test and composting without adding any specific kind of microorganisms. After 80 days, 50% of oxidized polyethylene has been mineralized in soil burial test where unoxidized part has been removed through solvent extraction. Composting does not show that much effective degradation level.[3] In another work, biodegradation of oxidized polyethylene films mixed with pro-oxidant like transition metals, lipids, and starch has been demonstrated by incubating with lignin degrading fungus *Phanerochaete chrysosporium* and *Streptomyces* sp. *Streptomyces* sp. shows 68% effective reduction in molecular weight in 4 weeks, whereas *P. chrysosporium* shows very little degradation. It may be possible that due to presence of starch in oxidized polyethylene, this lignin degrading fungus has not been able to degrade polyethylene.[7] Extensive biofilm formation has been observed on the surface of oxidized polyethylene containing pro-oxidant after incubation with *Rhodococcus rhodochrous* and *Nocardia asteroides* for 2 months under scanning electron microscope (SEM). But weight loss study has not been included in this study.[8] In another study, biodegradation of oxidized polyethylene films containing pro-oxidant by fungus isolated from soil has been reported through SEM image.[9] Bacteria from soil like *Rhodococcus* and *Pseudomonas* have been shown to have ability to grow on the surface of oxidized pro-oxidant containing polyethylene and this cell adhesion property of these two bacteria has been described as a result of the presence of carbonyl group in polyethylene and other oxidation product or may be due to production of biosurfactants by these two bacteria.[10] Oxidation of pro-oxidant containing polyethylene followed by biodegradation using soil bacteria is generally used in many methods for biodegradation of polyethylene.

But the main problem of these methods is that commercially available transparent polyethylene bags do not contain any pro-oxidant. On the contrary, antioxidant has been mixed with LDPE during production to reduce oxidation due to sunlight and to improve its longibility. Level of oxidation achieved for pro-oxidant mixed polyethylene after treatment with sunlight, heat, or UV after 1–6 months cannot be achieved for commercially available antioxidant mixed polyethylene. Subsequently, amount of bacterial growth on the surface of oxidized pro-oxidant mixed polyethylene is not possible in case of the polyethylene without any pro-oxidant. It is also observed that time required for soil bacteria or fungus to achieve detectable weight loss or reduction of molecular weight of oxidized pro-oxidant mixed polyethylene is 1–6 months depending on the conditions used for biodegradation study. Biodegradation of polyethylene waste should be economically cheap, less time consuming and direct oxidation of commercially available polyethylene without mixing any pro-oxidant followed by biodegradation is required. But methods described earlier have been costly and time consuming. Different fungi from soil, water, mangrove forest soil, and wood have been used to degrade oxidized polyethylene. In a recent study, a white rot fungus, *P. chrysosporium* mainly found abundantly in wood has been used for the degradation of oxidized starch mixed polyethylene. As this fungus is not able to grow on starch, no degradation of oxidized polyethylene has been observed. *P. chrysosporium* has been observed to degrade nylon-66 and nylon-6, another nondegradable plastic and presence of manganese has been reported to enhance degradation. As it is reported, manganese induces extracellular release of manganese peroxidase (MnP) from this fungus, this enzyme can be involved in nylon degradation.[11] Due to structural similarity between nylon-66 and nylon-6 with oxidized polyethylene containing keto group, this lignin-degrading fungus can be used for biodegradation of oxidized polyethylene. With their ability to depolymerize complex phenolic structure of lignin, lignin peroxidase (LiP) and MnP can be used to degrade oxidized polyethylene molecule in the presence of lignin. As it has been reported that the presence of ligninolytic material can enhance extracellular release of LiP and MnP, black liquor with added minerals can be used for the degradation study.[12] Black liquor is waste effluent from paper industry with high lignin content and strongly alkalic. Black liquor is also a major cause of water pollution including blackening of water body and reducing its chemical oxygen demand. If this fungus is able to degrade oxidized polyethylene with LiP and MnP in black liquor media, two major pollutants could be degraded simultaneously.

In this article, chemical oxidation using different concentration of potassium dichromate ($K_2Cr_2O_7$) and sulfuric acid was performed. Level of oxidation was evaluated by the absorbance in Fourier transform infrared spectroscopy (FTIR), by observing under SEM and by measurement of the lowering in the crystalline phase under X-ray diffractometer. Chemical oxidation was done as a primary stage of the biodegradation. Oxidized polyethylene then subjected to degradation study under various systems (Case I = N and Mn limited, Case II = N excess and Mn deficient, Case III = N limited and Mn excess, Case IV = N and Mn excess, and Case V with malt extract and glucose) by fungus *P. chrysosporium* with lignin to study degradation and to study whether LiP and MnP enzymes are involved in degradation process. In Case V, 70% weight loss has been observed. Further enzymatic analysis shows presence of both enzymes LiP and MnP is required for degradation of polyethylene. Biodegradation of oxidized film in lignin medium was demonstrated by observing it under SEM and by FTIR spectroscopic studies. FTIR study shows reduction of carbonyl peak which appeared after oxidation, while incubating oxidized polyethylene with this fungus. Black liquor's biodegradation was analyzed through UV spectroscopy by monitoring the decrease in lignin concentration and 40% decrease after 15 days has been observed.[8,13]

MATERIALS AND METHODS

Test Material

Daily used 0.01-mm-thick transparent colorless LDPE bags were collected from municipality waste bins. Bags were then cut into

rectangular pieces (5 × 5 mm^2) and rinsed in soap water and distilled water, respectively. Then, films were taken in a beaker and boiled at 100°C for 24 h in distilled water for removing soil and other oily substances attached to the surface of polyethylene. Rectangular pieces were then dried at 60°C overnight in a hot-air oven.

