Enhancement of in vitro high-density polyethylene (HDPE) degradation by physical, chemical, and biological treatments

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Abstract Partially degraded high-density polyethylene (HDPE) was collected from plastic waste dump yard for biodegradation using fungi. Of various fungi screened, strain MF12 was found efficient in degrading HDPE by weight loss and Fourier transform infrared (FT-IR) spectrophotometric analysis. Strain MF12 was selected as efficient HDPE degraders for further studies, and their growth medium composition was optimized. Among those different media used, basal minimal medium (BMM) was suitable for the HDPE degradation by strain MF12. Strain MF12 was subjected to 28S rRNA sequence analysis and identified as Aspergillus terreus MF12. HDPE degradation was carried out using combinatorial physical and chemical treatments in conjunction to biological treatment. The high level of HDPE degradation was observed in ultraviolet (UV) and KMnO₄/HCl with A. terreus MF12 treatment, i.e., FT10. The abiotic physical and chemical factors enhance the biodegradation of HDPE using A. terreus MF12.

Keywords Aspergillus terreus MF12 · Biodegradation · Biofilm formation · Ecosystem · FT-IR · High-density polyethylene · GC-MS · Medium optimization

Introduction

High-density polyethylene (HDPE) is one of the synthetic polymers of high molecular weight and is hydrophobic in nature. Natural biodegradation of HDPE is very slow in process. Microbes are not completely evolved to degrade the synthetic polyethylene in the atmosphere without the influence of abiotic factors. Due to the adverse effects caused by the polyethylene, it has to be removed from the natural ecosystem. Ever since polyethylene invention, it has undergone numerous changes in their forms to improve stability and degradability. For decades, researchers made significant changes in polyethylene to increase the stability. Nowadays, the many changes in polyethylene include the addition of per-oxidants, composite of cellulose, or starch molecules in the polyethylene during polymerization in order to enhance the biodegradability. Owing to a dramatic increase in HDPE consumption, the management of these HDPE wastes is critical and emerges as a parallel industry.

Disposal of HDPE into environment causes serious problems and, nevertheless, a threat to biological ecosystem. The HDPE accumulated in water bodies as a form of garbage waste could sometimes cause blockage in intestine of fish, birds, and marine mammals in marine ecosystem (Spear et al. 1995). Despite possessing deleterious effects, such as resisting degradation, coastal mangroves have been reported as favorable dumping sites for HDPE that potentially lead to pollution in the natural environment (Kathiresan and Bingham 2001). Direct disposal (littering or dumping) and incineration (burning) of these wastes is a common practice in the developing countries; both are ultimately harmful to the entire ecosystem. Burning of HDPE encourages air-borne pollution, the majority of which is extremely toxic and can cause health problems such as cancer, heart diseases, and respiratory disorder like asthma, etc. While HDPEs are yet to be considered to have significant disposal problem in many of the first-world
countries, organizations in developing countries have demonstrated considerable concern in regard to the detrimental effects of plastic products, notably the terminal waste generated by their disposal. But efficient polyethylene-degrading microbes are still needed to remove the polyethylene from the ecosystem by biologically sustained manner through natural recycler’s involvement of biodegradation process.

The objective of the study was to enhance the HDPE degradation by physical, chemical, and biological treatments. Moreover, the influence of abiotic factors like physical [temperature and ultraviolet (UV)] and chemical (KMnO4/HCL and citric acid) treatments in biodegradation of HDPE has been explained significantly.

Materials and methods

Substrate

Commercially available HDPE materials (40 μm in thickness) were used as substrate in this study, which is the grade of environmental pollution, rather than pure polyethylene in accessing the direct impact on environment and application-oriented solution. The composition of commercially available HDPE varies from pure polyethylene by the addition of additives like antioxidants and colorant.

Source of sampling

Partially degraded polyethylene wastes along with adhered soil samples were collected from various plastic waste dumping yard.

