9.1 Introduction

The unique characteristic of bacterial association with surfaces was evident from the very beginning of bacterial existence. In nature, all surfaces under natural and artificial conditions are covered by numerous microbial floras (Woese, 1987; Marshall, 1976; Angels et al., 1993; Gu, 2003). The process in which, a complex community of micro-organisms are established on a surface known bio-fouling or formation of biofilm. Biofilm, contains both micro-organisms and their extracellular polysaccharides (Geesey et al., 1990; Ford, 1993; Fletcher, 1996; Caldwell et al., 1997). Materials including metals, inorganic minerals & natural organic and synthetic polymers are susceptible for bio-film formation under humid conditions in tropical and subtropical climates (Gu et al., 2000d). The synthetic polymeric materials are very unique in physical forms, chemical composition and mechanical properties.

The synthetic polymers exhibit high versatility of the carbon to carbon and carbon to non-carbon (C - C, C – R and C – H) bonds and substituent groups, the possible configurations, stereo-chemistry and orientation provide basis for variations of chemical structures and stereo-chemistry (Odian, 1991). Very small variations in the chemical structures may result in large difference in terms of bio-degradability. Owing to this structural versatility, they are widely used in product packaging, insulation, structural components, protective coatings, medical implants, drug delivery carriers, slow release capsules, electronic insulations, aviation and space industries (Gu, 2003). But on the other hand the accumulation of plastics in the environment is a matter of great concern leading to long term environment, economic and waste management problems.
Since the photo/thermal initiators as pro-oxidant additives has caught the attention of researchers for the development of degradable polyethylene, especially the transition metals like Fe, Mn, Co in the form of carboxylates during the processing stage. They pose remarkable ability to decompose the hydroperoxides formed during the oxidation process of polyethylene.

The rate of bio-degradation of low-density polyethylene (LDPE) has been found very slow. Only 0.2% weight loss was reported for LDPE films buried in soil (Albertson et al., 1987). The total degradability of any substrate depends only on the formation of microbial colonies. The surface properties of the substance play a vital role in adhesion of microbial cells to initiate bio-degradation. The oxo-biodegradable additives render the polymer backbone week by initiating the abiotic oxidation which introduces new polar groups on the surface. These polar groups create a hydrophilic surface and facilitate the microbial colonization. Earlier, Comamonas acidovorans fy-1, Xanthomonas maltophilia, Sphingomonas paucimobilis (Wang et al., 2003a, b), Rhodococcus rubber Sa (Li et al., 2005a,b), Klebsiella oxytoca Sc, Methylobacterium mesophilicum Sr (Gu et al., 2004) have been reported for degrading plasticizers.

The present study aims to identify the efficiency of Lysinibacillus fusiformis strain Ma-Su CECRI-2 (Lys) for degrading low density polyethylene. In shake culture flasks with the isolate, the in vitro polyethylene degradation was observed for (July, 2010 to June, 2011) 12 months and reported. Here the polymer is supplied to the organism as a sole source of carbon. The changes in its physical, chemical and mechanical properties at different time intervals were monitored.
9.2 Materials and Methods

9.2.1 Preparation of low density polyethylene film

Virgin LDPE films were prepared using a film blowing machine (reported in chapter 5) using an extruder (Gurusharan Polymer Make) and LDPE film was designated as PE and films containing oxo-biodegradable additive were designated as BPE followed by a numerical suffix indicating the amount of additive added. LDPE films containing 10% and 20% oxo-biodegradable additive were designated as BPE10 and BPE20 respectively.

9.2.2 Abiotic treatment: Photodegradation and Thermooxidation procedure

Fifty micrometer thick blown film of PE, BPE 10 and BPE20 were irradiated with 40 W UV-B lamps generating energy between 280 and 370 nm with a maxima at 313 nm in air at room temperature (30±1 °C) on open racks positioned 5 cm from the lamp (Roy et al., 2006). Exposures were conducted uninterrupted, 24 h per day and the samples recovered in 21 days. The UV-treated samples were then followed the thermal aging in a hot air oven for 10 days at 70°C. The combined abiotic oxidation provides the initial changes in the polymer and it creates the effect of one year outdoor weathering (Nils et al., 2009).

9.3.3 Biotic Treatment

9.3.3.1 Bacterial system

The bacterial culture of *Lysinibacillus fusiformis* strain Ma-Su CECRI-2 (Lys) was isolated from Municipal compost yard Vellalore, Coimbatore, Tamilnadu. The mineral medium-C (Onoddera et al., 2001) was used for the culturing. The colonies were preserved at 4°C in 2% agar slants of medium B (5 % malt extract, 0.3% yeast extract and distilled water; pH 7±0.2) (Onoddera et al., 2001).
9.3.3.2 Chemical disinfection of films

The disinfection procedure used with each pre-treated and un-treated rectangular film strips (20 X 2.5 cm) were placed in the fresh solution containing 7ml Tween 80, 10 ml bleach and 983 ml of sterile water and stirring for 30-60 min. The films were removed and placed into a covered beaker filled with sterile water and stirred for 60 min at room temperature. The films were then aseptically transferred into ethanol solution 70% (Vol/Vol) and left for 30 min. The films were then placed into sterile petri dish and incubated at 45-50°C to dry overnight, allowed to equilibrate to room temperature (Sudhakar et al., 2008).

