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Research article

BIODEGRADATION OF LOW DENSITY POLYETHYLENE BY BACTERIA ISOLATED FROM OIL CONTAMINATED SOIL

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ABSTRACT: Plastics are composed of petroleum based materials that are resistant to biodegradation. The widespread applications of plastics are not only due to their favourable mechanical and thermal properties but also mainly due to the stability and durability. The most commonly used non-degradable solid waste is polythene which is a linear hydrocarbon polymers consisting of long chains of the ethylene monomers. Most shopping bags are made from polyethylene a chemically inert compound consisting of carbon and hydrogen. Burning of this plastic waste and burying of the plastics releases harmful toxic material which is a major pollutant in environment. Degradation of waste plastics through microorganism use represents one of the alternatives to deal with such problems. The present study aims to investigate the biodegrading potentials of bacteria isolated from oil contaminated soil. The extents of biodegradability of the untreated low density polyethylene film by the isolated bacterial strains were assessed *in vitro* in the medium containing polyethylene film as the sole carbon source. After 30 days of incubation period, the biodegradation of the polyethylene film was measured in terms of weight loss and physicochemical analysis by scanning electron microscopy and fourier transform infra red spectroscopy. The hydrophobicity of the bacterial isolates was evaluated by BATH test. The results depict that both the isolates were hydrophobic and were able to grow in a medium containing untreated polyethylene as a sole carbon source. Incubation of untreated polyethylene with bacterial isolate 1 and 2 (30 days, 37º c) reduces its mass by 1.29% and 1.3 % respectively. The smooth surface of the untreated polyethylene film became eroded as a result of biodegradation. The FTIR spectra showed changes in the chemical properties of the polyethylene film due to the biodegradation by the bacterial isolates.
Keywords: Biodegradation, LDPE, bacteria, SEM, FTIR, oil contaminated soil.

INTRODUCTION

Polyethylene is known for being a remarkably resistant polymer to degradation. Its chemical and biological inertness has fostered its application into various products from plastic bags and piping to the construction of fuel storage tanks. From an ecological point of view, the accumulation of plastic debris in the environment is a growing concern, as the rate of plastics manufacture goes over 25 million tons per year [16]. The use of polyethylene growing worldwide at a rate of 12% per year and about 140 million tons of synthetic polymers are produced worldwide each year [25]. In recent years there has been growing public concern over environmental deterioration associated with the disposal of conventional plastics. Biodegradation on the disposal site appears to be the best approach when compared to recycling, land filling and incineration (14). Biodegradation is the safest method of breakdown that possibly leaves behind less toxic residue and shows potentials of bio-geo chemical cycling of the substrate [18].

Polyethylene is highly hydrophobic and chemically inert, and microbes on the earth surface have not yet been fully evolved to digest the artificially made plastics. A lot of research has been carried out to alleviate the environmental burden by improving degradability of the waste polyethylene. Abiotic pre-treatment such as weathering, UV irradiation and thermal treatment was employed to raise the hydrophilicity of polyethylene by introducing polar groups such as carbonyl groups to the polyethylene backbone chain and thus facilitates the microbes to metabolize the unwieldy plastics [4].

It has also been known that microbes from various sources are responsible for the degradation of polythene. But efficient polythene degrading microbe is still need to be screened from all the sources. In-vitro biodegradation of plastic waste through microbial strains could offer a solution to this problem [8]. In the present study two bacterial strains capable of degrading low density polyethylene (LDPE) were isolated from a soil chronically contaminated with petroleum oil since plastics are mostly made from fossil resources such as petroleum, coal, and natural gas. In vitro Biodegradation assay of LDPE was performed with the isolated bacterial strains. After the period of incubation with bacterial strain weight reduction in LDPE was determined. Physicochemical analysis of treated LDPE was done by Scanning electron Microscopy (SEM) & Fourier Transform Infrared Spectroscopy (FTIR).