Isolation of HDPE-degrading microorganisms

Samples of 10 g of each soil and partially degraded polyethylene waste were inoculated along with a sole carbon source in 100 ml of synthetic media (SM) containing (per liter of distilled water): NH4NO3 - 1.0, MgSO4·7H2O - 0.2, K2HPO4 - 1.0, CaCl2·2H2O - 0.1, KCl - 0.15, yeast extract - 0.1 (Difco), all in gram per liter, and micronutrients for 1.0 mg l−1 of each of the following: FeSO4·6H2O, ZnSO4·7H2O, and MnSO4. The samples were incubated for 12 weeks at 30 °C. After 12 weeks of incubation, the mixtures of fungus were isolated and purified by spread plate technique and subsequent purified cultures were maintained on potato dextrose agar (PDA) slants.

Screening of HDPE-degrading fungi

Isolated individual fungal colonies were tested for HDPE-degrading efficiency according to Gilan et al. (2004). Briefly, flasks containing 50 ml of SM were amended with preweighed HDPE and isolated fungal strains separately. The dry weight of the HDPE was determined after 30-days of incubation at 30 °C; subsequently, the polyethylene samples were removed, washed, dried at 60 °C, and weighed. Furthermore, the degradation was confirmed with the aid of Fourier transform infrared spectroscopy (FT-IR).

Determination of HDPE residual dry weight

To facilitate accurate measurement of residual HDPE dry weight, the microbial biofilm was washed off from the HDPE surface with 2 % (v/v) of aqueous sodium dodecyl sulfate (SDS) solution for 4 h and further washed with distilled water (Gilan et al. 2004). The washed HDPE was placed on a filter paper and dried overnight at 60 °C before weighing. The weight loss was calculated using the following formula: Percentage of weight loss=[(Final weight−Initial weight) / Original weight]×100.

FT–IR spectroscopic analysis

Spectrum RX1 (Perkin-Elmer, USA) was used at a resolution of 2 cm−1, in the frequency range of 4,000–400 cm−1. The relative absorbance intensities of the ester carbonyl bond at 1,740 cm−1, keto-carbonyl bond at 1,715 cm−1, terminal double bond (vinyl) at 1,650 cm−1, and internal double bond at 908 cm−1 to that of the methylene bond at 1,465 cm−1 were evaluated using the following formulae (Albertsson et al. 1987): KETO-carbonyl bond index (KCBI)=I1715 / I1465; ester-carbonyl bond index (ECBI)=I1740 / I1465; terminal double bond index (TDBI)=I908 / I1465; internal double bond index (IDBI)=I908 / I1465. The crystallinity (%) of the HDPE was measured based on the method suggested by Zerbi et al. (1989) and calculated by the following formula: % of crystallinity=100−[(1−(Ia / Ib)) / 1.23(1−(Ia / Ib))]×100, where Ia and Ib are absorbance values from the bands at 1,474 and 1,464 cm−1 or at 730 and 720 cm−1, respectively.

Identification of MF12 strain

The total genomic DNA for 28S rDNA amplification was performed from efficient fungal isolate grown to the late exponential phase using a standard protocol (Amato et al. 2007). The ~500 bp rDNA of 28S rRNA fragment was amplified using high-fidelity polymerase chain reaction polymerase using primers of ITS1: TCCGTAGGTGAACCTGCGG and ITS4: TCCTCGGCTTATTGATATGC. After amplification of the isolated DNA fragments that were sequenced (Chromous Biotech Pvt. Ltd., Bangalore, India), the obtained sequence was analyzed using the BLASTN (http://www.ncbi.nlm.nih.gov/BLAST/) software. The phylogenetic tree using
Neighbor-Joining method was constructed by MEGA software version 5.