Experimental Procedure

Chemical Oxidation. Boiled and overnight dried rectangular polyethylene pieces were refluxed continuously with a varied concentration of potassium dichromate ($K_2Cr_2O_7$) and 10% sulfuric acid solution at 80°C for 5 days. Then, polyethylene films were recovered from the solution by filtering through a Whatman (no. 42) filter paper and washed vigorously with distilled water. Washed and reacted pieces of polyethylene were dried overnight at 60°C in a hot-air oven and later stored at room temperature.

FTIR analysis was carried out with attenuated total reflectance (ATR)-FTIR (model alpha, Bruker, Germany) spectrometer scanning from 4000 to 500 cm^{-1} at room temperature. The resolution was set at 4 cm^{-1} with 42 scans per spectrum. Carbonyl index (CI) was calculated using the ratio of the absorbance frequency of the carbonyl peak (1705 cm^{-1}) and that of the CH_2 group bending frequency (1463 cm^{-1}).[14,15]

Samples were sputter coated with gold layer by a Hitachi sputter coater (model-E1010 Ion Sputter), Japan. Photomicrographs were observed under SEM (EVO 18, Carl Zeiss, Germany).

X-ray diffraction study of chemically reacted polyethylene samples were recorded with an X-ray diffractometer (PANalytical, the Netherlands) at angle of 2θ of 3–50° and fixed scan rate of 1° min^{-1}.

Biodegradation. *P. chrysosporium* (MTCC No. 787, ATCC No. 24725) was used in this study. This white rot fungus was obtained from Institute of Microbial Technology, Chandigarh, India. Oxidized polyethylene films were washed with alcohol and kept at aseptic condition at 4°C. In this study, oxidized polyethylene was subjected to degradation under five different conditions denoted as Cases I–V. Cases I–IV contain glucose 10 g, potassium dihydrogen phosphate (KH_2PO_4) 1 g, magnesium sulfate ($MgSO_4$) 0.5 g, and calcium chloride ($CaCl_2$) 0.1 g in 1 L of diluted black liquor. Diluted black liquor was obtained by mixing 200 mL of concentrated black liquor (lignin concentration 30 g L^{-1}) with 800 mL of distilled water. Black liquor was obtained from Nagaland Pulp and Paper Company, Nagaland, India. In addition, 4 g of ammonium nitrate (NH_4NO_3) was added in Cases II and IV in 100 mL for N-excess media. Cases I and III were N-limited media without any added NH_4NO_3. Manganese was added as manganese sulfate ($MnSO_4$) with an initial concentration of 2.5 g in 100 mL in Cases III and IV and Cases I and II were maintained as manganese-deficient media. Media composition for Case V was glucose 10 g, malt extract 10 g in 1 L of diluted black liquor. All media were autoclaved under 120°C for 15 min and after inoculation media were incubated at 37°C for 15 days.

FTIR analysis of incubated oxidized polyethylene films was done in the ATR-FTIR (model alpha, Bruker, Germany) spectrometer scanning from 4000 to 500 cm^{-1} at room temperature.

For SEM imaging, oxidized polyethylene samples incubated for 5, 10, and 15 days with fungus were fixed with 2.5% glutaraldehyde in 0.1*M* phosphate buffer, followed by dehydrating in graded ethanol (50, 70, and 90%). Some polyethylene films incubated for 15 days were washed in ethyl alcohol to remove the vigorously grown fungus, followed by drying in hot-air oven.

Enzyme Assay. LiP assay by veratryl alcohol oxidation for colorless nutrient media could not be performed for diluted black liquor media as the darkening color of the culture due to presence of lignin molecule in the black liquor. Instead LiP assay was done by oxidation of Azure B dye. One milliliter of reaction mixture contained 32 μ*M* and 100 μ*M* H_2O_2 in 50 m*M* Na tartrate buffer at pH 4.5 at 25°C and 500 μL of culture. Decrease in the optical density was observed at 651 nm.[16] MnP assay was done by phenol red oxidation at 30°C. One milliliter of reaction mixture contained 25 m*M* lactate, 0.1 m*M* $MnSO_4$, 1 mg bovine serum albumin, 0.1 mg phenol red, and 500 μL of culture in 20 m*M* sodium succinate buffer at pH 4.5. Reaction was started by addition of 0.1 m*M* of H_2O_2 and was stopped after 1 min by 50μL of 10% NaOH. Optical density was measured at 610 nm. Control assay was done by omitting Mn salt from the reaction mixture. MnP activity was calculated by subtracting the value of phenol red oxidizing activity in the absence of Mn salt from the value of the same in presence on Mn salt. Activity was expressed as the increase in absorbance at 610 nm/(min mL).[17]

UV/Vis Spectrophotometric Analysis. Black liquor from incubation flask was collected after 5, 10, and 15 days and centrifuged to remove cell debris. Supernatant was collected and concentration of lignin in black liquor is measured by UV–Vis spectrophotometer (Optizen view, made in India) at 280 nm. Concentration was measured using Beer–Lambert law. Lignin concentration in concentrated black liquor is 30 g L^{-1} which was calculated by precipitating lignin by acid precipitation method.

$$A_{280\text{nm}} = \varepsilon l c$$

$A_{280\text{nm}}$ is absorbance at 280 nm, ε is absorption coefficient and it is a constant, l is cell path length, and c is the concentration.

