Studies on preparation, Characterization and Biodegradation Behavior of HDPE Natural polymers Blends

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INTRODUCTION

Polyethylene (HDPE) is widely used in various applications due to its chemical, physical, and biological inertness but its durability presents a great challenge when it is released in the environment. To reduce its adverse effect on environment, various efforts are being made to modify its properties using naturally occurring biodegradable polymers but still these modifications found to be costly and required biodegradability in polyethylene is not yet achieved. Therefore, an attempt has been made to develop biodegradable polyethylene blends using naturally occurring polymers.

Abstract

Polyethylene (HDPE) is widely used in various applications due to its chemical, physical, and biological inertness but its durability presents a great challenge when it is released in the environment. To reduce its adverse effect on environment, various efforts are being made to modify its properties using naturally occurring biodegradable polymers but still these modifications found to be costly and required biodegradability in polyethylene is not yet achieved. Therefore, an attempt has been made to develop biodegradable polyethylene blends using naturally occurring polymers. In this connection biodegradable high density polyethylene (HDPE) blends were prepared by thermally blending 2.0, 5.0 and 10.0 wt % amount of naturally occurring polymers such as; chitosan (CH), cellulose (CE), starch (ST), alginate (AL), pectin (PE), shellac (SH) and xanthan (XA). The observed biodegradability in HDPE blends might be due to the presence of hydrolysable linkages and stereo-favourable orientations of blended natural polymers. The added polymers have played a significant role in increasing the hydrophilicity in blended HDPE and acted as a bioassimilative nutrient for seeded microorganisms. The biodegradability of HDPE-polymer blends was evaluated in presence of various fungi such as; aspergillus niger, aspergillus terreus, fusarium solani, tricoderma hariziaum and tricoderma viride. The disinfected films of pristine HDPE and polymer blended HDPE were inoculated with these fungi and the extend of biodegradation was evaluated after a incubation period of three months at 28 ± 1°C. The biodegradability of HDPE-polymer blends was compared with pristine HDPE by evaluating their molecular weights, and weight percent loss in samples incubated for three months along with selected fungus. The biodegradation in pristine HDPE and its polymer blends was confirmed by comparing their FT-IR spectra and also by evaluating the variations in their mechanical and thermal properties. A significant variation in their morphologies in presence of fungi has confirmed biodegradation in HDPE-polymer blends in comparison to pristine HDPE films. These studies have provided sufficient evidences to confirm the role of added natural polymers in developing a biodegradable HDPE by blending various polymers such as; chitosan (CH), cellulose (CE), starch (ST), alginate (AL), pectin (PE), shellac (SH) and xanthan (XA).

Out of these polymers, the chitosan is found to be quite effective as it is acted better bioassimilative nutrient for microorganisms to cause biodegradation of HPEF in comparison to other polymers.

Key words: HDPE, Natural polymers, Polymer blend, Biodegradation, Fungus bioassimilative nutrient polymers.

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Polyethylene is synthesized easily without using expensive method or procedure (Honakar et al., 2005) and found to be useful in various applications such as; packaging materials for agricultural products (Li et al., 2015), food materials and other items (Fasoyoro et al., 2017). But non-judicious and unplanned usage of polyethylene or non-degradable polymers has generated hazardous non-degradable wastes in our surroundings, which become a threat to the very survival of human lives and biological systems (Singh and Sharma, 2008). In order to reduce the load of plastic waste accumulation in our environment, it is highly desirable to develop biodegradable polymers (Arvantiyannis et al., 1998) by modifying the properties of non-degradable synthetic polymers (Vallini et al., 1994). The biodegradable polymers are commonly produced either altering their structures (Soheb et al., 2018) or blending with naturally occurring biodegradable polymers (Anutchelvi et al., 2008; Kahar et al., 2017). Though naturally occurring polymers such as; 3-hydroxyl butyrate (PHB), its copolymers and some aliphatic polyester have been used frequently to develop biodegradable polymers but these modifications found to be costly (Avella et al., 2005; Nowak et al., 2010) in comparison to natural polymers (Bandyopadhyay et al., 2010). The best known and easily available natural polymers to develop biodegradable plastics are chitosan, pectin, starch, cellulose, shellac, and xanthane. The naturally occurring polymers are hydrophilic in nature (Kang et al., 1996), which is considered responsible for their biodegradability in comparison to hydrophobic synthetic polymers. Thus, considering these properties of naturally occurring polymers, the blends of polyethylene have been developed, which found to be quite susceptible for microbial biodegradation (Griffin, 1994) through enzymatic action of microorganisms. To study the microbial degradation of organic matters including polyethylene, the soil separated fungi has been used successfully (El-Shafei et al., 1998). The action of extracted enzymes in biodegradation of polyethylene is also evaluated by other workers (Mandels and Sternberg, 1976). In view of literature reports on application of naturally occurring polymers in developing biodegradable polymers, an effort has been made to prepare biodegradable polymer blends using commonly used synthetic high density polyethylene (HDPE) and naturally occurring biopolymers. In comparison to low density polyethylene (Satyalakshmi, 2016), the high density polyethylene (HDPE) is found to be highly resistant to microbiological degradation or disintegration; hence its blends were prepared using easily available...
biopolymers such as; chitosan(CH), cellulose(CE), starch(ST), alginate(Al), pectin (PE), shellac(SH) or xanthane(XA), which themselves are biodegradable due to the presence of sufficient hydrolysable linkages. The enzymatic action of various fungi (Skariyachan et al., 2018; Al-Jailawi et al., 2015) such as: aspergillus niger, aspergillus terrus, fusarium solani, tricoderma harziauum and tricoderma viride is found to be quite effective in biodegradation of polymer blends; hence, these fungi have been used to test the biodegradability of prepared blend of HDPE with natural polymers. The extent of biodegradation in pristine HDPE and HDPE-Polymer blends before and after inoculation with chosen microorganisms has been evaluated as per ASTM standard.