MATERIALS AND METHODS

Sample collection

The soil samples were collected from a chronically oil contaminated site located in Coimbatore, Tamilnadu. Soil was collected from a depth of 3-5cm, and sealed in plastic bags immediately after sampling, then transported in an ice chest and used for the isolation of bacteria.

Polyethylene Film

The LDPE films used for this study were obtained from local market where it is sold as 20 micron thick carry bags. LDPE films were cut into (3X3 cm) strips and then washed with 70% ethanol for 30 min, washed with distilled water, and air dried for 15 minutes in Laminar air flow chamber and was added to the medium.

Isolation of Polyethylene Degrading Bacteria

Isolation of the low density polythene (LDPE) degrading Bacterial strains were performed according to the method proposed by Hadad (10). The medium used for the isolation of LDPE degrading bacterial strains was composed of the following: NH_4NO_3 : 1.0g; $MgSO_4.7H_2O$: 0.2g; K_2HPO_4 : 1 .0g; $CaCl_2.2H_2O$: 0.1g; KCl : 0.15g;yeast extract : 0.1g; and 1.0 mg micro-elements: $FeSO_4.6H_2O$: 1.0 mg, $ZnSO_4.7H_2O$: 1.0 mg and $MnSO_4$: 1.0 mg; in 1000 ml distilled water. To the flask containing 100 ml SM medium, 1g of soil sample was added as inoculum source and the untreated low density polyethylene films were cut into small pieces (about 3x3 cm each), weighed at a concentration of 300 mg/100ml, disinfected in 70 % ethanol and air dried for 15 minutes in Laminar air flow chamber and was added to the medium. The medium was then incubated at 37°C for 7 days in a rotary shaker (150 rpm). Once for every 7 days, subcultures were done up to 35 days.

After the subcultures, the enriched culture broth was spread on the agar plates supplemented with 1 ml/l of emulsified hexadecane and incubated at 37°C for 1 week. Polyethylene degrading bacteria were selected based on the growth of the organism on the agar plates. The emulsified agar plate for the selection of the polyethylene degrading strains was prepared by adding 1 ml of hexadecane (Sigma) into 1L of the enrichment medium. LDPE degrading bacterial strains were selected based on the size of the clear zone.

Identification of Polyethylene degrading bacteria

The identification of isolated bacterial strains was performed on the basis of macroscopic and microscopic examination. The bacterial isolates were identified macroscopically by examining colony morphology- surface pigment, shape, size, margin, surface on nutrient agar plates and microscopic examination- Gram staining, to study the staining behaviour, shape and cell arrangement.

In-vitro biodegradation assay

LDPE degrading ability of the bacterial isolates was studied in the *in-vitro* biodegradable assay. Bacterial isolates were maintained on nutrient broth or nutrient agar media. Liquid cultures 50 ml were incubated in flasks (250 ml) on a rotary shaker (150 rpm) at 30° C for 30 days. The synthetic medium used for assaying biodegradation of polyethylene contained the following elements in 1000ml distilled water: NH_4NO_3 : 1.0g; $MgSO_4.7H_2O$: 0.2g; K_2HPO_4 : 1 .0g; $CaCl_2.2H_2O$: 0.1g; KCl : 0.15g; and yeast extract : 0.1g; and 1.0 mg; micro-elements: $FeSO_4.6H_2O$: 1.0 mg, $ZnSO_4.7H_2O$: 1.0 mg and $MnSO_4$: 1.0 mg.

Bacterial isolates were grown overnight in nutrient broth until the cultures attained log phase (absorbance of 0.6 at 600 nm). 10 % of this log phase culture was inoculated in 250 ml Erlenemeyer flask containing 50 ml of Synthetic medium and polyethylene films. Prior to transfer to liquid culture media, polyethylene films were cut into pieces (3x3 cm), weighed (300mg/100ml) disinfected (30 min in 70 % ethanol), air dried for 15 minutes in Laminar air flow chamber and added to flask. Biodegradation test were performed in triplicates. As for control, un-inoculated minimal broth supplemented with untreated LDPE films were maintained under similar conditions. The tests were performed in triplicate for each strain of bacteria. At several intervals (day 0, 3, 7, 10, 14, 17, 21, 24, 28 and 30) the culture broth were subjected to spectrophotometric analysis.