RESULTS AND DISCUSSIONS

Characteristic Analysis of Chemically Reacted Polyethylene Films

Washed and boiled polyethylene films were cut into pieces and subjected to chemical oxidation using different concentrations of $K_2Cr_2O_7$ (25, 30, 35, 45, and 50%). After 5 days, polyethylene films were washed with water, dried and were subjected to FTIR, SEM, and XRD analysis.

FTIR Analysis. In Figure 1, FTIR spectra of controlled unoxidized polyethylene film is compared to that of the polyethylene films reacted with various concentrations of $K_2Cr_2O_7$, that is, 25, 30, 35, 45, and 50% along with 10% of H_2SO_4. A broad absorbance peak between 1724 ± 5 cm^{-1} and 1678 ± 5 cm^{-1} with highest absorbance at 1705 ± 5 cm^{-1} appears in case of

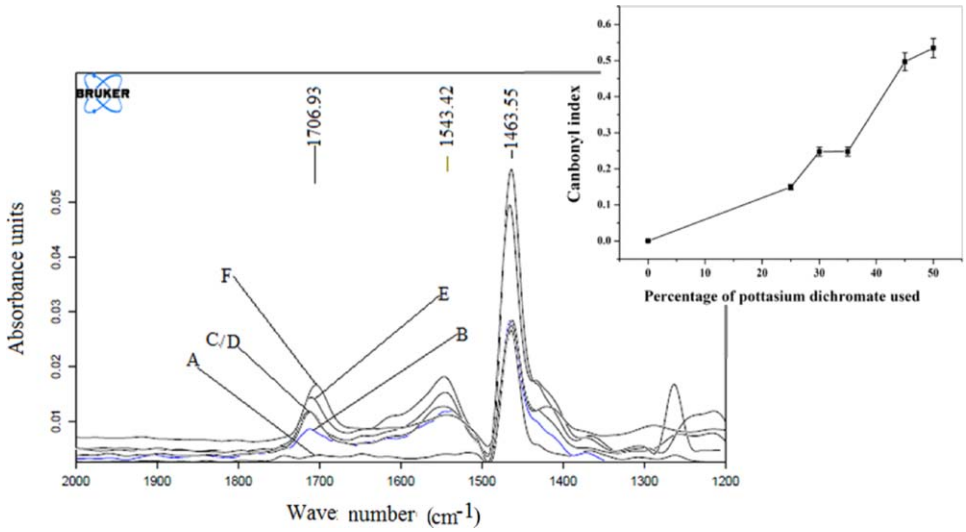

Figure 1. FTIR spectra of control and reacted PE films. (A) Control, (B) 25% $K_2Cr_2O_7$ used, (C) 30% $K_2Cr_2O_7$ used, (D) 35% $K_2Cr_2O_7$ used, (E) 45% $K_2Cr_2O_7$ used, and (F) 50% $K_2Cr_2O_7$ used. Inset: CI reduction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

oxidized polyethylene films. This broad absorbance peak in this region is responsible for the presence of various species of oxygenated carbonyl compound including carboxylic acid, ketone, and ketoacids.[14,15] Carbonyl peak absorbance increases with the increasing concentration of $K_2Cr_2O_7$. For the polyethylene reacted with 25% $K_2Cr_2O_7$, carbonyl peak absorbance is minimum compared to the reacted polyethylene with other higher concentration of $K_2Cr_2O_7$ and carbonyl peak absorbance is the highest in the polyethylene oxidized by 50% $K_2Cr_2O_7$. Appearance of carbonyl peak indicates the introduction of polar groups in the polyethylene backbone leading to a change in the hydrophobic nature into a hydrophilic one. Another peak appears at 1543 cm^{-1} in the FTIR spectra of the reacted polyethylene. Absorbance in this region is due to the presence of carboxylate ion.[18] Presence of both carbonyl and carboxylate groups indicates the oxidation of the polyethylene by $K_2Cr_2O_7$, which leads to a hydrophilic polymer. Absorbance at region 1463 cm^{-1} is due to the CH_2 group bending frequency which is a characteristic peak for polyethylene.

From Figure 1 (inset), it is apparent that CI of the polyethylene films oxidized by various concentrations of $K_2Cr_2O_7$ increases with the increasing concentration of $K_2Cr_2O_7$. CI of the polyethylene films oxidized by 25% $K_2Cr_2O_7$ is the lowest among the oxidized polyethylene by other concentrations of $K_2Cr_2O_7$. A sharp increase in the CI is observed when the concentration of $K_2Cr_2O_7$ increases from 35 to 45%. CI is the highest for the polyethylene film oxidized by 50% $K_2Cr_2O_7$. Increased value of carbonyl content in the oxidized polyethylene films indicates the introduction of carbonyl group in the polymer backbone and subsequent change in the hydrophobic nature of polyethylene to a hydrophilic one.

XRD Study. In Figure 2, XRD spectrum of control polyethylene is compared to the XRD spectra of polyethylene films oxidized by various concentrations of $K_2Cr_2O_7$. In the XRD spectra of control polyethylene film, peaks at 22° and 25° are the respective reflections from 110 and 200 planes of the orthorhombic crystalline phases and peak at 19° is a reflection from the 001 plane of the monoclinic crystalline phase.[19] These peaks are the characteristic peaks of polyethylene. When these peaks of control polyethylene are compared to that of the polyethylene films oxidized by 25% $K_2Cr_2O_7$, change in the intensity and broadness of these peaks are observed. Intensity of these peaks decreases with the increase in the level of oxidation. As observed from the FTIR spectra of oxidized polyethylene films, oxidation level is higher for the polyethylene films reacted with 50% $K_2Cr_2O_7$. Decreases in the intensity of XRD characteristic peaks with the increase in the oxidation level may be due to oxidative degradation of the sample resulting in change in the crystalline structure of oxidized polyethylene.[20] As it is well-known fact

