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Synergistic effects of pretreatment and blending on fungi mediated biodegradation of polypropylenes

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HIGHLIGHTS

- *Phanerochaete chrysosporium* NCIM 1170 (F1) and *Engyodontium album* MTP091 (F2) were tested.
- Starch blended (ST-PP) and metal ions blended polypropylene (MI-PP) are used.
- About 18.8% and 9.42% gravimetric weight loss were observed.
- About 79% and 57% TGA weight loss (at 400 °C) were observed.
- Extractable low-molecular weight hydrocarbons are high in UV treated MI-PP.

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ABSTRACT

Environmental issues raise concern on restrict the use of nondegradable polymers and encourage the development of degradable once. This study is carried out was to understand the rate of biodegradation of untreated and pretreated (100 °C or UV for 10 days) polypropylene (PP), pro-oxidant blended (MI-PP) and starch blended polypropylenes (ST-PP) with two different fungal strains, *Phanerochaete chrysosporium* NCIM 1170 (F1) and *Engyodontium album* MTP091 (F2). About 18.8% and 9.42% gravimetric weight loss and 79% and 57% TGA weight loss (at 400 °C) were observed with UV pretreated MI-PP in 1 year with F2 and F1 strains respectively. The amount of lacasse produced by the organism and biomass attached on the polymer surface are correlated with TGA weight loss (0.6–0.93). The formation of extractable oxygenated compounds and unoxidized low-molecular weight hydrocarbons are high in pretreated and blended samples. These results indicate blending and pretreatment strategy leads to an optimal waste-disposal strategy.

1. Introduction

Polyolefins have found widespread applications due to their physico-chemical properties and their recalcitrant nature. They represent 20–30% of the total polymer sales in United States and Canada in the year 2004 (Trishul and Doble, 2010). So it could be concluded that more than 50% of plastic waste in the environment could be polyolefins (Sivan et al., 2006). Degradation of polyolefins in nature is a very slow process leading to their accumulation in the environment at the rate of 25 million tons per year (Orhan and Buyukgungor, 2000). Formation of biofilm or attachment of microorganism on polypropylene is very poor because of its this hydrophobic nature (Singhania et al., 2012).

Several approaches for solving the pollution problem caused by polyolefins are developed in the past 20 years. These included blending with biopolymers, biodegradable polymers, or additives including starch and metal ions etc. (Jeyakumar et al., 2012; Arutchelvi et al., 2008; Williams and Bagri, 2003; Sebaa et al., 1993). There is a great interest in incorporating starch into conventional plastics because it is environmentally friendly (Lima and Estudo, 2005). Polyolefins that has been oxidized by metal ions are more susceptible to microbial degradation than the virgin ones since the former are more hydrophilic than the latter and also due to the formation presence of low-molecular weight degraded fragments (Sebaa et al., 1993). The rate of degradation of polyolefin can also be enhanced by pre-treatment which includes thermal treatment (TT) or ultraviolet radiation (UV) (Jeyakumar et al., 2012). These pre-treatments generate free radicals, which can oxidize the polymer resulting in the breakage of the chain. Oxidation leads to the formation of carbonyl, carboxyl and ester functional groups (Graeme and Mathew, 2000) and also decreases the hydrophobicity of the surface (Sudhakar et al., 2008) which helps in the formation of microbial biofilm. In earlier study synergistic

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interaction of pre-treatment strategy and blending such as starch and metal ions on the stability of High-Density Polyethylene has been reported (Jeyakumar et al., 2012). This study reports the synergistic interaction of blending with pretreatment on the biodegradation of PP by two fungi.

Fungi are potential organisms for biodegradation since they have a rich source of degrading enzymes and have the ability to survive in harsh environments under low nutrient and moisture conditions. They have the ability to extend and penetrate into cracks and crevices through the distribution of hyphae (Trishul and Doble, 2010). Use of *Aspergillus niger* for the degradation of PP has been reported (Cacciari et al., 1993). Biodegrdation of pretreated polycarbonate using two fungi namely, *Engyodontium album* and *Penicillium* spp. isolated from a plastic dump site along with a commercial white rot fungus, *Phanerochaete chrysosporium* NCIM 1170 has been reported (Trishul and Doble, 2010). The biodegradation of bisphenol A, a monomer of PC, by fungi has also been reported (Kang et al., 2006). In the earlier studies the synergistic effects UV and TT on the degradation of pretreated polypropylene has been reported with soil consortia (Ambika et al., 2009). The effects of environments such as soil, ocean and direct sunlight on the biodegradation of starch and catalyst blended HDPE and PP have been reported (Muthukumar et al., 2011). The biodegradation of starch and metal ions blended polypropylene by fugal sp is reported here. UV and thermally pretreated and unpretreated samples were exposed to *P. chrysosporium* (F1) NCIM 1170 and *E. album* (F2) MTP09 for one year.

2. Methods

Commercial PP films (PP) [Reliance Industries Ltd., Mumbai, India], Starch blended PP (ST-PP) [Biobags Ltd., Chennai, India], and Catalyst blended PP (MI-PP) [Symphony, Chennai, India] are received as gifts. Films of Size 8 × 2.5 cm and 0.05 mm thick were used in these experiments. All the chemicals were procured from (HIMEDIA Laboratories, India).