Determination of dry weight of residual polyethylene

To facilitate accurate measurement of residual polyethylene weight, bacterial biofilms were washed off the polyethylene surface with 2% (v/v) sodium dodecyl sulfate (SDS) overnight, followed by rinsing with distilled water (10).

$$\text{Weight loss \%} = \frac{\text{Initial weight-Final weight}}{\text{Initial weight}} \times 100$$

Evaluation of bacterial hydrophobicity

Bacterial cell-surface hydrophobicity was estimated by the bacterial adhesion to hydrocarbon (BATH) test proposed by Rosenberg (22) which is based on the affinity of bacterial cells for an organic hydrocarbon such as hexadecane. The more hydrophobic the bacterial cells, the greater their affinity for the hydrocarbon, resulting in transfer of cells from the aqueous suspension to the organic phase and a consequent reduction in the turbidity of the culture. For the BATH test, bacteria were cultured in NB medium until the mid- exponential phase, centrifuged and washed twice with phosphate–urea–magnesium (PUM) buffer containing (g l^{-1}): K_2HPO_4, 17; KH_2PO_4, 7.26; urea, 1.8 and $MgSO_4.7H_2O$, 0.2. The washed cells were resuspended in PUM buffer to an O.D. (400 nm) value of 1.0–1.2. Aliquots (1.2 ml each) of this suspension were transferred to a set of test tubes, to which increasing volumes (ranging: 0–0.2 ml) of hexadecane were added. The test tubes were shaken for 10 min and then allowed to stand for 2 min to facilitate phase separation. The turbidity of the aqueous suspensions was measured at O.D. 400 nm. Cell-free buffer served as the blank.

Fourier Transform Infrared Spectroscopy of polyethylene

Changes in the polyethylene structure after incubation with bacterial isolates in the medium containing untreated polyethylene as a sole carbon source were analysed by Fourier Transform Infrared Spectroscopy (Shimadzu).

Scanning Electron Microscopy of Polyethylene

The surface morphology of the PE film was analyzed through Scanning Electron Microscopy to check for any structural changes on the film. A piece of film was placed on the sample holder and was scanned at a magnification of 2500x, 5000x, 7500x and 10000x [13].

RESULTS

Low density Polyethylene degrading bacterial strains were isolated from oil contaminated soil. These isolates were capable of growing on a carbon free synthetic medium containing polyethylene films as sole carbon source which was seen by subsequent sub-culturing followed by plating on synthetic medium supplemented with hexadecane. These bacterial strains were designated as Isolate 1 and Isolate 2. Both the isolated bacterial strains were identified on the basis of their colony morphology, and Gram staining character is summarized in Table 1. From the results it can be inferred that Isolate 1 was rod shaped gram positive bacterium and Isolate 2 was Gram positive coccus.

Table .1 Characteristics of Low Density Polyethylene Degrading Bacterial Isolates

Characteristics	Bacterial Isolate 1	Bacterial Isolate 2
Shape	Irregular	Rhizoid
Size	Small	Large
Color	White	White
Surface	Rough shiny	Smooth shiny
Margin	Undulate	Filamentous
Gram stain	Gram +ve rod	Gm +ve cocci
Cell arrangement	Single rod	Cocci in pairs

In-vitro biodegradation assay

Biodegradation of polyethylene by the bacterial isolates was assessed in the medium containing untreated LDPE as the sole carbon source. When inoculated into the medium, the bacterial isolates were able to colonize on the surface within few days. The growth of the bacterial isolates in liquid synthetic media containing polyethylene as sole carbon source was monitored throughout the incubation period. At several intervals (days 0, 3, 7, 10, 14, 17, 21, 24, 28, 30) the broth culture was subjected to spectrometric analysis. Figure 1 shows the growth profile of the bacterial isolates during the in-vitro biodegradation assay.