Optimization of media

Basal medium (BM) in grams per liter [NaCl - 0.07, CaCl2·2H2O - 0.004, MgSO4 - 0.002, malt extract - 1.0]; basal minimal medium (BMM) in grams per liter [urea - 1.0, NaCl - 0.007, CaCl2 - 0.004, MgSO4·7H2O - 2.0]; mineral medium (MEM) in grams per liter [(NH4)2SO4 - 0.1, KH2PO4 - 1.0, urea - 1.0]; malt extract medium (MExM) in grams per liter [KH2PO4 - 1.0, malt extract - 1.0, ammonium chloride - 0.1, citric acid - 0.1]; minimal medium (MiM) in grams per liter [KH2PO4 - 0.2, CaCl2 - 0.1, MgSO4·7H2O - 0.1, NH4H2PO4 - 0.05, FeSO4 - 0.035, urea 1.0]; and minimal salt medium (MSM) in grams per liter [KH2PO4 - 0.1, K2HPO4 - 1.0, CaCl2·2H2O - 4.0, MgSO4 - 0.1, Na2SiO4 - 0.05, CuSO4 - 0.5, ammonium molybdate - 0.06, ZnSO4 0.7, urea - 1.0] were used in order to optimize the degradation of HDPE by Aspergillus terreus MF12. They were inoculated 1 ml of culture OD <1 in to above mentioned media with 3×3-cm-sized HDPE film as a carbon source and incubated at 30 °C for 30 days.

Biodegradation of pretreated HDPE

After optimization of media, the HDPE was subjected to pretreatment by physical (heat and UV), chemical (citric acid and KMnO4/HCl), and biological (microbial) treatments in different combinations in order to enhance HDPE degradation. The FT-IR spectroscopy was used before and after the pretreatments and microbial treatments to HDPE film.

Physical treatments

Heat treatment The HDPE film was exposed to heat at 50 °C for 72 h.

UV treatment HDPE films were subjected to partial photolysis in a QUV-accelerated weathering tester (Q-Panel, Cleveland, OH, USA). The polyethylene was subjected to a program of alternating exposure to UV (312 nm) and humidity for 5 cycles day−1 (four of 4 h each and one of 3 h at 70 °C) separated by 1-h intervals at 50 °C, during which water is condensed on the polyethylene surface. Unless otherwise specified, the overall cumulative UV irradiation time to which the HDPE samples were exposed was 60 h.

Chemical treatments

KMnO4/HCl treatment The HDPE film was immersed to KMnO4/HCl at concentration of 0.25/0.5 mol l−¹ at 45 °C for 8 h (Fa’varo et al. 2007).

Citrac acid treatment The HDPE film was submerged in 10 % citric acid for 8 h at 45 °C. Then the HDPE film was washed quickly with 37 % HCl to remove any oxidation residues from the HDPE surface.

Biological treatment

Microbial treatment After physical and chemical treatments were carried out, the pretreated HDPE was aseptically inoculated with A. terreus MF12 separately and consortia for 30 days of incubation at 30 °C for microbial treatments.

The HDPE was pretreated by different combinations of physical and chemical with microbial treatments in order to enhance the biodegradation of HDPE as follows: FT1 - control, FT2 - UV treatment+A. terreus MF12, FT3 - KMnO4/HCl+A. terreus MF12, FT4 - citric acid+A. terreus MF12, FT5 - heat treatment+A. terreus MF12, FT6 - UV treatment+KMnO4/HCl+A. terreus MF12, FT7 - UV treatment+citric acid+A. terreus MF12, FT8 - UV treatment+KMnO4/HCl+citric acid+A. terreus MF12, FT9 - UV treatment+citric acid+heat treatment+A. terreus MF12, FT10 - UV treatment+KMnO4/HCl+heat treatment+A. terreus MF12, FT11 - KMnO4/HCl+citric acid+heat treatment+A. terreus MF12, FT12 - KMnO4/HCl+heat treatment+A. terreus MF12, FT13 - KMnO4/HCl+citric acid+heat treatment+A. terreus MF12, and FT14 - citric acid+heat treatment+A. terreus MF12.

Analysis

Viability of fungal biofilm

Viability of the fungal biofilm was determined by the live/dead FungaLight yeast viability kit (Molecular Probes, Carlsbad, CA, USA), according to the manufacturer’s instruction. Live cells (green in color) were differentiated from dead cells, which emit red color. After 30 days, HDPE-containing biofilms were removed from medium, washed in sterile distilled water, and further viewed under an epifluorescent microscope (Chavant et al. 2002).