9.3.3.3 In-vitro flask Culture

Preweighted disinfected untreated and pretreated films (20X2.5cm) were aseptically added to 100ml aliquots of filtered (0.22mmMillipore filter paper) medium - C. Log phase seed culture 3% (V/V) was added to these aliquots and they were kept under shaking at 180rpm at 30°C. The numbers of films taken were such that samples could be withdrawn after 1, 3, 6, 9 and 12 months. Also, all the samples were prepared and run simultaneously in triplicate. Mineral medium-C containing polymer without the microorganism was maintained as positive controls (Doi et al., 1992). Streak culture (surface plating) method was used for identifying any contamination in the medium or any possible changes to the morphology of the organisms. The growth of the bacterial culture containing polymer was monitored by counting the colony forming units (CFU). Fresh filtered Mineral medium-C was added after 6 months to make up for the evaporation.

Three sets of polyethylene samples were analysed in each category of (a) PE, (b) BPE10 and (c) BPE20;

(a). PE
(i) Treated PE (TPE) unexposed to strain Lys [control] and (ii) Treated PE exposed to strain Lys (TPE+ Lys)

(b). BPE10

(i) Treated BPE10 (TBPE10) unexposed to strain Lys [control] and (ii) Pre-treated BPE10 exposed to strain Lys (TBPE10+ Lys)

(c). BPE20

[i] Treated BPE20 (TBPE20) unexposed to strain Lys [control] and (ii) Treated BPE20 exposed to strain Lys (TBPE20+ Lys)

9.3.3.4 Colony forming unit (CFU)

Bacterial culture growths for the 12 months were monitored by plating method. In this method 1 ml of bacterial culture was serially diluted and inoculated on the surface of nutrient agar solid media in sterile petridish and incubated for 24 h. viable colony counts were obtained by appropriately diluting the sample (Moat and Foster, 1995) at different incubation periods. Colonies were counted by using digital colony counter (Scigenics, Chennai, India).

\[
\text{Number of bacteria per mL} = \frac{\text{Number of CFU}}{\text{Volume plated (ml)} \times \text{dilution used}}
\]

9.3.3.5 Bacterial viability analysis

The TPE, TBPE10 and TBPE20 pre-treated films with Lysinibacillus fusiformis (Lys) were immersed for 5 min in 3% glutaraldehyde solution in order to fix the biofilm onto the polymer surface. Then, the films were gently rinsed with autoclaved and sterilized distilled water and two drops of 0.01% aqueous solution of acridine orange were added. These samples were kept in the incubator for 15 min (Chavant et al., 2002). The morphology of the biofilm was studied with a Nikon epi-fluorescence microscope (Model E200, Nikon, Tokyo, Japan).
9.4 Fourier transformed infrared spectroscopy (FT-IR)

The surface chemical modifications which occur in LDPE films upon thermo-oxidation were investigated using FTIR spectroscopy. The FTIR spectra were recorded using a Thermo Nicolet, Avatar 370 spectrophotometer in the spectral range between 4000-400 cm$^{-1}$.

9.5 TGA

TGA measurements were carried out in nitrogen atmosphere at a constant flow rate with Perkin-Elmer TGA 7/DX Thermal Analyzer instrument in the heating range between 40 upto 800°C (heating rate; 20°C/ min).

9.6 Differential Scanning calorimetry

Crystallinity studies were conducted using a DSC Q200 V24.4 Build 116 module under nitrogen atmosphere and the temperature ranges from -75°C to 300°C at constant raise in heating of 10°C / min. The instrument was calibrated using indium. Samples of 0.1-1.0 mg were sealed in aluminium pans and subjected to heating – cooling cycles. Previous thermal effects were minimized by initially heating samples until melting. The samples were then cooled to room temperature at a constant heating rate 5°C per minute to favour crystallization. Subsequently, the second heating was recorded at 10°C per minute in the temperature range of 30-200°C.

9.7 Contact angle and surface energy

Wettability determinations of film surfaces subjected to biodegradation were performed by contact angle measurements on samples using a video based contact angle meter OCA 20 attached to a camera. The wetting liquid used was Millipore grade distilled water (liquid surface tension ($\gamma_l$) = 72.8 mJ/m$^2$). The value was the average of five samples for each experiment.
Surface energy was calculated using equation of state, Schultz Method-2, using Data Physics SCA20 software (Version 2.01).