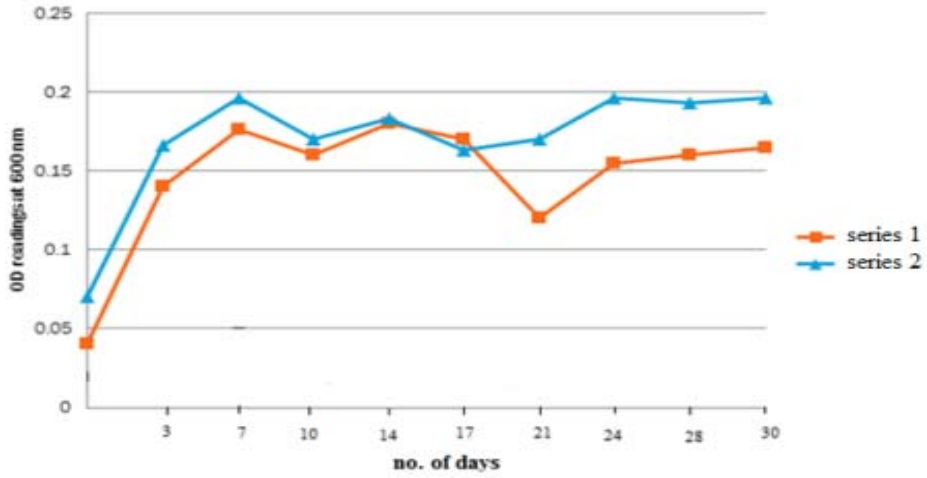

Fig 1 Growth curve of bacterial isolates during biodegradation assay

Series 1- Bacterial isolate 1 Series 2- Bacterial isolate 2

Determination of dry weight of residual polyethylene

After 30 days of incubation period, the percentage of weight reduction was estimated and it is shown in table 2. Low density polyethylene films incubated with bacterial Isolate 1 and isolate 2 showed weight loss of 1.29% and 1.31% respectively which was found to be greater than the weight loss obtained in control. Therefore, the observed percentage weight loss of polyethylene strips incubated on bacterial Isolates was not as result of chemicals in the mineral salt medium but because of a biological process.

Table.2 Mass of the low density polyethylene film after 30 days of incubation with bacterial isolates

Bacterial isolates	Initial mass of film(g)	Final mass of film(g)	% Reduction in mass
Control	0.15060	0.15040	0.13
Isolate 1	0.15040	0.14845	1.29
Isolate 2	0.15026	0.14830	1.31

Each data point represents the mean of three replicates.

Evaluation of bacterial hydrophobicity

The BATH assay (fig 2) clearly shows the higher hydrophobicity of Isolate 1 compared with that of Isolate 2. For Isolate 1, the adhesion of bacterial cells to hexadecane was evident even at the lowest concentration of the hydrocarbon, resulting in a reduction of more than 33% in the turbidity of the culture.

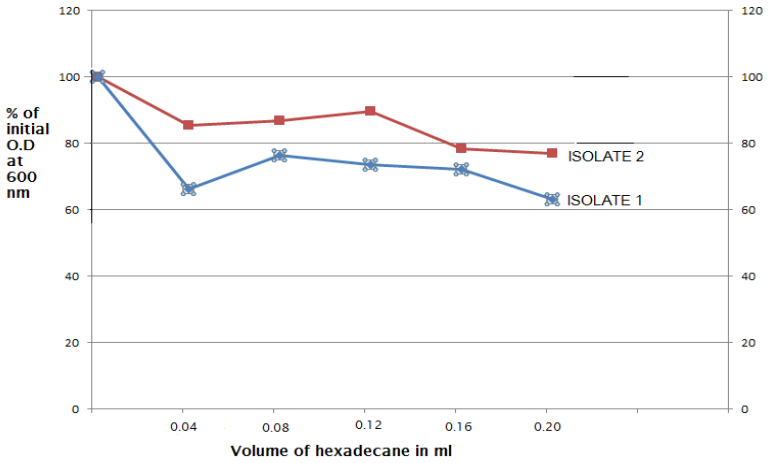

Fig. 2. Hydrophobicity of bacterial isolates determined by the bacterial adhesion to hydrocarbon test (BATH)

