6.1 Introduction

There is strong environmental pressure on industries to recycle waste polymers, particularly those used in packaging applications. Polyethylene (PE), in the form of low density polyethylene (LDPE) carry bags, currently commands more than half of the recycled polymer market. They form the major polymeric part of post consumer waste streams. (Mhumak *et al.* 2017 & Agrawal *et al.* 2015). Despite its wide usage, large amount of PE carry bags are discarded every year and become a source of pollution. Therefore, finding effective ways to reuse waste PE carry bags and improve the recycling rate is becoming very important for environmental sustainability. Recycling polyethylene is of vital importance for our environment. Over 60 million metric tons of PE is produced annually for use as packaging and containers, including plastic bags and bottles ordinary products consumers encounter everyday. (http://pvcenterprise.com/recycling-plastic-waste/polyethylene-recycling.html) Polyethylene recycling is a trend that's catching on.

Recycling is an approach for end-of-life waste management of plastic products. It makes increasing sense economically as well as environmentally and a considerable surge in the rate of recovery and recycling of plastic wastes is evident from the current trend. Polyethylene is recycled as it is generated in the largest volume of all the other plastics. (Hopewell *et al.* 2009) These trends are destined to continue, but some important provocations still exist from both technological factors and from economic or social behaviour issues relating to the collection of recyclable wastes, and substitution for virgin material. Recycling of a wider range of post-consumer plastic packaging, will further enable improvement in recovery rates of plastic waste and diversion from landfills.

Polyethylene bags are increasingly being recycled, but the majority still end up in landfills where they may take hundreds of years to break down. Increasing the recycled

PE content of new plastic bags is a way of using lesser natural resources and reducing the environmental impact of bags. (http://www.wrap.org.uk/content/carrier-bags-material-matters-0)

The worldwide market of LDPE is huge, around 80million tons per year. The amount of plastic used in plastic bags has reduced by around 70% in the last 20 years**.** For example, it is more cost efficient to produce a bag from recycled LDPE than to manufacture from 'virgin' plastic. LDPE is produced using considerable amounts of fossil fuels and it takes a total of 2.0 kg of petroleum to manufacture just 1kg of LDPE. Recycling a ton of plastic bags (about 450,000 bags) saves 11 barrels of oil.(https://earth911.com/recycling-guide/how-to-recycle-plastic-bags/). Many new products can also be manufactured using recycled LDPE, including grocery bags, thin packaging (bread, newspaper, dry cleaning, sandwich bags, etc),plastic film (i.e. cling wrap, saran wrap),squeeze bottles, six pack rings (for sodas),moisture barriers in construction, agricultural wrap, plastic laminate for cardboard milk and juice bottles. Biodegradation of recycled PE of various grades like Grade I recycled LDPE (green color), Grade II (red color) and Grade III (black color) has not been performed earlier. In the present study biodegradation of recycled polythylene carry bags using *Rhizopus oryzae* strain NS5 has been investigated in the batch system. The confirmation of degradation of LDPE films was done by the analysis of weight loss, universal tensile strength, FTIR, SEM, AFM and GC-MS results.

6.2 Result and Discussions

6.2.1 Characterization of Potential Strains and Phylogenetic Analysis: Fungal culture was isolated and characterized using the protocol described earlier (in section 3.1 and 4.2.1).

6.2.2 Measurement of weight loss

To quantify the recycled LDPE degradation efficiency of *Rhizopus oryzae* NS5 the change in weight was measured at different time intervals for 30 days incubation. There was a time dependent weight loss of rPE films. Over a period of 30 days 24.9±3 % of green, 28.1±3% of red and 30±3% of black rLDPE were found to be degraded by fungus (Fig.6.1a-c). ANOVA result indicates that weight loss with incubation time was significant. The value of was $F = 3031$ and $P<0.001$ for green rLDPE, for red rLDPE $F=3145$, $P<0.001$ and for black LDPE $F=3649$, $P<0.001$. No weight loss was observed in control experiment. Therefore these results showed the reduction in weight was due to the utilization of PE films as a sole carbon source by fungus. This confirms that *Rhizopus oryzae* NS5 is capable of degrading recycled LDPE films without any abiotic pretreatment. There are various literatures available on the fungal biodegradation of fresh LDPE films but biodegradation of rLDPE has not been not reported earlier. Hanaa *et al* explored the susceptibility of fungi to degrade the degradable polyethylene bags (Hanaa *et al*.1998). Similarly polyethylene was found to be degraded by *Aspergillus flavus*, isolated from sanitary landfills (Mendez *et al.*2007). *Penicillium simplicissimum* YK identified by Yamada *et al* could degrade the untreated high-density polyethylene (Yamada-Onodera *et al.*2001).

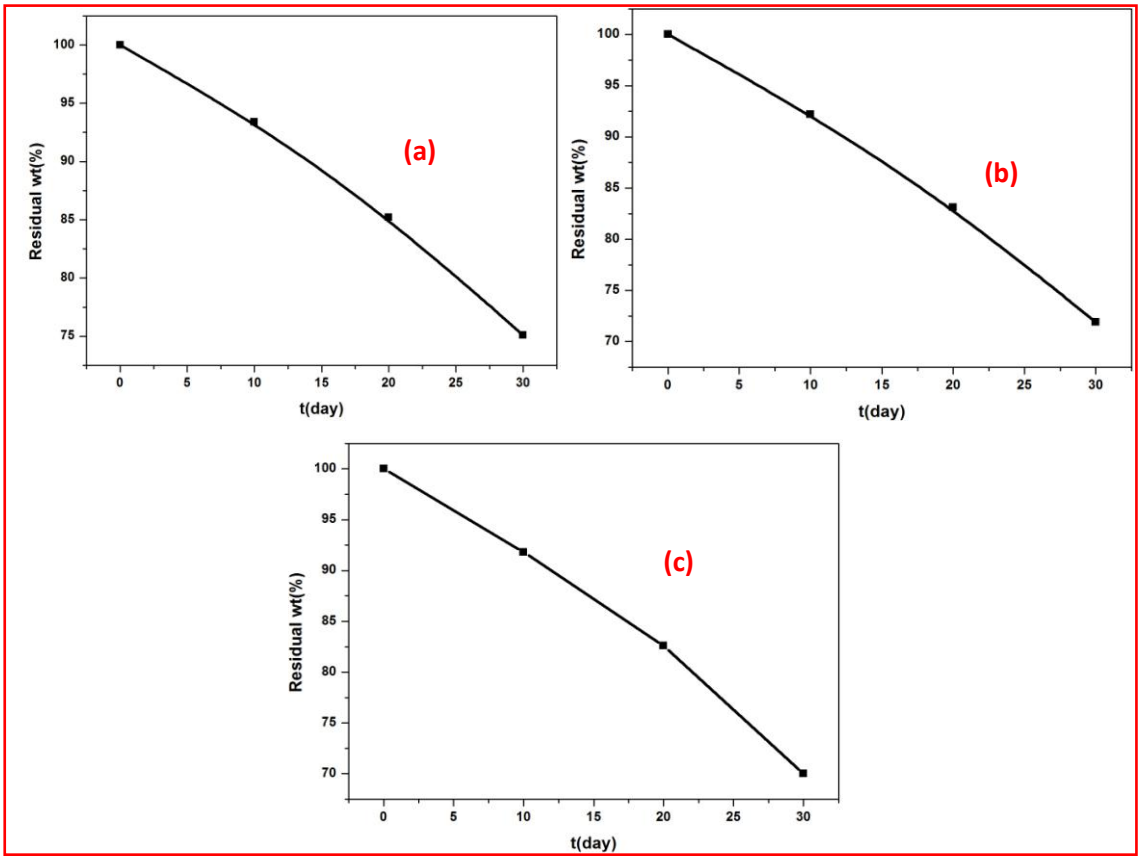


Fig.6.1 Change in wt of green (a), red (b) and black rLDPE (c) film

6.2.3 Measurement of change in tensile strength

A reduction in tensile strength of films after 30 days incubation with fungal isolate was observed. Tensile strength of green rLDPE got reduced to about 57%, of red LDPE by 74% and of black LDPE by 84% (Fig.6.2 a-c). ANOVA result indicates that the changes in tensile strength with incubation time were significant. The value of F was =208 and $P<0.001$ for green rLDPE, for red rLDPE F=219, $P<0.001$ and for black LDPE F=230, $P<0.001$. The reduction in tensile strength complements the reduction in weight of films after incubation as fungus utilises the rLDPE films as a source of carbon for its growth. The results are in accordance with the earlier studies (Awasthi *et al*. 2017, Hanaa *et al*. 1998, Lee *et al.* 1991 Nowak *et al*. 2011, Jakubowicz *et al*. 2006). Values represent the average of three independent experiments. Error bars indicate standard deviation

(±SD). Statistical significance between the groups was evaluated at p = 0.05 significance level. 61% loss in tensile strength after 1 month incubation with *A. niger* was reported by Mathur *et al.* . There was 18.48% reduction in TS of LDPE in 4 months by *A. glaucus* documented by Vijaya and reddy (2008).There are reports similar to our results by Hanaa *et al*., Lee *et al*. and Nowak *et al*. they reported that biodegradation reduces the percentage elongation of polyethylene films (Hanaa *et al*. 1998, Lee *et al.* 1991& Nowak *et al*. 2011). In a study by orhan *et al* 56% reduction in TS in 3 months was observed (orhan *et al.*2000). Our results are also in agreement with earlier studies by Jakubowicz *et al*. on thermally-treated-PE films, which showed a reduced elongation (%) after incubation with compost microorganism (Jakubowicz *et al*. 2006). It has been reported by konduri *et al* that 51% reduction in TS of polythene in 3 months with the incubation with *A. Oryzae* (konduri *et al.* 2011).

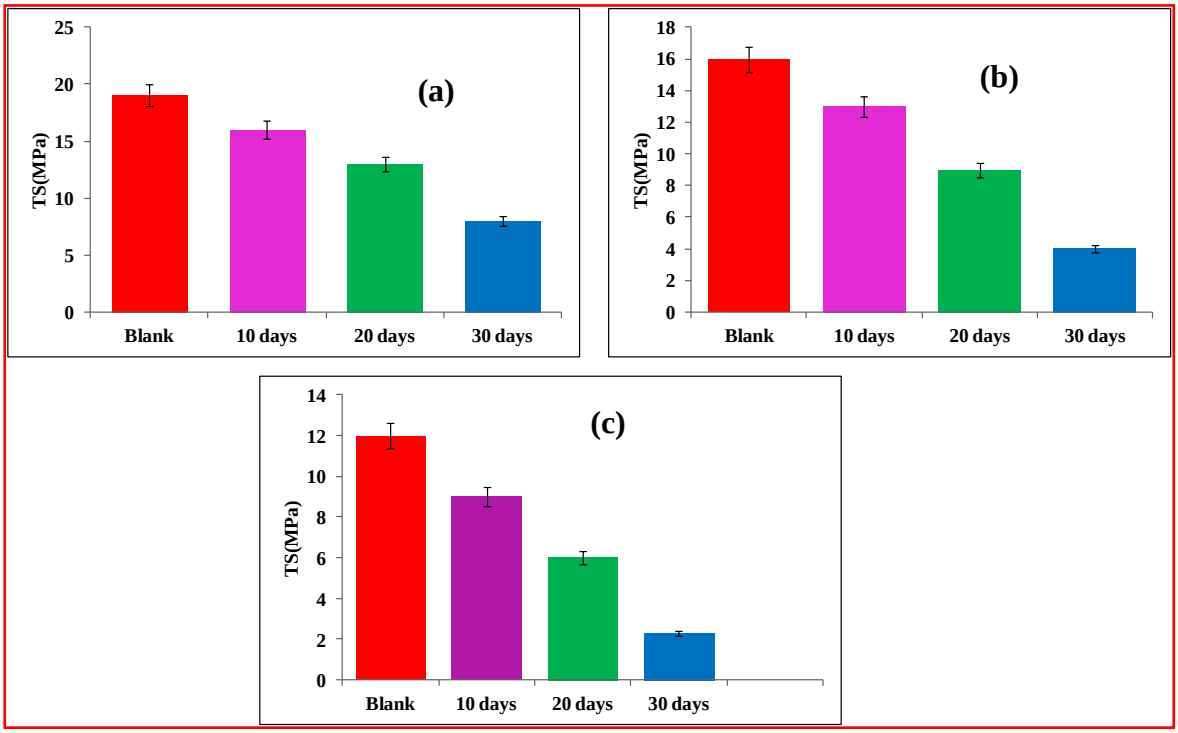


Fig.6.2 Tensile strength of green rLDPE (a),red rLDPE(b) and black rLDPE (c)

