Effect of Pro-Oxidants on Biodegradation of Polyethylene (LDPE) by Indigenous Fungal Isolate, *Aspergillus oryzae*

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Received 29 July 2010; accepted 3 October 2010
DOI 10.1002/app.33517
Published online 14 February 2011 in Wiley Online Library (wileyonlinelibrary.com).

ABSTRACT: Proxidant additives represent a promising solution to the problem of the environment contamination with polyethylene film litter. Pro-oxidants accelerate photo- and thermo-oxidation and consequent polymer chain cleavage rendering the product apparently more susceptible to biodegradation. In the present study, fungal strain, *Aspergillus oryzae* isolated from HDPE film (buried in soil for 3 months) utilized abiotically treated polyethylene (LDPE) as a sole carbon source and degraded it. Treatment with pro-oxidant, manganese stearate followed by UV irradiation and incubation with *A. oryzae* resulted in maximum decrease in percentage of elongation and tensile strength by 62 and 51%, respectively, compared with other pro-oxidant treated LDPE films which showed 45% (titanium stearate), 40% (iron stearate), and 39% (cobalt stearate) decrease in tensile strength. Fourier transform infrared (FTIR) analysis of proxidant treated LDPE films revealed generation of more number of carbonyl and carboxylic groups (1630–1840 cm^{-1} and 1220–1340 cm^{-1}) compared with UV treated film. When these films were incubated with *A. oryzae* for 3 months complete degradation of carbonyl and carboxylic groups was achieved. Scanning electron microscopy of untreated and treated LDPE films also revealed that polymer has undergone degradation after abiotic and biotic treatments. This concludes proxidant treatment before UV irradiation accelerated photo-oxidation of LDPE, caused functional groups to be generated in the polyethylene film and this resulted in biodegradation due to the consumption of carbonyl and carboxylic groups by *A. oryzae* which was evident by reduction in carbonyl peaks. Among the pro-oxidants, manganese stearate treatment caused maximum degradation of polyethylene. J Appl Polym Sci 120: 3536–3545, 2011

Key words: proxidants; *A. oryzae*; Biodegradation; Scanning Electron Microscope; FTIR

INTRODUCTION

Synthetic polymers (plastics) are widely used in industry and agriculture. Because of their high durability, they accumulate in the environment at a rate of 25 million tons per year.[1] Plastics are not normally biodegradable until they are degraded into low molecular products which can be assimilated by micro-organisms.[2,3] This shows that biodegradation must be preceded by an abiotic treatment that produces monomeric and oligomeric products. It has been shown that alcohols, aldehydes, ketones, carboxylic acids, etc., which are generated by oxo-biodegradation, can be utilized by the micro-organisms as nutrients for their growth.[4] In a study, it has been reported that linear paraffin molecules having a molecular weight below 500 Daltons,[5] or *n*-alkanes upto $C_{44}H_{90}$,[6] can be utilized as a carbon source by micro-organisms.

Commercial polyethylenes, which are widely used, are very much resistant to oxidation and biodegradation because they contain antioxidants and stabilizers. However, they can be made oxo-biodegradable by the use of pro-degradant additives.[7,8] The most active pro-degradants are those based on metal combinations capable of yielding two metal ions of similar stability and with an oxidation number differing by one unit, e.g., Mn^{2+}/Mn^{3+}.[9] Thus, the material degrades via a free radical chain reaction involving oxygen from atmosphere. The primary products are hydro peroxides, which can either thermolyse or photolyse under the catalytic action of a pro-degradant, leading to chain scission and the production of low molecular mass oxidation products, such as carboxylic acids, alcohols, ketones, and low molecular mass hydrocarbons.[10] Peroxidation also leads to

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Contract grant sponsors: IICT, Hyderabad, India, Jawaharlal Nehru Technological University, Hyderabad, India.

hydrophilic surface modification, which is favorable to micro-organisms.[11] In a study of a commercial photo biodegradable polyethylene[12] that low molar mass products are removed from the surface of the polymer by bio erosion without significant effect on the molar mass of the bulk polymer. The biodegradation of photo-degradable polyethylene begins at molecular weight 40,000 Daltons, and it was concluded that photo-initiated peroxidation is the rate determining step in the biodegradation of polyolefins in sunlight.

From a chemical perspective, we would expect polyethylene to be degradable, as linear alkenes are usually subject to biodegradation. However for polyethylene there is an inverse relationship between molecular weights and biodegradability. Linear hydrocarbon oligomers with molecular weights lower than 620 Daltons support microbial growth, while those having higher molecular weights are not utilized.[13,14] It is widely accepted that the resistance of polyethylene to biodegradation stems from its high molecular weight, its three-dimensional structure and its hydrophobic nature, all of which interfere with its availability to micro-organisms. Nevertheless, several studies have demonstrated partial biodegradation of polyethylene after UV irradiation,[15] thermal treatment[16] or oxidation with nitric acid.[17] Furthermore, a synergistic effect has been found between photo-oxidation and biodegradation of polyethylene.[18]

We have isolated indigenous fungal isolate, *Aspergillus oryzae* which was effective in degrading polyethylene sample (LDPE). The purpose of study is to correlate the generation of oxidation products on the surface of polymer with the action of pro-oxidants and UV light and also to show loss of oxidation products after incubation with *A. oryzae*. The degradation is monitored in terms of growth of micro-organisms[15] and change in mechanical properties of LDPE.[19] The degradation is described here with results of Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM).