9.8 Morphological analysis

Changes in the bacterial attachment due to biodegradation were investigated with SEM. (JEOL Model JSM - 6390LV) using a voltage of 15kv. Photomicrographs were taken at uniform magnification of 5000 fold and surface topography of the films analysed by AFM (Solver P 47 PRO, Russia) in the semi-contact mode, under normal laboratory air atmosphere with the scan rate of 0.5 Hz, using single crystal silicon antimony doped probe to evaluate the change in surface topography due to biodegradation.

9.9 Results and Discussion

9.9.1 Pre-treatment initiated abiotic degradation

The abiotic pre-treatment involved the exposure of LDPE films to UV-B radiation for total of 21 days and thermal oxidation for 10 days. After this period, pristine LDPE and LDPE with pro-oxidants undergoing the light and heat treatment, the polymer is chemically modified and is more susceptible for microbial attack (Bonhomme et al.,2003). The catalytic degradation in the presence of transition metal in polyethylene has been attributed to its ability to generate free radicals on the surface of polyethylene, which later react with oxygen to generate carbonyl groups which are visualized in FTIR spectra (Osawa et al., 1979; Osawa, 1988).

9.9.2 Bacterial growth

The colony count (CFU / ml) of Lysinibacillus fusiformis strain Ma-Su (Lys) pure culture colonies were quantified at different time intervals for a period of 12 months (Fig
9.1. The growth of micro-organism in the flasks showed a gradual increase in TPE, TBPE 10 and TBPE 20. The initial reduction of bacterial population is due to the time required for the bacteria to the new environment. The TPE showed a gradual growth from $3.1 \times 10^4$ CFU/ml up to 60 days. It attains maximum number $3.7 \times 10^4$ CFU/ml and then gradually to $4.1 \times 10^4$ CFU/ml after one year. Sample TBPE10 and TBPE20 also initiated with $3.3 \times 10^4$ CFU/ml and $3.9 \times 10^4$ CFU/ml on 15th day of incubation. Attain maximum growth at 2nd month owing to maximum utility of available polymer. The bacterial population maintains study stationary phase. After 12 months of time the population grows slowly to $4.6 \times 10^4$ CFU/ml and $5.7 \times 10^4$ CFU/ml. The similar biodegradation study was done by Sudhakar et al (2008) and found the pretreated TBPE10 & TBPE20 supports well the growth of the bacteria in liquid culture.

The abiotic-oxidation (photo and thermal) influenced the growth of *Lysinibacillus fusiformis* strain Ma-Su (C2) on the pre-treated films and was evident after 60 days of incubation. The reason may be due to the surface modification during abiotic-oxidation introduced new polar groups and made the surface hydrophilic by increasing its surface energy. The pre-treated LDPE uninoculated act as control and all the flasks were monitored for contamination at regular time intervals by plating technique.

### 9.9.3 Bacterial viability

The process of polyethylene biodegradation is a complex and slow. Even after prolonged exposure to microbial consortia of soil, the carbon mineralization is less than 1% (Albertson and Karlsson, 1990). The number of live organisms on the treated- TPE, TBPE10 and TBPE20 surfaces after one year incubation was determined using epi-fluorescence microscopy shown in fig 9.2. The numbers of live organisms are more in abiotically- treated samples and appear as green in colour, where as the un- treated samples showing more red
coloured spots indicating the dead cells after 12 months of duration. The abiotic pre-treatment increase the surface roughness and the introduction of new functional groups make the microbes to attach themselves more with TPE, TBPE10 and TBPE20 (fig.9.2). More number of viable cells are still found in pre-treated BPE20 sample holds the dynamic population of viable cells. Few bacteria in the pre-trained TPE samples starved to death due to unavailability of nutrients and accumulation of secondary metabolites (Sudhakar et al., 2008). Hence the added oxo-biodegradable additive is also playing some role in modifying the surface properties of the polymer along with abiotic oxidation.

9.9.4 FTIR studies

The IR spectra of pre-treated TPE, TBPE10 and TBPE20 control and with Lysinibacillus fusiformis (Lys) are shown in Fig. 9.3 (a & b). The focus here are the 1720, 1740, 1640 and 3050-3550 cm\(^{-1}\), corresponding to ketone carbonyl (-C=O-); ester carbonyl (-COO-), internal double bonds (-C=C-) and hydroxyl (-OH-) respectively.