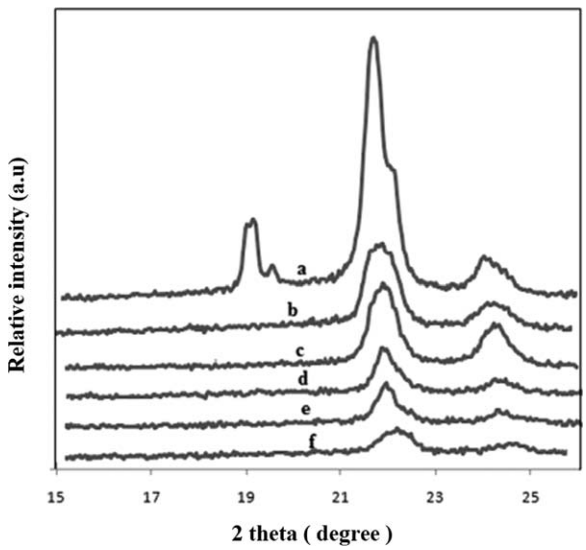

Figure 2. XRD spectra analysis of oxidized polyethylene: (A) control, (B) oxidized polyethylene by 25% $K_2Cr_2O_7$, (C) oxidized polyethylene by 30% $K_2Cr_2O_7$, (D) oxidized polyethylene by 35% $K_2Cr_2O_7$, (E) oxidized polyethylene by 45% $K_2Cr_2O_7$, and (F) oxidized polyethylene by 50% $K_2Cr_2O_7$.

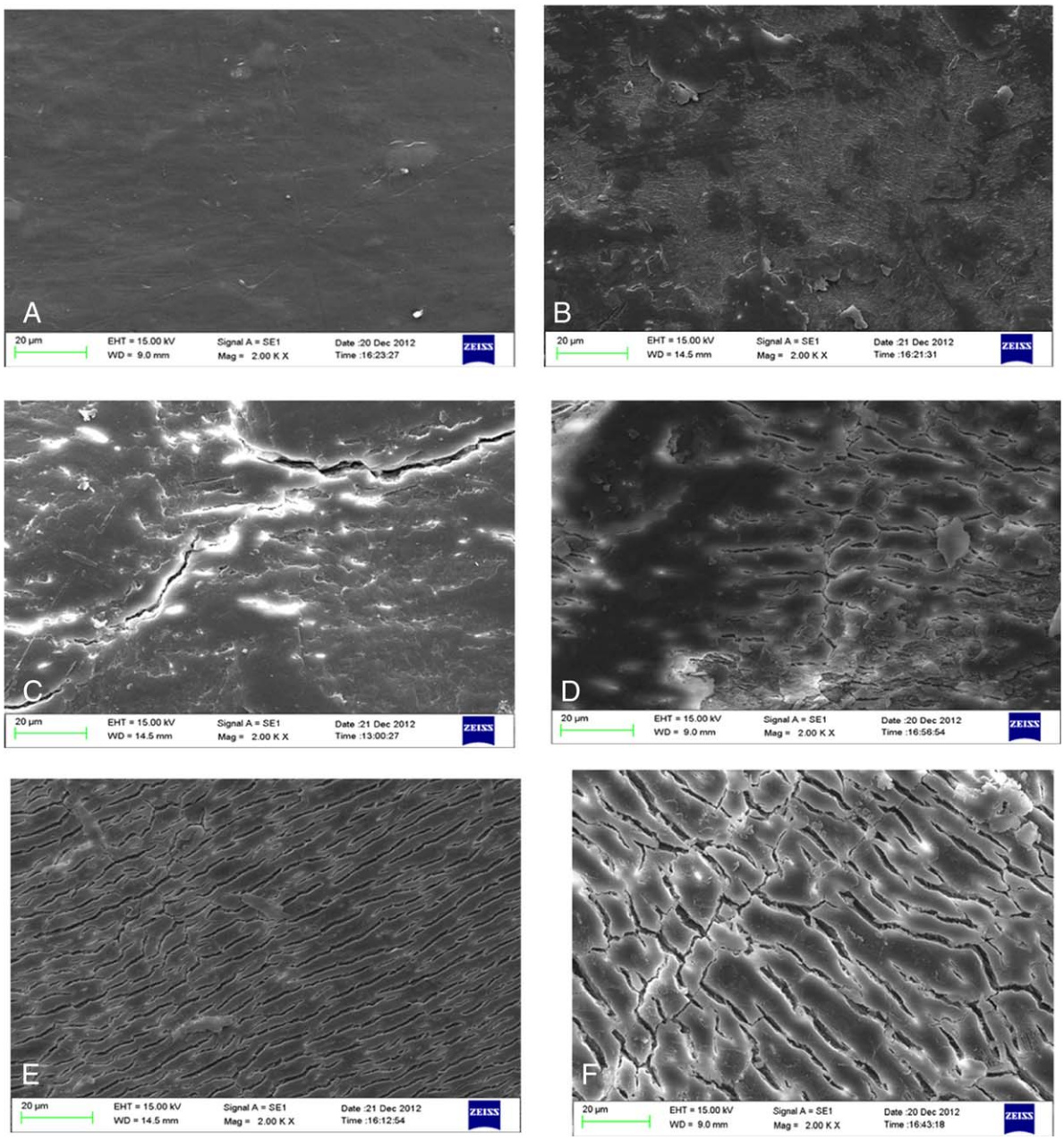

Figure 3. SEM after chemical oxidation at ×2000 magnification. (a) Control film, (b) oxidized PE films by 25% $K_2Cr_2O_7$, (c) oxidized PE films by 30% $K_2Cr_2O_7$, (d) oxidized PE films by 35% $K_2Cr_2O_7$, (e) oxidized PE films by 45% $K_2Cr_2O_7$, and (f) oxidized PE films by 50% $K_2Cr_2O_7$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that amorphous nature of the test material is represented by a broad peak, whereas the crystalline one by a sharp peak, decrease and broadening of the peak with increasing oxidation level may be due to change in the crystalline structure into amorphous one.