MATERIALS AND METHODS

Plastic samples

Polyethylene sample (LDPE) with average molecular weight of 1,80,000 Daltons and 8.7 PDI used in this study was purchased from Shalimar packs, Tenali, Guntur (India). All the chemicals used in this study were obtained from Merck.

Isolation and characterization of polyethylene degrading fungal isolate

Fungal strain, *A. oryzae* used in the study was isolated and characterized as reported earlier.[20]

Chemical treatment of polyethylene samples

Polyethylene film (LDPE) used in the study was chemically pretreated for 2 days in pro-oxidant solution which acts as a photo inducer before subjecting films to UV irradiation. Four types of proxidants viz. stearates of Fe, Co, Mn, and Tiwere used to study the effect of proxidants on the rate of biodegradation of polyethylene. The basis of pro-oxidants is transient metal ions, typically added in the form of stearate or other ligand complexes, most often stearates of Fe^{3+}, Mn^{2+} or Co^{2+} as pro-oxidants.[21,22] Prior to pretreatment polyethylene films were cut into pieces (about 10×10 cm^2 each), weighed, disinfected in 70% ethanol and air dried for 15 min in a laminar-flow head.

UV irradiation of polyethylene

To stimulate partial photolysis during the natural weathering of polyethylene exposed to sun, chemically pretreated LDPE samples were subjected to partial photolysis in a QUV accelerated weathering tester (Model-GJ-032), China. The polyethylene was subjected to a program of continuous exposure to UV (312 nm) for 50 h.

Biodegradation assay

The biodegradation assay was performed in 250-mL conical flasks by adding 100 μL of pure active *A. oryzae* culture into 100 mL of Czapek-Dox broth containing chemically pretreated and untreated polyethylene samples as a carbon source in separate conical flasks. The assay was performed with respective positive (Czapek-Dox broth + *A. oryzae* + chemically treated/untreated LDPE pieces) and negative (Czapek-Dox broth + pretreated LDPE pieces) controls. The flasks were incubated at 27°C at 120 rpm for 3 months with 12-h interval of shaking. Three replicates were prepared for each pretreated and untreated LDPE film.

Film harvest

After exposing to fungal isolates for 3 months LDPE pieces were harvested, washed in 70% ethanol to remove as much biomass as possible, dried at 45°C and equilibrated and weights were determined. Each of the films with and without chemical treatment was compared with the corresponding uncultured material (negative control) as well as with the cultured material.

Determination of weight loss

Recovered LDPE films were analyzed for degradation by weight loss before and after microbial

TABLE I
Effect of Prooxidants on Mechanical Properties and Weight of the LDPE Film Exposed to *A.oryzae* for 3 Months

Type of proxidant	Tensile strength (Mpa)	Breaking load (N)	Percentage of elongation	Weight (g)
Control (untreated and unexposed)	27.5	10.1	458	0.0604
UV treated	21.7	8.2	349	0.0495
Manganese stearate + UV treated	13.5	5.3	178	0.0319
Titanium stearate+ UV treated	15.1	6.2	252	0.0354
Iron stearate+ UV treated	16.5	6.6	261	0.0387
Cobalt stearate+ UV treated	16.8	6.8	271	0.0398
Abiotically untreated	26.9	10.02	443	0.0575

treatment using electronic balance, Type AX200, Shimadzu, Japan. The percentage weight loss of the inoculated LDPE samples is given by the formula:

$$\% \text{ weight loss} = (\text{Final weight} - \text{Initial weight})/\text{Original weight} \times 100$$

Mechanical tests

The mechanical properties of chemically pretreated and inoculated LDPE films were examined using Universal Testing Machine, Shimadzu, AGS 10 KN model. Thin film grips were used to avoid damage to the test samples at the contact surface between the grips and polymer. All tests were performed at 25°C using a crosshead speed of 10 mm/min with gauge length 5 cm. Three replicates were tested for each sample, and average values of the breaking load, tensile strength and % of elongation were determined.

FTIR spectroscopy

Changes in the polyethylene structure following prooxidant treatment, UV irradiation and subsequent incubation with *A. oryzae* were analyzed by using FTIR, spectrum 400 IR system, Perkin–Elmer, USA. The entire spectral range between 400 and 4000 cm^{-1} was scanned with a resolution of 2 cm^{-1}. Seven types of LDPE samples were analyzed: (i) untreated LDPE (control), (ii) UV irradiated LDPE, (iii) LDPE treated with iron stearate and UV irradiated, (iv) LDPE treated with manganese stearate and UV irradiated, (v) LDPE treated with cobalt stearate and UV irradiated, (vi) LDPE treated with titanium stearate and UV irradiated, and (vii) abiotically treated LDPE incubated with *A. oryzae*.

Determination of biomass concentration

The growth of *A. oryzae* in the presence of untreated and treated LDPE as a sole carbon source was determined to study the effect of pro-oxidants on the degradation of polyethylene. Five types of LDPE viz. (i) abiotically untreated LDPE, (ii) UV irradiated LDPE, (iii) manganese stearate treated and UV irradiated LDPE, (iv) titanium stearate treated and UV irradiated LDPE; iron stearate treated and UV irradiated LDPE, and (v) cobalt stearate treated and UV irradiated LDPE were used as a sole carbon source in the flasks containing medium for the growth of *A. oryzae*. The flasks were incubated at 27°C at 120 rpm for 3 months with 12-h interval of shaking. The biomass concentration of *A. oryzae* was determined after 3 months of incubation period using UV-visible spectrophotometer (ELICO) by measuring the absorbance at 600 nm.