Two different pretreatment strategies were employed on the three different films (MI-PP, ST-PP, and PP). In the first method they were thermally pretreated at 100 °C for 10 days to induce oxidation. In the other method they were subjected to UV radiation (UV-C, >300 nm wavelength) for 10 days. Then the samples were disinfected with ethanol, dried and weighed.

Samples are labeled as Pure PP [PP], starch blended PP [ST-PP], metal ions blended PP [MI-PP], UV pretreated pure PP [UV-PP], UV pretreated starch blended PP [UV-ST-PP], UV pretreated metal ion blended PP [UV-MI-PP], thermal pretreated pure PP [TT-PP], thermal pretreated starch blended PP [TT-ST-PP] and thermal pretreated metal ion blended PP [TT-MI-PP]. (1 g KH_2PO_4, 0.2 g NaH_2PO_4, 0.5 g $MgSO_4{\cdot}7H_2O$, 0.1 g $CaCl_2$, and 0.169 g (1 mM) $MnSO_4{\cdot}H_2O$, 1 g yeast extract, and 1 ml of vitamin solution was used in all the experiments (Orhan and Buyukgungor, 2000). About 20 mg of untreated or pretreated films in triplicate were incubated in 500 ml Erlenmeyer flaks contains 100 ml of this mineral salt medium in 1 l solution. Fresh mycelium of the previously grown fungus on potato dextrose agar plates was scraped and suspended in 10 ml of sterile water and vortexed. Around 1 ml of this suspension was inoculated into the flask. They were then incubated at 30 °C and at 200 rpm on an orbital shaker (Scigenics Pvt. Ltd., India). Samples were withdrawn every 4 months under aseptic conditions, washed in sterile water and air-dried before further analysis. Films without the fungus served as a biotic control, while flasks with the pure culture but without the polymer films served as the biotic control. All the controls were treated the same way as the test (samples and fungus) samples.

2.1. Measurement of biological properties

Total protein concentration in the supernatant was determined based on the method reported by Bradford, 1976 with Bovine serum albumin as the standard. The total carbohydrates were estimated as suggested by (Dubois et al., 1956) with glucose as the standard. Fungal biomass is a direct measure of its growth in the medium; and its amount is estimated as per a reported method (Trishul and Doble, 2010). Laccase activity was determined as per the reported method (Srinivasan et al., 1995).

2.2. Polymer characterization

Gravimetric weight loss of the polymer is a direct measure of its degradation and is estimated from multiple samples. During the process of degradation, formation or disappearances of carbonyl (1700 and 1800 cm^{-1}) and methyl groups (corresponding to 1377 cm^{-1}) with respect to methylene group (1456 cm^{-1}) are monitored (Sudhakar et al., 2008). It is assumed that no changes would take place in the methylene groups during the degradation process.

Changes in morphology of the surface of the polymer after biodegradation are observed with a Scanning Electron Microscope (SEM), with an AQ7 Nanoscope IV digital instrument (Veeco Technologies) equipped with ADCS controller. The temperature of the sample is raised from 50 °C to 200 °C at a heating rate of 20 °C min^{-1} under nitrogen atmosphere and the ensuring weight loss is measured (Hadad et al., 2005) with a TA-Q 500 (Thermal Analyzer, model 204, Netzsch, Germany).

A total of 0.5 g of sample polymer is cut into small pieces, mixed with 10 ml of chloroform in a glass vial, and sonicated in a Branson 2210 apparatus for 2 h in a hot-water bath held at 55 °C (Rodrigo et al., 2001). The extract obtained is concentrated by evaporation of the solvent at room temperature. Then, 2 ml of chloroform is added, the solvent layer is filtered through a 0.2-mm filter and is analyzed by GC–MS (Bruker EM640S) equipped with an HP 5MS capillary column of medium polarity. The oven temperature is programmed from 40 °C for 4 min and then increased to 250 °C at a heating rate of 5 °C min^{-1}, and then held at this temperature for 20 min. Degradation products were identified by comparing of the mass spectrum with the data available in the National Institute of Standards and Technology (NIST) database (http://webbook.nist.gov/chemistry/) (Jeyakumar et al., 2012).

2.3. Statistical analysis

All the experiments were carried out in triplicate and two sample *t*-test, ANOVA were performed with Minitab ver 14.0, and multiple regression analysis.

3. Results and discussion

3.1. Biological properties

The results of variation in the total protein from the extracellular supertant and on the surface of the polypropylene at the end of 12 months are shown in supporting Figure 1. There is an increase in the protein content as a function of time with both the strains (kinetic data not shown). Highest protein is observed in the supernatant of UV treated MI-PP with *E. album* MTP09 (F2). Protein con-

tent with both the strains is higher in thermally treated samples when compared to untreated samples. The protein in the extracellular supernatant is more than the amount on the polymer surface.