2.Experimental:

2.1 Materials:

High density polyethylene (HDPE) was supplied by BDH Chemical Company, UK in a granular form. Chitosan, cellulose, starch, alginate, pectin, and xanthane were obtained from Sigma-Aldrich Chemicals Company, UK. Three stains of mesophilic and aerobic fungi were isolated from soil and preserved in presence of optimum humidity at 5°C. The soil isolated fungi are aspergillus niger, aspergillus terrus, fusarium solani, tri codera harziauum and tricoderma viride. Other chemicals used in experimental work were of reagent grades in their purity and used without further purification.

2.2 Samples preparation:

High density polyethylene (HDPE) films of 0.5 mm thickness were prepared manually using thermo-press method at 110°C. To measure the tensile strength, dog bone shaped samples were prepared from pristine HDPE and polymer blended-HDPE films. The melt-blended samples of HDPE and polymers were used to prepare HDPE-Polymer blended films. The melt-blended samples of HDPE-Polymers were prepared separately by mechanically mixing (Damrongssakkul and Ngamsinlapasathian, 2002) 2.0, 5.0, and 10 wt % of each polymers with 2.0 g of pristine HDPE. The HDPE blends were prepared using chitosan, cellulose, starch, alginate, pectin, shellac, and xanthane.

2.3 Culturing of pristine HDPE and HDPE-Polymer blend films:

To evaluate the biodegradability of pristine HDPE and its polymer blends, samples were incubated along with chosen fungus in a media for optimized time of incubation. To carry out these experiments, a media was prepared and HDPE-Polymer blended samples were cultured in it.

2.3.1 Preparation of potato dextrose agar media and disinfection of the films:

The potato dextrose agar media (PDA) was prepared by taking 200 g potato, 20 g D-glucose, and 15 g agar in 1.0 L distilled water. The mixture was boiled for 20 min and disinfected by autoclaving (BS2646,1988,AUX 750 OIo) at 121°C for about 30 min at 1kg/cm pressure. After autoclaving the mixture, the pH of potato dextrose agar media was adjusted to 7.4 using 0.1 M solution of NaOH and using pH meter (Hanna PH211, Instrument Microprocessor pH meter). The chemical disinfection of pristine HDPE and HDPE-Polymer blended films was carried out by immersing films in a solution prepared freshly by mixing 7.0 mL of 10% sodium hypochlorite (bleach) in 1.0 L sterilised water. The films were kept for 30-60 min under mechanical stirring and after removing they were dried for about 60 min at room temperature before keeping for 30 min in 70% (v/v) solution of ethanol. These disinfected films were incubated in a sterilised petri dish at 45°C for overnight, and gradually cooled to ambient temperature before recording their weights (El-Shafie et al., 1998).

2.3.2 Culturing of microorganism and inoculation of HDPE-Polymer blended films:

The freshly germinated fungus was incubated homogenously using inoculation loop in a petri dish containing 2.0 mL potato dextrose agar media. After inoculation, the petri dish was incubated for one week in a autoclave maintained at 25 ± 1°C. The cultured fungus was finally preserved at 5°C (Kawai et al., 2004). The samples of other fungi were also cultured by following same procedure. The pre-weighed and disinfected HDPE-Polymer blended films were inoculated homogeneously on both sides with chosen fungus using disinfected loop. The fungus inoculated polymer films were kept vertically in potato dextrose agar media in a dish and then placed in an incubator. At the end of fixed period of incubation, the pristine and HDPE-Polymer blended films were harvested and washed gently using 70 % (v/v) solution of ethanol to remove adhered cell mass and biodegraded mass of the films. Finally, films were dried at 45°C for 24 h in a hot air oven.

2.4 Characterization of pristine HDPE and polymer blended films:

The pristine HDPE and polymer blended HDPE films were characterised for various parameters to confirm the enhanced biodegradation in fungi inoculated HDPE-Polymer blended films in comparison to pristine HDPE films.

2.4.1 Determination of molecular weight:

The viscometric average molecular weights (\( \overline{M}_v \)) of pristine HDPE and HDPE-Polymer blends were calculated by determining the intrinsic viscosity [\( \eta \)] of pristine HDPE and HDPE-Polymer blends in p-xylene using Ubbelohde type of capillary viscometer at 105°C. The viscometric average molecular weights (\( \overline{M}_v \)) of pristine HDPE and HDPE-Polymer blends were calculated (Dhiman et al., 2004) using following Mark-Houwink equation 1.

\[
[\eta]_i = \alpha \overline{M}_i^K
\]

Where, ‘K’ and ‘\( \alpha \)’ are characteristic Mark-Houwink constants.