Scanning electron microscope

SEM analyses on polyethylene films retrieved from biodegradation and abiotic degradation were performed using a scanning electron microscope, SEM Hitachi-S520 (Japan). The LDPE samples were metalized with gold (three discharges of 40 mA/50 s; each one, argon atmosphere), in a high vacuum metalizator (Bal-Tec SCD 050). Samples were analyzed in an electron microscope by means of secondary electrons, with an acceleration voltage of 10 kV and a work distance between 28 and 30 mm. The surface changes of polyethylene films viz., (i) LDPE treated with manganese stearate and UV light and (ii) LDPE treated with manganese stearate, UV light and incubated with *A. oryzae* were studied and were compared with (iii) untreated LDPE (control).

RESULTS

The fungal isolate used in the study was isolated from polyethylene film buried in the soil for 3 months and was characterized as *A. oryzae* as reported previously.[20]

Stearate salts of transition metals as photo inducers and UV irradiation as a photo oxidative treatments to biodegradation

Four different metal stearate salts viz. iron stearate, manganese stearate, cobalt stearate, and titanium stearate were used in chemical treatment of LDPE before subjecting to UV irradiation and

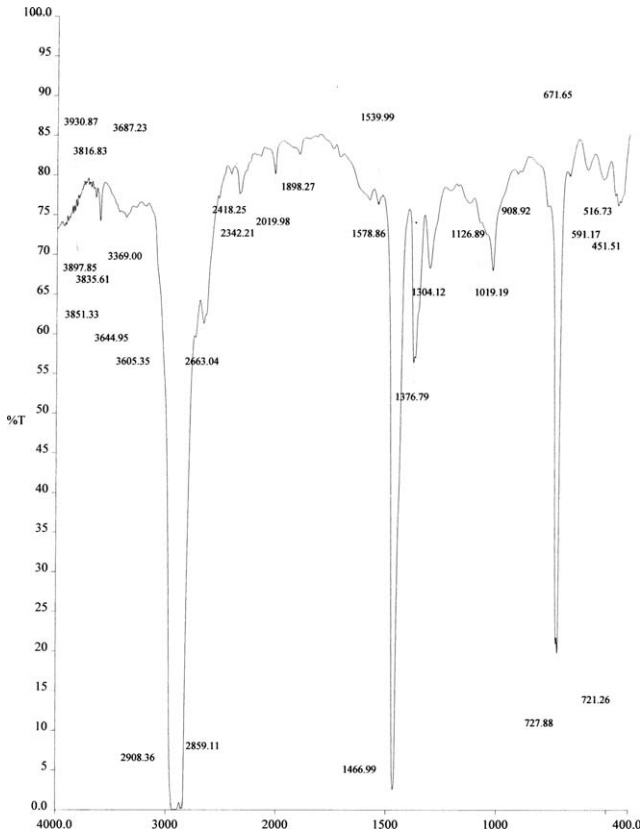

Figure 1 FTIR image of untreated LDPE (control).

biodegradation. Among all the four metal salts, manganese stearate treated LDPE undergone maximum degradation compared with other samples, which was measured in terms of weight loss (Table I). Chemical treatment of LDPE with manganese stearate followed by UV irradiation and incubation with *A. oryzae* for 3 months resulted in weight loss by 47.2%. Treatment with other metal salts viz. titanium stearate, iron stearate, and cobalt stearate resulted in weight loss by 41.6, 36.1, and 34%, respectively, compared with chemically untreated LDPE exposed to UV irradiation and *A. oryzae* showed only 18% weight loss; whereas abiotically untreated LDPE incubated with *A. oryzae* showed 5% weight loss compared with control (untreated and unexposed).

Determination of mechanical changes of the low density polyethylene (LDPE)

In most applications envisaged for films and fibers in contact with micro-organisms, loss in tensile properties is the most relevant practical criterion to determine its degradation.[19]

Both chemically treated and untreated LDPE samples exposed to UV irradiation and *A. oryzae* were tested for mechanical changes based on breaking load, tensile strength and elongation break to determine the effect of various transition metal stearates on biodegradation of LDPE.

Chemically treated LDPE samples exposed to UV irradiation and *A. oryzae* showed greater reduction in tensile strength, breaking load and percentage of elongation compared with untreated LDPE exposed to U V light and *A. oryzae* (Table I). Manganese stearate treated LDPE exposed to UV irradiation and *A. oryzae* showed 51% reduction in tensile strength compared with titanium stearate, iron stearate, and cobalt stearate treated LDPE which showed 45, 40, and 39% decrease in tensile strength, respectively. Whereas UV irradiated LDPE showed only 21% decrease in tensile strength.

Decrease in breaking strength of LDPE films was observed when treated with manganese stearate, titanium stearate, iron stearate, and cobalt stearate by 47, 39, 35, and 33%, respectively, compared with UV irradiated LDPE which showed 19% reduction in breaking load. Finally reduction in percentage of elongation was also observed when manganese stearate, titanium stearate, iron stearate, and cobalt stearate treated LDPE samples exposed to UV irradiation and *A. oryzae* by 62, 45, 43, and 41%, respectively, compared with UV irradiated LDPE exposed to UV irradiation and *A. oryzae* with only 24% reduction in percentage of elongation; whereas LDPE