6.2.4 Measurement of change in pH

Initial pH of the media was 5.2±0.27 while pH after 30 days incubation was measured as 4.0±0.30, 3.6±0.30 and 3.3±0.30 for green, red and black rLDPE (Fig.6.3a-c) while pH of the control remains unchanged after 30 days. Values represent the average of three independent experiments. Statistical significance between the groups was evaluated at p = 0.05 significance level. ANOVA result indicates that reduction in pH with incubation time was significant. The value was $F = 90.1$ and $P < 0.001$ for green rLDPE, For red rLDPE $F = 92.3$, $p < 0.001$ and for black LDPE $F = 95.5$, $p < 0.001$. pH of the media after biodegradation in the acidic range denotes that acids are produced after fungal activity on rLDPE films. The reduction in pH validates that the culture is still metabolically active and rLDPE films are utilized for their growth as a sole carbon source. Similar results were reported by Duddu *et al* (2015) for the biodegradation of LDPE with *streptomyces coelicoflavus* NBRC15399. LDPE biodegradation with *Bacillus amyloliquefaciens* results also shown reduction in pH of the media (Das *et al*.2015), Study by Awasthi *et al.*(2017) shows the similar findings after biodegradation of LDPE by *R. Oryzae* NS5.

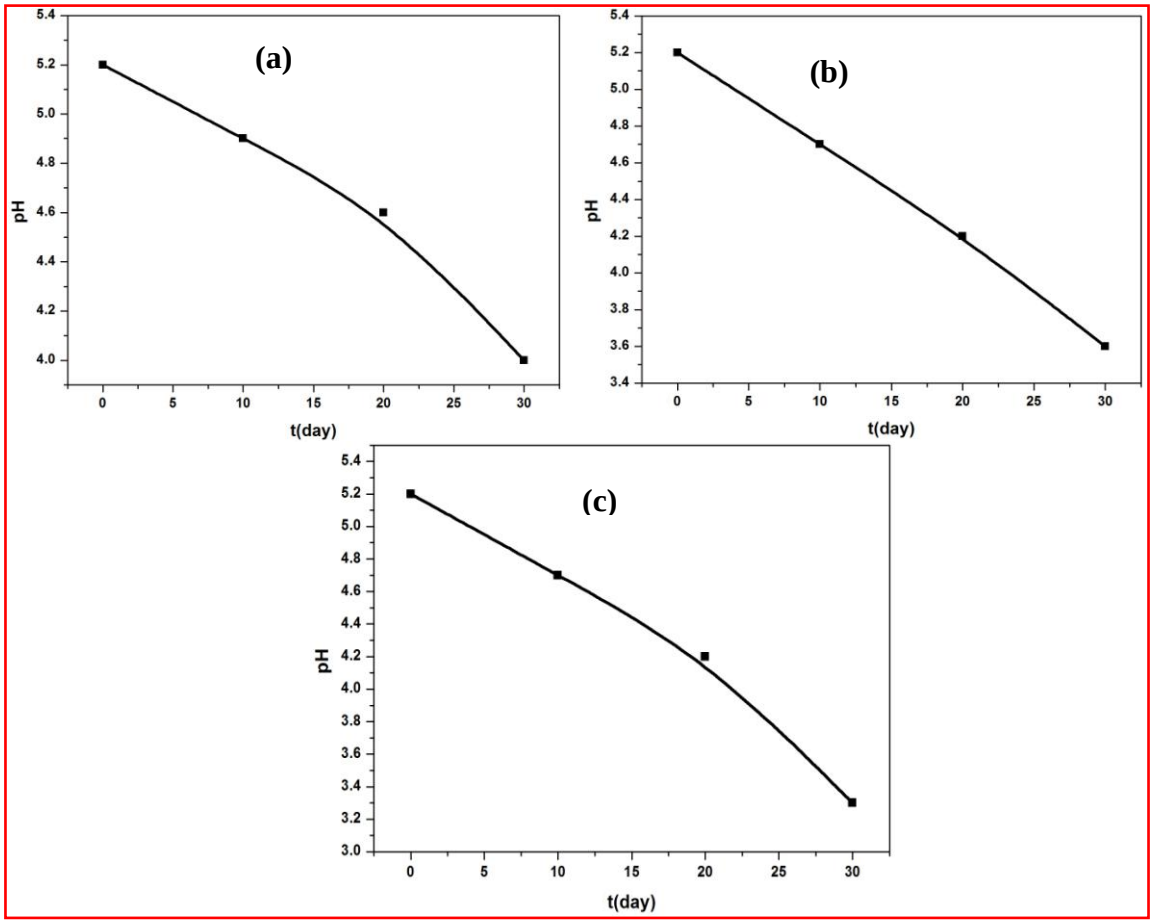


Fig.6.3 Change in pH of green (a), red (b) and black rLDPE (c)

6.2.5 Measurement of contact angle

The contact angles of the untreated rLDPE films were 95.3± 3.5, 93.3± 3.5 and 90.4 ±3.5^{o} (Fig. 6.4 a- c) which were decreased to 65.2± 3.5, 62.0± 3.5 and 52.3± 3.5^{o} (Fig. 6.5 a- c) after 30 days incubation with fungal strain. This lowering of the contact angle is an evidence of the increased hydrophilicity and wettability of the polyethylene surfaces. There was, though, no decrease in the contact angle for the control set of samples. This indicated that the polymer surface became relatively hydrophilic with increasing immersion period, which was also reported earlier by several authors (Awasthi *et al*. 2017, Sonak *et al*.1995 & Sudhakar *et al*. 2008).

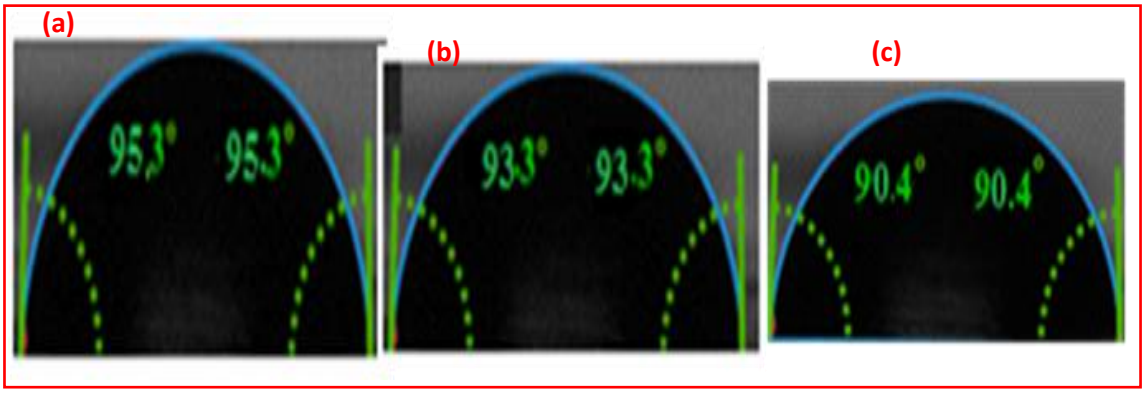


Fig.6.4 Contact angle of blank green (a), red (b) and black (c) rLDPE film

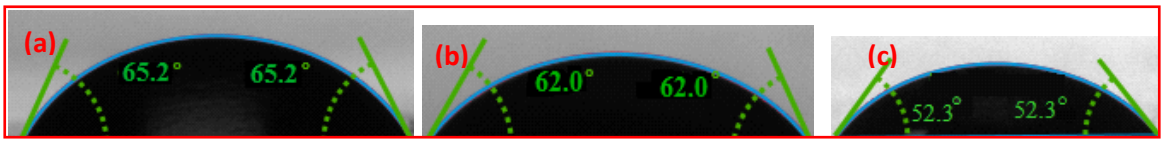


Fig.6.5 Contact angle of biodegraded green (a), red (b) and black (c) rLDPE film

6.2.6 Surface morphology

The surface morphology of recycled polyethylene films was observed by SEM and AFM micrographs.

6.2.6.1 Scanning Electron Microscopy (SEM)

Superficial growth of fungal hyphae on the recycled films can be seen in SEM image obtained after 30 days of incubation with *Rhizopus oryzae* NS5. While In the blank film fungal growth is absent (Fig. 6.6(a), 6.7(a) & 6.8(a)). Hyphal growth on the surface of polyethylene and degradation of the polyethylene around the fungal cells in the biofilm was seen which resulted in the formation of grooves in polyethylene (Fig. 6.6(b), 6.7(b) & 6.8(b)). It is possibly because of *Rhizopus* secretes lipase, (Coenen *et al*. 1997) tyrosinase, peroxidase (León-Santiesteban *et al.* 2008), laccase (Shinkafi *et al*. 2014) and manganese peroxidase enzymes capable of degrading polyethylene and consequence of such enzymatic activities is the grooves formation. There are several reports demonstrating that initial attack generally begins with the surface colonization (Moriyama *et al*. 1993). Scanning electron microscopy (SEM) allows direct observation of biodegradation. The

results obtained are in close association with earlier reports (Manzur *et al.* 2004 & Sepulveda *et al*. 2002).