Fourier transforms infrared spectroscopy of polyethylene

In the present study the changes in the polyethylene structure with subsequent bacterial inoculation were analyzed by Fourier Transform Infrared Spectroscopy (Shimadzu) in the frequency range of 4000 – 800 cm^{-1}. The FT-IR spectra of the untreated LDPE film and LDPE film incubated with Bacterial Isolates 1 and 2 for 30 days in liquid SM media containing polyethylene as sole carbon source are shown in Figure 3, 4 and 5 respectively.

Scanning Electron Microscopy of Polyethylene

The surface morphology of the treated low density polyethylene film was analyzed through Scanning Electron Microscopy to check for any structural changes on the low density polyethylene film. Figures 6 & 7 show the micrographs of the Scanning Electron Microscopy of low density polyethylene film before and after incubation with bacterial isolates 1 and 2 respectively**.** From this the structural changes and erosions on the surface of the polyethylene films were observed. Cavities were also observed on the polyethylene surface.

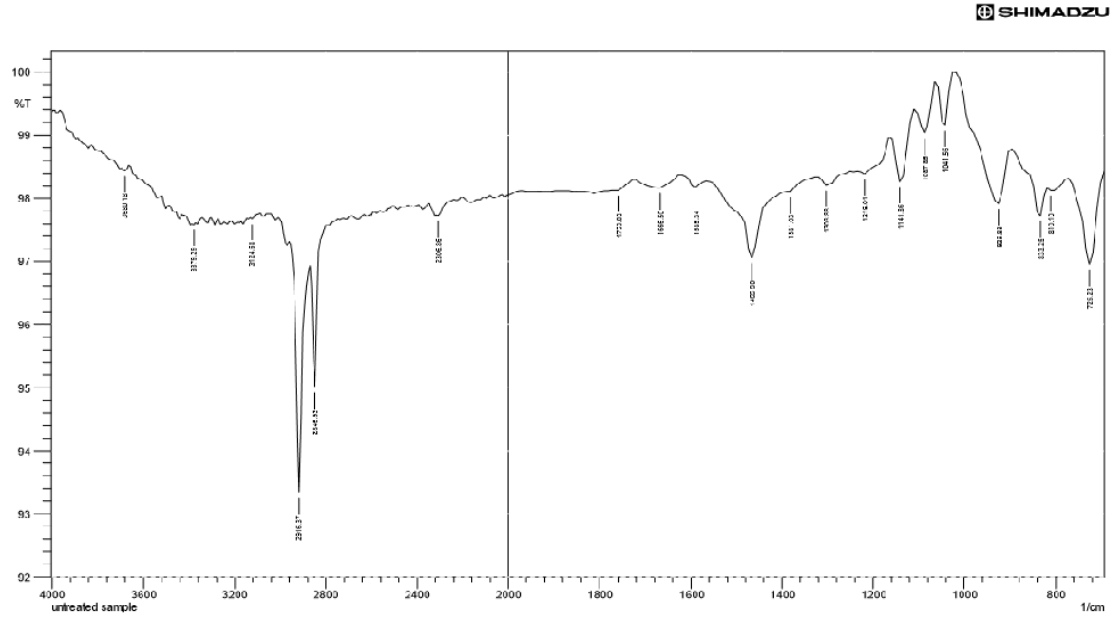

Fig.3 FTIR spectra of untreated low density polyethylene

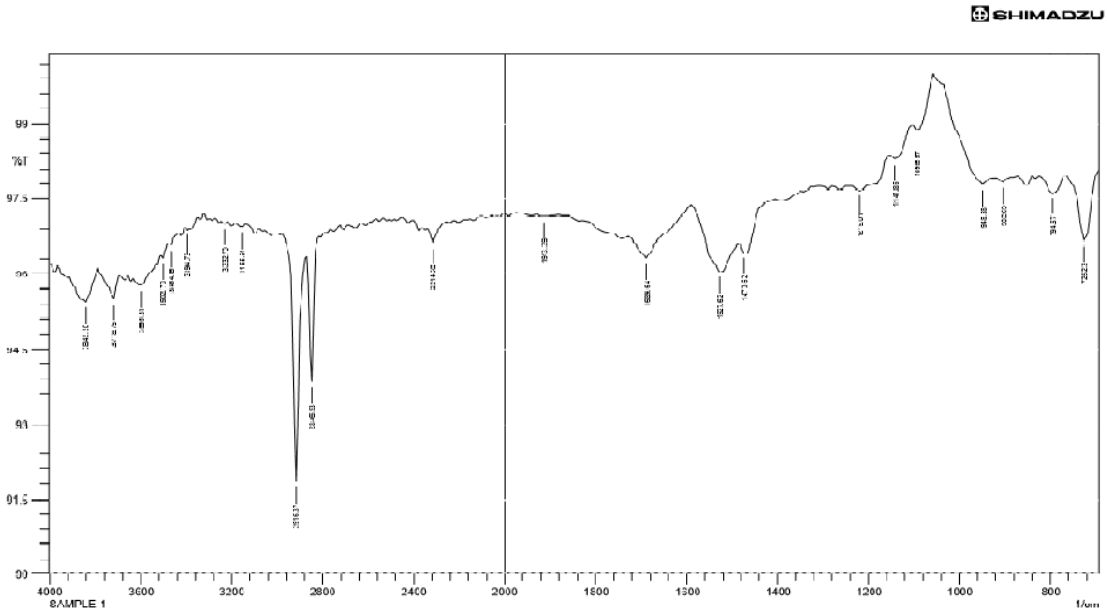

Fig.4 FTIR spectra of low density polyethylene after 30 days incubation with Bacterial isolate1

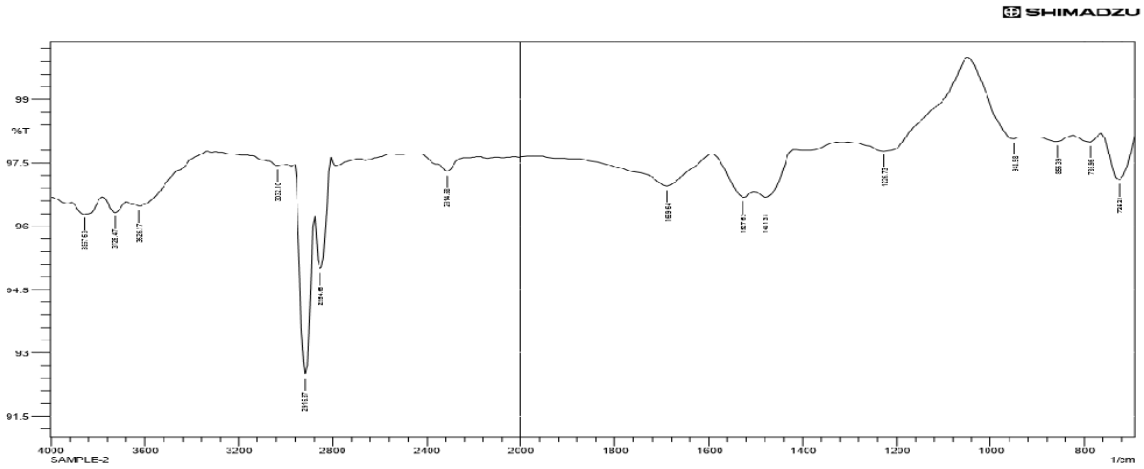

Fig.5 FTIR spectra of low density polyethylene after 30 days incubation with Bacterial isolate 2

