2.1 Introduction

Plastic is an artificial and synthetic polymer of great concern from the environmental safety point of view. Globally, about 100 million tonnes of plastic is produced annually according to a report of Greenpeace International (2014). Synthetic polymers including low density polyethylene and high density polyethylene are widely used in the industry due to their ease of processability and durability. Moreover, multipurpose, lightweight, strong, potentially transparent, inexpensive, exquisite oxygen/moisture barrier and non biodegradable nature makes them outstanding material for packaging (Andrady 2011). The most common polyethylene types are: Low Density Polyethylene (LDPE), High Density Polyethylene (HDPE), Linear Low Density Polyethylene (LLDPE) and Cross Linked Polyethylene (XLPE). They differ in their density, degree of branching and availability of functional groups on the surface (Table 2.1)

Table 2.1 Classification of polyethylene by density

PE Type	Density (g/cm^3)
Low	0.910 – 0.925
Medium	0.926 – 0.940
High	0.940 – 0.959
High density homopolymer	0.96 and above

Polyethylene rapidly accumulates in large quantity because of its recalcitrance to biological degradation. Its recalcitrant nature has been attributed to its complicated three-dimensional structure (Contat-Rodrigo and Ribes Greus, 2002, Nanda *et al.* 2010). Further, chemicals (e.g. bisphenol and phthalates) added in plastic materials to give it certain special features have notable adverse effects on the endocrine system of humans

as well as animals **(**Ashakura *et al.* 2004). Moreover, the leachates from polyethylene are very toxic and cause various environmental health problems (Lithner *et al.* 2012).It has adverse effects to humans and natural environment in both rural and urban areas. Due to various negative effects and considerable accumulation of plastic to the environment, its remediation is the need of hour, challenging environmentalists and chemical engineers. Biodegradation of polyethylene has accepted as a cost effective and eco-friendly approach for the management of polyethylene pollution as other treatment methods are not environment friendly and economical (discussed in section 1.3 of chapter 1). Biodegradation has been recognized as an intrinsic activity in the microbial world. It plays a crucial role in recycling of materials in the natural ecosystems (Albertsson *et al.* 1987). Microorganisms can utilize polymers as carbon and energy sources for their growth. The PE surface is highly Hydrophobic and it is necessary to oxidise it for making it hydrophilic to some extent for the attachment of microbes. There is a strong synergism between biodegradation and environmental factors, and biodegradation can, in practice, never be entirely separated from the purely physical and chemical ageing. Methods for the pretreatment of polyethylene which are in practice are presented below.

2.2 Methods of pretreatment

2.2.1 Physical treatment: Polyethylene is oxidised by photo-oxidation and thermal treatment. In photo oxidation PE films are exposed to UV-B radiation (320-280 nm) at different exposure times (Feuilloley *et al.* 2005, Li *et al.* 2000, Romo *et al.* 2015 & Sheik *et al.* 2015). Abrusci *et al.* tested the biodegradability of photo-degraded polyethylene through *Bacillus cereus, B. megaterium, B. subtilis and Brevibacillus borstelensis* at 30 and 45°C. The maximum carbon mineralization was found at 11.5% and 7–10% with *B. borstelensis* and MIX (a mixed culture of *Bacillus cereus, B.*

megaterium and B. subtilis), respectively, at 45 °C (Abrusci *et al.* 2011). In thermal oxidation films are kept in hot air oven at different temperatures for different times (Brown *et al.* 2004). Thermal or radiation treatments on polyethylene reduce the polymeric chain size and form oxidized groups such as carboxyl, carbonyl and hydroxyl which are more easily degraded by microorganisms (Albertsson *et al*. 1995).

2.2.2 Chemical oxidation: The chemical modification methods available include dry (corona, flame, plasma) as well as wet (acid, alkali, electrochemical) treatments, all of which results in the introduction of a variety of new functionalities (Briggs *et al.* 2003). These treatments modify the properties such as crystallinity level and morphological changes of the original polymer, and facilitate the polymer biodegradation (Lee *et al.* 1997).

2.2.3 Metal catalysed oxidation of Polyethylene: Manganese, iron and cobalt are used as prooxidants in polythene (Fontanella *et al.*2010). It reduces the strength of hydroxyls and carbonyls by changing their structure. Peroxidant additives are employed in polyethylene manufacturing for agriculturally used plastic, this type of polyethylene showing susceptibility to thermal and photochemical mineralization in vitro (Patahk *et al.* 2017).

2.3 Microenvironments for the study of PE biodegradation

There are various microenvironments which are used for the study of polyethylene biodegradation (Table 2.2). These are as follows:

2.3.1 Marine exposure conditions

Polyethylene are significantly persistent in the marine environment which results in exposure to physical, chemical and biological processes causing to their fragmentation

down into smaller pieces. polyethylene present in surface waters are more prone to degradation compared to those on the seafloor, for which decomposition is made longer because of the cold water temperature and reduced sunlight (UV) penetration (UNEP year book, 2014, Pegram and Andrady, 1989,Artham *et al.* 2009, Lobelle and cunliffe 2011, Albrtsson *et al*.1980, Albertsson *et al.* 1987 & Balasubramanian *et al*. 2010).

2.3.2 Soil burial conditions

Soil burial is one of the frequently used methods for the determination of biodegradability of polyethylene (Yang *et al.* 2005, Eya *et al*. 2002). In this method, biodegradation test is performed under natural conditions or laboratory conditions. Sample with definite weight and dimension is buried in specific depth under the soil for different time intervals. After a specified time, sample is taken out of soil, thoroughly rinsed with distilled water following immersion in distilled water and after that dried at 50^{o}C for 24 h in a vacuum oven after this it is allowed to equilibrate to ambient temperature and humidity for at least 24 h before measurement. In a study by Mumtaz *et al.* it was reported that soil-buried LDPE showed active microbial growth on LDPE in 7–9 months, and surface deterioration was confirmed within 17–22 months as determined by SEM analysis (Mumtaz *et al.* 2010).