Scanning electron microscopic (SEM) analysis

After 30 days of incubation, the HDPE film was taken and cut into 1×1 cm size and subjected to SEM in order to find out surface modification of HDPE film due to microbial degradation. The SEM micrographs for the samples were taken with deep vacuum and visualized by SEM using a JEOL JSM-35CF (JEOL, Japan). A sputter coater had used to precoat conductive gold on the surfaces of HDPE film before observing the micrographs (Huang et al. 1998).
Gas chromatography-mass spectrometry (GC-MS) analysis

After 30-day incubation of HDPE degradation process, 20 ml of liquid medium was added to equal volume of diethyl ether and the organic phase was separated with the help of a separating funnel. The organic layer was collected and added to equal volume of the Brine solution (saturated NaCl) and separated the organic phase of the solution. Then MgSO4 or NaSO4 was added to the collected organic phase until salt was free-floated in the organic phase in order to remove the water content. Finally, the organic phase was concentrated up to 5 ml by evaporation at room temperature. A Perkin-Elmer Model 8500 gas chromatograph with a split/split less injector was connected to a Perkin-Elmer ion trap detector (ITD) mass spectrometer. The column used was DB-WAX capillary column from J & W (30 m×0.32 mm I.D.). The column temperature was held for 1 min at 50 °C, then raised to 240 °C at 8 °C min⁻¹, and finally held for 15 min at 240 °C. Helium was used as a carrier gas. The samples were introduced in the split less injection mode at 225 °C (Albertsson et al. 1998).

Statistical analysis

Triplicates were maintained for all samplings. All data were subjected to analysis of variance (ANOVA) and the means were separated using Duncan’s multiple range test (Zar 1984). The data were calculated and expressed with standard deviation (±). SPSS version 17 was used for statistical analysis.

Results and discussion

Partially degraded polyethylene wastes with adhered soil samples were collected from plastic waste dumping yard. HDPE-degrading fungi were isolated from the collected samples after the incubation period and purified by spread plate technique. The fungal isolates were screened for the efficiency by weight loss and FT-IR analysis. In the screening of fungi capable to grow on a Carbon-free SM supplemented with HDPE film, a few fungal colonies were shown to utilize and degrade the HDPE, as was evident from the weight loss and FT-IR analysis. One such fungus, designated as MF12 isolate, showed potential growth in SM containing HDPE films as the sole carbon source. HDPE weight loss (9.4±0.1 %) was observed by MF12 isolate. In addition, MF12 showed high KCBI, ECBI, TDBI, IDBI, and crystallinity in HDPE as 0.17±0.009, 0.183±0.008, 0.122±0.01, 0.7±0.024, and 32.6±0.1, respectively. Based on 28S rRNA sequence was of 535 base pairs, MF12 showed 99 % similarity with A. terreus (Fig. 1). The sequence of isolate A. terreus MF12 was deposited in GenBank sequence repository (NCBI GenBank accession number: JF431429). A. terreus MF12 showed better biodegrading ability without any pretreatments than the previously reported work in Aspergillus like Aspergillus niger (Volke et al. 2001), Aspergillus ornatus, Aspergillus nidulans, Aspergillus flavus, Aspergillus cremeus, Aspergillus candidus, Aspergillus oryzae, and Aspergillus glaucus were capable of polyethylene-degrading fungi (Kathiresan 2003; Vijaya and Mallikarjuna Reddy 2008). Yamada-Onodera et al. (2001) suggested that pretreatment of HDPE prior to their cultivation of fungal strain Penicillium simplicissimum YK makes degradation more competent. Gamini et al. (2006) explained that HDPE biodegradation occurs in concurrence to biofilm formation by Penicillium frequentans and Bacillus mycoides. Similarly, A. niger, Penicillium funiculosum, Paecilomyces variotii, and Gliocladium virens were also reported to degrade thermally oxidized HDPE (Weiland et al. 1995). Most of the above mentioned Aspergillus sp. was reported to degrade the pretreated HDPE by thermal or UV irradiation at low level. However, A. terreus MF12 has shown up to 9.4±0.1 % of HDPE degradation rate without any prior treatments.