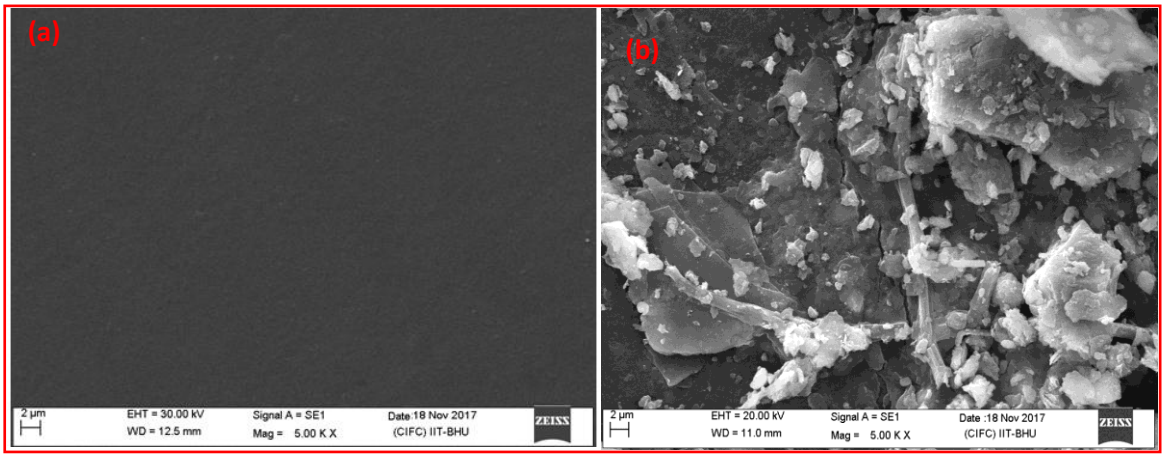


Fig.6.6 SEM image of blank (a) & biodegraded (b) green rLDPE

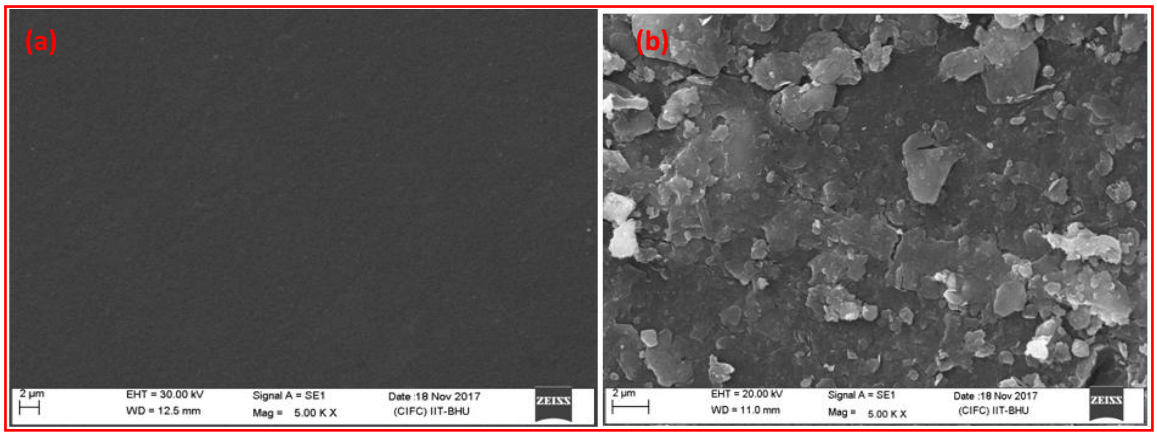


Fig.6.7 SEM image of blank (a) & biodegraded (b) red rLDPE

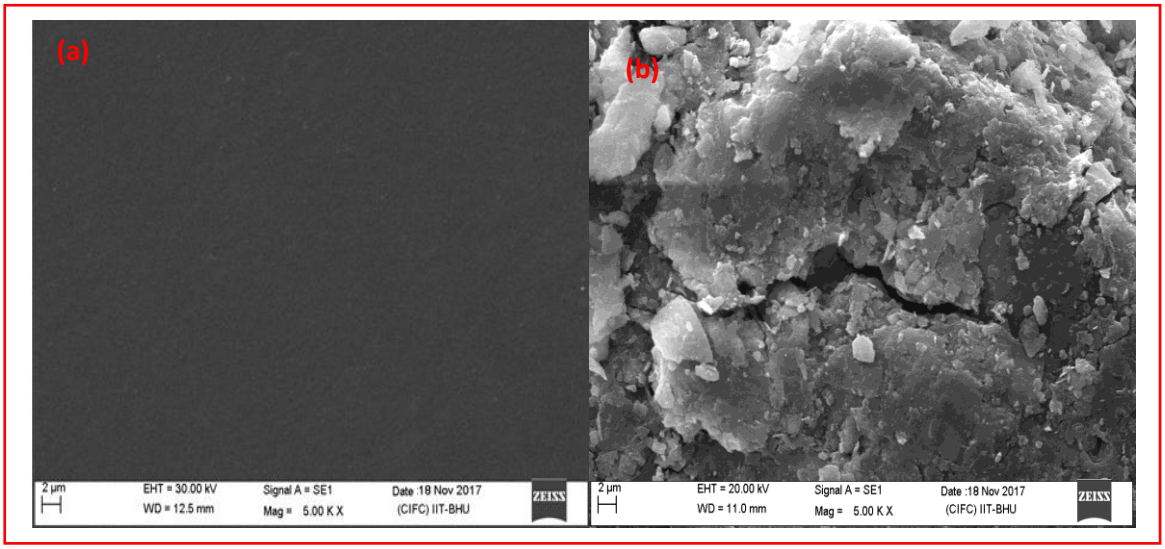


Fig.6.8 SEM image of blank (a) & biodegraded (b) black rLDPE

6.2.6.2 Atomic Force Microscopy (AFM)

It was observed that the surface of the rLDPE films treated with the fungal isolate showed roughness, development of cracks and grooves. These morphological changes are presented in AFM images of rLDPE films (Fig.6.9 (a-b), 6.10(a-b) and 6.11(a-b)). In contrast, the film that was not treated (control) with the fungus retained a smooth surface even after 30 days of incubation under the same conditions. This change in growth could be a cellular response to the change in surface topography of the PE films during degradation whereby pits are formed on the film surface due to enzymatic digestion. Similar changes in surface topography have also been noticed from AFM analysis (Tribedi *et al*. 2013). Microorganisms colonize the polymer surface and adhere by extracellular polymer production.

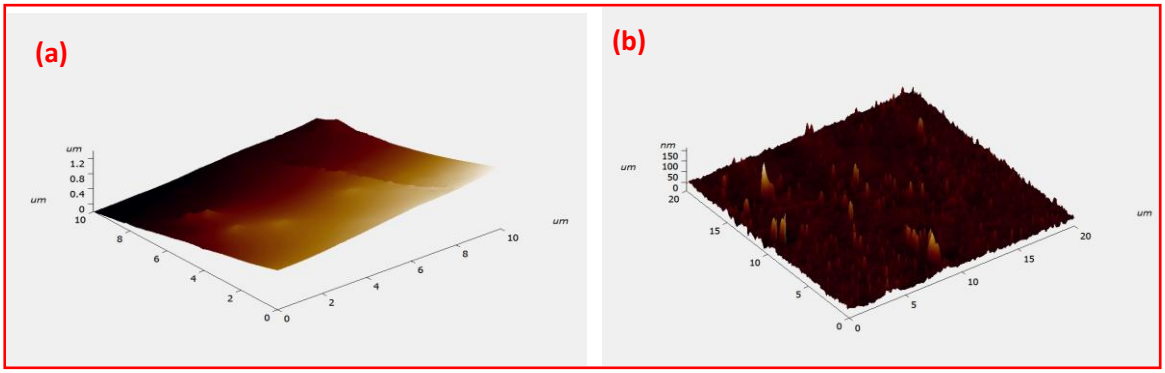


Fig.6.9 AFM image of blank (a) & biodegraded green rLDPE (b) films

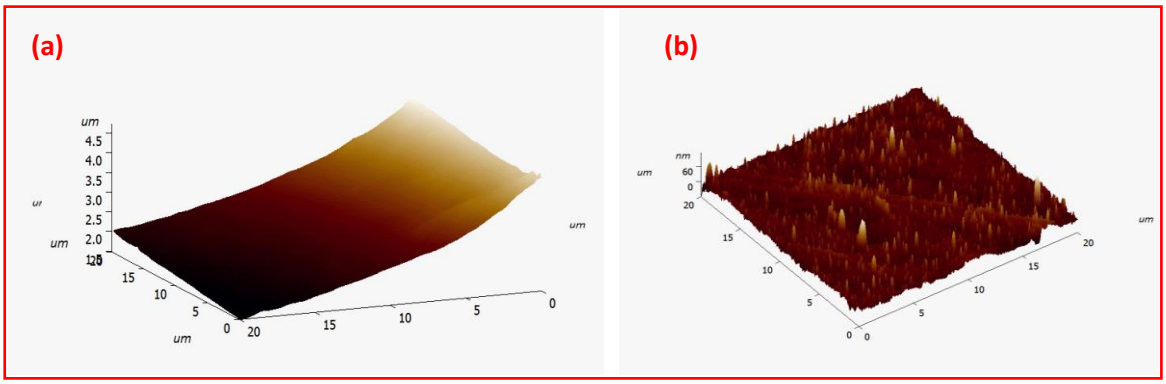


Fig.6.10 AFM image of blank (a) & biodegraded red rLDPE (b) films

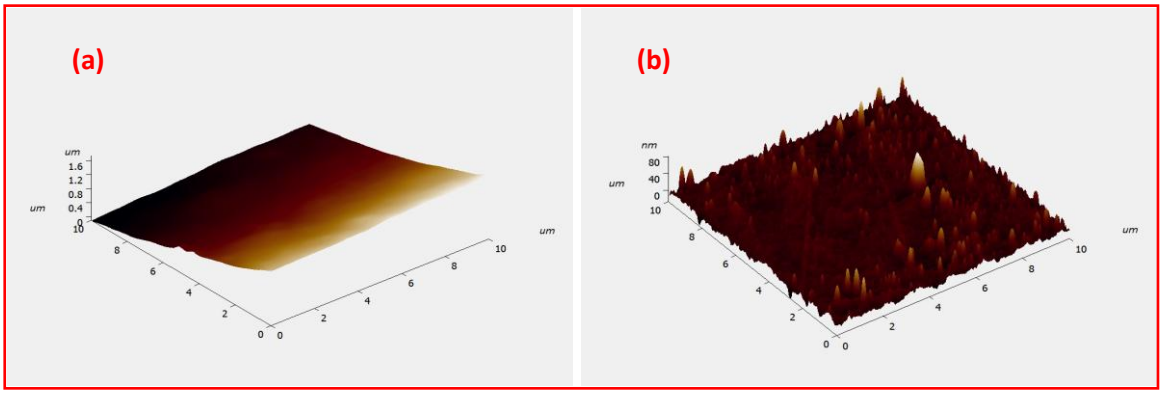


Fig.6.11 AFM image of blank (a) & biodegraded black rLDPE (b)

6.2.7 Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR spectra of green, red and black rLDPE have been depicted in Fig. 6.12 (a-b), 6.13(a-b) and 6.14 (a-b). A number of peaks are present in the control rLDPE of different grades (i.e. green, red and black) manifesting the complex nature of the recycled carry bags (fig. 6.12a, 6.13a & 6.14a). In case of green PE film characteristic bands are present at 476 cm^{-1}, 719 cm^{-1}, 1372 cm^{-1} (N-O stretching= presence of nitro compound), and 1469 cm^{-1}. After biodegradation new peaks at 820 cm^{-1}, 1025 cm^{-1} carboxylic acid and 3612 cm^{-1} are noticed. After incubation with fungal isolate there is an alteration in the band intensities in different regions. Peaks appear in the range of 3400-3600 cm^{-1} which indicate the depolymerisation activity of the microbial isolate (Awasthi *et al.* 2017). Absorbance in the range of 3000–2800 cm^{-1} corresponds to C-H stretch and presence of alkanes (Shah *et al.* 2007). The change in the peak values of almost all functional groups confirming the configurational change on polymer surface. (Das *et al*. 2015, Gilan *et al*. 2004, Drímal *et al*. 2007, Hadad *et al*. 2005**,** Arboleda *et al*. 2004). In red film groups are present at 463 cm^{-1},720 cm^{-1} (CH2 group), 1372 cm^{-1},and 1462 cm^{-1}, new peaks have been observed at 882 cm^{-1} (C-C stretching), 1017 cm^{-1} (C-O strtching), and 3512 cm^{-1} . In Green film characteristic bands were present at 476 cm^{-1}, 719 cm^{-1}, 1372 cm^{-1}, 1469

cm^{-1}. A shift in peak was observed from 470 to 476 cm^{-1} and new peaks at 820 cm^{-1}, 1025 cm^{-1} and 3612 cm^{-1} were noticed. Peaks in the range of 3400-3600 cm^{-1} support the depolymerisation activity of the microbial isolate (Awasthi *et al.* 2017).

In red rLDPE peaks are present at 463, 720, 1372 and 1462 cm^{-1} after 30 days incubation new peaks at 1154 and 3512 cm^{-1} appeared affirming the oxidation of film by fungal strain.

In the control sample of black film, the characteristic absorption bands were present at 528 cm^{-1}, 710 cm^{-1} (C–H bend-mono) and 1451 cm^{-1} (C=C stretch), and 2919, 2850 cm^{-1} (both due to C–H stretch). After biodegradation appearance of new peaks at 878 cm^{-1}, 1020 cm^{-1} and 3644 cm^{-1} happens. The absorbance range of 700–900 cm^{-1} corresponds to − C = C− stretching and the presence of alkene group (Esmaieli *et al.* 2014), absorbance range of 1500–1400 cm^{-1} corresponds to − CH_2 stretching and presence of aromatics (Negi *et al.* 2011) and 3644 cm^{-1}. Peak at 1451 cm^{-1} gets broader after biological incubation. These new peaks were observed due to the vibrations in the stretching of the O-H bond in alcohols and phenols. These results are similar to the findings of Ojha *et al*. (Ojha *et al*. 2017).