2.3.3 Compost conditions

In this method, the definite weight of the dry polyethylene is subjected to the mixture of definite amount of mature compost and then incubated at 58^{o}C with maintained moisture content at 65%. Biodegradation is measured based on the amount of material carbon converted to gaseous carbon dioxide (Corti *et al.* 1992). Nature of compost affects the degree of degradation (Yang *et al.* 2004, Bellia *et al.* 1999 & Pandey

et al. 2003). For example both thermophilic bacteria and thermophilic actinomycetes (thermophilic microorganism thrive above 45^{o}C and some live at or even above the boiling point of water) are fewest in the compost stored at 20^{o}C as expected, indicating that thermophilic microbes are more susceptible to stress in the freezing conditions than the mesophilic ones (Yang *et al.* 2004). Activity of the extracellular enzymes plausibly excreted by the microbes in the compost decreased as a result of the storage (Yang *et al.* 2004). Shape of the plastic sample (Yang *et al.* 2005) and additives in the compost (Jang *et al.* 2002) effect the plastic degradation in the compost. Vermiculite, a clay mineral, can be activated and used as a solid matrix in place of mature compost. The composting test method with activated vermiculite is a comprehensive system for the evaluation of the environmental impact of biodegradable polyethylene. Biodegradation rate as well as the final biodegradation level are not affected by activated vermiculite. Alternatively, possible metabolic intermediates and polymeric residues left after biodegradation can be recovered more effortlessly from activated vermiculite than from mature compost, a very complex organic matter (Innocenti *et al.* 2001).

Table 2.2 Different microenvironments used in the study of polyethylene biodegradation

Microenvironment	Reference
Marine exposure conditions	Pegram and Andrady, 1989,Artham *et al*. 2009, Lobelle and cunliffe, 2011 Albrtsson, 1980, Albertsson *et al*. 1987
Soil burial conditions	Karlsson *et al*.1988, Albertsson and karlsson 1990, Orhan and Buyukgungor, 2000
Composting conditions	Husarova *et al.* 2010a Chiellini *et al*. 2003, Mumtaz *et al*. 2010, Nowak *et al*. 2011,Chiellni *et al.* 2003

2.4 Microorganisms involved in polyethylene degradation

Biological agents, both prokaryotic (bacteria) and eukaryotic (fungi, algae and plant), are involved in the bioremediation process.

2.4.1 Algae

Scenedesmus dimorphus, Anabaena spiroides , Navicula pupula have been reported by Kumar *et al.* for the biodegradation of polythene (Kumar *et al.* 2017). 15 algal taxa, including *Chaetophora, Coleochaete scutata, Coleochaete soluta, Aphanochaete, Gloeotaenium, Oedogonium, Oocystis, Oscillatoria, Phormidium, Chroococcus, Aphanothece, Fragillaria, Cocconis, Navicula and Cymbella* were identified by Das *et al.* (Das *et al.* 2012) (Table 2.3)

Table 2.3 Algal strains associated with polyethylene biodegradation

Algal strain	Targeted Polyethylene	Brief conclusion of the experiments by authors	Reference
Scenedesmus dimorphus	Polythene	3.74% (+/-0.26) weight loss	Kumar *et al.* 2017
Anabaena spiroides	Polythene	8.18% with +/-0.66 weight loss	Kumar *et al.* 2017
Navicula pupula	Polythene	4.44% (+/-0.82) weight loss	Kumar *et al.* 2017
Chaetophora, Coleochaete scutata, Coleochaete soluta, Aphanochaete, Gloeotaenium, Oedogonium, Oocystis, Oscillatoria, Phormidium, Chroococcus, Aphanothece, Fragillaria, Cocconis, Navicula Cymbella	Polythene	colony proliferation on the surface of polythene by SEM study	Das *et al.* 2012

2.4.2 Bacteria

Most of the studies done on bioremediation of polyethylene waste employed bacteria for the degradation purpose owing to its very fast growth and easy adaptation in changing environmental conditions. *Pseudomonas, Bacillus subtilis, Staphylococcus aureus, Streptococcus lactis, Proteus vulgaris, Micrococcus luteus Pseudomonas, Streptomyces, Corynebacterium, Nitrosomonas sp., Nitrobacter winogradkyi*, *Burkholderia* sp., *Methylobactor* sp., *Methylococcus capsulatus, Methylocystic* sp. and *Methylocella* sp. Other bacterial sps utilising PE for their growth are listed in the Table 2.4.