Oxidized Polyethylene Under SEM. In Figure 3, surface modifications of the oxidized polyethylene by various concentrations of $K_2Cr_2O_7$ are compared to the surface of control unreacted polyethylene. It is evident from the SEM images that after oxidation, polyethylene surface disintegrates rapidly. Depending on the concentration of $K_2Cr_2O_7$ used for the oxidation of the polyethylene, different types of fissures are observed on the polyethylene surface. In Figure 3(a), very little change is observed on the surface of the control polyethylene prior to subjecting it to oxidation by $K_2Cr_2O_7$. Fissures are observed after oxidation and with the increase in the oxidation level, surface disintegration increases. When concentration of $K_2Cr_2O_7$ is 25%, surface disintegration is very low [Figure 3(b)]. Cracks on the surface of polyethylene oxidized by 25% $K_2Cr_2O_7$ are small in size and less in numbers. Surface disintegration is much less in the polyethylene oxidized by the 30% $K_2Cr_2O_7$ [Figure 3(c)]. Only very few deep cracks can be seen on the surface. As the concentration of $K_2Cr_2O_7$ increases to 35%, fissures are more in

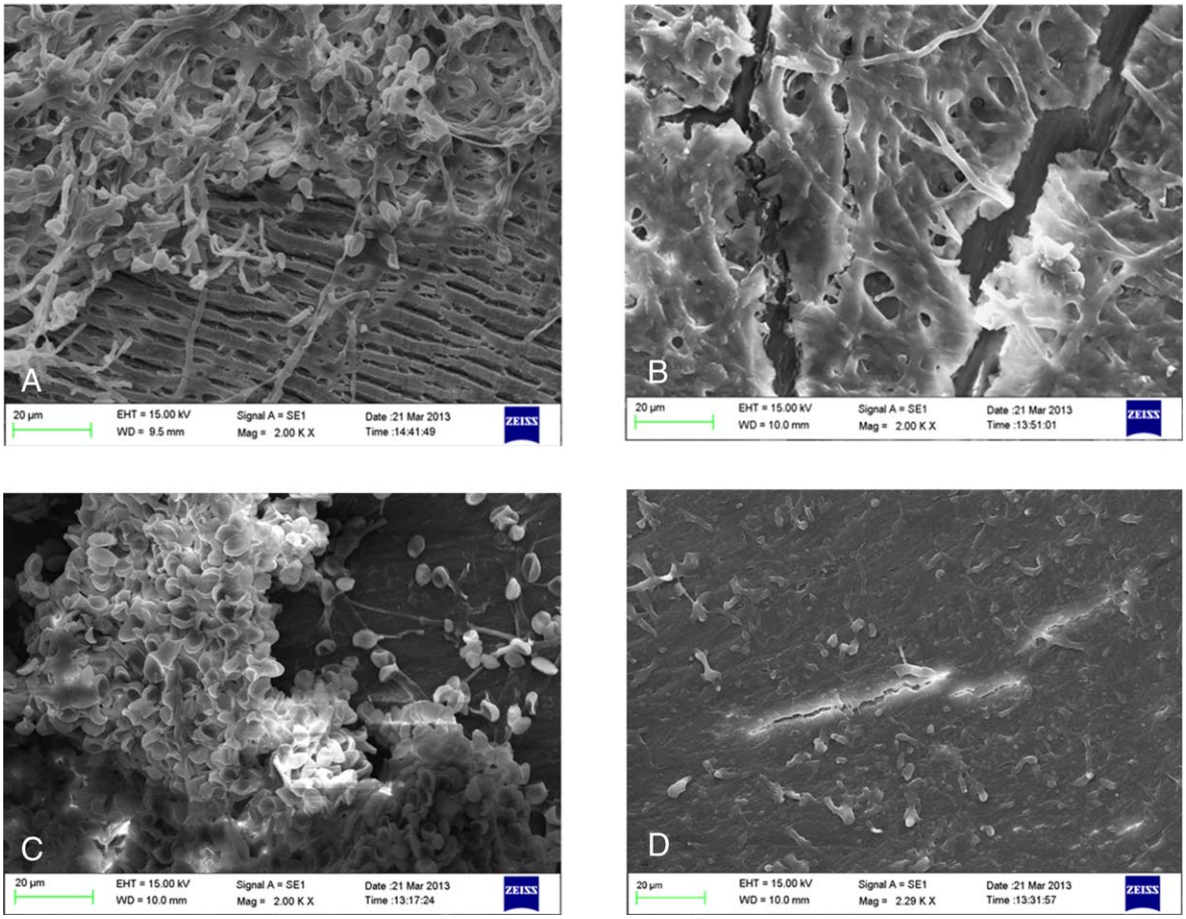

Figure 4. SEM after fungal degradation. (a) After 5 day glutaraldehyde fixed, (b) after 10 day glutaraldehyde fixed, (c) after 15 day glutaraldehyde fixed, and (d) after 15 day without fixing with glutaraldehyde. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

number and are present in some area of the surface as observed in Figure 3(d). For polyethylene oxidized by 45% $K_2Cr_2O_7$, fissures are smaller than that of the polyethylene oxidized by higher concentration of $K_2Cr_2O_7$ and present throughout the surface [Figure 3(e)]. In Figure 3(f), fissures are more in number and are distributed throughout the surface of polyethylene oxidized by 50% $K_2Cr_2O_7$. Surface disintegration is highest for this oxidized film.