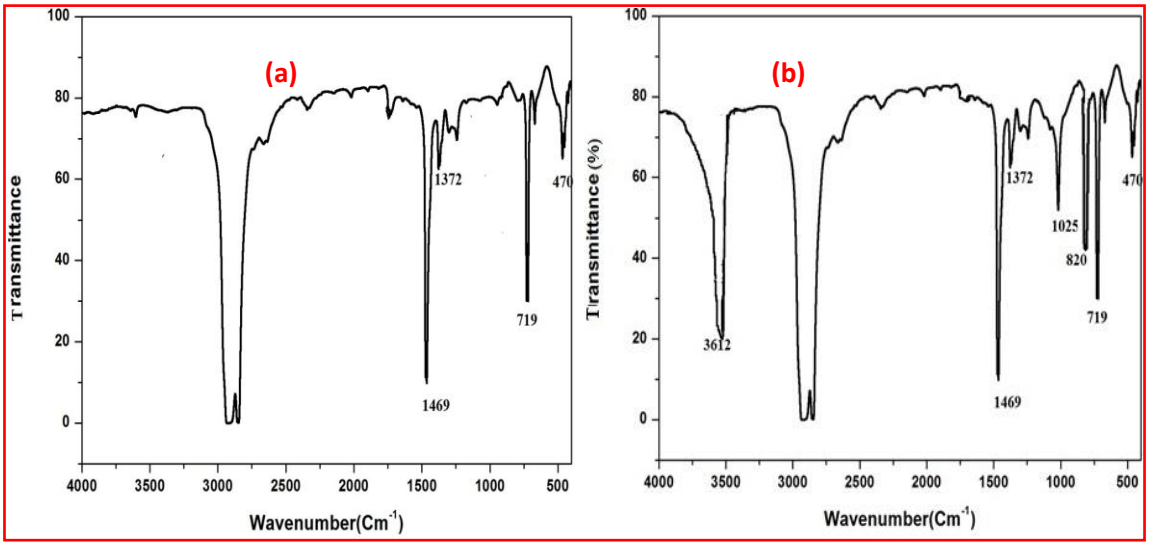


Fig.6.12 FTIR of green blank (a) and biodegraded film(b)

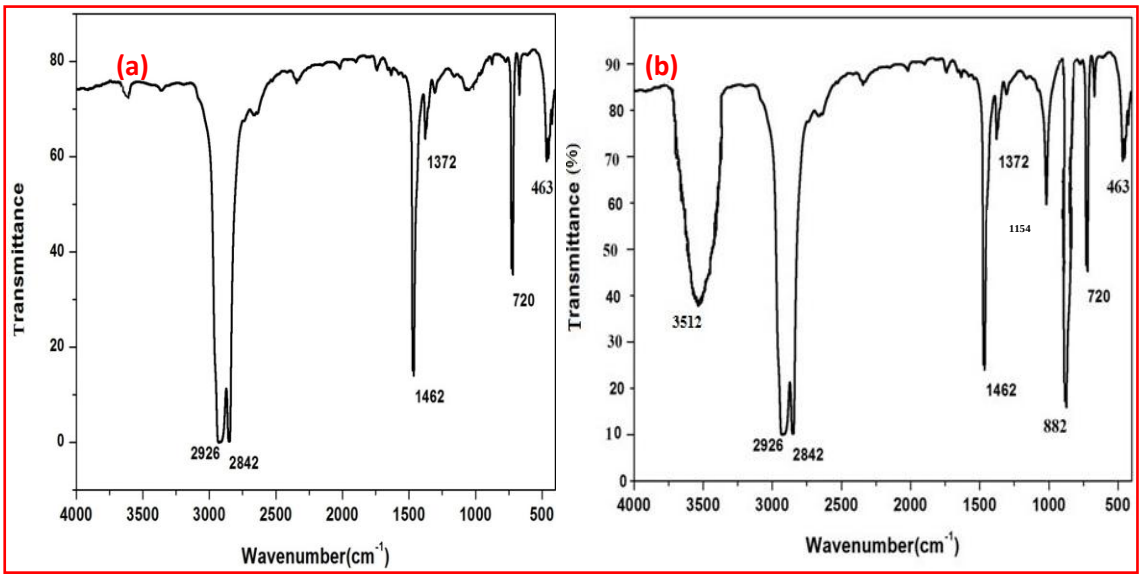


Fig.6.13 FTIR of red blank (a) and biodegraded film (b)

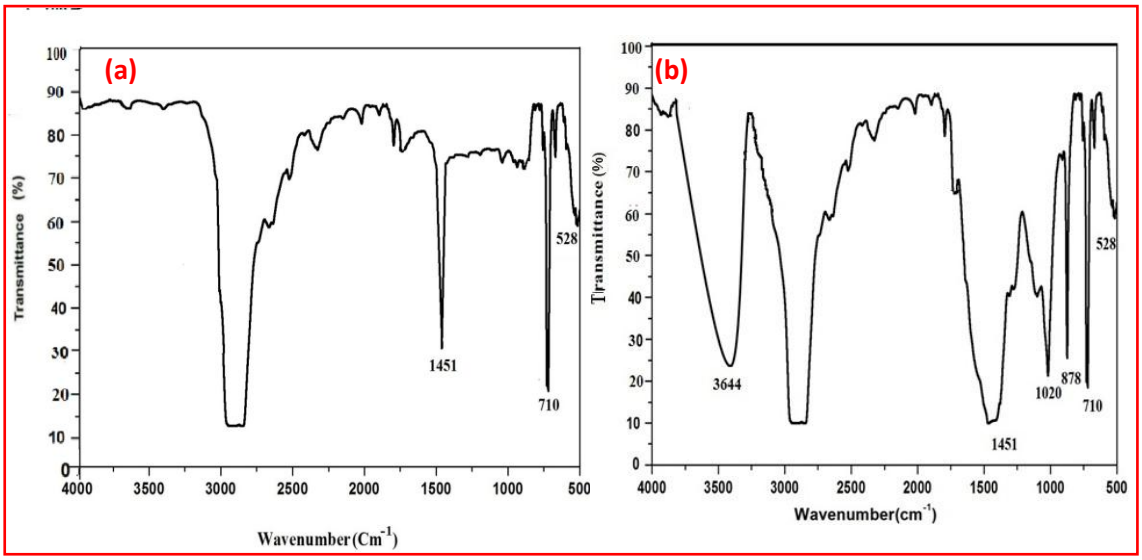


Fig.6.14 FTIR of black blank (a) and biodegraded film (b)

6.2.8 GCMS

The analysis of polyethylene degradation products (PEDP) has been performed to know the by-products/intermediates formed. According to Sivan *et al*. *Rhodococcus rubber* (C208) uses polyethylene as a carbon source and produces polysaccharides and proteins(Sivan *et al*. 2006) . As per Hakkarainen *et al.* 2-heptanone, 2-octanone, 2-

nonanone, formic acid, acetic acid, propanoic acid, butanoic acid, pentanoic acid, and hexanoic acid resulted in the pyrolysis of polythene at high temperature (Hakkarainen *et al*. 2003). Two important products alk-1-enes and n-alkanes in the range C_6-C_{23} were reported during the thermal degradation of polythene which was confirmed by GC-MS analysis (Sojak *et al*. 2006). In the current study, four kinds of by-products like fatty acid (Carboxylic acid), plasticizer (dibutyl thalate), benzene (Benzene, 1-methoxy-4-(2-Propenyl), Benzene 1 ,2-methylene[dioxy]4-propenyl-[E], Benzene,(1,5 dimethyl)-4-methyl-, Benzeneacetic acid-2- methyl propyl ester, 1,2-Benzene dicarboxylic acid dibutyl ester), and alcohol (2,2-dimethyl, 4,6- hepta diene-3 – ol) were observed in the PEDP culture supernatant which was extracted with distilled ether. Our results are in accordance with the previous reports (Kyaw *et al*. 2012); Aswale (2010) also reported a number of polythene biodegraded products such as Ergosta-5, 22-dien-3-ol, acetate (3,22 E), 1-monanalinoeoglycerol trimethylsilyl ether, betamethasone acetate, Azafrin, 9,12,15-octadecatrienoic acid, 2,3-bis [(trimetylsilyl) oxy] propyl ester, (Z,Z,Z)- C27H52O4Si2). Kyaw *et al*. 2012 reported 22 different biodegraded products from the polythene but identified only 18 compounds as benzene, methyl, tetrachloroethylene, benzene, 1,3-dimethyl, octadecane, 7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, hexadecanoic acid, ethyl ester, eicosane, octadenoic acid, docosane, 3-chloropropionic acid, heptadecyl ester, tricosane, octadecanoic acid, butyl ester, 1-nonadecene, tetracosane, pentacosane, 1,2-benxenedicarboxylic acid, di-iso-ostyl ester, and hexacosane (Kyaw *et al*. 2012). Mahalakshmi *et al*. (2012) reported PEDP using GC-MS by the action of *Bacillus* and *Pseudomonas* and reported octadecadienoic acid, octadecatrienoic acid, benzene dicarboxylic acid, and cyclopropanebutanoic acids as the main by-products. Pramila and Ramesh (2015) studied the biodegradation of

polyethylene (LDPE) with *A. baumannii* and with GC-MS analysis recorded 2-butene, 2-methyl, acetone, and ethene.

6.2.8.1 GCMS of green PE (blank)

Compounds present in blank green PE are shown in table 6.1and fig.6.15a.

6.2.8.2 GCMS of biodegradation byproducts of green PE

The Intermediates formed after biodegradation of recycled green LDPE are as: Benzene, 1-methoxy-4-(2-Propenyl), Phenol, 2, 6 bis-(1,1-dimethyl ethyl)-4- methoxy methyl, Pthalocyanin, Hexadecanoic acid ester, Benzene,1 ,2-methylene[dioxy]4-propenyl-[E]. The pthalocyanin is the byproduct of pigment material which is added in the polythene for color (Fig 6.15b). The structure of the compounds is presented in table 6.2.

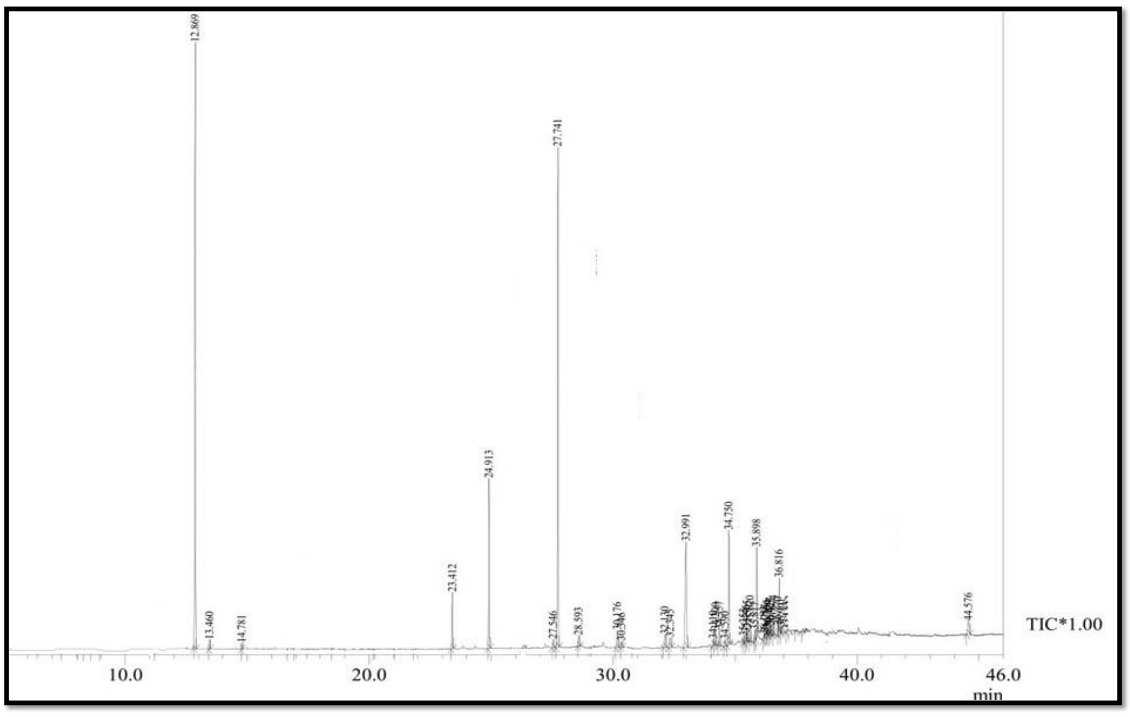


Fig.6.15 (a) GC-MS of green rLDPE (blank)