There are significant differences in the behavior of the MI and ST blended polymers to fungi treatment. The former polymer shows higher protein content than the later or pure PP. The metal ions in the polymer generates free radicals on the surface of the polymer, which reacts with the oxygen to produce carbonyl groups (Williams and Bagri, 2003). In contrast, starch in the PP directly oxidizes the polymer which leads to insertion of oxygen to form carbonyl groups (Osawa et al., 2003). Metal ions, including Co, Mn, Cr, Ni, Mo and Fe on Al_2O_3 or SiO_2 are highly oxidative (Williams and Bagri, 2003). These are also called photodegradable polymers. Oxidized polymers are highly mineralized than the unoxidized polymers (Sudhakar et al., 2007). The oxidation helps in adhesion of microorganism on the polymer due to the decrease in its hydrophobicity (Arutchelvi et al., 2008). The contact angle of the polymer decreased by 10° during the study period. Higher amount of biomass, carbohydrates and proteins are observed in the biofilm in strains that are grown on UV-treated PP than others.

The amount of total carbohydrates in terms of reducing sugars (such as glucose) increased throughout the study period with both the strains. The highest carbohydrate is observed in UV treated MI-PP with *P. chrysosporium* (F1). It is higher in thermally treated samples when compared to untreated samples with both the strains. The carbohydrates in the medium was higher than on the polymer surface. The carbohydrate in the medium in the presence of the polymer was higher than in its absence (−ve control) indicating that the polymer was used by the organisms for their growth.

Fungal biomass is a direct measure of the growth of the strains in the medium and it will contain live and dead cells. There is an increase in the biomass as a function of time with both the strains. Biomass of *E. album* attached on the surfaces is generally higher than the biomass of *P. chyrsoporium*. Highest biomass is observed in supernatant of UV treated MI-PP with *E. album* (F2) after 12 months (40 mg/l). Biomass produced by these strains are significantly influenced by the pretreatment strategy and additives used. Pretreatments of the polymer leads to its oxidation and subsequent breakdown assisting in the easy assimilation by the fungus. Hence UV or thermal treatment, can be effectively used as a strategy before subjecting the polymer to biodegradation. (Trishul and Doble, 2010; Sudhakar et al., 2007). Also the additives (starch and metal) have significant effect on the oxidation process (Jeyakumar et al., 2012). Hence the biomass content is high in the pretreated samples than in the un-pretreated samples.

In all the cases laccase activity was highest (1.8 nanokatals ml^{-1}) with *P. chrysosporium* (F1) than with *E. album*. The highest activity is seen with UV-treated followed by thermally treated MI-PP polymer. In the case of starch blended polymer, laccase activity is higher with thermally treated polymer than with UV-treated one. Laccase activity was much lower with unblended PP when compared to other blended PPs. The *P. chrysosporium* (F1) strain used in this study is a white-rot fungus. It is a known source for laccases (phenol oxidases) and this strain is reported to degrade a number of recalcitrant xenobiotics including polycyclic aromatic hydrocarbons, synthetic dyes (Novotný et al., 2004), synthetic polymers, and lignin (Sutherland et al., 1997). Similar pattern in the production of laccase and hemicellulytic enzyme is also reported with several white- and brown rot fungi (Machuca and Ferraz, 2001).

3.2. Physical properties

3.2.1. Weight loss

Gravimetric weight loss of pretreated samples are higher than of the untreated PP after 12 months (Fig. 1) 18.8% weight loss is observed in UV treated MI-PP with *E. album* and 9.42% with *P. chrysosporium*. The weight loss decreases in the order of catalyst blended PP, starch blended PP and Pure PP with both the cultures. These results suggest that UV treatment and metal ions additives have highest influence on the biodegradation of polypropylene. 10% gravimetric weight loss is reported with starch blended PP when exposed to direct sunlight (Muthukumar et al., 2011) 2.5%, 1.5% and 0.5% gravimetric weight loss is reported with unblended LDPE, HDPE and PP deployed in marine waters for a period of 6 months, respectively (Sudhakar et al., 2007). *E. album* used in the current study is isolated from a plastic dump site and successfully tested for the degradation of polycarbonate (Trishul and Doble, 2010).

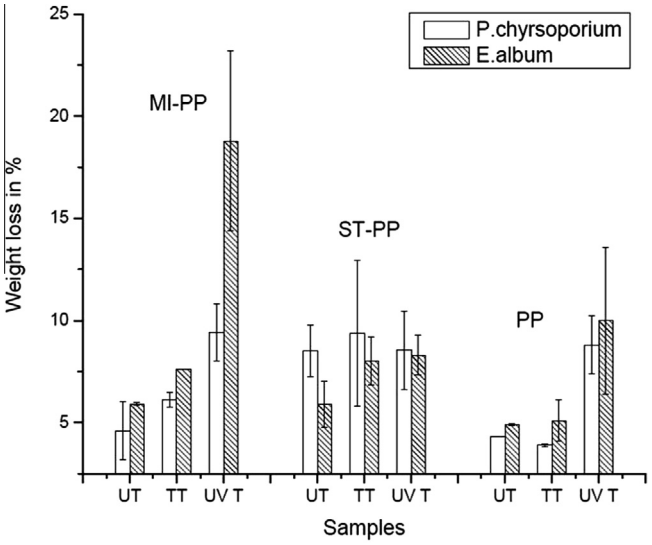

Fig. 1. Percentage weight loss of the pretreated and untreated MI-PP, ST-PP and PP incubated with F1 and F2 at the end of 12 months.