2.4.2 Determination of biodegraded weight loss in pristine HDPE and HDPE-Polymer blended films:

The observed weight loss (%) in pristine HDPE and polymer blended HDPE films after incubation with selected fungus has been used to evaluate the extent of biodegradation and assimilation of films by incubated microorganisms (Ghazaki et al., 2005). The films weight loss (%) was calculated using following realation (Equation 2) (Krystyna et al., 2003; Labuzek et al., 2004) and by determining weight accurately using digital precision balance (AND HR-200).

\[
\text{Weight loss ( % )} = \frac{W_i - W_f}{W_i} \times 100
\]

Where, \( W_i \) and \( W_f \) are the weights of the films before and after biodegradation by selected fungus.

2.4.3 FT-IR characterization of pristine HDPE and polymer blended HDPE-films:

FT-IR spectra of pristine HDPE and polymer blended HDPE films were recorded before and after seeding with microorganisms. The FT-IR spectra of films were recorded with the help of film holder supplied for IR measurements with FT-IR spectrophotometer (Tensor Co. Brucker, 2003, Germany FT-IR spectrophotometer) (Orhan and Buyukgungor, 2000).
2.4.4 Mechanical properties of pristine HDPE and polymer blended HDPE films:

Tensile strength of HDPE films was evaluated by using dog bone shaped samples at ambient temperature by following ASTM D638 standard and using universal testing machine (Gunt Wp 300-20). The samples with a gauge length of 38 mm, and a width of 8 mm were used for analysis. Young’s modulus (E) was calculated using Hooke’s law (Equation 3) (Ghosh, 2003).

\[ \sigma = \frac{F}{A} \]

Where, \( \sigma \) is stress (force per unit area, N/mm²), \( \epsilon \) is strain (elongation in length, %) and \( E \) is Young's modulus corresponding to slope values of stress-strain curves.

2.4.5 Thermal studies of pristine HDPE and polymer blended HDPE films:

To determine the extent of fungal biodegradation in polymer films, the variation in thermal properties of pristine HDPE and polymer blended HDPE films before and after inoculation with selected fungus was determined by recording their TG and DTG thermograms under inert atmosphere using TG analyzer (Extra TG/DTA 6300) at a heating rate of 10°C/min within a temperature range from 25-550°C (Negi et al., 2009). To supplement the TG and DTG analysis, the differential scanning calorimetric (DSC) analysis of pristine HDPE and polymer blended HDPE films was also carried out.

2.4.6 Scanning electron micrograph of pristine HDPE and polymer blended HDPE films:

To analyse the extent of biodegradation on morphological behavior of pristine HDPE and HDPE-polymer blends on incubation with selected fungus, the scanning electron micrographs (SEM) of pristine HDPE and HDPE-Polymer blends were recorded (LEO435VP-England). To record SEM micrographs, the samples were mounted on aluminium studs using double adhesive tapes and vacuum coated by exposing to a gold ion beam sputter (PELCO.S.C.6) at a current of 25 mA for about 40 s. To visualise the microstructural variation on microbial biodegradation (Labuzek et al., 2004) in the samples, the SEM micrographs were recorded at an optimized magnification.

RESULTS AND DISCUSSION

HDPE is an indispensable material for domestic and industrial applications but its durability and chemical resistance is a main drawback for producing non-composable waste in the environment. Therefore, to sustain its domestic applications, its properties should be modified to make it eco-friendly and biodegradable. Considering the useful properties of HDPE it is difficult to discard its importance in commercial and industrial applications; hence, some efforts have been made to make biodegradable polyethylene using natural polymers (Avella et al., 2005). To produce biodegradable HDPE, various naturally occurring polymers have been used in making melt-mix blends of pristine HDPE and effect of blended natural polymers on biodegradation of HDPE has been evaluated by studying their properties after seeding with selected fungus. The results of fungus seeded HDPE-Polymer blends have indicated that microbial biodegradation was first initiated on HDPE blended polymers and after that it continued to biodegrade of long chains of HDPE by enzymatic action. The biodegraded products were finally used up by seeded microorganism as bioassimilable nutrients. The extent of biodegradation of pristine HDPE is found to be lower in comparison to polymer blended HDPE due to significant decrease in hydrophobicity of HDPE and due to the presence of hydrolysaes linkages in HDPE-Polymer blends.

3.1 FT-IR Characterization:

FT-IR spectra of pristine HDPE and natural polymers were recorded separately to confirm the blending of polymers with HDPE (Table 1). FT-IR spectra of polymer blended HDPE samples having 10 wt% of polymers were recorded before and after (Fig. 1) seeding with selected fungus for three months to confirm biodegradation in polymers blended HDPE by seeded microorganism. On comparing the FT-IR spectra of HDPE-Polymer blends before and after incubation with selected fungus for 3 months (Table 2 & Fig. 1), it is clear that incubation of HDPE-Polymer blends with selected microorganism has either shown a significant shift in characteristic functional groups of HDPE-Polymer blends (Table 2) or produced some new functional groups (Table 2 & Fig. 1) on biodegradation of polymer blended HDPE. The appearance of strong absorption band between 1650–1760 cm⁻¹ (Fig. 1) after inoculation with selected fungus for three months has suggested the formation of new carbonyl compounds (Table 2 & Fig. 1) such as esters, aldehydes or carboxylic acid (Orhan and Buyukkungor, 2000).

| Functional groups | Wave number (cm⁻¹) |
|-------------------|-------------------|
| HDPE              | PS                |
| C=O              | 2920-2850         |
| C-H               | 2912              |
| O-H               | 3150              |
| C=O              | 1543-1605         |

Table 1: IR frequencies of HDPE and polymers

Table 2: IR frequencies of HDPE-Polymer blends

Seeding time, 3 months; Films thickness, 0.5 mm; HDPE blended with 10 wt% polymer.