Table 2.4 Bacterial strains associated with polyethylene biodegradation

Bacterial strain	Targeted Polyethylene	Brief conclusion of the experiments by authors	Reference
Klebsiella pneumoniae CH001	HDPE	18.4% wt loss in 60 days	Awasthi *et al*. 2017
Lysinibacillus fusiformis	Polyethylene	Wt. loss of 2.97% in 30 days	Mukherjee *et al*. 2017
Comamonas, Delftia, and Stenotrophomonas	Untreated PE	46.7% decrease in viscous area	Peixoto *et al.* 2017
Stenotrophomonas pavanii CC18	modified LDPE [blend of titania (TiO2) and starch].	25% wt loss after 56 days in starch containing LDPE	Mehmood *et al.* 2016
Lysinibacillus fusiformis VASB14	Polyethylene	21.87 ± 6.37 % wt loss after 2 months	Shahnawaz *et al.* 2016
Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas burkhplderia, Flavobacterium dominate	LDPE	1.5% wt loss in 150 days	Veethahavya *et al.*2016
Marine microbes	Commercial PE bags & biodegradable PE	1.5% wt loss in 90 days	Nauendorf *et al*. 2016
Burkholderia sp	HDPE	15%–20% HDPE degradadtion in 3 months	Muenmee *et al*. 2016
Bacillus Licheniformis *Lysinibacillus fusiformis*	Polyethylene	3.0% wt loss in 3 months 2.9% wt loss in 3 months	Mukherjee *et al*. 2016
Achromobacter Denitrificansstrain S140 S1 20	LDPE	40% wt loss in 2 months 60% wt loss in 2 months	Devi *et al.*2015
Bacillus amyloliquefaciens	LDPE	% mineralization of 14.7% in 60 days	Das *et al.* 2014
Pseudomonas aeruginosa	Low molecular weight	40.8% of the carbons of LMWPE into CO_2 after	

E7	Polyethylene (LMWPE)	80 days of biodegradation in compost at 37 °C	Jeong *et al*. 2015
Methylococcus capsulatus,Acinetrobacter spp. and *Flavobacteria* spp	HDPE, LDPE	Wt. loss of HDPE and LDPE was15%, 4.96% in 1 year, k=00.128y^{-1},0.048y^{-1} for HDPE and LDPE respectively	Muenmee *et al*. 2015
Aspergillus flavus VRKPT2 *tubingensis* VRKPT1	HDPE	6% wt loss 8.51 ± 0.1% wt. loss	Devi *et al*. 2015
P. fluorescens	Polyethylene	8.06%loss in 1 mnth in lab conditions,16% loss in 12 mnth in field condition.	Thomas *et al.* 2015
Pseudomonas sp. AKS2	LDPE	Enhanced microbial growth with 26% surface hydrophobicity and 31% hydrolytic activity	Tribedi *et al.* 2015
Bacillus amyloliquefaciens	LDPE	16% wt loss in 60 days	Pal *et al.* 2015
Pseudomonas citronellolis	LDPE	17.8% in 4 days	Bhatia *et al.* 2014
Lysinibacillus xylanilyticus	LDPE	7.6% wt loss in untreated PE, 8.6% in UV treated PE	Esmaeili *et al*. 2013
Kocuria palustris M16	LDPE	1% reduction in wt. In 30 days	Harshvardhan *et al*. 2013
Pseudomonas sp. E4	LMWPE	28.6, 14.9, 10.3 and 4.9% carbon mineralization in 80 days	Yoon *et al.* 2012
Brevibacillus parabrevis PL-1, *Acinetobacter baumannii* PL-2, *A. baumannii* PL-3 *P. citronellolis* PL-4	LDPE	Wt loss of 0. 7042% 1. 0603% 1. 0604% 0. 5706%	Pramila *et al.* 2012
Cladosporium cladosporoides	Photodegradable Polyethylenefilms (Plastor®)	20% reduction in Avg MWt. reduction in 30 days	Santo *et al.* 2012

M. paraoxydans *P. aeruginosa*	LDPE	61.0% wt loss in 2 months 50.5% wt loss in 2 months	Rajandas *et al.* 2012
Bacillus borstelensis *Bacillus cereus* *Bacillus* megaterium *Bacillus subtilis*	Thermal and photodegraded LDPE	11.5% mineralization 7-10% mineralization	Abrusci *et al.*2011
B. mycoides, B.cereus, B. amyloliquefaciens, B. Pumilus	LDPE modified with Bionelle	98% reduction in TS	Nowak *et al.* 2011
Bacillus thuringiensis	LDPE modified with Bionelle	Microbes were attached even after 225 days,	Nowak *et al,* 2011
Bacillus amyloliquefaciens	LDPE modified with Bionelle	Microbes were attached in the beginning of the biodegradation process	Nowak *et al.* 2011
Acinetabacter baumanii	LDPE modified with Bionelle	Molecular weight was changed	Nowak *et al.* 2011
Rhodococcus rhodochrous	HDPE,LDPE and LLDPE with prooxidants	Oxidation level of prooxidant film was increased	Fontanella *et al.*2010
Diplococcus, Micrococcus, Moraxella	HDPE & LDPE	HDPE 16-20% reduction in TS , LDPE 12-13% reduction in TS in 4 months	Vijaya *et al.* 2008
Arthrobacter sps	HDPE and LDPE	Reduction in Carbonyl index	Balasubramanian *etal.* 2010, Satlewal *et al.*2008, sudhakar *et al.* 2008, Roy *et al.* 2008
Rhodococcus r*uber*	Polyethylene films	7.5% reduction in presence of mineral oil,	Gilan *et al.* 2004
Nocardia asteroids	Green film containing TDPA®	Reduction in mol wt	Bonhomme *et al.* 2003