A positive correlation is seen between the biomass attached on the polymer surfaces and weight loss at the end of the study period, (correlation co-efficient of 0.64 for *E. album* and 0.6 for *P. chrysosporium*. Although *P. chrysosporium* produces more laccase than *E. album*, the extent of biodegradation (gravimetric weight loss) is more with the latter organism. *E. album* produces more biomass than the other organism. This data indicates that several factors take part in the degradation process. A very strong correlation between laccase activity of *E. album* and gravimetric weight loss ($r = 0.93$) is seen here, while correlation with *P. chrysosporium* is weak ($r = 0.49$) indicating that the decrease in the weight loss with the latter organism is probably due to the production of several other enzymes. *P. chrysosporium* is known to produces several enzymes.

3.2.2. Fourier transform infrared spectroscopy (FTIR) analysis

The FTIR spectrum of the treated and untreated PP films inoculated with *P. chrysosporium* (F1) and *E. album* (F2). The UV treated MI-PP shows strong broad peaks at 1700–1800 cm^{-1} corresponding to ketone and at 1300–1400 cm^{-1} for ester. This indicates presence of an intermolecular hydroxyl group in the backbone of the polymer during its oxidation (Albertsson et al., 1995) suggesting the formation of simpler functional groups, including COOH (Ambika et al., 2009). Similar observations have been made by others (Albertsson et al., 1995; Jeyakumar et al., 2012; Sudhakar et al., 2008; Ambika et al., 2009) which clearly indicates the oxidation of the polymer.

The changes in the carbonyl index ($COi_)$ (ketone and Ester) for all the polymers with *P. chrysosporium* (F1) and *E. album* (F2). The UV treated PP samples show higher CO*i* followed by thermally

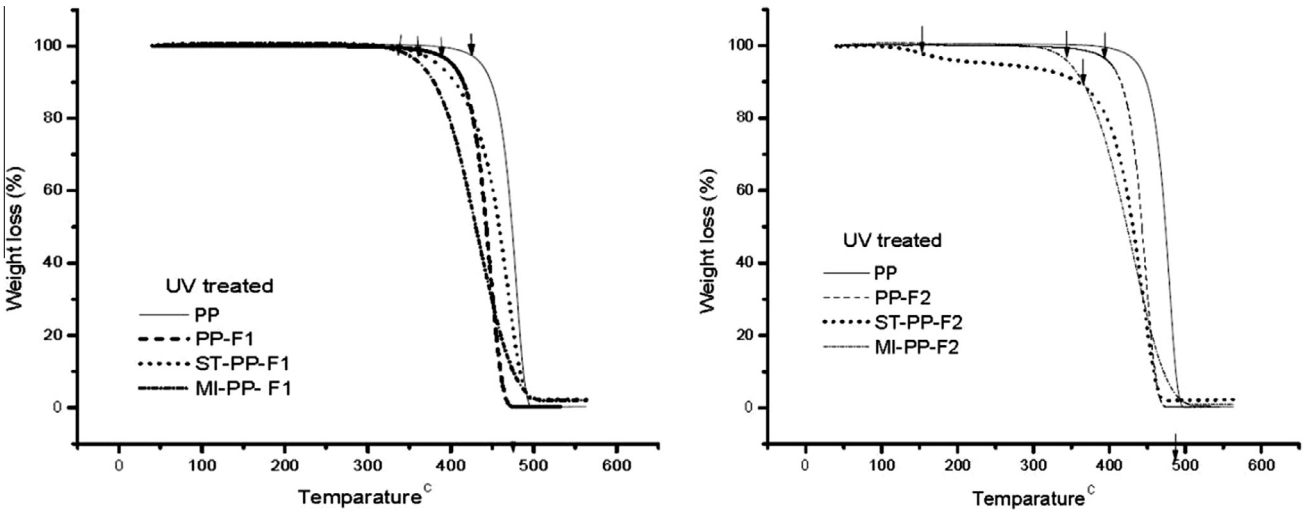

Fig. 2. Thermo gravimetric analysis of various PP films after one year of treatment with *P. chrysosporium* (F1) and *E. album* (F2) (arrows indicate on set of weight loss).