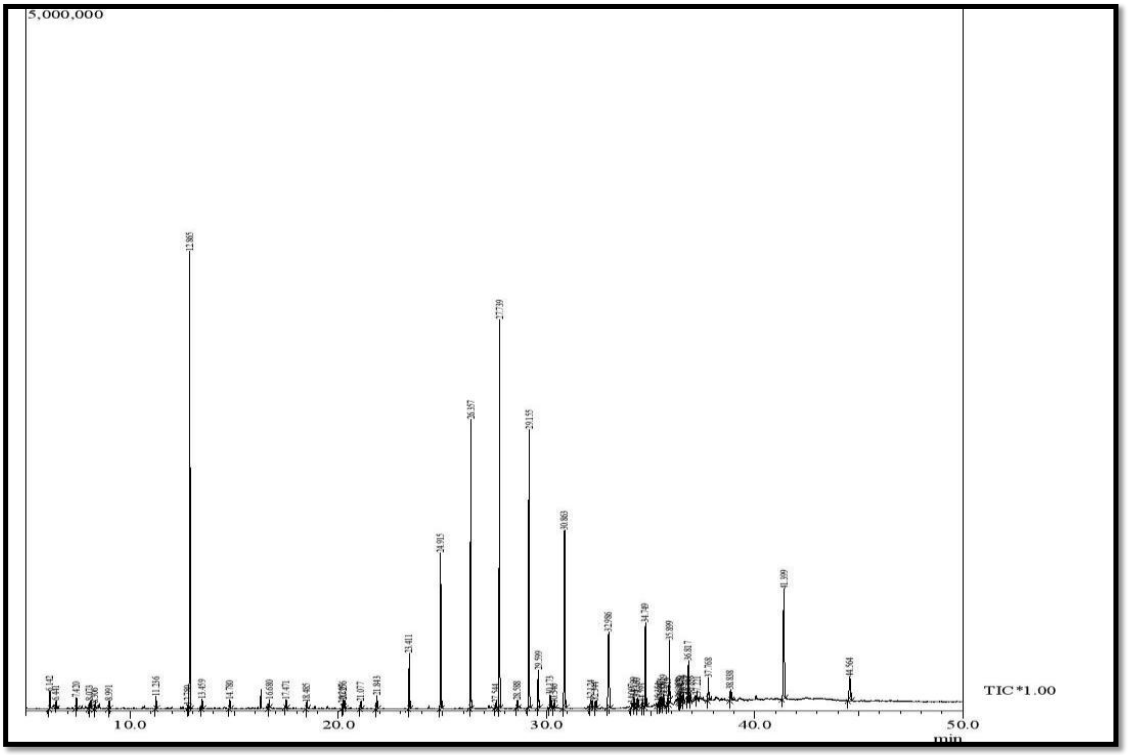


Fig. 6.15(b) GC-MS of green rLDPE after biodegradation

6.2.8.3 GCMS of blank red LDPE

Intermediates obtained before biodegradation of red PE film are listed in table 6.1. Fig.6.16 (a) shows the chromatogram of the film.

6.2.8.4 GCMS of biodegradation byproducts of red LDPE

Biodegradation by-products resulted from red PE film are mainly oxidised compounds, shown in table 6.2 and in chromatogram (Fig.6.16 b).

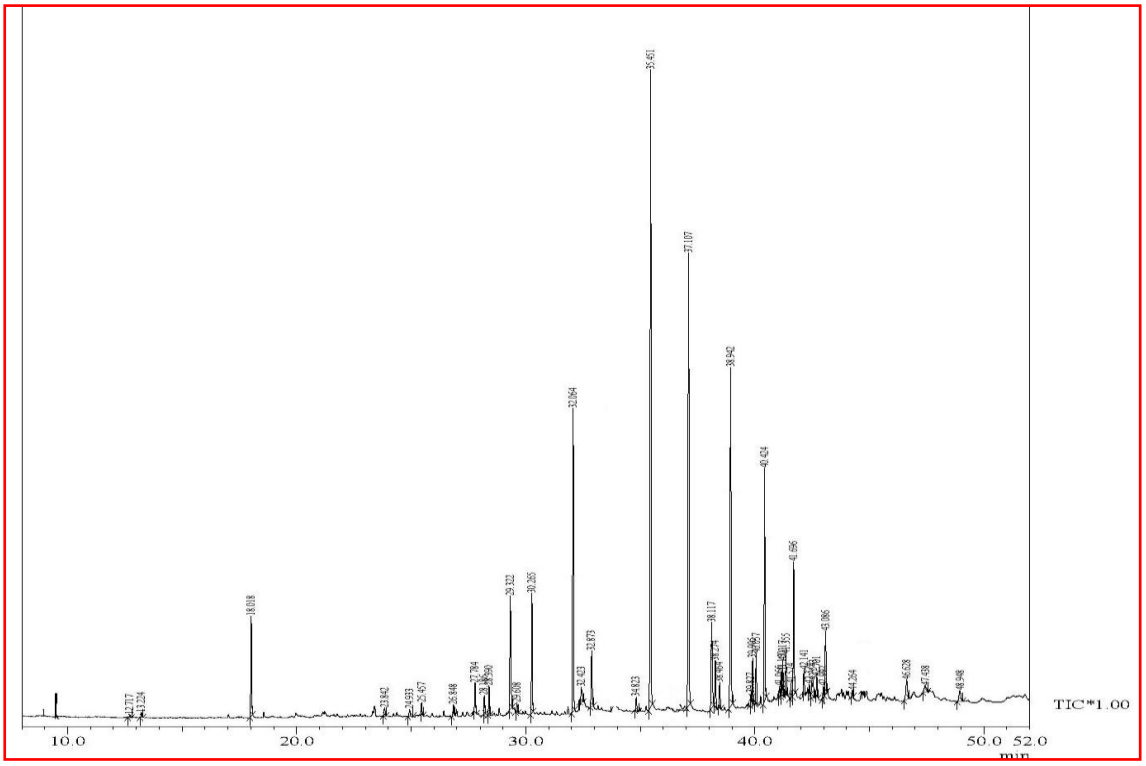


Fig. 6.16 (a) GC-MS of red rLDPE (blank)

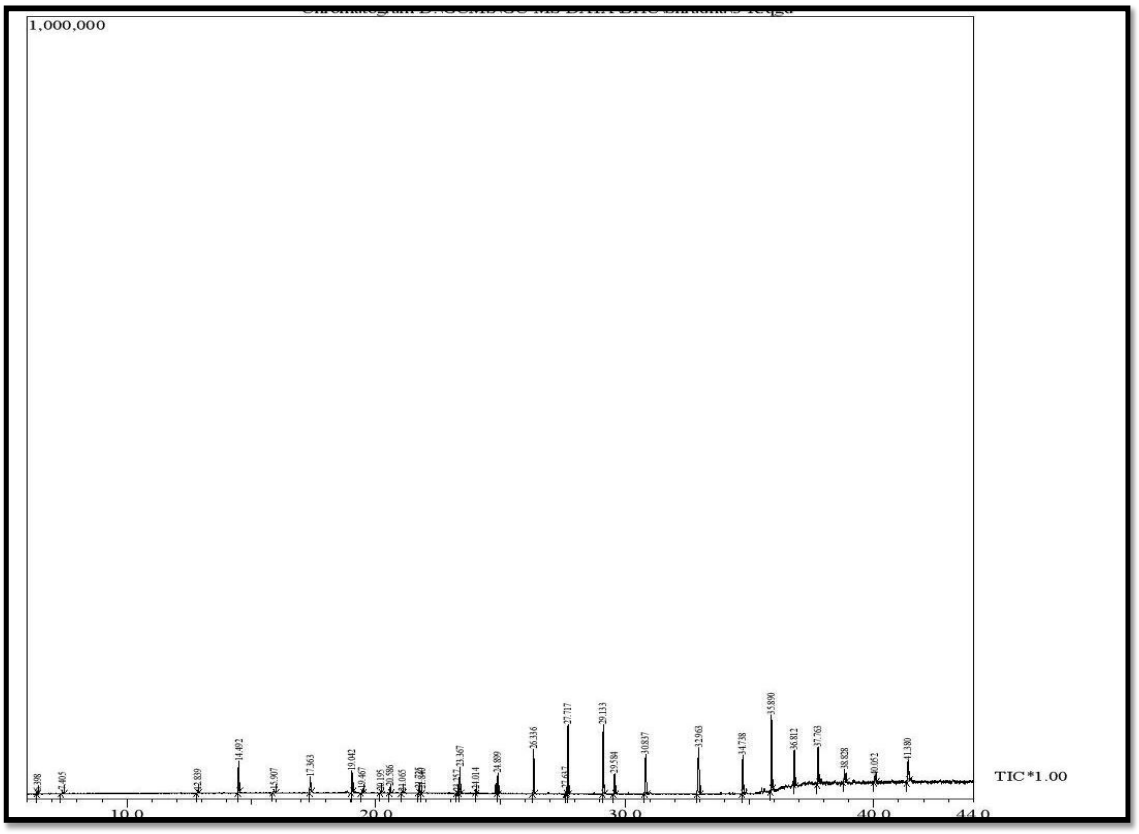


Fig. 6.16(b) GC-MS of red PE after biodegradation

6.2.8.5 GCMS of black LDPE (blank) film

The compounds present in black LDPE film are alkanes and their derivatives shown in table 6.1 and respective chromatogram (Fig.6.17a)

6.2.8.6 GCMS of biodegraded black LDPE

Black LDPE film degradation by fungal culture resulted into formation of different compounds. Intermediates formed after biodegradation of films are oxidised compounds like acids, esters and short carbon chain alkanes. The structure of compounds is presented in table 6.2 and chromatogram in fig.6.17b.