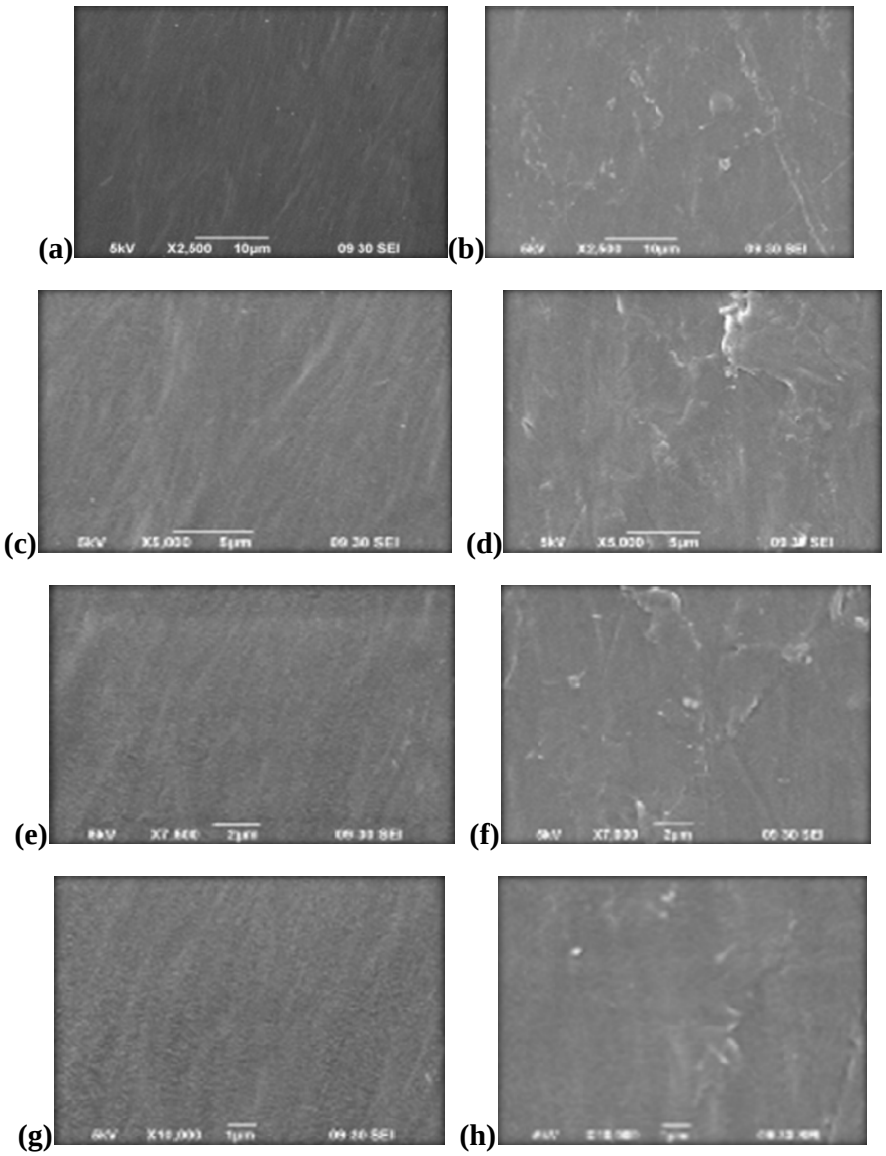

Fig .6 (a), (c), (e) and (g) untreated low density polyethylene at x2500, x5000, x7500 and x10000 respectively; (b), (d), (f) and (h) – low density polyethylene after 30 days incubation with Bacterial isolate 1 at x2500, x5000, x7500 and x10000 respectively.