In the present study the carbonyl peak increases (1718 cm\(^{-1}\)) initially and prolonged incubation with Lysinibacillus fusiformis (Lys). In control sets of TPE shows the presence of 1463 cm\(^{-1}\) peak which is responsible for CH\(_3\) deformation. The increase in the 1718 cm-1 peak indicates the –C=C- stretching. It shows increase in peak intensity at 12 months of degradation. The Uninoculated pre-treated samples showed a higher carbonyl indexes, showing the evidence of propagation of free radical initiated reaction. The initial carbonyl rise is due to oxidation occurring initially at extruder during PE film manufacturing. In TPE10 and TPE20 controls showing the broadening of peaks from 900 cm\(^{-1}\) to 1600cm\(^{-1}\). It attributes for the overlapping of gamma lactones and alcohols.

The abiotic pre-treatment initiate’s the process of polyethylene oxidation in TPE, TPE10 and TPE20. It derives the oxygen from the ambient and it introduces the carbonyl
groups on the PE. The addition of pro-oxidant additives significantly contributes the initiation and the propagation of radical reaction. The transient metal ions, typically added in the form of stearate of Fe$^{3+}$, Mn$^{2+}$, or Co$^{2+}$ complex plays a role in photo-oxidation process as a source of radicals for reaction initiation (Jakubowicz, 2003; Weiland et al., 1995). The auto-oxidation of polyolefins occurs when they exposed to sunlight or at elevated temperatures. Formation of carbonyl groups is dependent on various environmental factors and the additives present in the polyolefins.

In the spectra of TPE, TPE10 and TPE20 cultured with *Lysinibacillus fusiformis* (*Lys*) forms the carbonyl groups at 1720 cm$^{-1}$. As the degradation prolongs, the accumulation of more carbonyl compounds found. In TPE20, the 1723 and 1750 cm$^{-1}$ introduced in prolonged biodegradation. After the formation of oxidised compounds on the surface, the slow reduction in peak intensity was absorbed. It is due to utilization of those carbonyl compounds by bacteria. It is probably due to biodegradation (biotic) through Norrish mechanism or through formation of ester. The rate of decrease in carbonyl indexes for TBPE20 is faster comparatively with TPE and TBPE10 with Lys strain.

When the pro-oxidants catalyze they decompose peroxides and leads to chain session. The cleaved chains are most frequently terminated by carboxylic groups and other functionalities like esters, ketones and alcohols. The carboxylic acids formed react with coenzyme-A (CoA) and remove two carbon fragments, acetyl-CoA enters in the citric acid cycle and produces carbon-di-oxide and water as the final degradation products (Albertson et al., 1987). The thermal and photo-oxidation leads to the session of main chain in the polymer. It breaks down the polymer in to fragments of lower molecular weight compounds. Embrittlement and hydrophilicity due to the introduction of carbonyl groups further promotes the biodegradation of the polymer. During microbial degradation, a marginal decrease in the carbonyl group was noted.
9.9.5 Wettability analysis: contact angle and surface energy measurements

The surface modification of pre-treated control and test TPE & TBPE samples are investigated by contact angle measurement. Figure 9.4, shows the variation in the contact angle of different samples at different time intervals.

Once the free radical reaction was initiated by abiotic treatment the pre-treated PE samples undergo oxidation using the dissolved oxygen from the media. The Uninoculated (control) samples of TPE, TBPE10 and TBPE20 (fig 9.4) showing a gradual reduction in contact angle from 100.12°, 103.42° and 104.09° to 94.05°, 93.59° and 90.32° respectively. The reduction was attributed to the formation of a little amount of polar groups such as –OH, C=O, COOH and COO- on the main chain of the polymer matrix. Here the pro-oxidant additive plays the role for introducing new groups during prolonged biodegradation period.

The extent of surface modification was evident in the samples of TPE, TBPE10 and TBPE20 inoculated with bacteria (Lys), combining the bacterial degradation of polyethylene and with the catalytic action of pro-oxidant additive. The initial contact angles of TPE, TBPE10 and TBPE20 inoculated with bacteria (Lys) were 100.12°, 103.42° and 104.09°. But it markedly reduced to 85.29°, 81.26° and 79.10° respectively. The decrease in contact angle showed the formation of hydrophilic groups in (pre-treated) TPE, TBPE10 and TBPE20 incubated with Lys strain film surfaces. On absorption of energy in the form of light & thermal, the components present in the pro-oxidant additive forms free radicals. These species can combine with oxygen from media, which generates the introduction of polar groups. The reduction in contact angle may due to initiation of the process of auto-oxidation due to long time incubation and the activation of pro-oxidant additive in liquid media. sudhakar et al., (2007) reported the same decrease in contact angle of polypropylene in marine environments.
The surface energy (fig 9.5) was interestingly increasing in pre-treated bacterial inoculated PE films than the control and untreated bacterial inoculated PE sample films. The samples of TPE, TBPE10 and TBPE20 incubated with (Lys) strain shows the increase of 31.34 mJ/m², 34.76 mJ/m² and 36.04 mJ/m² from the initial of 22.67 mJ/m², 20.07 mJ/m² and 20.81 mJ/m² respectively. But the uninoculated pre-treated samples TPE, TBPE10 and TBPE20 attains the surface energy of 26.56 mJ/m², 26.89 mJ/m² and 28.33 mJ/m² form the initial value of 22.67 mJ/m², 20.07 mJ/m² and 20.81 mJ/m² respectively.