Bacillus pumilus, Bacillus halodenitrificans cereus	LDPE	8.4 ± 1.37% weight loss	Roy *et al.* 2008
Bacillus subtilis	LDPE	9.26% wt loss in 30 days	Satlewal *et al.* 2008
Bacillus cereus, B. Halodenitrificans	LDPE	8.4% in 2 weeks	Roy *et al*. 2008
B.Pumilus, B.arthrobacter, B.Cereus	LDPE & HDPE	22.7% wt reduction in 2 weeks(HDPE),21.3% wt reduction in 2 weeks(LDPE)	Satlewal *et al*. 2008
B. mycoides	LDPE	0.01% wt loss	Seneviratne *et al*. 2006
Arthrobacter paraffineus	LDPE + starch/Fe stearate Thermal pretreatment LDPE + starch, Mn stearate, + Styrenbutadien co polymer Thermal pretreatment	Low mol wt compounds formed	Albertsson *et al*. 1995,1998
Bacillus pumilus M27, *cereus* & *halodenitrificanus*	LDPE	Decrease in CI (1.29-0.31)	Roy *et al.* 2008, Satlewal *et al.* 2008 , Kawai *et al*. 2004
Bacillus cereus	LDPE HDPE	19% in LDPE (TT) & 9% in HDPE (TT) 10%,3.5% (untreated), Change in contact angle	Sudhakar *et al.* 2008,Nowak *et al.* 2004,Hadad *et al.* 2005,Kounty *et al.* 2009
Brevibacillus brostelensis	LDPE	11% decrease in gravimetric wt & 30% decrease in mol. wt	Hadad *et al.* 2005
Flavobacterium asteroides	Commercial environmentally degradable polyethylene	Erosion of film, Formation of polysachharide, Decrease in Carbonyl index	Bonhomme *et al*.2003
Rhodococcus ruber	Polyethylene	8% wt loss in 30 days	Gilan *et al.* 2004,

		0.86% wt loss per week	Sivan *et al.*2006
Rahnella aquatilis	Pre-aged modified PE films	Decrease in Carbonyl index in 225 days	Nowak *et al.* 2011
Pseudomonas aeruginosa *fluorescens*	Oxidised LDPE	50.2% reduction in wt of nitric acid pretreated LDPE 16% decrease in 72 months	Koutny *et al.* 2009, Rajandas *et al.* 2012, Thomas *et al.* 2015
Staphylococcus epidermidis *Cohnii* *Xylosus*	LDPE	Growth & cracks on the film Consortia resulted in tensile strength reduction, no wt loss observed	Chatterjee *et al.* 2010, Nowak *et al.* 2011
Nocardia asteroides	Green film (LC) containing TDPA®	Erosion of the film, Oxidation products formation andreduction in mol wt	Bonhomme *et al.* 2003, Koutny *et al.* 2006b

2.4.3 Fungi

The advantage of fungus used for polyethylene degradation over bacteria is due to increased cell to surface ratio, fungi have a greater physical and enzymatic contact with the environment. The fungus extracted extracellular enzyme is advantageous in tolerating high concentration of the toxicants. *Z. maritimum*is, is capable of utilizing PE, and causes a decrease in both mass and size of the pellets (Paço *et al.* 2017). *Aspergillus niger*, *Aspergillus glaucus***,** Actinomycetes sp. and *Saccharomonospora* (Swift *et al.* 1997) are also efficient in biodegradation of PE. Other fungal sps. utilising PE for their growth are listed in the Table 2.5

Table 2.5 Fungal strains associated with the polyethylene biodegradation

Fungal strain	Targeted Polyethylene	Brief conclusion of the experiments by authors	Reference
Rhizopus oryzae	LDPE	8.4±0.3% wt. Loss of LDPE in 30 days	Awasthi *et al*. 2017
Zalerion maritimum	LDPE	Increased acidic groups, No quantitative data	Paço *et al*. 2017
Penicillium chrysogenum NS10 *Penicillium oxalicum NS4*	HDPE and LDPE	55.598% wt loss of HDPE, 34.35% wt loss of LDPE 55.34% wt loss of HDPE, 36.60% wt loss of LDPE	Ojha *et al.* 2017
A. clavatus (strain JASK1)	LDPE	35% wt loss in 90 days, 2.32 g l^{-1} CO_2 evolution in 1 month From LDPE degraadtion	Gajendiran *et al*. 2016
Aspergillus sp. *Paecilomyces lilacinus* *Lasiodiplodia theobromae*	LDPE	decrease in mol wt 595.22 & viscosity (pose) 0.0125 898 &0.0204 pose 1704 &0.0388 pose	Sheik *et al.*2015
Streptomyces coelicoflavas 15399T	LDPE	30% wt loss of LDPE in 4 months	Duddu *et al.* 2015
Aspergillus terreus MF12	HDPE	9.4± 0.1% wt loss in 30 days	Balasubramanian *et al*. 2014
Aspergillus Niger *Glaucus* *Candidus* *Ornatus* *Nidulans* *Flavus* *Cremeus* *Oryzae* *Aspergillus oryzae*	HDPE HDPE and LDPE LDPE	3.44% in 30 days, 61 % TS reduction 18.48% TS reduction in HDPE , 12.15% in LDPE in 4 months 51% reduction in TS in 3 months	Mathur *et al*. 2011 Vijaya & Reddy. 2008 Konduri *et al*. 2011

Aspergillus Niger *versicolor* *Flavus* *Aspergillus versicolor* *Aspergillus sp*	Commercial polyethylene	0.5% mineralization , Amorphicity decreases Amorphicity decreases Mol. Wt. not decreased CO_2 evolution 3.8g/L in 1 week 4g/L in 1 week	Raghavan and Torma, 1992 Volke-Sepulveda *et al*. 2002, Manzur *et al*.2004, Karlsson *et al*.1988, Pramila and Ramesh 2011
Chaetomium Globosum	Polyethylene	7.5% wt loss in autoclaved, 21% in UV treated PE in 3 mnths	Sowmya *et al*. 2014
Cladosporium Cladosporiaides	Commercial environmentally degradable polyethylene	Growth of biofilm on the surface	Bonhomme *et al*. 2003, Koutny *et al*. 2006b
Fusarium Redolens	HDPE and LDPE	0.5% CO_2 evolution in 2 years	Albertsson *et al*.1980, karlsson *et al*. 1978, Albertsson and karlsson, 1990
Penicillium simplicissimum *Aspergillus niger* *Penicillium pinophilum*	LDPE	3.26% mineralization	Manzur *et al*. 2004
Martierella alpina	oxidized polyethylene film containing prooxidant additives	Cracks were developed on the surface	Kounty *et al*. 2006b
Mucor circinellaides	LDPE	5.9g/l CO_2 in 30 days	Pramila And Ramesh, 2011a
Phanerochaete Chrysasporium *Phanerochaete pinophilum* *Aspergillus niger* *Gliocladium nirens* *P. chrysosporium*	LDPE+starch blended film LDPE	56% reduction in TS in 3 months Decrease in MPt. & Crystallinity	Orhan and Buyukgungor *et al*.2000 Manzur *et al*. 2004