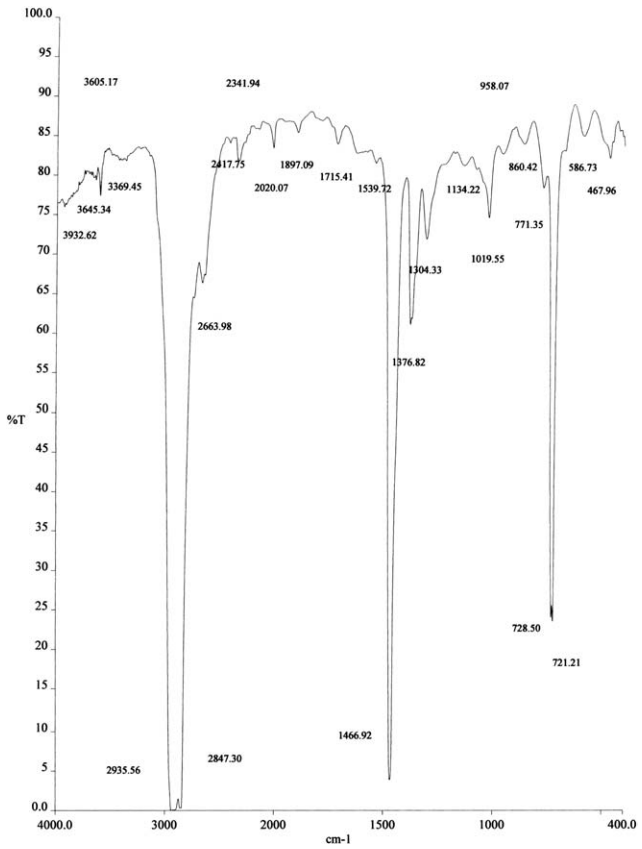

Figure 2 FTIR image of UV irradiated LDPE.

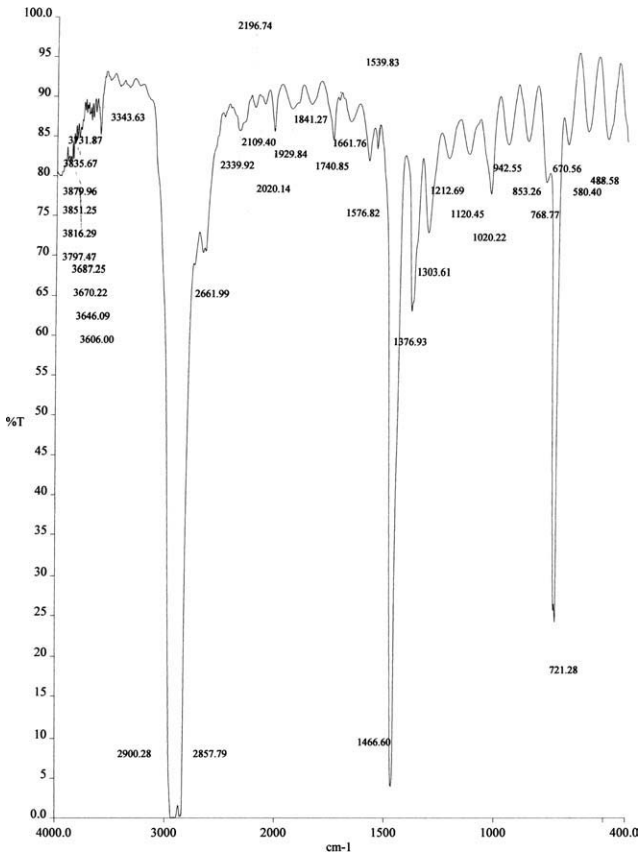

Figure 3 FTIR image of manganese stearate and UV irradiated LDPE.

sample which was not chemically treated and UV irradiated showed only 2.2, 1.2, and 3.2% reduction in tensile strength, breaking load, and percentage of elongation, respectively, after incubation with *A. oryzae* for a period of 3 months.

FTIR spectroscopy

As mentioned earlier, the structural changes in the polymer (LDPE) were determined by FTIR. FTIR image of untreated piece of LDPE (control) is shown in Figure 1. In comparison of FTIR image, UV treated LDPE (Fig. 2) with control peaks were found to be generated in UV treated film between 1630 and 1840 cm^{-1}. These peaks correspond to presence of carboxylic acids and its derivatives and carbonyl groups which includes aldehydes and ketones. Pretreatment with pro-oxidants like manganese stearate, titanium stearate, iron stearate, and cobalt stearate before UV irradiation resulted in more number of peaks between 1630 and 1840 cm^{-1} compared with UV treated film as shown in Figures 3–6, respectively. Comparison of FTIR images of LDPE treated with various pro-oxidants revealed that LDPE treated with manganese stearate has undergone maximum degradation which was evident from presence of more number of peaks in the carbonyl region especially peak at 1226.89 cm^{-1}(O—C stretching) corresponds to carboxylic acids absent in any other pro-oxidant treated LDPE sample. The peaks that were formed in the carbonyl region due to abiotic treatments were found to be reduced and some are completely degraded after biodegradation with *A. oryzae* which is shown in Figure 7. This indicates the breakdown of polymer chain and presence of oxidation products of LDPE which were later consumed by *A. oryzae*.