9.9.6 Thermal analysis

(ii) TGA

Thermogravimetric analysis (TGA) measures the weight loss of samples heated to a particular temperature and hence determines their thermal stability. The TGA curves of pre-treated and untreated PE subjected to biodegradation with *Lysinibacillus fusiformis* (*Lys*) show that a considerable weight loss has occurred at 450°C and 500°C in the thermal behaviour of low density polyethylene (LDPE) was given in Table.9.1.

The thermal behaviour of PE has widely studied before and after biodegradation of treated and untreated samples and occurs as a single stage of decomposition (fig.9.6). The uninoculated (control) TPE sample initially showed T_on at 452.1°C and after 12 months of exposure decreases to 450.9°C. After 12 month of incubation they showed a increase in weight loss percentage from 6.15 to 11.34 % at 450°C. It implies the oxidation and catylitic action of bacterial enzymes on polyethylene holds the responsibility for losing its thermal stability. The pre-treated samples (TPE) in the presence of (Test) *Lysinibacillus fusiformis* (*Lys*) tend to lose its weight almost 12.13 % at 450°C. But the presence of oxidised products and due to microbial erosion the Ton was decreased to 446.7°C just below the initial degradation temperature of treated uninoculated sample. The polymer without pro-oxidant
additive is not much affected by the bacterial degradation, as it does not easily lose its integrity (Corti et al., 2010; Roy et al., 2008).

In the sample of uninoculated pre-treated TBPE10 (control), on zero day it showed its $T_{on}$ at 462.6 °C and 10.58 % weight loss at 450°C and after 12 months of incubation without *Lysinibacillus fusiformis* (*Lys*) affect the weight loss percentage (7.81%) but decrease in $T_{on}$ 453.7°C with residue of 5.9% as impurities. The treated samples TBPE10 inoculated with *Lysinibacillus fusiformis* (*Lys*) showed $T_{on}$ 432.5°C with residue of 2.2% due to incorporated pro-oxidant additive at 650°C. Hence the thermal stability of treated (TBPE10) sample is slightly reduced during biodegradation reduced when compared with 12 months treated TBPE10 without bacteria. Thus the abiotic treatment and the pro-oxidant additive plays a vital role in enhancing the reduction of thermal stability of the polymer by oxidation mechanism and after 12 months of biodegradation with bacteria, make it susceptible for degradation. The same results were reported by Corti et al (2010) by fungal biodegradation of LDPE with pro-oxidant additive.

The samples of TBPE20 initially after abiotic oxidation read the $T_{on}$ at 453.1°C with weight loss of 13.96 % at 0-day of biodegradation. After 12 months in the absence of *Lysinibacillus fusiformis* (*Lys*) it showed $T_{on}$ at 451.3°C with weight loss of 12.53%. Along with *Lysinibacillus fusiformis* (*Lys*), when the TBPE20 samples were subjected to biodegradation it, showed $T_{on}$ at 447.8°C with weight loss of 17.32 %. The residue at 650°C was 14.4% for TBPE20 with bacterial inoculum, indication the residual effect of pro-oxidant additive. Thus the thermal stability of inoculated (Test) pre-treated samples showed reduction in thermal stability when compared pre-treated without bacteria (control).

The decrease in $T_{on}$ for the 0-day pre-treated 10% and 20% additive added PE samples was observed (Corti et al., 2010). The maximum degradation temperature is not only a measure of polymer chain length but also a function of % crystallinity, molecular weight
distribution, presence of additives, degradation pattern of microbe involved and the components of additive added to it (Sudhaker et al., 2008; Chiellini et al., 2007).

9.9.7 Differential scanning colorimetry (DSC)

The DSC curves obtained for the pre-treated and untreated samples of PE before and after 12 months of biodegradation with Lysinibacillus fusiformis (Lys) are shown in figure 9.7.

The table 9.2 showing the melting temperature (Tm °C), heat of fusion (ΔHf) and percentage of crystallinity of pre-treated control and test TPE & TBPE samples before and after 12 months of biodegradation with Lysinibacillus fusiformis (Lys). DSC thermograms of 0-day TPE and 12-months TPE given in (fig) showed endotherms corresponding to the melting transition of the samples at 119.3 °C and 119.5°C respectively. No difference in melting temperature was found even after 12 months of oxidation in the media. 12-months TPE sample subjected to biodegradation with Lysinibacillus fusiformis (Lys) showing change in the melting temperature of 120.79°C and shown increase in percentage crystallinity of 33.85 from 22.21% initial value of treated sample zero day. A marked rise in heat of fusion of 96.50 J/g also observed. In earlier biodegradation study Suthaker et al (2008) reported the same results.