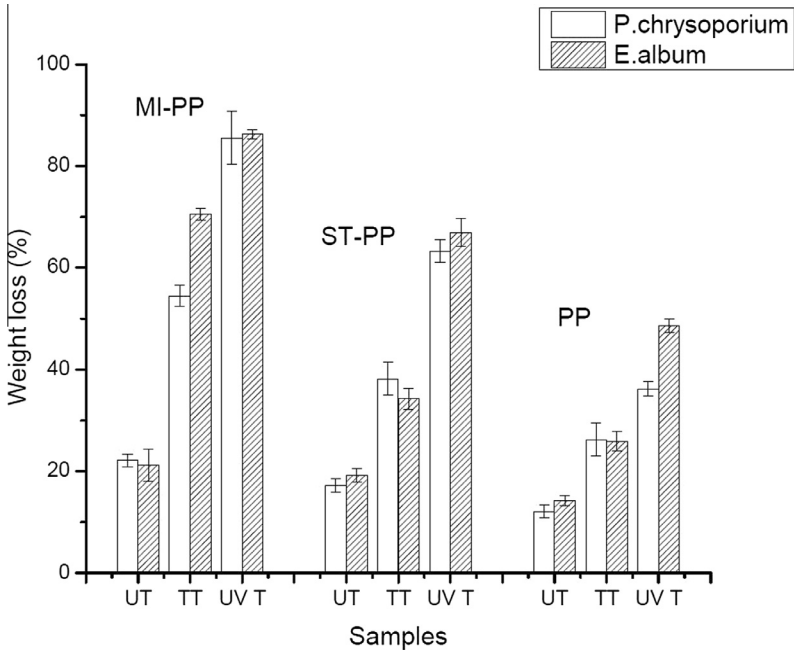

Fig. 3. Percentage TGA weight loss (measured at 400 °C) of the pre-treated and untreated (MI-PP, ST-PP and PP) samples incubated with both the strains for 12 months.

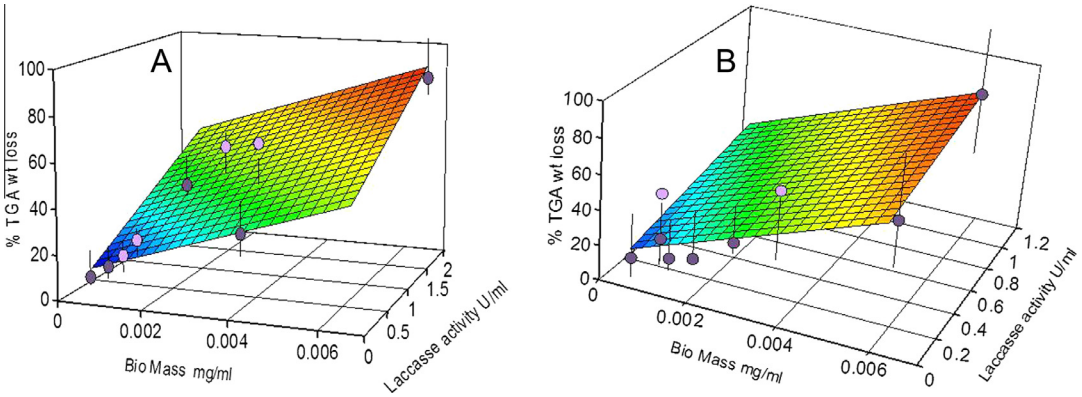

Fig. 4. Comparison of multiple linear regression model (relating TGA weight loss with amount of attached biomass and amount of lacasse produced) and experimental data (A) *P. chrysosporium* (F1) and (B) *E. album* (F2).

Table 1
Possible degradation products from *P. chrysosporium* F2 for a period of 12 months were made based on the GC–MS results by comparing the mass spectra with the NIST database.

S. no.	Compound name	MW	Molecular formula	UV treated PP			Temp treated PP			Untreated PP		
				PP	MI-PP	ST-PP	PP	MI-PP	ST-PP	PP	MI-PP	ST-PP
	Hydrocarbons											
1	1-Tricosene*	322.611	$C_{23}H_{46}$		+	+		+	+			
2	5-Dodecyne*	166.303	$C_{12}H_{22}$	+		+			+			
3	Hexadecane*	226.441	$C_{16}H_{34}$			+		+				
4	Tridecane	184.361	$C_{13}H_{28}$		+			+				
5	Propylene	42.08	C_3H_6	+	+	+	+	+	+			
6	1,19-Eicosadiene	278.516	$C_{20}H_{38}$		+			+				
7	Octane	114.229	C_8H_{18}	+	+	+	+	+	+		+	+
8	Pentane	72.1488	C_5H_{12}	+	+	+	+	+	+	+	+	+
9	7-Octadecyne,2-methyl	264.489	$C_{19}H_{36}$		+	+		+				
10	9-Eicosyne*	278.516	$C_{20}H_{38}$	+	+	+	+	+	+	+	+	+
11	Acrylonitrile	53.0626	C_3H_3N		+			+			+	
12	3-Dhloropropene	110.97	$C_3H_4Cl_2$		+	+		+	+			
	Esters and Acids											
13	Octanoic acid,6,6-dimethoxy-methyl ester*	218.29	$C_{11}H_{22}O_4$	+	+	+	+	+	+		+	+
14	Epoxides					+			+			
15	Octanoic acid ethylester	172.265	$C_{10}H_{20}O_2$		+	+		+				
16	Decyl methyl phthalate*	320.423	$C_{19}H_{28}O_4$		+			+				
17	Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester*	250.376	$C_{16}H_{26}O_2$			+			+			
18	Ppyridyl-methyl pentadecanoate	278.344	$C_{16}H_{22}O_4$		+			+				
19	Dibutyl isophthalate	278.344	$C_{16}H_{22}O_4$			+			+			
20	6,9-Tetracosadiene*	334.622	$C_{24}H_{46}$	+	+			+			+	+
21	Butanoic acid, methylester	102.132	$C_5H_{10}O_2$			+			+			
22	5,9-Undecadien-2 one, 6,10-dimethyl*	194.313	$C_{13}H_{22}O$		+			+			+	
23	10,12-Octadecadiynoic acid*	276.414	$C_{18}H_{28}O_2$	+	+	+	+	+	+			+
24	9,12-Octadecadiynoic acid, methyl ester*	290.44	$C_{19}H_{30}O_2$		+	+		+	+			+
25	1,2,3-Propanetricarboxylic acid tris(trimethylsilyl) ester	176.124	$C_6H_8O_6$		+	+		+	+	+		+
25	Propanoic acid	74.0785	$C_3H_6O_2$		+	+		+	+			
27	Decanoic acide	172.2646	$C_{10}H_{20}O_2$					+	+			
28	Tricarballylic acid	176.1241	$C_6H_8O_6$		+	+		+	+			
29	Acetoacetate	101.08	$C_4H_5O_3$	+	+	+		+	+			
	Metal ions											
30	Tetramethoxysilane	151.3724	$C_4H_{12}O_4Si$		+			+				
31	Dimethoxydimethylsilane	120.222	$C_4H_{12}O_2Si$		+			+				
	Ketons											
32	6-Methyl, 2-octanone	142.239	$C_9H_{18}O$	+								
33	3-Nonanone	142.239	$C_9H_{18}O$		+			+				
34	N-hexylacetamide*	143.227	$C_8H_{17}NO$	+	+	+		+	+			
35	2-Tricosanone*	338.611	$C_{23}H_{46}O$	+	+	+	+	+	+			
36	1,2-Epoxypropane	58.08	C_3H_6O		+	+	+	+	+	+		
37	9-Heptadecanone	254.4513	$C_{17}H_{34}O$		+	+	+	+	+	+		
	Alcohols											
38	1-Octanol*	130.228	$C_8H_{18}O$			+			+			
39	3-Hexanol*	102.175	$C_6H_{14}O$	+		+			+	+		+
40	1,2-Propanediol*	160.168	$C_7H_{12}O_4$			+						
41	1,10-Decanediol*	174.281	$C_{10}H_{22}O_2$			+			+			
42	Isopropyl alcohol	60.095	C_3H_8O			+						
43	Propargyl alcohol	56.0633	C_3H_4O			+						
44	1,12-Dodecanediol*	202.334	$C_{12}H_{26}O_2$	+		+			+	+		+
	Aldehyde											
45	Hexadecanal	240.425	$C_{16}H_{32}O$		+	+			+			
46	2,4-Undecadienal*	166.26	$C_{11}H_{18}O$			+						
47	Tetracosanal*	352.637	$C_{24}H_{48}O$			+			+			
48	Heptadecanal	254.451	$C_{17}H_{34}O$	+	+	+			+			+