Determination of biomass concentration

Increase in the biomass concentration when polyethylene is used as a sole carbon source is one of the criterions, which can be used to determine the extent of biodegradation. Generation of carbonyl groups and carboxylic acids in the LDPE due to abiotic activity is the rate limiting step for the growth of micro-organisms leading to biotic degradation.[23]

From the Figure 8, it was found that *A. oryzae* grown in medium containing manganese stearate treated and UV irradiated LDPE exhibited maximum increase in biomass concentration (16%)

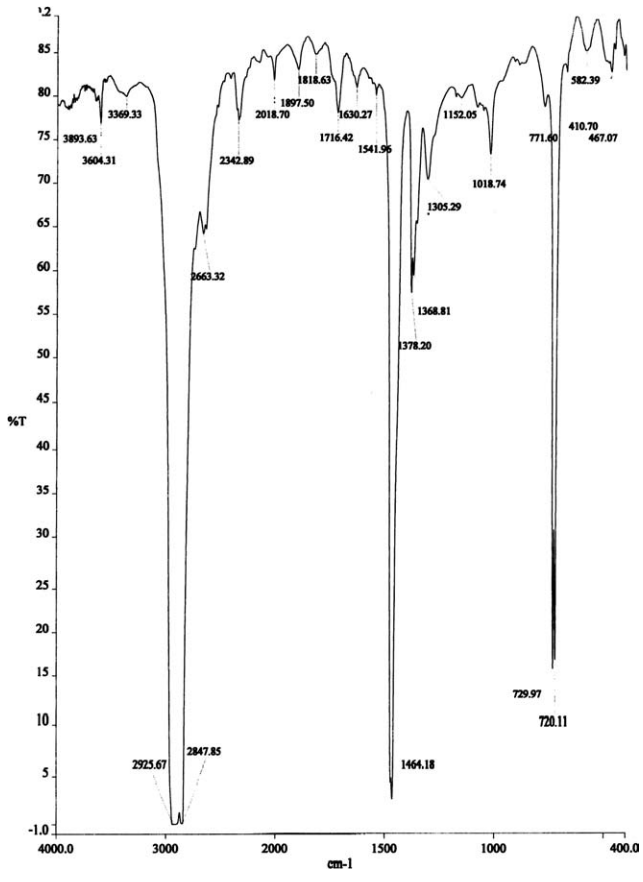

Figure 4 FTIR image of titanium stearate and UV irradiated LDPE.

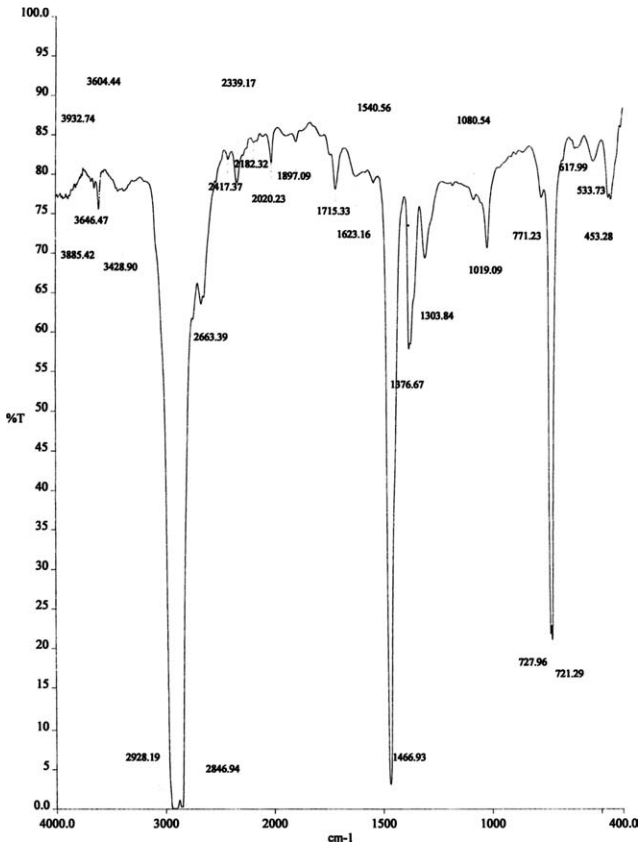

Figure 5 FTIR image of iron stearate and UV irradiated LDPE.

compared to increase in biomass concentration (4.7%) of *A. oryzae* when grown in medium containing UV irradiated LDPE. Whereas *A. oryzae* grown in medium containing titanium stearate, iron stearate, and cobalt stearate treated LDPE showed 11, 8, and 8% increase in biomass concentration, respectively. *A. oryzae* grown in medium containing untreated (control) LDPE showed negligible growth (0.6%).

Scanning electron microscope

From the Figure 9, it was evident that after abiotic treatment (manganese stearate and UV treatment) the films exhibited degradation because fissures and cavities appeared which were absent in control; this evidence was more notable as treatment time lengthened. The effect is increased when abiotically treated LDPE incubated with *A. oryzae* for 1 month. Deep cavities are formed with a clear indication of further breakdown of LDPE.

DISCUSSION

Fungal isolate used in the study was isolated from HDPE film buried in soil for 3 months and was identified as *A. oryzae*, which was capable of utilizing polyethylene as a sole carbon source as reported previously.[20] LDPE samples used in the study were treated abiotically by exposing them to various pro-oxidants (manganese stearate, titanium