Analysis of Oxidized Polyethylene Films Cultured with *P. chrysosporium*

Polyethylene films oxidized by 45% $K_2Cr_2O_7$ were taken for fungal degradation study. The study was performed continuously for 15 days by incubating *P. chrysosporium* in Case V media with oxidized polyethylene film at 37°C under constant shaking. Films were taken out in interval of 5 days for FTIR, SEM, and weight loss studies. For observing change in concentration of lignin, 4 mL of medium was taken out every 5 days interval and centrifuged at 5000 rpm to remove cells. Supernatant was taken for studying the change in lignin concentration by observing absorbance at 280 nm. After studying degradation by *P. chrysosporium*, oxidized polyethylene films were subjected to degradation study in Case I–IV media for study enzyme involved in degradation of polyethylene.

Weight Loss Study. In Figure 6, weight loss of oxidized polyethylene films are compared to that of the control unoxidized polyethylene films after 5, 10, and 15 days of incubation with *P. chrysosporium*. It is observed that weight loss of the oxidized polyethylene increases with an increase in the number of days, reaching 70% of weight loss after 15 days. But no weight loss is observed even after 15 days of incubation in case of the unoxidized control polyethylene.

SEM Analysis. In Figure 4, biofilm formation on the surface of the oxidized polyethylene is observed under SEM after 5, 10, and 15 days of incubation. In Figure 4(a), biofilm formation can be observed. Fungus growth is formed all over the surface including on the fissures. In Figure 4(b), it is observed that after 10 days of incubation fungus mucous network is formed all over the surface. In Figure 4(c), after 15 days of incubation, lumps of fungus cells can be observed. Under these lump of cells, oxidized polyethylene surface without any fissures can be seen. When these oxidized polyethylene films without fixing of gluteraldehyde is observed after 15 days of incubation, a surface with a lesser number of fissures is visible compared to the oxidized polyethylene films before fungal incubation [Figure 3(d)].

FTIR Characterization. In Figure 5, absorbance frequency of the carbonyl peak of the oxidized polyethylene films after 5, 10,

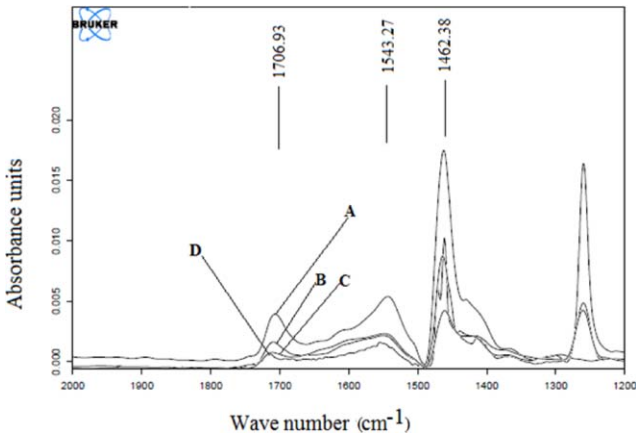

Figure 5. FTIR spectra after 15 day fungal degradation. (A) Control PE films 50% $K_2Cr_2O_7$ without any incubation. (B) Oxidized films after 5-day incubation, (C) after 10-day incubation, and (D) after 15-day incubation. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and 15 days of incubation is compared to that of the same oxidized polyethylene films before incubation. After fungal incubation, a decrease in the carbonyl peak absorbance is observed. Absorbance of carbonyl peaks of incubated oxidized polyethylene films decreases with the increase in the incubation days. From this decrease in the carbonyl absorbance and observing images of oxidized polyethylene films incubated with *P. chrysosporium*, it is apparent that during incubation, fungus is able to degrade oxidized and fissured surface. Therefore, degrading oxidized products present on the polyethylene surface. From 5 days to 15 days of incubation, fungal growth increases with time leading to degradation of oxidized polyethylene. After 15 days of incubation, fungus is able to degrade most part of the cracked surface of oxidized polyethylene, leaving its inner layer with lesser number of fissures exposed.

CI of the oxidized cultured polyethylene films after fungal degradation reduces from that of the oxidized control polyethylene films with an increase in the number of incubation days. This decrease in the CI after 5, 10, and 15 days of fungal degradation is in accordance with the weight loss (%) of the respective oxidized and incubated polyethylene films for respective incubation

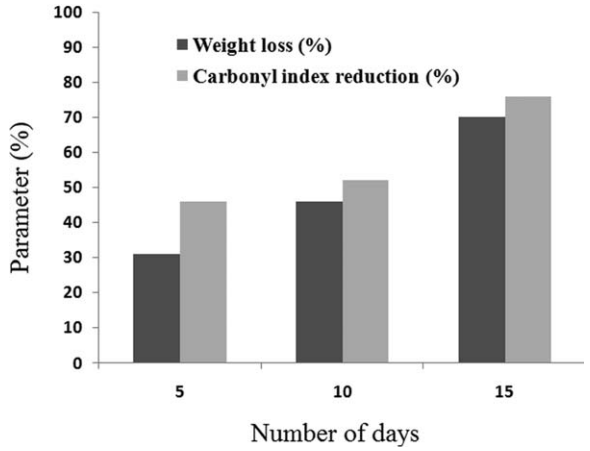

Figure 6. Comparison between weight loss (%) and CI reduction.