The inoculation of microorganism for three months with HDPE-Polymer blends has shown a clear shift in absorption bands specially for O–H str and C=O str bonds, which provided an evidence for the microbial attack on polymer blended with HDPE (Table 2 & Fig. 1). Thus, appearance of new absorption bands after incubation with selected fungus has provided enough proof for the biodegradation of HDPE-Polymer blends and for the formation of new compounds as byproducts of biodegradation. In addition to appearance of new absorption bands and variation in absorption frequency of functional groups of blended polymers, the inoculated fungus has also caused a complete disappearance of absorption bands belonging to blended polymers (Table 1 & Fig. 1), which also provided an evidence to confirm that blended polymers in HDPE have acted as a source of nutrition to incubated fungus.
Fig. 1a: FT-IR Spectra for HDPE blends with 10% w/w cellulose recorded before and after seeding with selected fungus.

Fig. 1b: FT-IR Spectra for HDPE blends with 10% w/w of starch recorded before and after seeding with selected fungus.

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The HDPE chains were found to be a main source of nutrition for germinated colony of microorganism after enzymatic biodegradation of blended polymers in HDPE. The HDPE-b-CH blends (Table 2) before fungal inoculation have shown absorption band for C=O str at 1640 cm\(^{-1}\) corresponding to amide group but after inoculation with selected fungus for three months, the amide absorption band of chitosan was disappeared totally indicating a complete consumption of chitosan by inoculated fungus on HDPE-b-CH blends. The germinated colonies of fungi on HDPE-Polymer blends were subsequently able to cause enzymatic biodegradation of long chains of HDPE. The enzymatic biodegradation of long chains of HDPE and formation of oxidation byproducts with ketonic and alcoholic groups was confirmed by FT-IR analysis. The FT-IR spectra have shown new absorption bands at 1825 cm\(^{-1}\) and 3380 cm\(^{-1}\) corresponding to ketonic carbonyl (C=O str) and alcoholic (O–H str) bonds respectively. The HDPE-b-CE blends with 10 wt% cellulose after incubation for three months with selected fungus were totally free from characteristic bands of cellulose at 1060 cm\(^{-1}\), 2902 cm\(^{-1}\) and 3445 cm\(^{-1}\) (Table 1, Fig.1a), which suggested for a complete biodegradation of blended cellulose in HDPE-b-CE samples. The appearance of strong band at 1820 cm\(^{-1}\) (C=O str) and broad band at 3447 cm\(^{-1}\) (O–H str) has further confirmed the formation of ketonic and alcoholic byproduct on biodegradation of HDPE blended with cellulose. The FT-IR spectra of HDPE blended with other polymers have also provided sufficient evidences for the formation of new compounds and enzymatic biodegradation of HDPE by germinated colonies of inoculated fungi. These results have also suggested that the extent of biodegradation has shown a significant dependence on type of blended polymers and microorganism used for seeding with polymer blended HDPE samples.

3.2 Molecular weight degradation:

The molecular weight (\(\overline{M}_V\)) of pristine HDPE samples after seeding with fungi for three months is found to be almost same as it was before seeding with microorganism, which clearly suggested that pristine HDPE was highly resistant to microbial attacks. But on blending HDPE with polymers, the HDPE has shown a significant decrease in its \(\overline{M}_V\). The initial \(\overline{M}_V\) of pristine HDPE was 654 kg mol\(^{-1}\), which remained almost constant after seeding with fungi for three months. But HDPE in polymer blended samples has shown a decreasing trend on seeding with different fungus for three months. The \(\overline{M}_V\) of HDPE in polymer blended samples after seeding for three months has shown a systematic order such as HDPE-b-CH< HDPE-b-PE< HDPE-b-CE< HDPE-b-ST< HDPE-b-XA< HDPE-b-b-SH for the \(\overline{M}_V\) of HDPE as 316, 350, 444,451, 480, 586, 598 kg mol\(^{-1}\) respectively in these blends. The seeded fungus has first used up the blended polymer as its nutrient and after that it caused biodegradation of HDPE chains through enzymatic action by germinated colonies of microorganism, which ultimately has reduced the \(\overline{M}_V\) of HDPE in polymer blended HDPE. The HDPE blended with chitosan has shown a significant decrease in \(\overline{M}_V\) of HDPE (316 kg mol\(^{-1}\)), whereas \(\overline{M}_V\) of HDPE in shellac blended HDPE (HDPE-b-SH) samples has shown least biodegradation (598 kg mol\(^{-1}\)) after seeding with selected fungus. This has suggested that chitosan acted as a suitable nutrient to seeded fungi and was able to germinate more colonies of microorganism to cause an effective enzymatic biodegradation of HDPE in chitosan blended HDPE samples. The variation in extend of biodegradation of HDPE in polymer blends might be due to the difference in chemical and stereostructures of polymers used for blending with HDPE. The hydrophilicity, stereo-orientation and chain flexibility of polymers have all together played a significant role in controlling the permeability of fungus and their biodegradative action of polymers (Kawai et al., 2004). Amongst selected polymers, the chemical and stereo-structures of chitosan and pectin were found to be more effective in causing biodegradation or in decreasing the \(\overline{M}_V\) of HDPE in polymer blends in comparison to other polymers used for blending with HDPE and seeded for three months with selected fungus. The molecules of chitosan and pectin are more flexible and able to form a layer of hydrogels through hydrogen bonding at HDPE surfaces that give rise an adhesive property to HDPE in the blends. This has provided a significant help to seeded fungi for their attachment, germination and in releasing a sufficient amount of enzymes for cleaving C–C bonds of HDPE chains to ultimately decrease the \(\overline{M}_V\) of HDPE in polymer blends. In addition to chitosan and...
pectin, the cellulose, starch and xanthane have also shown a decreasing trend in $M_v$ of HDPE on seeding with selected fungus due to the presence of microbial friendly functional groups in these biopolymers. However, alginate and shellac have shown poor biodegradation of HDPE in comparison to other polymers, due to their acidic properties, which might have prevented the germination and colonization of fungus on the surface of HDPE in polymer blends, hence alginate and shellac have shown a minimal decrease in $M_v$ of HDPE in polymer blends after incubation with selected microbiota.