Fig. 1 Neighbor-joining tree of selected 28S rRNA gene sequences of the genus Aspergillus terreus MF13 obtained from BLAST search of the MF12 strain sequence for phylogenetic inference. Values represent the branch length
Various microbial enrichment media of basal medium (BM), basal minimal medium (BMM), mineral medium (MEM), malt extract medium (MExM), minimal medium (MiM), and minimal salt medium (MSM) have been used to the optimization of HDPE biodegradation by *A. terreus* MF12. The media optimization enhances the biodegradation rate of HDPE by *A. terreus* MF12. The composition of all media was changed to find the suitable nitrogen source and mineral nutrients for the degradation of the HDPE by *A. terreus* MF12. *A. terreus* MF12 was degraded, and the degradation rate of HDPE was observed up to 9.54±0.12, 16.7±0.159, 10.26±0.086, 11.26±0.09, 10.4±0.68, and 9.44±0.085 % using BM, BMM, MEM, MExM, MiM, and MSM, respectively (Fig. 2a). In FT-IR analysis, the high amount of HDPE degradation by *A. terreus* MF12 was observed in BMM as 0.39±0.017, 0.4±0.026, and 29.4±0.03 in ECBI, TDBI, and crystallinity, respectively (Fig. 3). Among various medium compositions, BMM supported to maximum extent in HDPE degradation by *A. terreus* MF12. Therefore, BMM was selected as a suitable medium for HDPE degradation by *A. terreus* MF12. The biodegradation rate of HDPE was increased from 9.4 to 16.7 % by *A. terreus* MF12 (Fig. 2a), when using BMM. BMM might be suitable for both the growth of *A. terreus* MF12 and enhance the degradation of HDPE. An added advantage in this medium is that urea served as an initial carbon and nitrogen sources for the initial adaptation of *A. terreus* MF12 to the medium. Hence, they were economically cheaper. In contrast to these results, Iioshi et al. (1998) has suggested that the nitrogen limitation may facilitate the biodegradation of complex, slowly biodegradable polymers such as lignin; this tempted us to explore the role of nitrogen source in the media composition of polymer-degrading microorganisms. FT-IR results proved that BMM media was suitable for the growth of *A. terreus* MF12 and the degradation of HDPE. KCBI, ECBI, TDBI, IDBI, and crystallinity of HDPE have increased, when compared to other media (Fig. 3a–e).

The HDPE was pretreated by using physical and chemical agents with different combinations to enhance the HDPE degradation by *A. terreus* MF12. After the pretreatment of HDPE, it brought under microbial treatments (*A. terreus* MF12). The high amount of HDPE degradation was observed in FT10 as 20.8±0.1 % by weight loss analysis (Fig. 2b). In FT-IR analysis, pretreated HDPE were treated with *A. terreus* MF12, the HDPE’s KCBI was increased in all treatments, whereas FT3, FT4, and FT6 were reduced as 0.24±0.001, 0.069±0.0009, and 0.216±0.0008, respectively (Fig. 4a). ECBI of pretreated HDPE of FT3, FT6, and FT12 were reduced after *A. terreus* MF12 treatment as 0.22±0.001, 0.22±0.001, and 0.138±0.0007, respectively (Fig. 4b). *A. terreus* MF12 was reduced the TDBI in FT3, FT4, and FT6 as 0.24±0.001, 0.18±0.0012, and 0.207±0.0008, respectively (Fig. 4c). *A. terreus* MF12 degraded and reduced the IDBI of HDPE in all treatments except of FT12; 0.66±0.001 (Fig. 4d). FT1 (27.9±0.1), FT4 (27.1±0.101), FT7 (29.75±0.79), FT8 (32.03±0.125), FT10 (29.93±0.093), and FT12 (30.44±0.105) has showed increased levels of crystallinity after *A. terreus* MF12 degradation of pretreated HDPE (Fig. 4e). Nevertheless, the rest of the treatments were also observed as decreased level of crystallinity. HDPE degradation rate was observed high in FT10 as 20.8±0.1 % by weight loss analysis (Fig. 2b). FT-IR analysis explains that the high amount of pretreated HDPE degradation was obtained in FT10 treatment when compared to control and other treatments (Fig. 4). Initially, the degradation of HDPE was started by abiotic treatments (physical and chemical pretreatments) that led to oxidation of HDPE. These eventually form carboxylic groups, which subsequently undergo β-oxidation (Albertsson et al. 1987) and might totally degrade via the TCA cycle, resulting in the formation of CO₂ and H₂O. β-oxidation and TCA cycle were carried out by *A. terreus* MF12 and combined treatments. Monitoring the formation or disappearance of acids (1,740 cm⁻¹), ketones (1,715 cm⁻¹), and double bonds (1,640 and 915 cm⁻¹) by using FT-IR analysis.