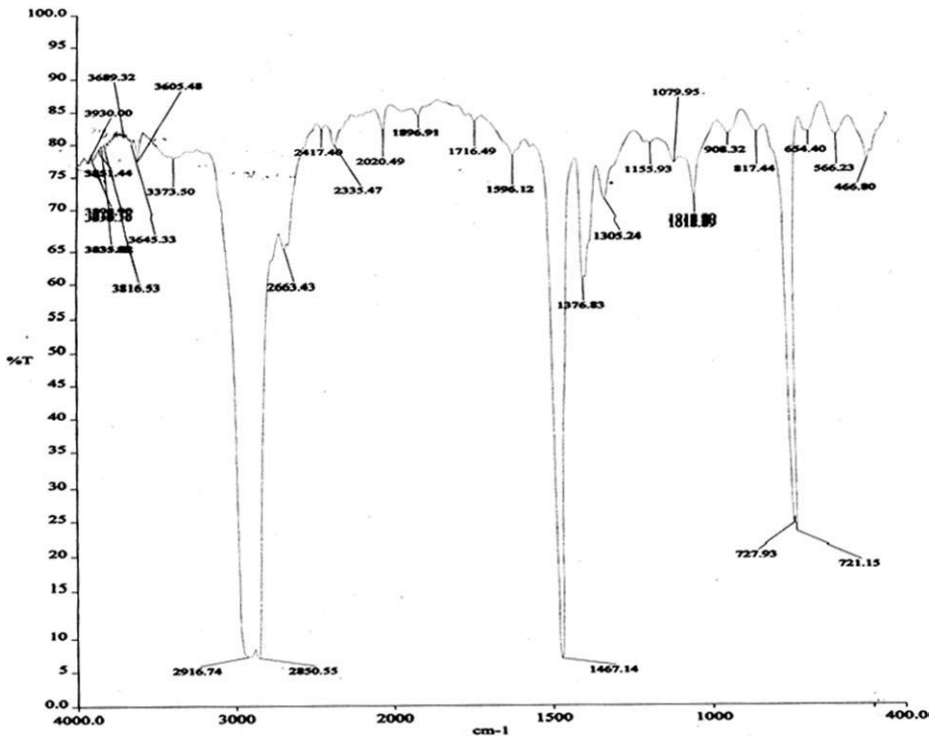

Figure 6 FTIR image of cobalt stearate and UV irradiated LDPE.

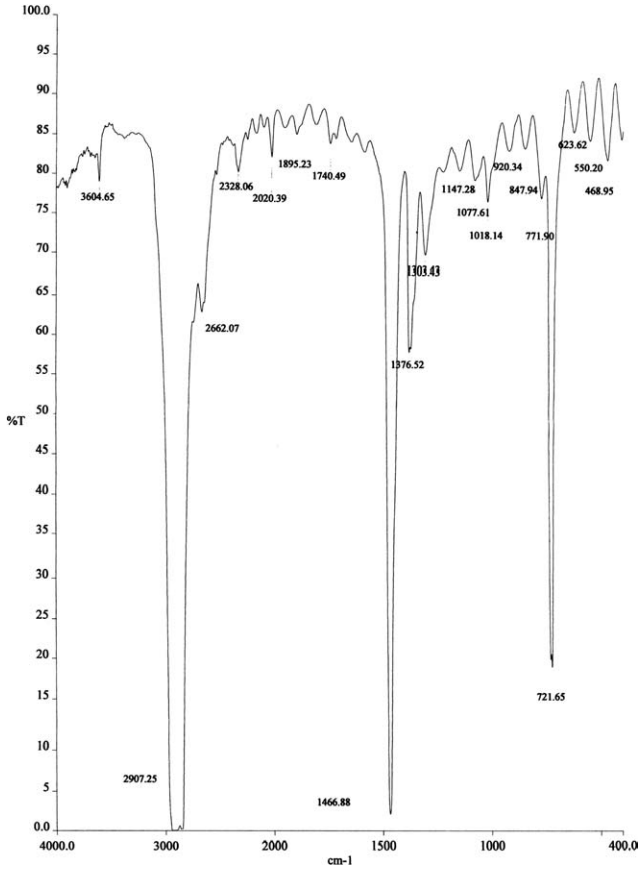

Figure 7 FTIR image of abiotically treated LDPE incubated with *A. oryzae*.

stearate, iron stearate, and cobalt stearate) acts as UV sensitizers followed by UV treatment. Heuck[23] pointed out that polyethylene need to undergo some nonbiotic degradation before microbial attack because of its hydrophobicity and its large molecular dimensions. Albertsson et al.[18] concluded that UV light or oxidizing agents such as UV sensitizers are needed at the beginning of biodegradation of inert materials such as polyethylene. These pretreated polymers were then applied to microbial treatment for 3 months using *A. oryzae* in a Czapek-Dox medium containing abiotically treated polymers (LDPE) as a sole carbon source. *A. oryzae* had grown well in the medium containing pro-oxidant treated films with a maximum of 16% increase of biomass when grown in manganese stearate treated LDPE containing medium compared to UV treated and untreated LDPE films with 4.7 and 0.6% increase of biomass, respectively. This indicates the generation of more number of functional groups in polyethylene film due to pretreatment with pro-oxidants before UV irradiation and *A. oryzae* grown in biomass by consuming the functional groups generated during abiotic process. As already noted, a significant amount of low molecular weight compounds is released to aqueous media from oxidized polyethylene film. It was shown that the compounds could be consumed by micro-organisms. Kounty et al.[24] followed release of low molecular weight compounds to water media from thermo and photo-oxidized HDPE and LDPE samples by NMR. These substances are subsequently consumed by *Rhodococcus rhodochrous* strain during 4 days of cultivation. This indicates biodegradation rate can be increased by subjecting the polyethylene to abiotic treatment initially. As expected it was also reported that with