3.3 Effect of polymer amount and type of microorganism on biodegradation of polymer blended HDPE:

In order to determine the effect of weight percent of blended polymer on biodegradation of HDPE by microorganism, the HDPE-Polymer blends with different amount (%) of polymers were prepared and incubated with selected fungus. The HDPE-Polymer blends having 2, 5, and 10 wt % of each polymer were prepared separately by melt-mix method and used to evaluate their biodegradability by seeding for three months with different fungi. The effect of weight percent of polymers on extent of biodegradation of HDPE was evaluated by determining their weight loss by gravimetric method after incubation with different fungi and results are given in Table 3. The data shown in Table 3 have clearly indicated that the weight loss of HDPE in polymer blends has shown an increasing trend on increasing the weight percent of polymers from 2-5 wt% and shown a maximum weight loss in HDPE in blends prepared with 10 wt % of polymers. Therefore, HDPE blends with 10 wt% polymers were used to evaluate their properties.

Table 3: Effect of polymer amount and type of microorganism on biodegradation of blended HDPE

| Blends codes | Weight loss in HDPE (%) in blend with 2 wt % polymer | Weight loss in HDPE (%) in blend with 5 wt % polymer |
|--------------|---------------------------------|---------------------------------|
| A. niger     | A. terreus                      | T. harzi-aum                    | F. solani |
| HDPE-b-CH    | 1.64                            | 0.84                           | 3.80      | 2.33   | 4.25   | 1.85   | 4.35   |
| HDPE-b-CE    | 1.52                            | 0.78                           | 2.53      | 2.22   | 2.31   | 1.59   | 3.87   |
| HDPE-b-ST    | 0.94                            | 0.65                           | 2.42      | 1.32   | 1.86   | 1.14   | 3.45   |
| HDPE-b-Al    | 0.32                            | 0.28                           | 2.26      | 0.74   | 1.78   | 1.10   | 3.08   |
| HDPE-b-PE    | 1.62                            | 1.54                           | 2.73      | 2.21   | 3.96   | 1.82   | 3.24   |
| HDPE-b-SH    | 0.71                            | 0.45                           | 1.92      | 0.83   | 1.04   | 0.65   | 2.33   |
| HDPE-b-XA    | 1.05                            | 0.82                           | 3.61      | 1.94   | 3.52   | 0.94   | 4.02   |

Fig. 2: The percentage weight loss of HDPE polymer blended with different % (w/w) natural polymers after seeding for 3 months with (A) A. niger, (B) A. terreus, (C) T. harzi-aum, (D) F. solani.

The 10 wt % amount of polymer seems to be sufficient to cover the surface of blended HDPE and was sufficient to facilitate the germination and colonization of microorganism on blended HDPE. The HDPE blends with 10 wt% amount of polymer were able to show enhanced biodegradation of HDPE in comparison to HDPE blends with low (< 10 wt %) amount of polymers. The type of fungus has also caused a significant effect on weight loss (%) of HDPE in polymer blends (Table 3, Fig. 2). The variation in weight loss with type of fungus might be due to the differences in their β-oxidation system, which is an essential part of cell membranes (Kawai et al., 2004). The chitosan and pectin blends have shown maximum weight loss (%) in HDPE due to sufficient similarity in their chemical structures.

3.4 Mechanical properties of HDPE and polymer blended HDPE:

To determine the extent of biodegradation of HDPE in polymer blended HDPE on seeding with selected fungus, the mechanical properties of pristine HDPE and its polymer blends was determined before and after seeding with fungi for three months. The results have indicated that pristine HDPE was having high yield stress and low elongation at break (%) due its crystalline nature. The observed high Young’s modulus (E) in pristine HDPE has indicated for its stiffness, which shown a decreasing trend on blending with polymers and on biodegradation on seeding with selected fungi (Table 4, Fig. 3). The decrease in the value of Young’s modulus of HDPE films on blending with polymers is attributed to its plasticization by blended polymers and due to the presence of weak interfacial interactions between polymer and hydrophobic long chains of HDPE (Pal et al., 2008). However, high volume fraction of HDPE and homogeneous dispersion of...
polymers in HDPE blends have played a significant role in overcoming the phase separation and controlling the mechanical properties of polymer blended HDPE (Dhiman et al., 2004; Lu et al., 2009). The lowering of Young’s modulus of blended HDPE films than pristine HDPE (Table 4) has suggested that blended polymers were homogenously intermingled with long chains of HDPE to cause an effective plasticization of HDPE. The mechanical properties of polymer blended HDPE has shown a significant variation with type of polymers used for blending with HDPE (Table 4 & Fig. 3), which may be due to differences in interfacial thickness of anchored polymers with HDPE chains and compatibilization of HDPE in polymers blended HDPE. The properties of polymers and large surface area of HDPE have likely controlled the interfacial thickness in melt-mixed polymer blended HDPE (Wu and Liao, 2005).