treated and untreated. MI-PP shows higher carbonyl index than ST-PP which is higher than with virgin PP. The CO*i* of MI-PP reach 0.0321, 0.011 and 0.018 at the end of 12 month of biodegradation with UV treated, thermal treated and untreated samples, respectively. This indicates that UV treatment oxidizes the surface more effectively than the thermal treatment. Several reports state that carbonyl groups in PP increases when exposed to abiotic environment and decreases when exposed to biotic environment (Hadad et al., 2005). No such decrease was observed in the case of PP-UT. The absorbance at 1377 cm^{-1} is due to the methyl group, and in the present study its intensity decreases as a function of time, which indicates that the oxidation takes place at the primary position of the polymer chain (Ambika et al., 2009) which can further decompose to produce ketones and esters (Gijsman and Hennekens, 1993). These products are observed in the current study when analyzed with GC–MS (described later).

3.2.3. Surface changes

The surfaces of the untreated PP films are smooth without cracks and free from any defects.The surfaces of treated PP films shows cracks and grooves due to the abiotic treatment. Clear crack initiation points are seen, indicating that the polymer has become brittle. Also, the microbial propagation has been initiated from these cracks. Such colonization and adhesion by microorganisms are a fundamental prerequisite for biodegradation of the polymer. Colonization of F2 on the polymer are relatively more than of F1.

Table 2
Possible degradation products from *E. album* F2 for a period of 12 months were made based on the GC–MS results by comparing the mass spectra with the NIST database.