However, microorganisms having high degradation ability for PE is missing in the literature. Results show that Biodegradation depends upon polymer characteristics, organism type and pretreatment by abiotic means (Shah *et al.* 2008). There are various reports showing biodegradation of HDPE and LDPE in terms of weight loss without any pretreatment (Table 2.6).

Table 2.6 Weight loss percentage due to biological action for HDPE & LDPE in various environments without pre-oxidative treatment

Substrate	Environment	Time (d)	% of weight loss	Reference
LDPE	Water coal	225	-0.26	Nowak *et al*. 2011
	Forest soil	225	-0.13	Nowak *et al*. 2011
	Crater soil	225	-0.28	Nowak *et al*. 2011
	Sea water	365	-1.9	Artham *et al*. 2009
	Soil + *Fusarium redolens*	3650	-0.2	Albertsson and Karlsson, 1990
	Soil	800	-0.1	Albertsson, 1980
	Mineral media + *Rhodococcus ruber*	56	-7.5	Sivan *et al*. 2006
	Mineral media + *Rhodococcus ruber*	30	-2.5	Santo *et al*. 2012
	Mineral media + *Brevibacillus borstelensis*	30	-2.5	Hadad *et al*. 2005
	Mineral media + *Pseudomonas* sp	45	-5	Tribedi and Sil, 2013
	Sea water	365	-1.6	
HDPE	Soil	800	-0.4	Artham *et al*. 2009 Albertsson, 1980

2.5 Increased biodegradation by blending: Addition of readily biodegradable compounds such as starch to polyethylene enhances the degradation of carbon-carbon backbone. *P. chrysosporium* and *Streptomyces* sp. are known to degrade starch-blended polyethylene (Flavel *et al.* 2006). Similar results were reported by Psomiadou *et al.* for

starch-blended LDPE degradation by means of a reduction in mechanical properties (Psomiadou *et al.* 1997). Arvanitoyannis *et al.* attempted to make biodegradable blended LDPE with starch and found that starch 10% (w/w) content in blended LDPE enhanced its biodegradation rate by altering the mechanical properties. (Flavel *etal.*2006) reported biodegradation of starch-blended polyethylene by *P. chrysosporium* and *Streptomyces* sp.(Arvanitoyannis *et al.*1998). Husarova *et al.* investigated biodegradation of calcium carbonate (prooxidant) containing LDPE (Husarova *et al.* 2010a).They found that prooxidant containing LDPE degraded (carbon mineralization) 16% in 80 days, while samples that did not contain any prooxidant were mineralized 7% in 13 months under soil and 23% in compost conditions. Mehmood *et al.* isolated bacterial strains from a solid waste dump and identified *P. aeruginosa* CA9, *Burkholderia seminalis* CB12 and *Stenotrophomonas pavanii* CC18; these strains were tested for modified LDPE [blend of titania (TiO_2) and starch]. Out of three strains, CC18 showed enhanced viability and degradation (25% wt loss in 56 days) (Mehmood *et al.* 2016).

2.6 Surfactants/Biosurfactants enhanced biodegradation of polyethylene wastes

Nonionic surfactants can promote the polymer biodegradation by increasing the hydrophilicity of the polymer, which helps in the adhesion of microorganisms on the polymer (Hadad *et al.* 2005). Hydrocarbon solubilisation ability of surfactants has been utilised for the deterioration of polyethylene by Mukherji *et al* they reported that PE degradation was higher (7.006 ± 0.05%, 1.76 ± 0.05% and 0.83 ± 0.05%) with 6%, 8% and 10% SDS oxidised PE film with *L. fusiformis* respectively at 60^{O}C for 1 month. Oxidation level of polyethylene treated by surfactant was higher as the availability of soluble oxygen and chain scission increased due to the attachment of surfactant to the polyethylene surface. Oxidative enzymes released by *Lysinibacillus fusiformis* (Mukherji

*et al.*2016). Duddu *et al.* studied 83 microbial isolates for biosurfactant production from oil-contaminated sites. Out of 83 isolates, the NDYS-4 isolate was identified as *Streptomyces coelicoflavas* 15399T and selected for LDPE degradation, showing 30% weight loss in 4 months and metabolic activity of isolates evaluated by TTC reduction test (Duddu *et al.*2015).