![Fig. 2](a) Optimization of HDPE degradation rate by weight loss using *Aspergillus terreus* MF12. (b) Weight loss of pretreated HDPE after 30 days of incubation with *A. terreus* MF12 (The values with common letter(s) are not significantly different at 5 % level according to DMRT. ±value represent standard deviation at 5 % level of significance.)

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which is essential to elucidate the mechanism of HDPE biodegradation process (Balasubramanian et al. 2010). Initially, carbonyl index has increased due to oxidation of HDPE by physical and chemical treatments (abiotic factor) in all pretreated HDPE. The 30-days incubation of microbial treatments with *Aspergillus terreus* MF12 led to a decrease in carbonyl

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**Fig. 3** (a) KCBI; (b) ECBI; (c) TDBI; (d) IDBI; (e) Crystallinity of degraded HDPE using FT-IR by *Aspergillus terreus* MF12 for optimization of media. (The value with common letter(s) are not significantly different at 5 % level according to DMRT. ± value represent standard deviation at 5 % level of significance.)
index of pretreated HDPE. The disappearance/or utilization of carbonyl groups might be due to the enzymatic attack of *Aspergillus terreus* MF12 through Norrish-type mechanism (Fig. 4a and b). Dolezel (1967) has also observed that the amount of carbonyl groups decreased with prolonged exposure to a biotic environment. Albertsson et al. (1987) and Weiland
et al. (1995) observed that a reduction in the carbonyl group after 150 days of incubation with a mixed fungal culture. Norrish type II reaction led to the formation of double bonds in the polymer chain. Ester- and keto-carbonyls were also reported as the major products formed during abiotic oxidation of polymer under thermal oxidation or in the presence of enzymes such as oxidoreductase (Albertsson et al. 1987; Karlsson and Albertsson 1998).

A negligible increase in carbonyl index was observed for all samples during the thermal pretreatment at 70 °C for 10 days, which was similar to the observations made by Khabbaz et al. (1998, 1999). The carbonyl and double-bond indices were higher in physical and chemical pretreated HDPE degradation than the unpretreated samples, when they were exposed to A. terreus MF12. This could be due to the formation of loose HDPE chain fragments during the heat treatment, and when later exposed to A. terreus MF12, they underwent higher oxidative products leading to the formation of carbonyl and double bonds. The fraction of internal double bond (—CH=CH—) was higher than that of the terminal/vinyl double bond (—CH=CH₂). However, similar observations were observed in the treatment of FT10 (Fig. 4c and d).

After a month of incubation period, the HDPE was subjected to test for the biofilm viability. The treatments of FT2, FT4, FT7, FT10, and FT14 were observed to have massive growth of fungal biofilm expressed in green color, which indicated viable fungal cells on the surface of pretreated HDPE surface, when compared with all the other treatments (Fig. 5). The high number of viable fungal mycelia in biofilm was observed in FT10 treatment. FT10 treatment showed high amount of pretreated HDPE degradation, because the physical and chemical pretreatments enhanced the HDPE degradation by A. terreus MF12. In the fungal biofilm viability test, the green and red colors indicated live and dead cells, respectively. Physical and chemical treatments made HDPE available to A. terreus MF12 that led to increases in viable fungal population in biofilm, and the pretreated HDPE became the sole carbon source for the growth of A. terreus MF12 (Fig. 5). The fungal mycelia were formed as biofilm on the surface of the HDPE in all treatments and were expressed as green in
color. It showed that viable fungal biofilm was attached on surface of HDPE film during degradation (Sudhakar et al. 2008).