In Fig. 9.7.b, the thermograms of 10% additive added pre-treated TPE10 control and test samples before and after biodegradation were plotted. The 0-day TBPE10 shown the melting temperature of 121.67°C and the percent crystallinity of 23.97% and after it underwent oxidation in media for 12-months TBPE10 shown increase in crystallinity of 27.64% and heat of fusion 78.78 J/g but there was no any marked change in melting temperature. When the sample TBPE10, subjected biodegradation with Lysinibacillus fusiformis (Lys) the heat of fusion increased to 83.56 J/g and increase in crystallinity of 29.31%. The 0 day pre-treated TBPE10 also displays the weight loss peak associated with the
ethylene chains of poly (vinyl alcohol) is significantly modified as because of biodegradation. The most accessible ethylene chains easily disappear and less accessible ethylene chains remains, probably by the influence of vinyl alcohol derivatives (Moriana-Torro et al., 2008). The increase in the crystallinity is an indirect result of the chain session of the polyethylene molecule in the amorphous region (Roy et al., 2009).

The fig .9.7.c , the thermograms of 20% additive added pre-treated control and test TBPE20 samples before and after biodegradation were shown increase in percent crystallinity and heat of fusion after biodegradation. The 0-day TBPE20 showed 119.68°C, 78.28 J/g and 27.46% of melting temperature, heat of fusion and crystallinity respectively. After 12 months of oxidation in media it showed increase in heat of fusion and crystallinity of 104 J/g and 36.49 % respectively without any change in melting temperature. The biodegraded TBPE20 sample after 12 months showed an increase in crystallinity and heat of fusion, 38.59% and 110.3 J/g respectively without shifting melting point. The pre-treated TBPE20 at 0 day also displays the weight loss peak associated with the ethylene chains of poly (vinyl alcohol) is significantly modified as because of biodegradation. The most accessible ethylene chains easily disappear and less accessible ethylene chains remains, probably by the influence of vinyl alcohol (Moriana-Torro et al., 2008) It is generally understood that during biodegradation, the amorphous fraction of the polyethylene has higher mobility and hence higher accessibility to oxygen and microorganisms, is exposed to attack (Thakore et al., 2001). Therefore the microbial degradation results in an increase in overall degree of crystallinity of the polyethylene sample ( Luckachan et al., 2006; Raghavan et al., 1992)
9.9.8 Mechanical Properties

The Figure 9.8, showing the tensile strength of the pre-treated and untreated samples of PE before and after 12 months of biodegradation with *Lysinibacillus fusiformis* (*Lys*). The initial tensile strength of 0-day treated (TPE) sample is of 15.96 MPa. After 12 months of incubation in media without microbe does not show any marked reduction and ends in 15.23 MPa. But in case of TPE with *Lysinibacillus fusiformis* (*Lys*) showed a little reduction to 14.75 MPa. The corresponding reduction in elongation at break of 431.0 from the initial value of 564.72 (Fig 9.9).

The PE blended with 10% additive, on 0-day TBPE10 showed 15.53 MPa with elongation at break at 682.2. After 12 months without bacteria in medium the sample exhibit 14.98 MPa tensile strength and 461.9 in elongation at break. The test samples inoculate with *Lysinibacillus fusiformis* (*Lys*) showed the reduction of 13.42 Mpa of tensile strength and 275.83 in elongation at break. The PE added with 20% additive, on 0-day TBPE20 showed 15.91 MPa with elongation at break at 671.8. After 12 months (control) without bacteria in medium the sample exhibit 14.76 MPa tensile strength and 423.27 in elongation at break. The test samples inoculate with *Lysinibacillus fusiformis* (*Lys*) showed the reduction of 12.39 Mpa of tensile strength and 265.9 in elongation at break. The abiotic exposure governs the losing of its tensile strength slowly when compared to biotic tensile strength reduction (Haded *et al.*, 2005). The abiotic oxidation produces random chain session and it was further degraded by microbial oxydoreductase and catalase enzymes to render the polyethylene to lose its physical strength (Roy *et al.*, 2008). The decrease in mechanical strength of the additive added polymer after 12 months of incubation with *Lysinibacillus fusiformis* strain Ma-Su (C2) was due to the initial absorption of energy in the form of light and the pro-oxidant catalyses the chain session by forming free radicals and thereby facilitating the microbe to degrade the polymer back bone (Saucedo *et al.*, 2008).
9.9.9 Surface Morphological & bacterial attachment studies