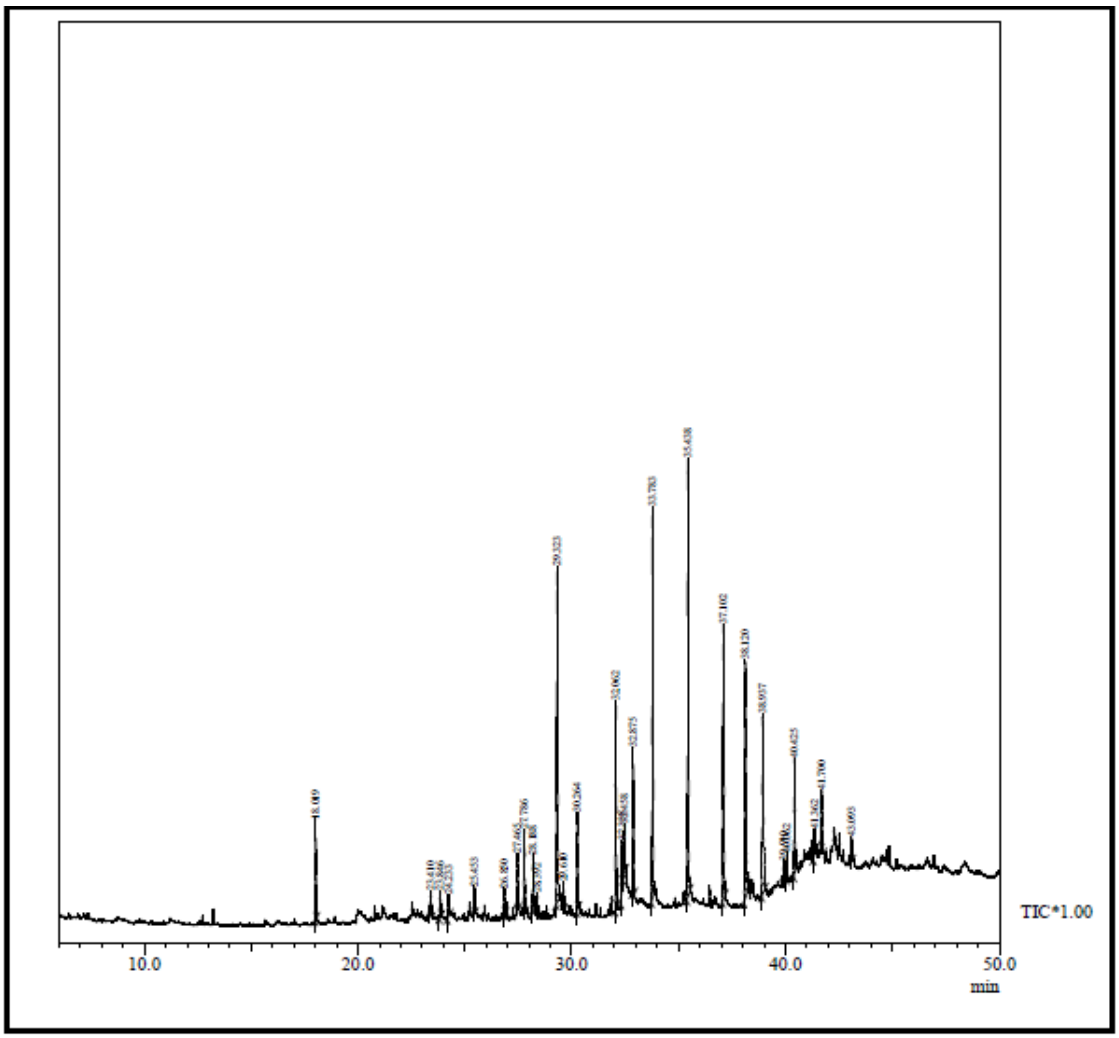


Fig. 6.17(a) GC-MS of black rLDPE (blank)

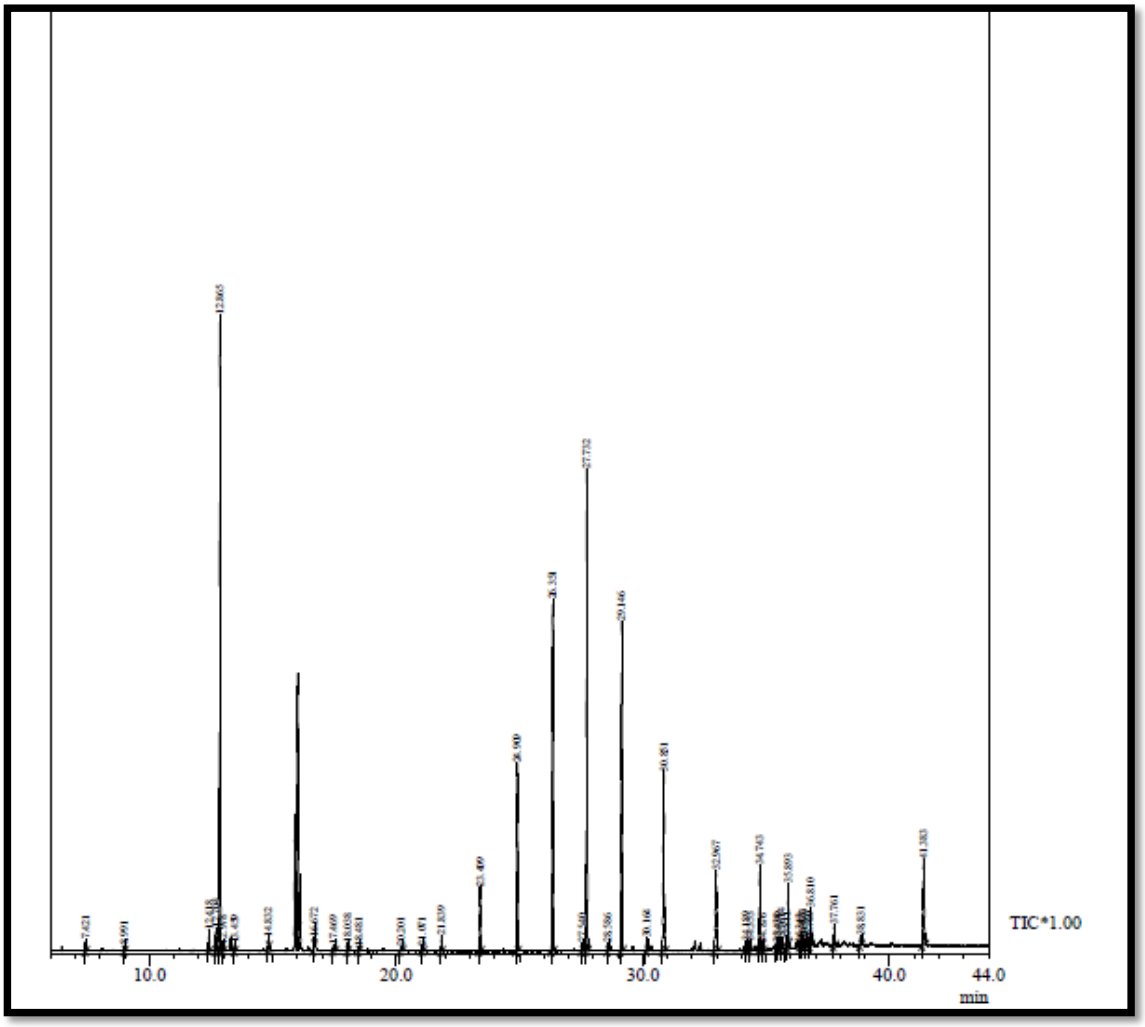


Fig. 6.17(b) GC-MS of black rLDPE after biodegradation

Table 6.1 Showing compounds in blank rLDPE films

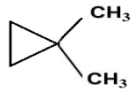
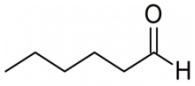
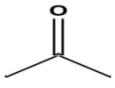
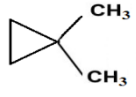
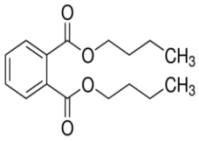
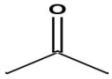
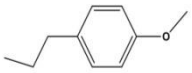
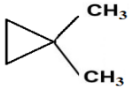
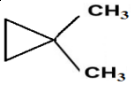
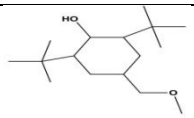
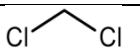
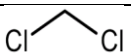
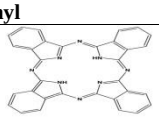
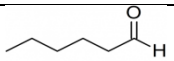
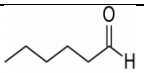
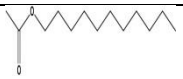
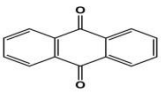
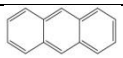
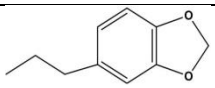
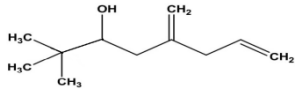
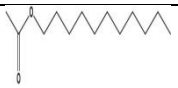
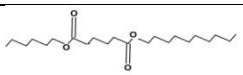
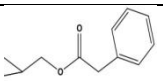
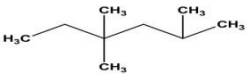
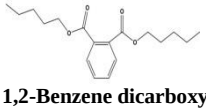
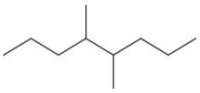
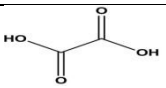
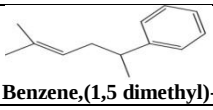
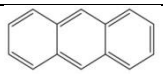
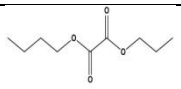
Compound	Green	Red	Black
 **Cyclopropanyl1,1-dimethyl-**	Present	Present	
 **Hexanal**			Present
 **2-Propanone**	Present		Present
 **Cyclopropanyl1,1-dimethyl-**	Present	Present	Present

Table 6.2 showing main by products resulted after biodegradation of rLDPE films

Green	Red	Black
 Dibutyl thalate	 2-Propanone	 2-Propanone
 Benzene, 1-methoxy-4-(2-Propenyl)	 Cyclopropanyl1,1-dimethyl-	 Cyclopropanyl1, 1-dimethyl-
 Phenol,2,6Bis-(1,1-dimethyl ethyl)-4-ethyl	 Methane di chloro-	 Methane di chloro-
 Pthalocyanin	 Hexanal	 Hexanal
 Hexadecanoic acid	 Anthraquinone	 Anthracene
 Benzene,1 ,2-methylene[dioxy]4-]	 2,2-dimethyl, 4,6- hepta diene-3 – ol	 Acetonyl decyl ether
	 Hexanedioic acid, dioctyl ester	 Benzeneacetic acid-2- methyl propyl es
	 Hexane-3,3,4-trimethyl-octane,4,5-dimethyl	 1,2-Benzene dicarboxylic acid dibutyl (
	 Octane, 4, 5- methyl	 Oxalic acid
	 Benzene,(1,5 dimethyl)-4-methyl-	 Anthracene
		 Butyl propyl ester

6.2.9 Optimization of parameters responsible for the biodegradation of rLDPE films

6.2.9.1 Optimization of parameters for green rLDPE

The effect of pH on the ability of *Rhizopus oryzae* NS5 to utilize polyethylene as a sole source of carbon was determined at different pH values (pH 3-10).The reduction in weight was minimum (0.8, 0.9 and 1.1 and 1.3%) at pH 3,4,9and10 respectively, as these are the extreme conditions. Maximum weight loss obtained (24.9%) was at pH 6 (Fig.6.18 a).

Temperature plays a very important role in biodegradation as metabolic activities of fungus very much depend on temperature. Optimization of temperature was done from 25 -50^{o}C and it was observed that weight loss increased from temperature 30 to 40^{o}C very drastically. At 40^{o}C weight loss was maximum as laccase activity is maximum at 40-50^{o}C (Chefetz *et al.*1998). Weight loss decreases at temperatures below 40 and above 40^{o}C it might be probably due to unfavourable temperature for the metabolic and enzymatic activities. (Fig.6.18 b).

Weight loss also depends on the agitation speed of rotary shaker. Experiments were performed at varying speeds (100-160 rpm). It was observed as the maximum wt. loss (24.2%) was found at 120 rpm while it was slightly less i.e. 21.6% and 20.1% at 110 rpm and 130 rpm respectively. Wt. loss was lowest at 100 and 160 rpm (1.6% and 1.8%). Thus optimum pH, temperature and agitation speed for the green polyethylene was found to be 6, 40^{o}C and 120 rpm with *Rhizopus oryzae* NS5.