The surface modification of HDPE due to physical and chemical pretreatments in conjunction with microbial degradation was studied using SEM when compared to control (FT1). The HDPE treatment with *A. terreus* MF12 had shown that all the treatments have surface modification, among which FT10 showed a high amount of HDPE degradation (Fig. 6b). Strong effect in the oxidative process has increased on the surface morphology of modified HDPE by KMnO₄/HCl (Fa’varo et al. 2007), heat, and UV treatments. The pretreated HDPE surface has been modified and corroded. It has undulated surface which may be due to an enzymatic attack by *A. terreus* MF12. Pretreated HDPE supported the fungal growth as a sole carbon source and helped the making of biofilm on the surface of HDPE, which leads to utilization/degradation of HDPE by *A. terreus* MF12 (Fig. 6a and b). After physical and chemical treatments, the HDPE was easily availed by *A. terreus* MF12. The functional group was added (Fig. 4a–d) in the HDPE during the abiotic (physical and chemical) treatments which led to the utilization of HDPE

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**Fig. 6** (a) SEM Photomicrographs showing surface modification of pretreated HDPE by *Aspergillus terreus* MF12 (corroded area indicated by arrow mark). (b) SEM Photomicrographs showing surface modification of pretreated HDPE by *Aspergillus terreus* MF12 (corroded area indicated by arrow)
by *A. terreus* MF12. Microbial attack led to the corrosion on the surface of HDPE.

Pretreated HDPE-containing liquid medium was subjected to GC-MS analysis in order to find the mechanism of HDPE biodegradation. Based on the results obtained from weight loss, FT-IR, biofilm viability, and SEM analysis, the selected treatments (FT1, FT2, FT3, FT4, FT5, and FT10) were subjected to GC-MS analysis. After GC-MS analysis, the organic compounds were detected and correlated with the MS library (Table 1). In control, all single organic compounds were found except the solvent peak (diethyl ether). However, in all other treatments, the organic compounds were found as carboxylic acids and alkanes (Fig. 7). The carboxylic acids were n-deconic acid, docosanoic acid, undeconic acids, n-deconoic acid, hexadecanoic acid, propanoic acids, oleic acids, oxalic acids, cyclopropanetetradecanoic acid, benzene dicarboxylic acid, phthyllic acid, octadecatrienoic acid, acetic acid, hexanoic acid, octadecanoic acid, butanoic acid, oxamimidic acid, undecanonic acid, docosanoic acid, tetracanoic acid, and i-propyldecanoic acid (Fig. 7). The addition of carboxyl groups in the HDPE resulted to the production of carboxylic acids, which was confirmed by FT-IR analysis (Fig. 4a-b). Then the carboxylic acids underwent further degradation by *A. terreus* MF12 and produced alkane compounds such as ethane, pentane, decane, tetradecane, pentadecane, hexadecane, heptadecane, octadecane, undecane, dodecane, and nonadecane (Fig. 7).
With the aid of GC-MS analysis, the mechanism of HDPE biodegradation was elucidated. The degradation of HDPE by the abiotic factors are useful either as a synergistic factor or as to initiate the biodegradation process with addition or deletion of carbonyl groups, but the formation of CO₂ was absent (Jakubowicz et al. 2006). In the biodegradation of HDPE, an initial abiotic step involves oxidation of the polymer chain due to the presence of dissolved oxygen, which is present at ambient, leading to the formation of carbonyl groups added in the HDPE. Norrish I-type reaction can cause cleavage of the yields as carbonyl radical, which can react with an alkoxy radical on the polyethylene chain. A peak appears at 1,740 cm⁻¹ in the IR spectrum if this ester formation occurs. When carbonyl groups have formed, the abiotic sample evidently did not undergo Norrish II degradation, as no double-bond peak could be found in the IR spectra from abiotically degraded polyethylene. These are eventually formed carboxylic acids by A. terreus MF12 degradation, same with the