The surface morphology of LDPE films were investigated using scanning electron microscope. The SEM micrographs indicate the bacterial adhesion and biofilm formation on the polymer surface films exposed to (Fig.9.10 a & b) biotic environment. The pre-treated TPE and TBPE samples inoculated with Lysinibacillus fusiformis strain Ma-Su (C2) show the evidence of microbial growth on the film surfaces. The pre-treated TPE showing poor bacterial adhesion and the pro-oxidant additive added TPBE10 and 20 showed gradual growth which is supported by bacterial viable count. But the control samples without bacteria have shown to form larger grooves on the polymer surface due to prolonged oxidation. The biological attack of polymer generally began with the colonization of bacteria on polymer film surfaces. The adhesion was scattered, not uniform, indicating that the amorphous region of the polymer was more susceptible to microbial adhesion and degradation. It has been reported earlier that the microbial growth is mainly concentrated around the fissures, which suggests that the low molecular nutrients migrate to the surface from the bulk of the polymer (Felsenstein, 2005).

9.9.10 Surface topological analysis: AFM

The surface topology of the three samples before and after biotic/abiotic degradation were analysed using AFM images (fig.9.11). The pre-treated control samples of TPE, TBPE10 and TBPE20 initially exhibits surface roughness of 10.82 nm, 11.7nm and 11.23nm respectively. After 12 months of abiotic degradation the surface roughness of TPE, TBPE10 and TBPE20 increases to 11.64nm, 12.37nm and 14.66nm respectively. The increase in roughness is due to extended period of abiotic degradation and the samples of TBPE10 and 20 may be accelerated by the pro-oxidant additive. The surface roughness of TPE sample undergone 12 month biodegradation with Lysinibacillus fusiformis (Lys) strain (fig 9.11.b)
was 11.82 nm. The pro-oxidant additive added samples TBPE10 and TBPE20 showed a marked increase in surface roughness from 15.02 nm and 17.60 nm respectively. The increased peaks like structures were found more in number in BPE20 than in the other two samples. The results show the positive sign of the influence of biodegradation by the *Lysinibacillus fusiformis* (*Lys*) strain. The increase in surface roughness in biodegradation process is probably due to surface degradation and deterioration.
Figure 9.1 CFU count for various LDPE samples inoculated with *Lysinibacillus fusiformis* (*Lys*)
Figure 9.2 Live and dead cells attached on LDPE surfaces after one year (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 exposed to *Lysinibacillus fusiformis* (*Lys*)
Figure 9.3.a. FTIR spectra of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 unexposed to **Lysinibacillus fusiformis** (*Lys*)
Figure 9.3.b. FTIR spectra of (a1) pre-treated TPE, (b1) TBPE10 and (c1) TBPE20 exposed to *Lysinibacillus fusiformis* (*Lys*)
Figure 9.4 Changes in contact angle of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 (control) unexposed to *Bacillus cereus* (Bac) and (a1) pre-treated TPE, (b1) TBPE10 and (c1) TBPE20 (test) exposed to *Lysinibacillus fusiformis* (Lys)
Figure 9.5 Changes in surface energy of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 (control) unexposed with (test) exposed to *Lysinibacillus fusiformis* (*Lys*)
Figure 9.6 Thermogravimetric curves of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 samples unexposed (control) and exposed to *Lysinibacillus fusiformis* (*Lys*).
Figure 9.7 DSC curves of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 samples unexposed (control) and exposed to *Lysinibacillus fusiformis* (*Lys*).
Figure 9.8 Tensile strength of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 samples unexposed (control) and exposed to *Lysinibacillus fusiformis* (*Lys*).
Figure 9.9 Percentage elongation of (a) pre-treated TPE, (b) TBPE10 and (c) TBPE20 samples unexposed (control) and exposed to *Lysinibacillus fusiformis* (*Lys*).
Figure 9.10.a. Comparative initial (a) and final (b) SEM micrographs of (1) pre-treated TPE, (2) TBPE10 and (3) TBPE20 samples unexposed (control) to *Lysinibacillus fusiformis* (*Lys*) for one year.
Figure 9.10.b. Comparative final (c) SEM micrographs of (1) pre-treated TPE, (2) TBPE10 and (3) TBPE20 samples exposed (test) to *Lysinibacillus fusiformis* (*Lys*) after one year.
Figure 9.11.a. AFM images of initial (a) and final (b) pre-treated (1) TPE, (2) TBPE10 and (3) TBPE20 samples unexposed (control) to *Lysinibacillus fusiformis* (*Lys*) for one year.
Figure 9.11.b. AFM images of (1) pre-treated TPE, (2) TBPE10 and (3) TBPE20 samples exposed (test) to Lysinibacillus fusiformis (Lys) after one year.
9.12. Diagrammatic representation of changes occurred in various parameters of treated polyethylene sample with and without bacteria after a year.
Table 9.1 Thermal properties of treated and untreated PE incubated with *Lysinibacillus fusiformis* (Lys)