The variation in mechanical properties of pristine HDPE and polymer blended HDPE after seeding with A. niger for three months was found to be significantly high in comparison to mechanical properties of samples before seeding with A. niger (Table 4 & Fig. 3).

In comparison to polymer blends prepared with other polymer, the HDPE-Polymer blends prepared with chitosan, pectin and xanthane have shown a significant decrease in Young’s modulus, ultimate tensile strength (TS) and elongation at break (EB) after seeding with A. niger for three months (Table 4, Fig. 3). This might be due to the enhanced biodegradation of HDPE chains and due to a significant loss in crystalline properties of HDPE after seeding for three months with selected fungus. The HDPE-Polymer blends with chitosan, pectin, and xanthan after seeding for three months with selected fungus have produced highly amorphous HDPE due to enhanced enzymatic biodegradation of HDPE than the HDPE blended with other polymers. Thus the enhanced decreasing trends in mechanical properties of HDPE blends prepared with chitosan, pectin and xanthan have clearly suggested that chitosan, pectin and xanthan have acted as suitable nutrients to selected fungus (A. niger) and helped in enzymatic degradation of HDPE due to enhanced germination and colonization during the seeding period of three months. The HDPE blends prepared with other polymers have also shown biodegradation of HDPE but in these blends, the microorganism were less active with fungus for three months, which indicated that shellac was not a suitable nutrient for selected fungus, hence it failed to cause significant enzymatic biodegradation of HDPE in shellac blended HDPE.

3.5 Evaluation of biodegradation of HDPE by thermal study of polymer blended HDPE:

To confirm the biodegradation of polymer blended HDPE on seeding with selected fungus, the thermal stability of seeded HDPE-Polymer blends was determined and compared with thermal stability of pristine HDPE. The thermal stability of polymer blended HDPE is evaluated as a loss in weight (%) at temperature by thermogravimetric (TG) analysis. The differential scanning calorimetric (DSC) analysis has been used to determine phase transition temperature and enthalpy of phase transition (T) in polymer blended samples before and after biodegradation. The HDPE-Polymer blends having 10 wt% amount of polymer was used for seeding with selected fungus and after seeding their thermal properties was compared with pristine HDPE. The results of TG and DSC analysis of seeded pristine HDPE and polymer blended HDPE with different polymers are presented in Table 5. The pristine HDPE after seeding with selected fungus for three months is found to be thermally stable with an initial decomposition temperature (IDT) of 470 °C and with a weight loss of ~10%, whereas the final decomposition temperature (FDT) was found to be 503 °C with a total weight loss of 98%. The rate of decomposition at maximum decomposition temperature (Tmax) of 491 °C (Table 5, Fig. 4) was found to be highest (4.3 mg min⁻¹). The seeded pristine HDPE has shown a first phase transition at 138 °C with a heat of fusion of 181 mJ mg⁻¹ and second phase transition was being observed at 491 °C with a heat of fusion of 329 mJ mg⁻¹ (Fig. 4).

Table 4: Mechanical properties of HDPE and polymer blended HDPE

| Blends codes | Before incubation | After incubation | Before incubation | After incubation |
|--------------|------------------|-----------------|------------------|-----------------|
| HDPE         |                  |                 |                  |                 |
| HDPE-c-CH    | 13.9             | 10.4            | 17.5             | 15.0            | 11.2            | 19.4            |
| HDPE-c-CE    | 11.0             | 7.6             | 16.4             | 7.9             | 4.8             | 9.5             |
| HDPE-c-ST    | 8.8              | 6.8             | 17.6             | 7.1             | 4.0             | 10.2            |
| HDPE-c-AJ    | 11.5             | 8.1             | 15.1             | 6.3             | 5.8             | 8.3             |
| HDPE-c-PF    | 14.2             | 10.7            | 18.0             | 3.6             | 2.4             | 5.1             |
| HDPE-c-SH    | 18.5             | 13.8            | 1.0              | 17.4            | 13.0            | 2.5             |
| HDPE-c-XA    | 12.7             | 9.5             | 15.7             | 4.0             | 3.1             | 5.5             |

Sample gauge length, 38 mm; Sample width, 8 mm; Thickness, 0.5 mm; Temp., 22 °C; Seeding time, 3 months; A. niger was used as microorganism.

Fig. 3: Stress-strain curves of pristine HDPE and its blends using 10 wt% of different polymers after seeding with fungi (A. niger) for three months.
Fig. 4: DSC and TG analysis of pristine HDPE after seeding with fungus for three months.

The weight loss (%) and phase transition temperatures (Tm1 and Tm2) of seeded HDPE were found to be almost same as were found with pristine HDPE without seeding with selected fungi. Similarly, the heat of fusion (ΔHTR) of seeded HDPE is found to be almost same as of HDPE without seeding with selected fungi. These results have given an indication for a minimal biodegradation in HDPE after seeding with selected fungus due to the presence of high crystallinity in pristine HDPE (Thaweegan et al., 2005). But on comparing the weight loss decomposition temperatures (IDT and FDT) and heat of fusion (ΔHTR) of fungus seeded polymer blended HDPE with fungus seeded pristine HDPE, it is clear that decomposition temperatures (IDT and FDT) of polymer blended HDPE were found to be significant low in comparison to pristine HDPE (Table 5).