Table I. Comparison of MnP Activity Between Case I and Case V

Types of culture	MnP activity (ΔA_{610}, mL^{-1} min^{-1})
Case I	0.001
Case II	0.066
Case III	0.36
Case IV	0.402
Case V	0.103

days (Figure 6). CI reduction is higher for the oxidized and incubated polyethylene films incubated for 15 days.

LiP and MnP Assay. LiP activity is observed through decrease in the O.D. at 651 nm due to oxidation of Azure B dye. LiP activity is higher in this system (Figure 7). MnP activity is also high as its activity is measured through phenol red oxidation in the presence of Mn (Table I). The growth of this fungus is also high in this system. Presence of both the enzymes LiP and MnP is mainly due to presence of ligninolytic material. In this case, it is diluted black liquor containing lignin.[12]

UV Study. In Figure 8, reduction in lignin concentration of black liquor medium in (%) during 5, 10, and 15 days of incubation is compared to initial lignin concentration. It is observed that concentration of lignin decreases with the increase in the incubation days. After 15 days of incubation, 40% decrease in the concentration of lignin is observed. From this observation, it is apparent that *P. chrysosporium* is able to degrade simultaneously both the lignin from black liquor and oxidized polyethylene. Thus, a waste material (black liquor) can be useful for alkaline fungal degradation of oxidized polyethylene.

Optimization of Culture Media for Biodegradation

From weight loss study, FTIR, and observing under SEM, it was evident that *P. chrysosporium* can degrade oxidized

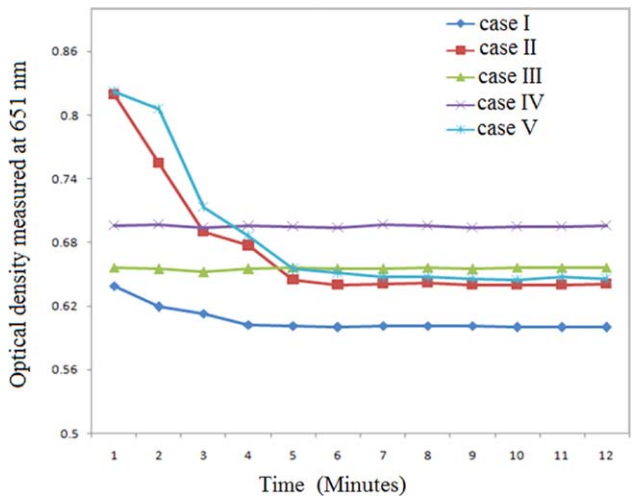

Figure 7. Comparison between decrease of O.D. at 651 nm with time which represent LiP activity of Cases I–V. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

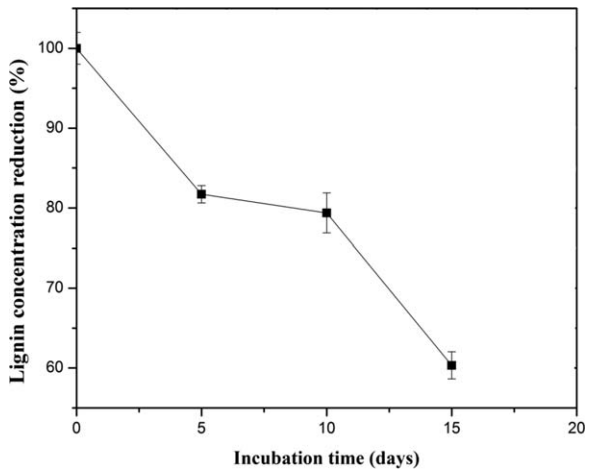

Figure 8. UV study of lignin of the black liquor after incubation with fungus.

polyethylene in the presence of ligninolytic material. In this media, that is, Case V, after 15 days of incubation with *P. chrysosporium*, both the enzymes are detected. To know, which of the enzymes are responsible for the degradation, oxidized polyethylene is subjected to degradation study under four different conditions, that is, Cases I–IV. After 15 days of incubation, samples were collected and culture was taken for enzyme assay.

Table II. Comparison of Weight Loss Percentage Between Case I and Case V

Types of culture	Weight loss in % after 15 days of incubation
Case I	1
Case II	35
Case III	1
Case IV	1.5

LiP and MnP Assay. LiP assay was done by Azure B oxidation, thus continuous decrease of O.D. at 651 nm with time is observed. LiP activity is comparatively higher in Case II than Case I. But no LiP activity is detected in Cases III and IV (Figure 7). LiP activity is low in Case I. This is due to the low growth level of *P. chrysosporium* in this N-limited medium. But high level of fungal growth is observed in Case II system [Figure 9]. But no LiP activity is detected in Cases III and IV due to presence of Mn. It is already reported that presence of Mn represses expression of LiP.[21] MnP is detected in all the systems except for the Case I (Table I). This is due to very low level of growth. MnP activity in Cases III and IV is much higher than the MnP activity in Case II. This is due to the presence of Mn in Cases III and IV which enhances MnP release and activity. For Case II, MnP is released due to presence of lignin