<table>
<thead>
<tr>
<th>Polymers</th>
<th>( % ) of weight loss</th>
<th>$T_{on}$ (°C)</th>
<th>Residual weight at 650°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>350 °C</td>
<td>450°C</td>
<td></td>
</tr>
<tr>
<td>0 – day TPE</td>
<td>0.43</td>
<td>6.15</td>
<td>452.1</td>
</tr>
<tr>
<td>12 – months TPE</td>
<td>12.22</td>
<td>11.34</td>
<td>450.9</td>
</tr>
<tr>
<td>12 – months TPE + Lys</td>
<td>3.42</td>
<td>12.13</td>
<td>446.7</td>
</tr>
<tr>
<td>0 – day TBPE10</td>
<td>4.8</td>
<td>10.58</td>
<td>462.6</td>
</tr>
<tr>
<td>12 – months TBPE10</td>
<td>2</td>
<td>7.81</td>
<td>453.7</td>
</tr>
<tr>
<td>12 – months TBPE10 + Lys</td>
<td>6.5</td>
<td>29.0</td>
<td>432.5</td>
</tr>
<tr>
<td>0 – day TBPE20</td>
<td>4.38</td>
<td>13.96</td>
<td>453.1</td>
</tr>
<tr>
<td>12 – months TBPE20</td>
<td>5.27</td>
<td>12.53</td>
<td>451.3</td>
</tr>
<tr>
<td>12 – months TBPE20 + Lys</td>
<td>2.23</td>
<td>17.32</td>
<td>447.8</td>
</tr>
</tbody>
</table>
Table 9.2 DSC data showing melting temperature, heat of fusion and percent crystallinity of TPE, TBPE10 and TBPE20 samples.

<table>
<thead>
<tr>
<th>Polymers</th>
<th>T&lt;sub&gt;m&lt;/sub&gt; °C</th>
<th>ΔH&lt;sub&gt;f&lt;/sub&gt; (J/g)</th>
<th>( % ) Crystallinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – day TPE</td>
<td>119.37</td>
<td>63.3</td>
<td>22.21</td>
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<tr>
<td>12 – months TPE</td>
<td>119.50</td>
<td>84.61</td>
<td>29.68</td>
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<tr>
<td>12 – months TPE + Lys</td>
<td>120.79</td>
<td>96.50</td>
<td>33.85</td>
</tr>
<tr>
<td>0 – day TBPE10</td>
<td>121.67</td>
<td>68.34</td>
<td>23.97</td>
</tr>
<tr>
<td>12 – months TBPE10</td>
<td>120.90</td>
<td>78.78</td>
<td>27.64</td>
</tr>
<tr>
<td>12 – months TBPE10 + Lys</td>
<td>119.68</td>
<td>83.56</td>
<td>29.31</td>
</tr>
<tr>
<td>0 – day TBPE20</td>
<td>119.68</td>
<td>78.28</td>
<td>27.46</td>
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<tr>
<td>12 – months TBPE20</td>
<td>119.06</td>
<td>104.0</td>
<td>36.46</td>
</tr>
<tr>
<td>12 – months TBPE20 + Lys</td>
<td>119.25</td>
<td>110.3</td>
<td>38.59</td>
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</table>
9.10 Summary

The present research work dealt with the investigation of the effect of microbial degradation of pristine polyethylene and pro-oxidant additive blended polyethylene after their abiotic oxidative modification. The microbial growth on polymer is progressive in pre-treated BPE20 than pre-treated TPE samples (fig 9.12). The prooxident additive, catalysis the degradation by the generation of free radical. The abiotic oxidation modifies the polymer, weakened the polymer back bone and introduces new functional groups on surfaces. Thus the attachment of microbe is facilitated there by speeding up bio-degradation. The little reduction in carbonyl peak in bio-degradation suggests the utilization of these groups by the microbes. The gradual reduction in contact angle proves the surface changes and the nature of polymer are being changed to hydrophilic. The TGA curves showed the samples undergoing biodegradation loses its thermal stability and the DSC endotherm showed increase in heat of fusion and percent crystallinity. The AFM images showed the increase in surface roughness of the biodegraded samples than the control. The loss of physical and mechanical strength is evident in reduction of tensile strength. The bacterial adhesion in SEM micrograph indicates that the pre-treatment facilitates the microbial adhesion on the surface of polymer. But on the other hand, the uninoculated control polyethylene is little effective in the progress of biodegradation. Thus the output of this research concludes,

1. The abiotic treatment of polyethylene initiates the physio-chemical modifications.
2. Pro-oxidant additive added in the PE accelerates the rate of degradation initially than the pristine PE.
3. The weakened PE becomes easily accessible for the microbe to proceed further biodegradation. The bacterial isolate Lysinibacillus fusiformis strain Ma-Su (Lys) shows the positive sign of polyethylene biodegradation.