<table>
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<th>Name of the compounds</th>
<th>Control</th>
<th>FT2</th>
<th>FT3</th>
<th>FT4</th>
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results of Albertsson et al. (1987) who observed that the long chain of HDPE was cut into small pieces as alkanes and carboxylic acids formed from HDPE degradation by microbes that could be analyzed with the help of GC-MS (Fig. 7). It led to the production of carboxylic acids like n-deconic acid, docosanoic acid, undeconic acids, n-deconoic acid, hexadeconoic acid, propanoic acids, oleic acids, oxalic acids, etc. (Fig. 7 and Table 1). So the degradation of HDPE was artificially initiated by abiotic factors like temperature, UV, and chemical treatments in order to enhance the polyethylene biodegradation. In a similar study, the polyethylene materials were treated by abiotic factors like UV light and nitric acid. That pretreated polymer was then subjected to microbial degradation by Fusarium sp. AF4 in a mineral salt medium contained pretreated plastic as a sole source of carbon and energy. As already noted, a significant amount of low molecular weight compounds was released to aqueous media from oxidized polyethylene film. It has shown that microorganisms could consume those compounds. Koutny et al. (2006) have followed the release of low molecular weight compounds.
compounds to water media from thermo- and photo-oxidized HDPE and LDPE samples. These substances are subsequently consumed completely by *Rhodococcus rhodochrous* strain during 4 days of incubation. LDPE without oxidation pretreatment did not release any substance. Konduri et al. (2010) has explained that the biodegradation rate could be enhanced by exposing HDPE to manganese stearate (photo inducer) and UV irradiation treatment and followed by microbial treatment. Polyethylene was also treated with nitric acid at 80 °C for 6 days before cultivation with inserted functional groups that were susceptible to microorganisms (Yamada-Onodera et al. 2001). Fa’varo et al. (2007) explained that increasing the concentration of KMnO4/HCl solution was the main factor in action in the chemical and morphological changes, the surface chemical characteristics, and the changes in thermo-gravimetric characteristics of HDPE.

Further, the alkanes and carboxylic acids were utilized by *A. terreus* MF12, and only the low molecular weight compounds like ethane, pentane, decane, acetic acid, n-deconic acid, propanoic acids, oleic acids, and oxalic acids were easily uptaken into the cells (Fig. 7 and Table 1). Those uptaken alkanes and carboxylic acids were oxidized/degraded by β-oxidation. Oxidase enzymes degrade long-chain hydrocarbons and fatty acids by a β-oxidation mechanism (Schlegel 1979). Fatty acids are degraded by a stepwise oxidative and two removal carbon atoms, as a result of oxidative attack at the β-carbon atom. The initial reaction has involved the activation of the fatty acid by combination with coenzyme A. The resulting fatty acetyl-CoA is then subjected to a sequence of reactions, the overall effect of which is to shorten the carbon skeleton by two carbon atoms, producing acetyl-CoA as one product and a shortened fatty acetyl-CoA. Repeated attacks on the latter will result to the complete oxidation of a long-chain fatty acid yielding acetyl-CoA. The two carbon fragment acetyl-CoA enters the citric acid cycle, from which carbon dioxide and water are released (Albertsson et al. 1987). Based on the results obtained, the mechanism of HDPE biodegradation by *A. terreus* MF12 was illustrated in Fig. 8.

![Schematic illustration for the mechanism of HDPE biodegradation by Aspergillus terreus MF12](image-url)
Conclusion

It was known that HDPE is resistant to microbial degradation and biodegradation of HDPE is very slow process. Nevertheless, the present study indicates that microbes are adapting toward the HDPE by natural evolution, since HDPE act as a carbon source for these microorganisms. In the process, on the absence of other carbon sources, microbes were forced to remodel their metabolic pathway and the enzyme system for the biodegradation of synthetic polymers such as HDPE for carbon quenching. The biofilm formation on the surface of the HDPE was viable and a high number in population abides the preceding theory. The degradation rate of HDPE using _A. terreus_ MF12 was gradually enhanced from 9.4±0.1 to 16.7±0.159 % by BMM media and, finally, up to 20.8±0.1 % of HDPE biodegradation rate in FT10 treatment. HDPE degradation was observed high in FT10 treatment (UV treatment + KMnO4/HCl + citric acid + heat treatment + _A. terreus_ MF12) among all the other treatments, indicating that environmental factors (physical and chemical) play a major role to initiate the HDPE degradation and also support the _A. terreus_ MF12 to degrade HDPE.

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