The HDPE-Polymer blends prepared with cellulose (HDPE-b-CE), starch (HDPE-b-ST), Schellac (HDPE-b-SH) and with xanthane (HDPE-b-XA) have shown high IDTs and almost a constant weight loss (~12 wt%) in comparison to HDPE-chitosan (PDPE-b-CH), and HDPE-pectin (HDPE-b-PE) blends (Table 5). This has suggested that chitosan and pectin were able to cause more biodegradation of HDPE on seeding with selected fungus in comparison to other polymers (Table 5). The enhanced biodegradation of HDPE in chitosan and pectin blended HDPE has caused a significant decrease in thermal stability of HDPE blends prepared with chitosan (HDPE-b-CH), and pectin (HDPE-b-PE), hence these blends were able to show thermal degradation at low IDT and FDT values in comparison to blends prepared with other polymers (Table 5 & Figs. 5 & 6).

| Blends codes | TG analysis | DSC analysis |
|--------------|-------------|--------------|
|              | IDT/°C      | FDT/°C       | Weight loss/°C | Weight loss % | Tm1/°C | Tm2/°C | ΔHf/ mJ mg⁻¹ | ΔHf/ mJ mg⁻¹ |
| Pristine HDPE| 470         | 503          | 98             | 138           | 181    | 491    | 329          | -            |
| HDPE-b-CH    | 300         | 360          | 97             | 135           | 143    | 458    | -1860        | -3220        |
| HDPE-b-CE    | 330         | 470          | 95             | 137           | 121    | 452    | -            | -            |
| HDPE-b-ST    | 390         | 455          | 93             | 139           | 136    | 437    | -3150        | -3470        |
| HDPE-b-PE    | 310         | 370          | 96             | -             | -      | 440    | 35.6         | 276          |
| HDPE-b-SH    | 340         | 425          | 97             | 139           | 195    | 488    | 276          | -            |
| HDPE-b-XA    | 325         | 460          | 94             | 134           | 120    | 438    | -3470        | -            |

Table 5: TG and DSC analysis of HDPE-Polymer blends after seeding for three months with selected fungus.

Fig. 5: DSC and TG analysis of HDPE-b-CH blend after seeding with fungus for three months.
The thermogram (TG) for fungus seeded HDPE-b-CH blend (Fig. 5) has shown a maximum rate of decomposition \((T_{\text{max}})\) at 300 °C for residual chitosan and at 360 °C for HDPE in biodegraded blends. On the other hand, the HDPE-b-PE blends were able to show maximum rate of decomposition \((T_{\text{max}})\) at 310 °C and at 370 °C respectively for residual pectin and HDPE in seeded samples (Table 5 & Fig. 6).

This has suggested that seeded fungus in pectin blended HDPE has consumed a very small amount of pectin, than the amount of chitosan consumed by fungus in chitosan blended HDPE, hence due to insufficient growth of fungi in pectin blended HDPE, the rate of biodegradation of HDPE was slower (1.83 mg min\(^{-1}\)) than blends (HDPE-b-CH) prepared with chitosan (2.77 mg min\(^{-1}\)). This has clearly suggested that the properties of blended polymer play important role in controlling the biodegradability of chemically inert HDPE by seeded fungi. Like HDPE-b-PE blend, the HDPE blends prepared with schellac and starch have also shown maximum rate of decomposition at 425 °C and 455 °C respectively as was confirmed from their TG curves (Table 5). The fungus seeded HDPE blends of schellac and starch have also shown phase transitions as was shown by HDPE blends prepared with other polymers, which is clear from the observed heat of fusion \((\Delta H)\) in their DSC thermograms (Table 5 & Figs. 7 & 8).
The rate of decomposition of polymer blended HDPE at Tmax is also used to understand the effect of seeded fungus on biodegradation of HDPE in HDPE-Polymer blends. The decreased rate of decomposition at Tmax in these samples is possibly due to the decrease in rate of decomposition of biodegradable shorter chains or oxidized long chains of HDPE as reported by other workers (Santonja et al., 2007). The long chains of HDPE decompose faster at Tmax in comparison to biodegraded shorter chains of HDPE, which decomposed at slower rate at low temperature as clear on comparing the rate of decomposition of seeded pristine HDPE (4.3 mg min\(^{-1}\)) and of fungus seeded blends (HDPE-b-SH) of shellac (3.15 mg min\(^{-1}\)) blends in which the rate of decomposition is found to be higher at 491 \(^\circ\)C and 437 \(^\circ\)C respectively for HDPE in fungus incubated pristine HDPE and fungus seeded blends (HDPE-b-SH) of shellac (Figs. 4 & 8). The trends in heats of fusion in fungus seeded HDPE-b-CH and HDPE-b-PE blends have suggested that chitosan and pectin were able to enhance the extend of biodegradation of HDPE on seeding with selected fungus in comparison to HDPE blends prepared with other polymers (Table 5 & Figs. 8). In comparison to fungus seeded pristine HDPE, the fungus seeded HDPE blends (HDPE-b-CH) of chitosan (Table 5 & Fig. 5) have shown exothermic phase transitions relatively at low temperatures (Tm1 and Tm2) for the decomposition of residual chitosan and fungal oxidized chains of HDPE. The TG and DSC analysis of fungus incubated blends of cellulose (HDPE-b-CE) and blends (HDPE-b-ST) of starch (Table 5) has indicated that cellulose was able to show enhanced biodegradation of HDPE in comparison to starch as ITD of fungus seeded HDPE-b-ST is found to be higher (390 \(^\circ\)C) than ITD of fungus seeded blends (HDPE-CE) of cellulose (330 \(^\circ\)C). Similar trends were observed for the heat of phase transition in these blends (Table 5). The rate of decomposition is found to be low (1.37 mg min\(^{-1}\)) at 437 \(^\circ\)C for HDPE-b-ST in comparison to rate of decomposition for HDPE-b-CE (2.24 mg min\(^{-1}\)) at 452 \(^\circ\)C. This has indicated that HDPE in fungus seeded HDPE-b-CE blends were having undecomposed long chains of HDPE in comparison to HDPE chains in fungus seeded blends (HDPE-b-ST) of starch. The appearance of single Tmax in fungus seeded HDPE blends of starch (HDPE-b-ST), pectin (HDPE-b-PE), and shellac (HDPE-b-SH) has given an indication that in these blends, the blended polymer was used up totally as nutrient by seeded fungus and enzymatically degraded residual amorphous HDPE subsequently decomposed relatively at a slower rate at Tm1 during TG analysis. In comparison to incubated blend of pectin (HDPE-b-PE), the incubated blend of shellac(HDPE-b-SH) and xanthane(HDPE-b-XA) have shown high Tmax and IDT suggesting that HDPE in fungus seeded blends of shellac and xanthane were less biodegraded than pectin; hence, HDPE in shellac (3.08 mg min\(^{-1}\)) and xanthane (2.77 mg min\(^{-1}\)) was able to show high rate of decomposition relatively at high temperatures as 488 \(^\circ\)C and 458 \(^\circ\)C respectively. Thus, considering the trends of Tmax and rate of decomposition of HDPE in polymer blends, the blends prepared with pectin is also found to be efficient in controlling the biodegradation of HDPE as similar to chitosan, cellulose and starch. Though xanthane was able to induce biodegradation in HDPE comparable to chitosan (Fig. 5) but lower than pectin and starch.