S. no.	Compound name	MW	Molecular formula	UV Treated PP			Temp Treated PP			Untreated PP		
				PP	MI-PP	ST-PP	PP	MI-PP	ST-PP	PP	MI-PP	ST-PP
	Hydrocarbons											
1	5-Octadecyne	250.4626	$C_{18}H_{34}$		+			+	+			
2	7-Octadecyne, 2-methyl*	264.4891	$C_{19}H_{36}$		+	+		+				+
3	1-Eicosene	280.5316	$C_{20}H_{40}$						+			
4	1-Docosene	308.5848	$C_{22}H_{44}$	+	+		+	+	+			
5	1,2-Epoxypropane	58.08	C_3H_6O	+	+		+	+	+			
6	1-Eicosyne	278.5157	$C_{20}H_{38}$		+							
7	1-Tricosene*	322.6113	$C_{23}H_{46}$		+	+		+	+			
8	Eicosane	282.5475	$C_{20}H_{42}$	+			+					
9	5-Dodecyne*	166.3031	$C_{12}H_{22}$			+		+				+
10	1-Pentacosene	350.6645	$C_{25}H_{50}$					+	+		+	
11	3-Octadecyne	250.4626	$C_{18}H_{34}$		+							
12	Hexadecane*	226.4412	$C_{16}H_{34}$			+						
13	Propylene	42.08	C_3H_6		+				+			
14	9-Eicosyne*	278.5157	$C_{20}H_{38}$	+		+	+	+	+		+	+
15	1,19-Eicosadiene	278.5157	$C_{20}H_{38}$		+			+				
16	1-Pentadecene, 2-methyl-	224.4253	$C_{16}H_{32}$		+							
	Esters and Acids											
17	Octanoic acid,6,6-dimethoxy-methyl ester*	218.29	$C_{11}H_{22}O_4$	+	+				+			
18	Diethyl phthalate	222.2372	$C_{12}H_{14}O_4$		+		+	+			+	
19	Dibutyl isophthalate	278.3435	$C_{16}H_{22}O_4$			+			+			
20	4-Butyl benzoic acid, pentyl ester*	248.3606	$C_{16}H_{24}O_2$		+						+	+
21	Butanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	222.3233	$C_{14}H_{22}O_2$	+	+							+
22	Acetoacetate	101.08	$C_4H_5O_3$			+		+				
23	Octanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	292.4562	$C_{19}H_{32}O_2$				+					
24	Hexanoic acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester	250.3764	$C_{16}H_{26}O_2$						+			
25	Propanoic acid	74.0785	$C_3H_6O_2$	+	+	+	+	+		+	+	+
26	2-Oxopropyl	197.26	$C_5H_9O_4S_2$		+		+					
27	Decanoic acid, 2,3-dihydroxypropyl ester	246.3431	$C_{13}H_{26}O_4$	+	+		+	+		+		+
28	13-Docosenoic acid, methyl ester	352.5943	$C_{23}H_{44}O_2$	+	+	+	+	+		+	+	+
29	6,9-Tetracosadiene H	334.622	$C_{24}H_{46}$			+	+	+	+		+	+
	Metal ions											
30	Tetramethoxysilane	151.3724	$C_4H_{12}O_4Si$		+			+				
31	Dimethoxydimethylsilane	120.222	$C_4H_{12}O_2Si$		+			+				
	Ketons											
32	2-Tricosanone*	338.6107	$C_{23}H_{46}O$	+	+			+	+			
33	Octadecyl methacrylate	338.5677	$C_{22}H_{42}O_2$			+	+					
34	N-hexylacetamide*	73.0938	C_3H_7NO			+		+			+	
35	N-butyl Propionate	130.0993	C7H14O2		+		+			+		
36	N-butyl-2-hydroxy-3-methyl-4-pentenamide*	185.141	$C_{10}H_{19}NO_2$	+					+			
37	3-Nonanone	142.2386	$C_9H_{18}O$	+					+		+	
	Alcohols											
38	3-Hexanol*	102.1748	$C_6H_{14}O$	+		+			+		+	
39	Propen-2-ol	58.0791	C_3H_6O			+		+				+
40	Polypropanal	58.0791	C_3H_6O			+			+			+
41	1-Octanol*	130.2279	$C_8H_{18}O$		+	+	+					
42	Isopropyl alcohol	60.095	C_3H_8O			+			+			+
43	1,2-Propanediol*	160.1678	$C_7H_{12}O_4$			+			+			
44	Cyclopropanol	58.0791	C_3H_6O			+					+	+
45	Propargyl alcohol	56.0633	C_3H_4O	+		+		+	+			
	Aldehyde											
46	9-Octadecenal	266.462	$C_{18}H_{34}O$	+	+	+	+	+	+		+	+
47	2,4-Undecadienal*	166.26	$C_{11}H_{18}O$			+			+			
48	Tetracosanal*	352.6373	$C_{24}H_{48}O$			+						
49	Polypropanal	58.0791	C_3H_6O	+	+			+				

Also the extent of colonization on UV treated are more than on thermal treated PP films. The colonization is more on MI-PP than on ST-PP. These results indicate a synergy between treatment and type of polymer. Pretreatment induces oxidation and, hence, the polymer becomes brittle, which eventually leads to cracks due to the action of fungi. Capsular structures are also observed on the thermally treated samples. Microorganisms that colonize the polymer surface can probably adhere by means of extracellular polymeric substances, which mainly constitutes of polysaccharides. This forms a sheath that is bonded to the polymer. This plays an important role in transporting the depolymerising enzymes to its surface (Sepúlveda et al., 2002). In the previous study similar results are observed with the same fungi on polycarbonate (Trishul and Doble, 2010). These changes are probably due to surface degradation.

3.2.4. Thermo gravimetric analysis (TGA)

Thermo gravimetric analysis (TGA) measures the weight loss of PP film, as a function of temperature when it is heated from 30 to 600 °C, hence it determines its thermal stability. The thermo gram of the UV treated PP films after one year of incubation with F1 and F2 are shown in Fig. 2.

Initial weight loss percentages of the all three different polymers are similar. The sudden drop in weight with increasing temperature occurs between 150 °C and 450 °C. The weight loss based on (TGA) at 400 °C are 84.2% and 86.3% for UV treated MI-PP with F1 and F2, respectively. 57% and 79% weight loss are observed with thermal treated MI-PP with F1 and F2, respectively. The weight loss of starch blended PP is 63.3% and 38.2% in UV and thermal treated, respectively with *P. chrysosporium* and 66% and 34%,

respectively with *E. album*. Thermal decomposition of polypropylene is less than that of starch (Walter et al., 2012).