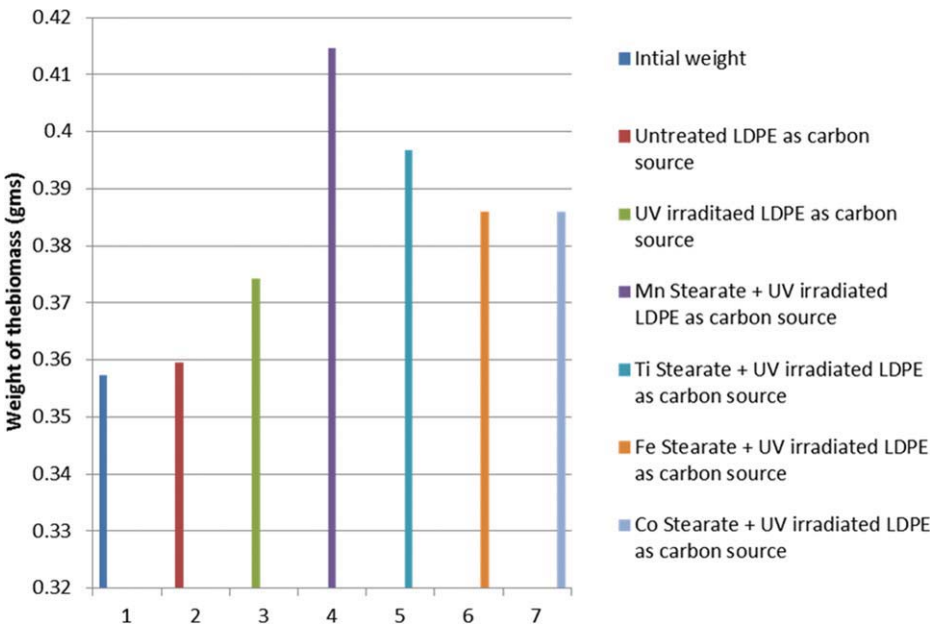

Figure 8 Increase in weight of biomass (*A. oryzae*) after incubation for 3 months. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com].

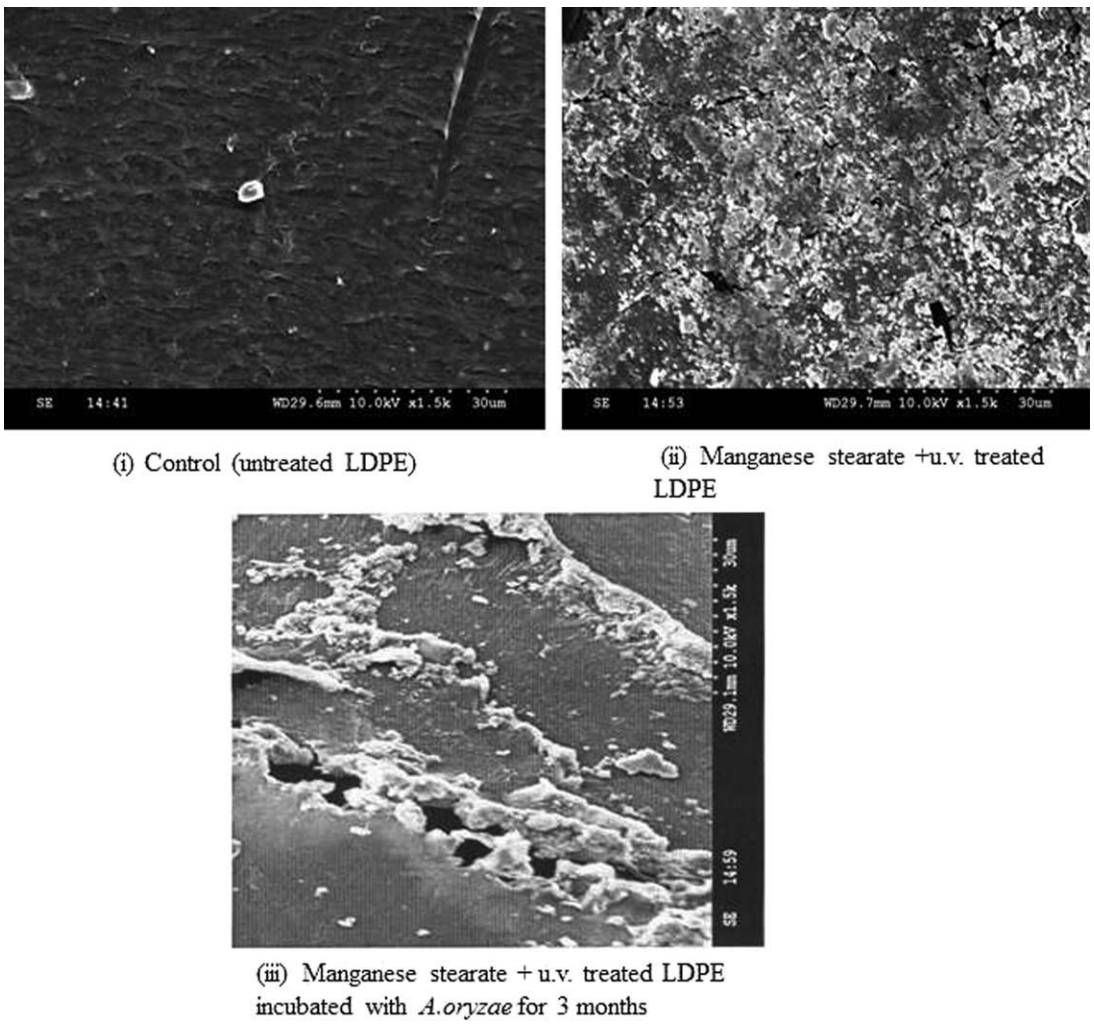

Figure 9 SEM photographs of (i) Control (untreated LDPE) (ii) Manganese stearate + UV treated LDPE (iii) Manganese stearate + UV treated LDPE incubated with *A. oryzae* for 3 months.