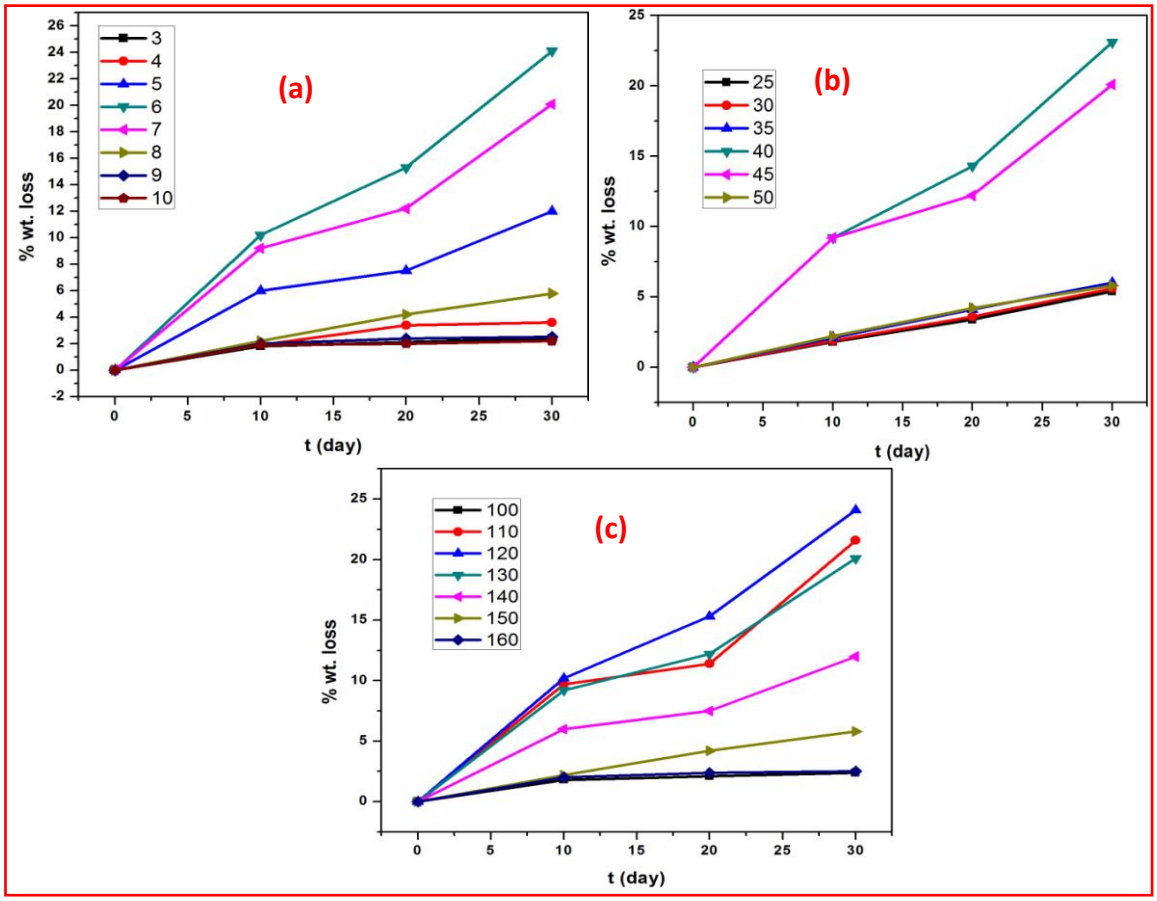


Fig.6.18 Optimization of pH(a),temp.(b) and agitation speed(c) for the biodegradation of green rLDPE in Potato Dextrose Broth. Conditions: shaking condition, 10% inoculums size

6.2.9.2 Optimization of parameters for red rLDPE

The reduction in weight was minimum (0.9, 1.0, 1.7 and 1.9%) at pH 3, 4, 9 and 10 respectively. Weight loss was maximum (28.1%) at pH6. It might be possibly because optimum pH for the growth of *R. oryzae* is 3.4 to 6.0 (Kurniawati *et al.* 2014). Weight loss was 0.80, 0.87, 0.92 and 1.4% at temp. 25, 30, 45 and 50^{o}C respectively. Maximum decrease in weight was observed at 40^{o}C it might be due to activity of laccase and manganese peroxidase is higher at 40^{o}C in comparison to lower temperatures. Percent decrease in the wt. was 0.7, 1.2, 1.3 and1.4% at 100,140,150 and 160 rpm respectively. There was an increase in weight loss above pH 5, temp 30^{o}C and agitation speed 100 rpm.

Maximum weight loss obtained (30.0%) was at pH 6, temperature 40^{o}C and 120 rpm agitation speed (Fig.6.19 a-c).

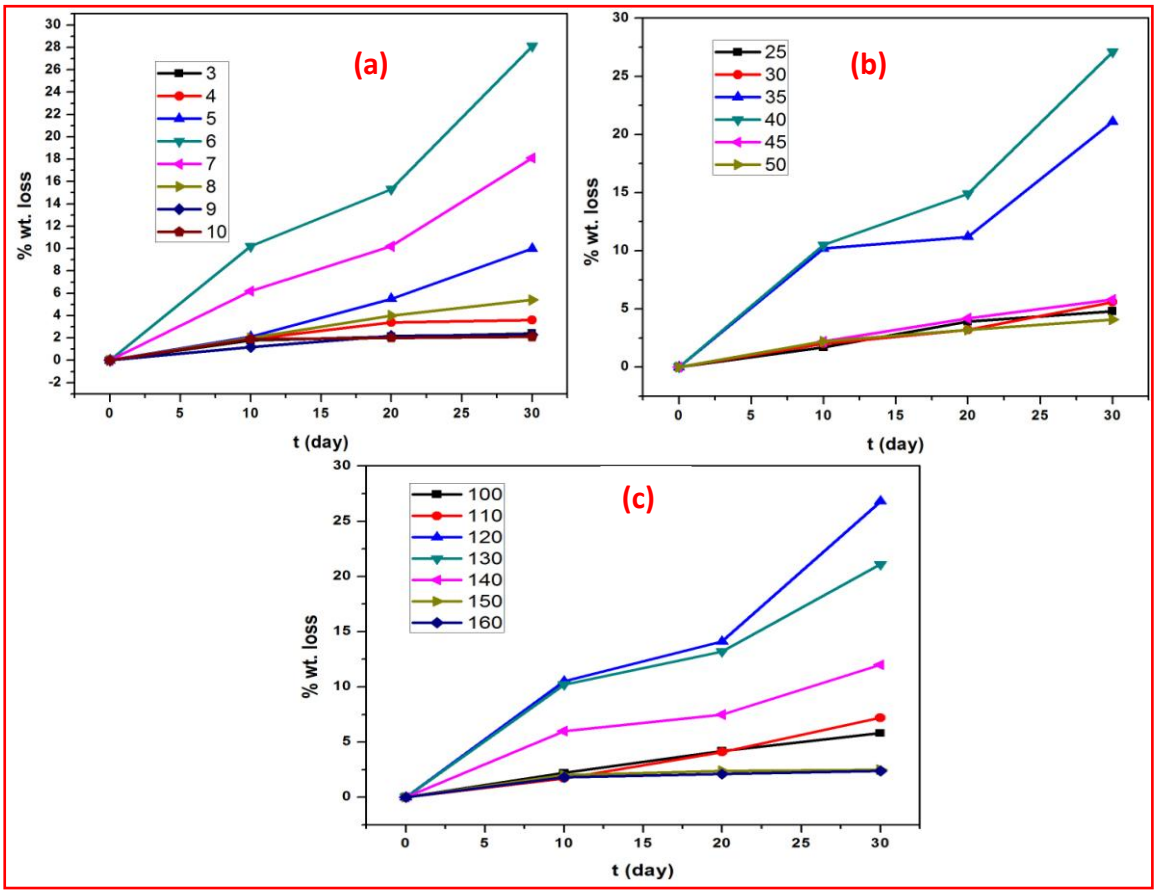


Fig.6.19 Optimization of pH (a), temp. (b) and agitation speed (c) for the biodegradation of red rLDPE

6.2.9.3 Optimization of parameters for black rLDPE

The reduction in weight was minimum (0.9, 1.0, 1.7 and 1.9%) at pH 3, 4, 9, 10 respectively. Weight loss was 0.9, 0.97, 0.98 and 1.5% at temperature 25, 30, 45 and 50 ^{o}C respectively. Decrease in the wt. was 0.5, 1.2, 1.5 and 1.6% at 100,140,150 and 160 rpm respectively. There was an increase in weight loss above pH 5, temp 30^{o}C and agitation speed 100 rpm. Maximum weight loss (30.0%) was at pH 6, temperature 40^{o}C and agitation speed 120 rpm (Fig.6.20 a-c).

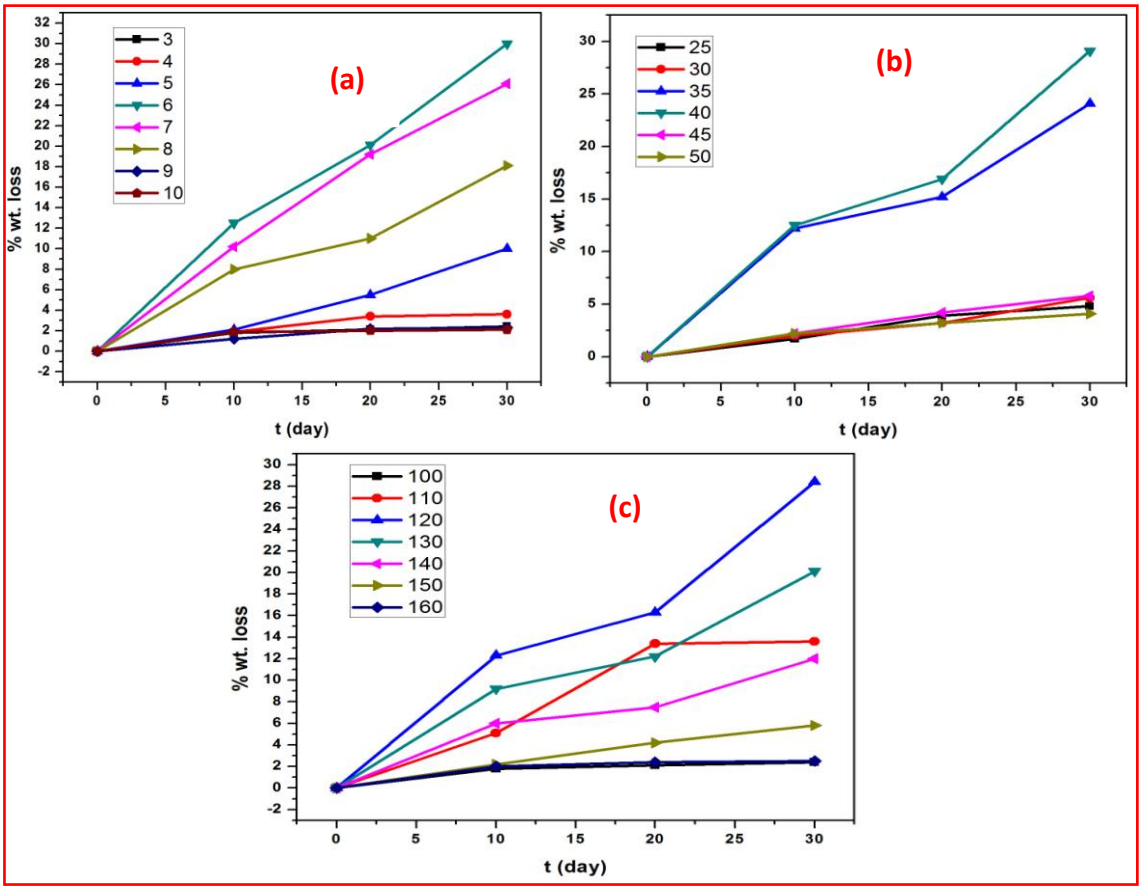


Fig. 6.20 Optimization of pH (a), temp. (b) and agitation speed (c) for the biodegradation of black rLDPE

6.2.10 Kinetics of the biodegradation of rLDPE films by *Rhizopus oryae* NS5

Increase in the cell concentration and decrease in the weight of the polyethylene films of different grades were studied at different time intervals. It has been observed that there was an increase in the cell biomass with time and this increase was complemented by a decrease in the weight of polyethylene films. This confirms that fungus utilises the PE films as carbon source for its growth. The fungal cells were viable for 30 days on the PE surface after 30 days there was very negligible increase in cell biomass and decrease in weight of PE films as well. Cell growth kinetics in a batch process can be represented by the eqn.

$$dX/dt = \mu X \quad \text{.....Eqn 6.1}$$

The specific growth rate of fungal cells in the presence of different grades LDPE films were determined from the slope of $\ln(X/X_0)$ against time, during the exponential growth phase as shown in Figure (6.21-6.23), here X_0 represents the initial cell biomass, X is the cell biomass at different time (days) intervals. The results show that the specific growth for green PE is 0.048 day^{-1}, for red PE 0.0493 day^{-1} and for black film is 0.0513 day^{-1}. A good fitting was obtained as shown in Figure 6.21(a-b), 6.22 (a-b) and 6.23(a-b). The parameters with their values are shown in Table 6.3(a-c) with R^2 of 97%. It has been observed that there is a decrease in the weight of the LDPE films with time. Slope of the graph was calculated and found to be 0.002, 0.002 and 0.003 $gday^{-1}$ for green, red and black LDPE films respectively.