Incubation with F2 shows higher TGA weight loss than with F1 with all the polymers. The change in the TGA weight loss indicates that the polymer has undergone degradation (Fig. 3). Degradation temperature is not only a measure of polymer chain length but also a function of percentage crystallinity, molecular weight distribution, presence of additives, degradation pattern of the additives and blends added to it (Sudhakar et al., 2008; Chiellini et al., 2007). So TGA weight loss is one of the factors that is affected during biodegradation. The correlation coefficient between these two weight losses (gravimetric and TGA) are 0.79 and 0.56, respectively with *E. album* and *P. chrysosporium*. Generally it is observed that the organism attacks the amorphous region of the polymer thereby decreasing the percentage crystallinity (Ambika et al., 2009). The TGA weight loss of the polymer incubated with F2 is highly correlated with attached protein, carbohydrate and biomass (correlation coefficient = 0.85, 0.72 and 0.85, respectively). Similar correlation coefficients for polymers incubated with F1 are 0.76, 0.88 and 0.86, respectively. The TGA weight loss is also correlated with the amount of Lacasse produced (0.65 and 0.9 with F2 and F1, respectively).

Two multi linear regression models are developed as shown below relating biomass attached on the polymer (mg/ml) and lacasse activity (U/ml) with TGA weight loss (%).

The equation for *E. album* (F2) is

$$[\text{TGA weight loss}] = 1 + 8972.2[\text{biomass}] + 17.92[\text{lacasse}]$$

with $R^2 = 0.76$, $R^2_{adj} = 0.68$, $F = 9.5$, $p < 0.05$

The equation for *P. chrysosporium* (F1) is

$$[\text{TGA weight loss}] = 11.1 + 6174.3[\text{biomass}] + 21.3[\text{lacasse}]$$

with $R^2 = 0.95$, $R^2_{adj} = 0.93$, $F = 55.9$, $p < 0.01$

The statistical parameters indicate the goodness of the data fit. Fig. 4. (A) and (B) show the model and experimental data for both the organisms and Figure 2 in supporting information show the two parity plots. All the data lie within the 95% prediction band once again indicating the quality of the two models developed.

3.2.5. Gas chromatography–Mass spectroscopy (GC–MS)

Abiotic peroxidation of polypropylene produces vicinal hydroperoxides. This process is particularly favored in polypropylene, due to the susceptibility of the tertiary carbon atom to hydrogen abstraction via a hydrogen-bonded intermediate (David and Gerald, 2006). A major proportion of the peroxidic products are hydrogen-bonded vicinal hydroperoxides they break down to small biodegradable molecules including carboxylic acids, alcohols and ketones (Lindström 2005; Albertsson et al., 1995). Longer chain oxygen modified products are also formed, which biodegrade slowly, The low molar mass products of this degradation process are small biodegradable molecules including acetic acids. The alkoxyl radicals formed by decomposition of the hydroperoxides contain weak carbon carbon bonds in the positions adjacent to the hydroperoxide groups, which lead to the formation of low molecular weight aldehydes and alcohols (Lindström, 2005). The later rapidly oxidize further to carboxylic acids. These are biodegradable species, similar to products formed by hydrolysis of aliphatic polyesters.

The GC–MS chromatograms of the volatile and the semi-volatile products extracted from UV exposed virgin PP, MI-PP and ST-PP incubated with *P. chrysosporium* F1 and *E. album* F2 for a period of 12 months. The chromatograms show more than 40 separated GC peaks. Qualitative analyses of the degradation products were made by comparing the mass spectra with the NIST database (Tables 1 and 2). The number of hydrocarbons and esters are highest in the UV treated MI-PP. Two different metal ions were identified only in the MI-PP. Ketons, aldehydes are next highest products in the MI-PP (similar compounds are reported by others (Scott, 2003)). Alcohols and acids are comparatively high in ST-PP than in MI-PP. Acids groups are highest in ST-PP which may be due to the presence of starch molecules as reported in the earlier study (Jeyakumar et al., 2012). Propylene, 1,2-epoxypropane which is seen here is reported in the metabolism of propylene *Xananobacter sp*. Py2 (Small and Ensign, 1995), 2-propyln-ol (seen here) is probably formed due to the metabolism of propionate and the latter is the of the propylene (Lehninger, 2005). Oxidation products of untreated samples are much lower than the products from the pretreated ones. More oxidation products are seen with polymers treated with *E. album* than with *P. chrysosporium*.

4. Conclusion

The study highlights the effects of fungus and pre-treatments on pure PP, MI-PP and ST-PP. Highest rates of oxidation and degradation were observed with UV treated MI-PP samples by both the strains when compared to ST-PP and Pure PP. Thermal treated samples are oxidized more than un-treated samples. A higher biomass, total protein, and total carbohydrate content in pretreated samples imply easy colonization when compared to untreated samples. Pretreatment and blending strategy proves to accelerate the biodegradation process. Metal ions are a more effective degradant than starch under UV conditions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2013.08.074.

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