2.7 Mechanism of PE biodegradation

Biodegradation of polyethylene is complex and not fully understood. In order to elucidate the potential mechanisms, two different strategies have been followed in the literature. In the first approach, degradation studies have been performed using pure strains able to degrade polyethylene (Pometto *et al.* 1992, Albertsson *et al*. 1995, Yamada-Onodera *et al.* 2001, Volke Sepulveda *et al.* 2002, Gilan *et al*. 2004, Hadad *et al*. 2005, Sivan *et al.* 2006, Koutny *et al*. 2006b, Balasubramanian *et al*. 2010, Fontanella *et al*. 2010, Rajandas *et al.* 2012, Santo *et al.* 2012, Yoon *et al*. 2012 & Tribedi *et al.*2013). This approach has the advantage of using pure strains, which is a convenient way to investigate metabolic pathways or to evaluate the effect of different environmental conditions on polyethylene degradation. A disadvantage of this approach is that it ignores the possibility that polyethylene biodegradation can be the result of a cooperative process between different species. These limitations are avoided by the second approach, in which the use of complex environments and microbial communities are applied (Albertsson, 1980, Albertsson *et al.*1987, Karlsson *et al.*1988, Pegram and Andrady, 1989, Albertsson and Karlsson, 1990, Orhan and Büyükgüngör, 2000, Chiellini *et al.* 2003, Artham *et al.* 2009, Mumtaz *et al.* 2010, Lobelle and Cunliffe *et al.* 2011, Nowak *et al.*2011). Abiotic factors play an important role in increasing the surface availability for microbial growth on polymers; these factors include thermal oxidation (Fig.2.1) photo-oxidation (fig.2.2),

physical disintegration and hydrolysis that cause decreasing molecular weight (Singh *et al*. 2008) which are further degraded by microbes (Fig.2.3).There is proposed mechanism of biodegradation of polyethylene by Albertsson *et al.* According to which abiotic factors (heat and light) oxidise the polyethylene to carbonyl groups Figure 2.1 shows the traditional mechanism for the photooxidation of PE. Initially, UV radiation is absorbed which leads to radical formation. Eventually, oxygen is absorbed and hydroperoxides are formed, the end products being carbonyl groups. Additional exposure to UV radiation causes the carbonyl groups to undergo the Norrish type I and/or II degradation.The photooxidation can be initiated by impurities. UV degradation can also begin at locations of trace hydroperoxide or ketone groups, introduced during the manufacturing processing or fabrication. The oxidative degradation of polyolefins can be followed by measuring the level of carbonyl group adsorption by infra-red spectroscopy (IR).The formation of carbonyl groups is increased by photooxidation, but also by increasing stress even after storage in an abiotic environment. If Norrish type I or II degradation (or both) occur, additional peaks are observed in the IR spectrum of the polymer. For example, a terminal double bond appears at 905-915cm $^{-1}$ and it is also possible to trace ester formation in abiotically-treated LDPE films. Norrish type I cleavage yields a carbonyl radical which can react with an alkoxy radical on the PE chain shown in Fig.2.2. A peak appears at 1740cm^{-1} in the IR spectrum. After incubation with microbes the abiotically oxidised chain is oxidized further to a carboxylic acid and the resultant acid undergoes β-oxidation which, by reaction with coenzyme A, removes two carbon fragments from the carboxylic molecule. The two carbon fragments, acetyl-SCoA, enter the citric acid cycle, from which carbon dioxide and water are released and these oxidised polyethylene chains are used by microorganisms, further these alkane chains are oxidized to a carboxylic acid and the resultant acid undergoes β-oxidation which, by reaction with coenzyme A, removes two

carbon fragments from the carboxylic molecule. The two carbon fragments, acetyl-SCoA, enter the citric acid cycle, from which carbon dioxide and water are released (Fig.2.3) (Albertsson *et al.*1987).