3.6 Morphological studies by scanning electron microscopy of chitosan blended HDPE films before and after seeding with fungus:

The HDPE-films blended with 10 wt \% of chitosan were used to record their SEM micrographs for analysing the morphological properties before (Fig. 9A) and after seeding with T. harziauum fungus (Fig. 9 B, C, & D). The SEM micrographs of chitosan blended HDPE films have confirmed a homogeneous surface morphology (Wu, 2003) before seeding with fungus (Fig. 9A) but after seeding with fungus for one month, the cracked surface morphology (Fig. 9B) was observed, which confirmed the biodegradation of blended chitosan (Fig. 9B). The SEM micrographs with white small sized spots morphology (Fig. 9 C) have suggested the biodegradation of long chains of HDPE in the blends by the enzymatic action of germinated colonies (Oake et al., 1995).

Fig. 9: SEM micrographs of HDPE blend with 10 wt % chitosan (A) before seeding; T. harziauum (Fig. 9 B, C, & D).

SEM micrographs of HDPE blends after seeding with fungus (T. harziauum) for one month (B), two months (C), and three months (D).
CONCLUSIONS AND FUTURE SCOPE OF WORK

1. The microbial biodegradation of synthetic polymer depends on its degree of hydrophilicity, hence blending of HDPE with chitosan and pectin has produced hydrophilic HDPE, which is suitable for seeding of fungi. As natural polymers, they are hydrophilic in nature, hence further studies may be carried out using other natural polymers having better chain flexibility and structural orientation suitable for the growth of seeded fungi (Kawai et al., 2004).

2. The blended polymers with HDPE should also act as a better nutrient for the growth and colonization of seeded fungi. Out of selected polymers, the chitosan and pectin have acted as a suitable nutrient, hence the extent of biodegradation of HDPE was more with these polymers in comparison to other polymers.

3. The microbial biodegradation of HDPE is only possible, if well developed colonies of fungi are formed on blended polymers. The biodegradation of long chains of HDPE takes place when optimum amount of enzymes are released by highly populated colonized microbes on the surface of HDPE.

4. The acidic environment may decrease the germination of fungi over the surface of HDPE, hence there should be no acidic product to observe biodegradation in HDPE.

5. The variation in degree of biodegradation of HDPE with different type of fungi was due to the difference in β-oxidation system, which is an essential part of cell membranes (Kawai et al., 2004).

6. The variation in Young’s modulus may be used to confirm the blending of polymers with HDPE on the basis of plasticization (Pal et al., 2008) as well as to confirm the biodegradation of HDPE by seeded fungi.

7. The difference in thickness of interface layer of blended polymers on HDPE might also be a reason for the difference in biodegradation capacity of blended polymers with HDPE. This might be the reason for low biodegradability of HDPE in presence of shellac.

8. Finally, the extent of biodegradation might be evaluated by recording the variation in physical and thermal properties of fungi seeded HDPE and also by recording the morphological changes with SEM micrographs.

9. The results of these studies contribute significantly to the existing knowledge for the synthesis and characterization of biodegradable polymers by blending the naturally occurring polymers.

CONFLICT OF INTERESTS

The authors declare no competing financial interest.

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