increase of pro-oxidant treatment and UV irradiation time resulted in a parallel increase of degradation of polyethylene followed by biodegradation.[25] In most of the studies the authors observed a period of fast growth on the beginning of incubation caused by consumption of eventual low molecular oxidation products of PE resulting in loss of weight and mechanical properties. After this first initial phase the metabolic activity dropped down and further progress of biodegradation became very uneasy to detect. With the help of ATP and ADP determination it was shown that during many months after initial fast growth period micro-organisms still gained energy from oxidized PE film, however, apparently at rather low rate.[26]

Among all the pro-oxidant treatments, manganese stearate treated LDPE incubated with *A. oryzae* for 3 months has undergone maximum degradation in terms of weight loss (47.2%) and percentage of elongation (61%). This decrease in percentage of elongation and weight loss is due to exposure of polymer to photosensitizer before UV irradiation which weakened the bonds present in the polymer thereby making the groups present in the LDPE available for *A. oryzae* which further decreased the mechanical strength of the polymer. Manganese stearate can induce oxidation of polyethylene even in the absence of light there by generating peroxides, participates in chain scissoring process which resulted in maximum degradation of PE.[24]

This biodegradation level is higher than the values reported for polyethylene incubated in soil for 10 years ranging from 3.5 to 8.4% reported by Albertsson and Karlsson.[27] Otake et al.[28] observed biodegradation of LDPE and HDPE buried in soil for 32–37 years, which was promoted by UV irradiation.

In earlier report, it was shown that polyethylene exposed to UV light for 60 h resulted in only 39% degradation. HDPE incubated in compost bags under controlled soil conditions for 1 month showed only 5.33% tensile strength loss.[29] In a recent report, tensile strength of starch blend LDPE and HDPE

which were exposed to *B. sphericus* showed only 29 and 30.5% tensile strength loss.[30]

In the biodegradation of polyethylene, an initial abiotic step involves oxidation of polymer chain due to the dissolved oxygen or that which is present in the ambient leading to formation of carbonyl groups. These eventually form carboxylic groups, which subsequently undergo β-oxidation[18] and are totally degraded via the citric acid cycle resulting in the formation of CO_2 and H_2O. Monitoring the formation or disappearance of peaks at 1710–1740 cm^{-1} (C=O stretching), 1210–1320 cm^{-1} (O—C stretching) and at 1630–1840 cm^{-1} (C=C stretching) using FTIR is necessary to elucidate the mechanism of biodegradation process.

Figures 5–9 show the various FTIR indices for untreated and abiotically treated LDPE. It was observed that in case of chemically (pro-oxidants) pretreated and UV irradiated LDPE more number of peaks appeared between 1710–1740 cm^{-1} and 1630–1840 cm^{-1} range compared with UV treated LDPE; whereas peaks are completely absent in untreated film. This indicates that chemical pretreatment and UV irradiation of LDPE resulted in generation of carbonyl groups, carboxylic acids and its derivatives. The peaks that were generated between 1710–1740 cm^{-1} and 1210–1320 cm^{-1} and 1630–1840 cm^{-1}were later found to be reduced after microbial treatment which was shown in Figure 7. This decrease in peaks is due to consumption of carbonyl and carboxylic acid derivatives by the micro-organisms indicating the breakdown of polymer chain.[31]

Prolonged exposure to organism leads to decrease in carbonyl index due to biodegradation (biotic) through Norrish type mechanism or through the formation of ester.[20] FTIR as a tool for differentiating between abiotic and biotic degradation of LDPE has also been reported by Albertsson et al.[18] They have observed that samples stored in air increased their carbonyl index with time, but all samples in contact with soil showed a decrease of carbonyl index with time. Others have also observed a continuous increase in amount of carbonyl compounds with exposure in an abiotic environment as against a decrease in the biotically aged samples[25,32–34] also observed that the amount of carbonyl groups decreased with prolonged exposure to a biotic environment.

Torres et al.[35] observed surface changes in LDPE starch films by the presence of fissures and cavities compared with virgin LDPE. Similarly in this study, presence of fissures and cavities were noted on the surface of abiotically treated LDPE which were not found on untreated LDPE (control). Further incubation with *A. oryzae* for 3 months resulted in deepening of cavities and fissures on the surface of LDPE.

CONCLUSION

The biodegradation of LDPE by *A. oryzae* was reported here under *in vitro* conditions in the Czapek-Dox medium. Chemical (proxidants) and UV pretreatment seems to play a vital role in enhancing the rate of biodegradation. Pretreated LDPE film exhibits a higher weight loss when compared with untreated films. *A. oryzae* grew better in medium containing pretreated film than in medium containing untreated film. The decrease in tensile strength, percentage of elongation, and elongation break of LDPE was also more for proxidant treated films when compared with UV treated and untreated films indicating the effect of proxidants on mechanical properties of HDPE. The decrease in the absorbance corresponding to carbonyl groups and carboxylic acid derivatives that were generated during pretreatment suggest that some of the groups were consumed by fungal isolate. Scanning electron micrographs of LDPE films used in the study also revealed the effect of pro-oxidants on biodegradation of LDPE. The results of this study indicated that biodegradation rate could be enhanced by exposing LDPE to proxidants (photo inducers) and UV irradiation and followed by microbial treatment. This encourages in the sense that further studies in abiotic and biotic treatments with modified procedures can enhance the rate of degradation of polyethylene.

The authors are grateful to Bapatla Engineering College, Bapatla, Andra Pradesh, India for providing facilities to carry out this project.

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