The mechanissm of Thermal Oxidation of polythene

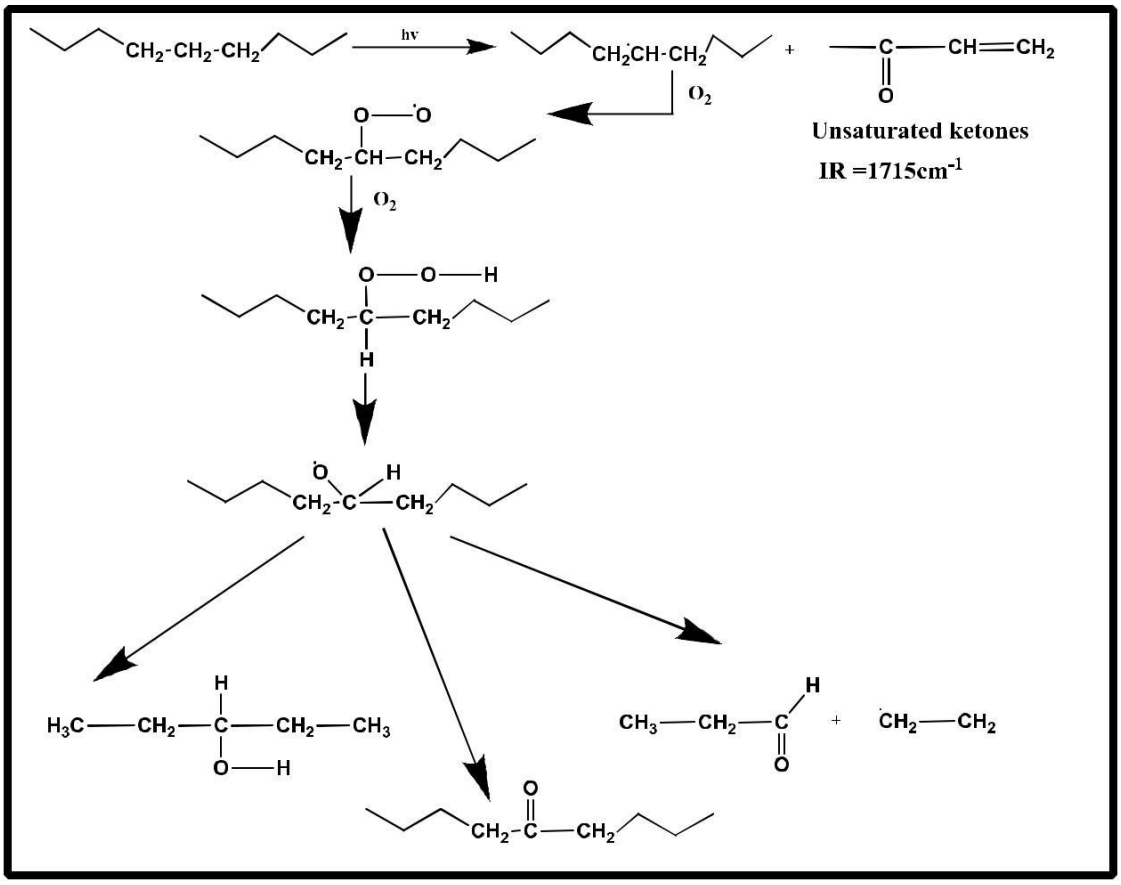

Fig. 2.1 Mechanism of thermal oxidation of Polyethylene

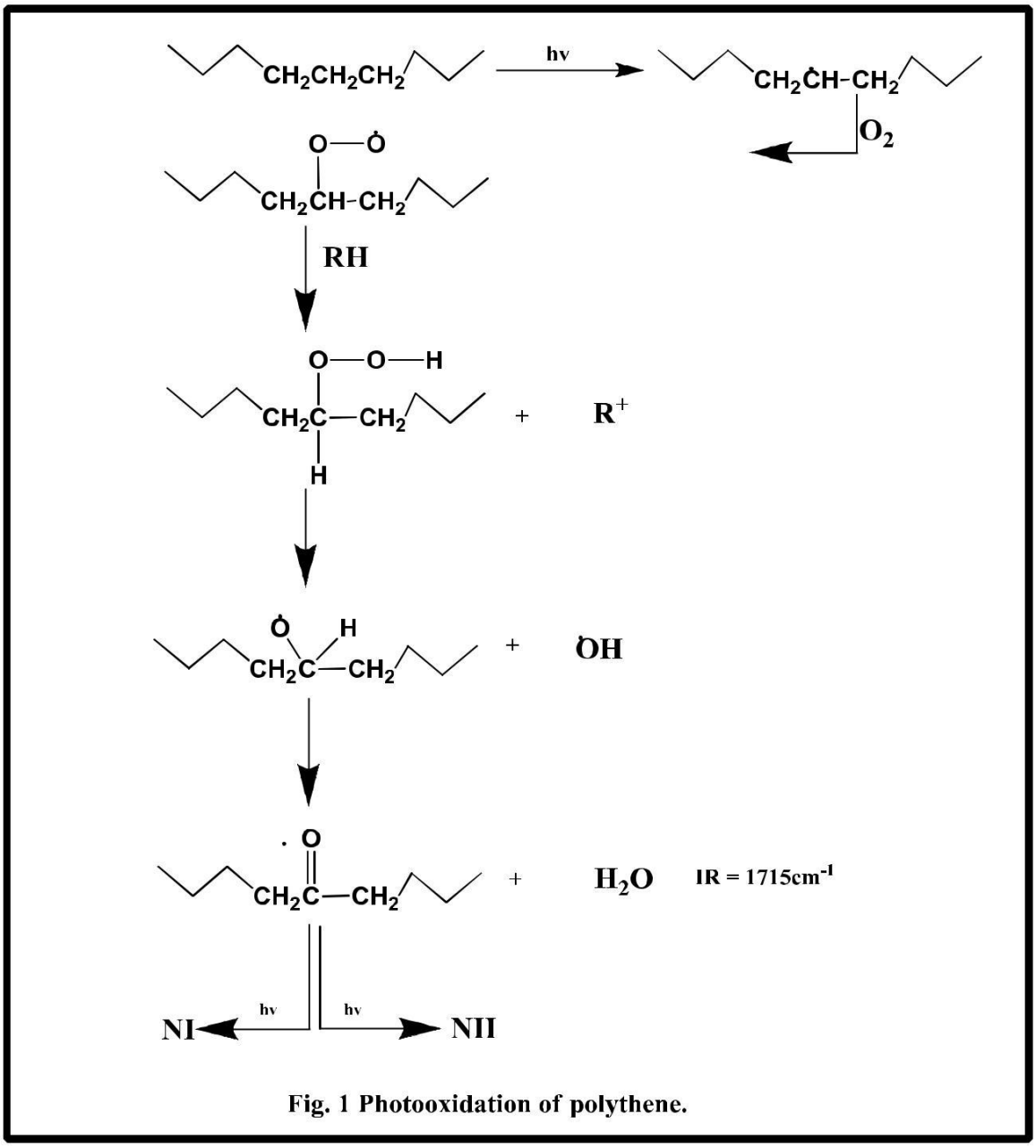

Fig. 2.2 mechanism of photooxidation of Polyethylene

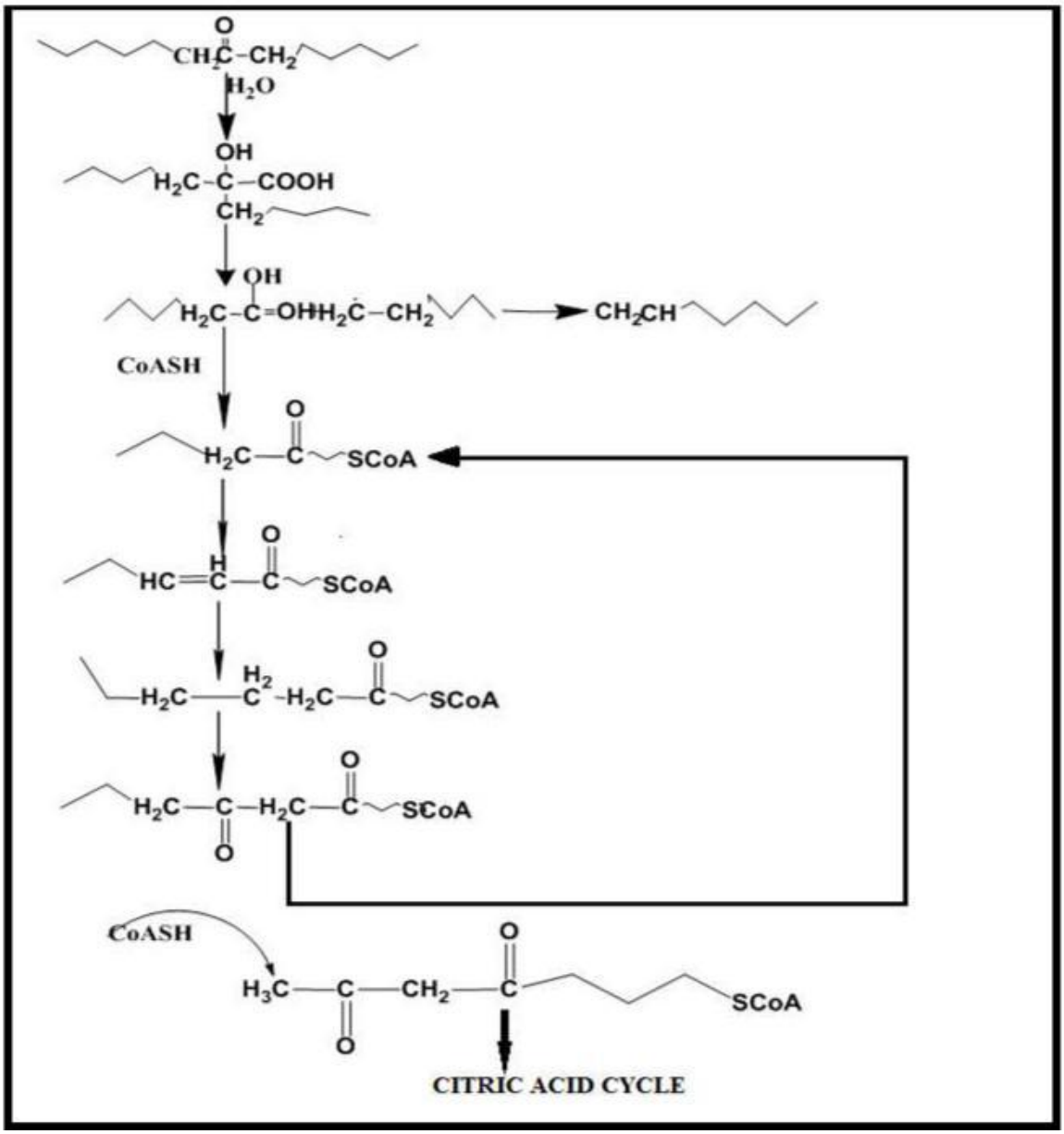

Fig. 2.3 Proposed mechanism for the biodegradation of polyethylene

2.8 Enzymes involved in biodegradation of PE

The enzymes responsible for polyethylene degradation are categorized into two groups, i.e. extracellular depolymerase and intracellular depolymerase (Gu *et al.* 2003). Exoenzymes are generally involved in the degradation of polyethylene to simple units like monomers and dimers. These are further exploited by microorganisms as energy and carbon sources. Lipase, (Coenen *et al.* 1997) tyrosinase, peroxidase (León-Santiesteban *et*

al. 2008) and laccase (Shinkafi *et al.*2014). Amylase, lignin /manganese peroxidase and laccase. Santo *et al.* reported bacterial originated copper-binding laccase from *R. ruber* for enzymatic degradation of polythene (Santo *et al.* 2013). Sheik *et al*. reported *Aspergillus* sp., *P. lilacinus* and *L. theobromae* as endophytic fungi for laccase production and LDPE degradation (Sheik *et al.* 2015).

2.9 Effect of microbial activity on polyethylene

Microorganisms able to colonize the surfaces of polyethylene have diverse effects on its properties; seven different characteristics are usually monitored for change in order to establish the extent of biodegradation of the polymer: molecular weight, functional groups on the surface, hydrophobicity/hydrophilicity, crystallinity, surface topography, mechanical properties, molecular weight distribution.

2.10 Objective of the present work