6.2.10.1 Kinetics of biodegradation of green LDPE film by *Rhizopus oryae* NS5

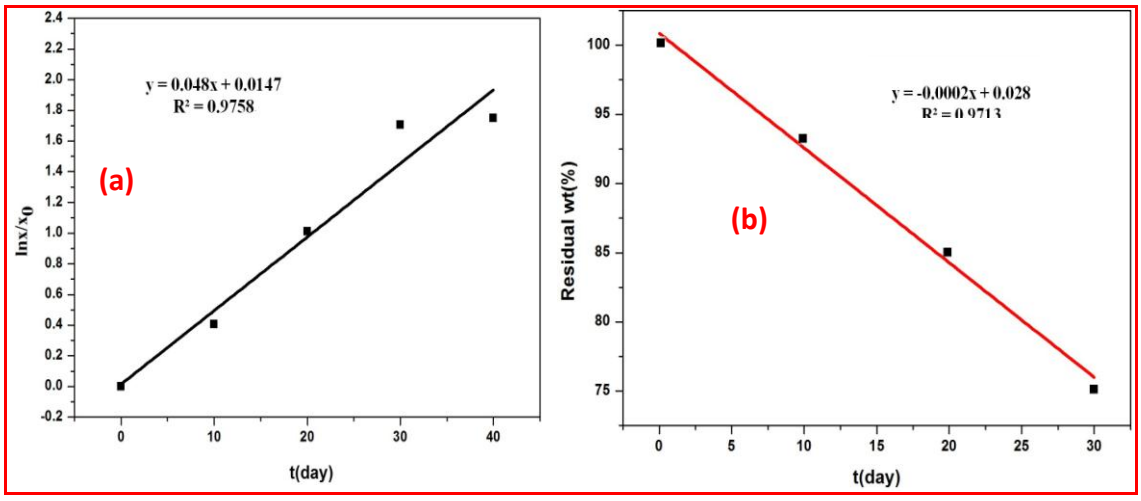


Fig.6.21 increase in cell growth (a) and decrease in weight of green PE film (b) with time

The values are the means of three replicates with the standard deviation which was within 5% of the mean.

Figure 6.21(a), 6.22(a) and 6.23(a) show the variation of biomass concentration with time. Biomass concentration increases with time which indicates that the microbes

utilized the PE films as energy and carbon source. A period of exponential growth phase was observed between 10 to 30 day, where the maximum biomass concentration reached and correspondingly maximum weight loss is observed. After 30 days following the exponential growth, a death phase was observed where increase in the biomass gets ceased.

Increase in cell biomass is maximum between day 20 and 30 (exponential phase) which is complemented by maximum decrease in weight of PE fim (Fig. 6.21(a-b), 6.22 (a-b) and 6.23(a-b).

Table 6.3(a) Rate constants for cell growth and degradation of green film

k(gday^{-1})	μ(day^{-1})	r^2(film)	r^2 (Fungal growth)
-0.0002	0.048	0.9713	0.9758

The values are the means of three replicates with the standard deviation which was within 5% of the mean.

6.2.10.2 Kinetics of biodegradation of red LDPE film by *Rhizopus oryae* NS5

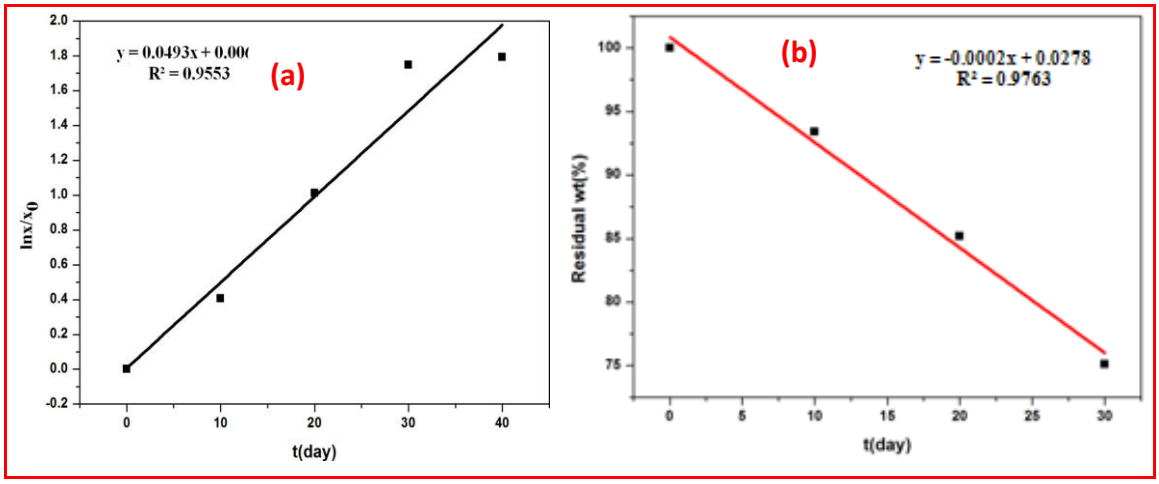


Fig. 6.22 increase in cell growth (a) and decrease in weight of red PE film (b) with time

Table 6.3(b) Rate constants for cell growth and degradation of red film

k ($gday^{-1}$)	μ(day^{-1})	r^2(film)	r^2 (Fungal growth)
-0.0002	0.0493	0.9763	0.9553

The values are the means of three replicates with the standard deviation which was within 5% of the mean.

6.2.10.3 Kinetics of biodegradation of black LDPE film by *Rhizopus oryae* NS5

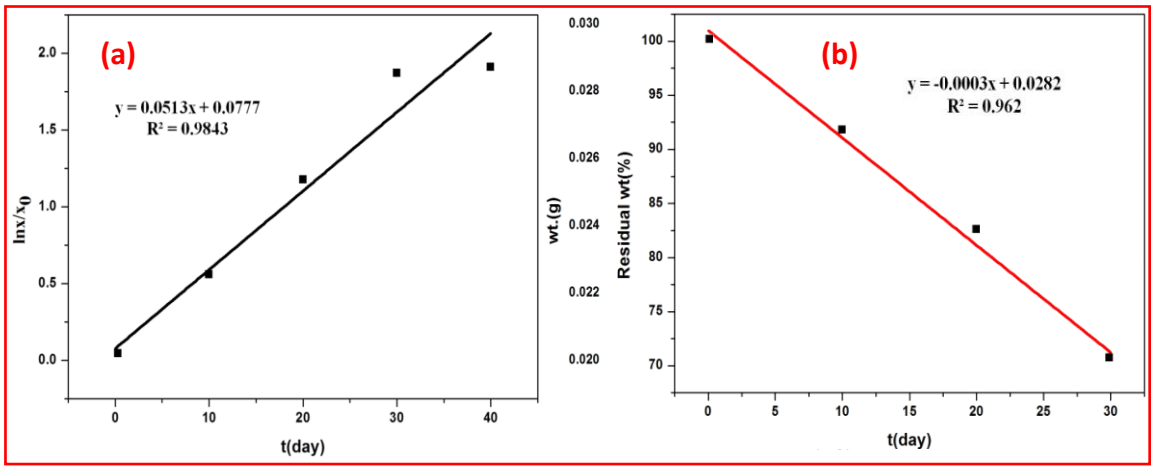


Fig.6.23 increase in cell growth (a) and decrease in weight of black PE film (b) with time

Table 6.3(c) Rate constants for cell growth and degradation of black film

k ($gday^{-1}$)	μ(day^{-1})	r^2(film)	r^2 (Fungal growth)
-0.0003	0.0513	0.962	0.9843

The values are the means of three replicates with the standard deviation which was within 5% of the mean.

6.2.11 Enzymatic activity of Laccase and Manganese peroxidase rLDPE films by *R. Oryzae* NS5

Rhizopus oryzae NS5 gives positive results for laccase and manganese peroxidase production. Enzyme activity was calculated at regular time interval and an increasing pattern of enzyme activity with time for both of the enzymes was observed. Maximum enzyme activity was observed between 20 and 30 day (Table 6.4(a-b), 6.5 (a-b) & 6.6(a-b)) which was consistent with the increase in weight of fungal mycelia and correspondingly decrease in wt. of rPE films (fig. 6.1(a-c)).

Table 6.4(a) Laccase activity for green rLDPE

Days	10	20	30
Enzyme activity(IU/ml)	0.00043±0.0003	0.00059±0.0001	0.00106± 0.0002

Table 6.4(b) Manganese peroxidase activity for green rLDPE

Days	10	20	30
Enzyme activity (IU/ml)	0.00047±0.0001	0.00063±0.0004	0.00113±0.0002

Table 6.5(a) Laccase activity for Red rLDPE

Days	10	20	30
Enzyme activity (IU/ml)	0.00045±0.0003	0.00061±0.0001	0.00112± 0.0002

Table 6.5(b) Manganese peroxidase activity for Red rLDPE

Days	10	20	30
Enzymeactivity (IU/ml)	0.00051±0.0001	0.00067±0.0004	0.00117±0.0002

Table 6.6(a) Laccase activity for Black rLDPE

Days	10	20	30
Enzyme activity(IU/ml)	0.00047±0.0003	0.00065±0.0001	0.00114± 0.0002

Table 6.6(b) Manganese peroxidase activity for Black rLDPE

Days	10	20	30
Enzyme activity (IU/ml)	0.00055±0.0001	0.00069±0.0004	0.00123 ±0.0002

Activity of manganese peroxidase (0.00113 ±0.0002 IU/ml) was more compared to laccase activity (0.00106± 0.0002 IU/ml) for green rLDPE (Table 6.4 (a-b) after 30 days incubation with green LDPE, it was again higher for red LDPE (0.00117 ±0.0002 IU/ml) than laccase (0.00113 ±0.0002 IU/ml) (Table 6.5 (a-b)).Similarly Activity of manganese peroxidase (0. 0.00123 IU/ml) was more than laccase activity (0.00114 IU/ml) (Table 6.6 (a-b) after one month of incubation with black LDPE. It shows that manganese peroxidise is more responsible for the biodegradation of recycled carry bags. Results obtained for the biodegradation of waste polyethylene films by Awasthi *et al* (2017) and showed similarity with our findings. Sawmya *et al* reported biodegradation of polythene by fungal consortia and reported that activity of manganese peroxidase was more in comparison to laccase after 3 months (Sawmya *et al.* 2014).There is no study on the enzymatic activity of microbe biodegrading recycled PE films.

6.3 Conclusion:

In the present study, lab isolate fungal strain, *Rhizopus oryzae* NS 5 (ITCC no. KT160362), capable of degrading recycled LDPE carry bags of different grades without any pretreatment. The degradation has been confirmed by morphological changes,

increase in surface roughness, weight loss, and changes in mechanical properties and functional groups. Intermediates formed were analysed by GC-MS. The existing observation gives a subsidiary to the corroborations of biodegradation of recycled LDPE. It shows that there is a remarkable possibility of biodegradation of other types of recycled plastics by this fungal strain. Further studies are in progress for the isolation of microorganisms with the potentiality of degrading untreated LDPE. Presently our efforts are focused on expounding the pathway for the degradation of recycled low density polyethylene carry bags and developing a new bioremediation strategy using this fungus.