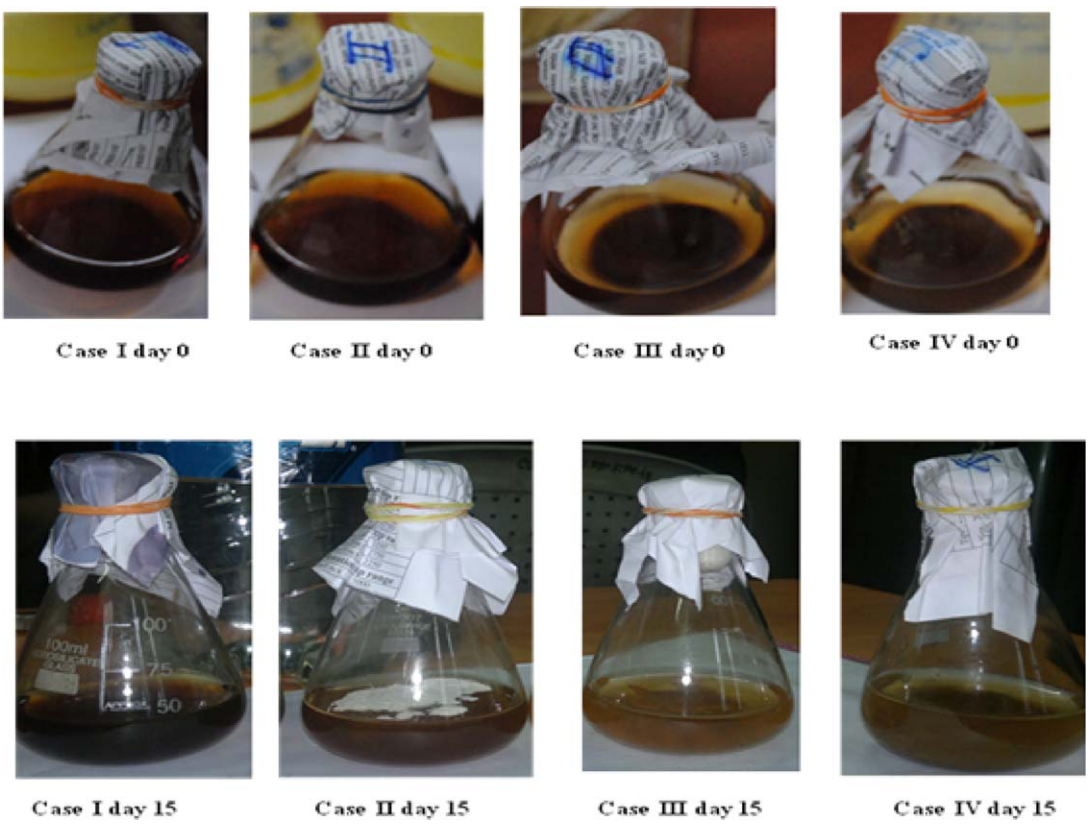

Figure 9. Comparison of growth between Case I and Case IV (day 0 and day 15). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

in the media and high amount of growth of the fungus was observed.[12]

Weigh Loss Study. Except for Case II, almost no weight loss is observed for all other systems, that is, Cases I, III, and IV (Table II). For Case II, weight loss percentage is 35% which in turn is much less than the weight loss observed in Case V. Almost no degradation is observed for all those systems, where either LiP or MnP is detected, not both. But for Cases II and V, where weight loss is observed, both the enzymes LiP and MnP are detected in high amount. Thus, it can be remarked that for successful degradation of oxidized polyethylene, both of LiP and MnP enzymes are required to be present in the medium in which oxidized polyethylene is immersed.

CONCLUSIONS

Oxidation level of the chemically oxidized polyethylene by various concentrations of potassium dichromate (25, 30, 35, 45, and 50%) along with 10% sulfuric acid is high as characterized through FTIR and XRD studies. An absorbance peak for the carbonyl group appears in the oxidized polyethylene films and absorbance of this respective peak increases as the concentration of $K_2Cr_2O_7$ increases. CI of chemically oxidized polyethylene films increases with an increase in the concentration of $K_2Cr_2O_7$. CI is highest for the polyethylene films oxidized by 50% $K_2Cr_2O_7$. Another peak at 1543 cm^{-1} appears in the oxidized polyethylene. This is due to the introduction of carboxylate ions in the polymer backbone. Appearance of both carbonyl and carboxylate absorption peaks in the FTIR spectra indicates introduction of these groups into polymer backbone, leading to a hydrophilic polymer. As observed in XRD spectra, on increasing the concentration of $K_2Cr_2O_7$, peaks at 22° and 25° become broader and intensity of these peaks decreases. This indicates the respective change in the crystalline phase into amorphous one in certain plain. Rapid disintegration of the oxidized polyethylene surface is also observed under SEM. From the above-mentioned change in the property after chemical oxidation of polyethylene, it can be concluded that the polyethylene films after oxidation become hydrophilic, amorphous and surface of polyethylene becomes disintegrated. These properties are all favorable conditions for biodegradation. *P. chrysosporium* is able to degrade oxidized polyethylene in malt extract–glucose media containing lignin from black liquor with a weight loss of 70% after 15 days of incubation. In this media, both the major enzymes, that is, MnP and LiP responsible for lignin degradation are detected. But further subjecting degradation under different conditions (N and Mn limited, N excess and Mn limited, N limited and Mn excess, and N and Mn excess), it is observed that *P. chrysosporium* is degrading oxidized polyethylene only if both the enzymes are present in the medium; the presence of either of this two enzymes cannot degrade oxidized polyethylene. CI reduction after 15 days of fungal degradation is observed to be 76% from that of the oxidized control polyethylene films. Black liquor which is used as medium for the fungus to degrade polyethylene is also successfully degraded by the fungus, showing 40% reduction in lignin concentration after 15 days of incubation. Thus, in this study, one waste material is used to degrade another waste material and this process is very quick and effective on both ways.

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