Degradation of virgin and recycled plastics is a challenge due to increasing white pollution. From the literature review it is observed that significant work has been reported on the biodegradation of polyethylene. Though biodegradation is advantageous for overcoming the white pollution to some extent but, for the industrial processes, final selection depends on various factors such as; energy consumption for the overall process and its optimization and economy issues for the complete mineralization of polyethylenes.

Use of a pure culture system permits the distinction between chemical and biological degradation of a polymer by providing necessary controls and also facilitates the experimental replication needed to obtain statistical evaluation of the data.

Development of hybrid pathways through genetic manipulation of microorganisms is one of the promising techniques which would drastically improve the process of bioremediation is still an economic challenge.

Based on the above discussion, it is observed that there is paucity of literature on biodegradation of recycled polyethylene carry bags thus there is a need for much more as well as continuous research to achieve an economical route for the bioremediation of recycled polyethylene carry bags and waste virgin polyethylene bags as compared to existing processes.

Based on the above discussion, following objectives have been set for the present work:

(a) Collection of soil samples from the plastic/garbage contaminated sites

(b) Isolation of the microbes from the contaminated soil samples

(c) Identification of the isolated strains using 16S rDNA/18S rDNA gene sequence analysis

(d) Comparative biodegradation study of waste LDPE and HDPE by pure isolated cultures (bacterium and fungus)

(e) Optimization of parameters responsible for the efficient degradation of waste polyethylene and recycled polyethylene