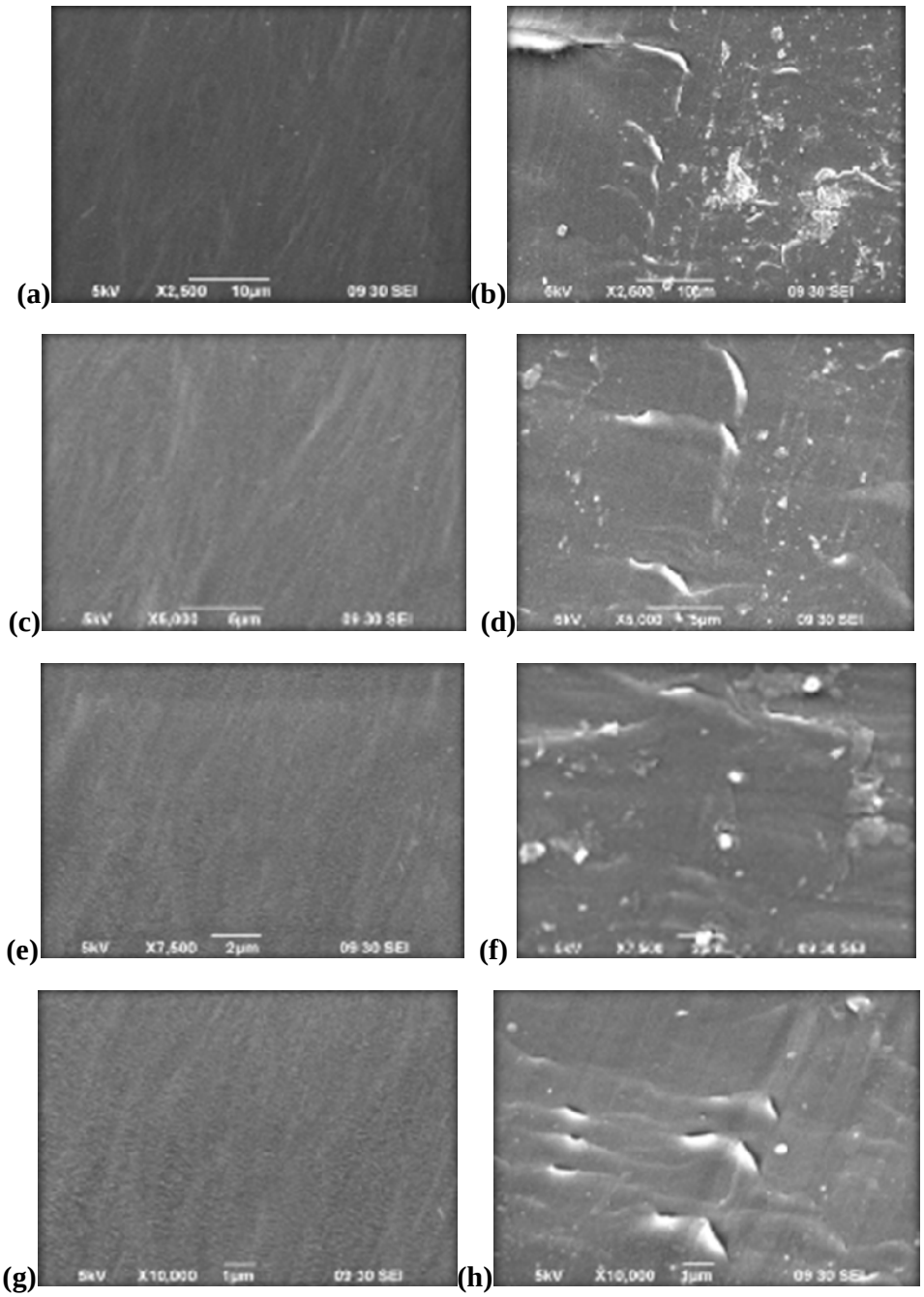

Fig.7 (a), (c), (e) and (g) untreated low density polyethylene at x2500, x5000, x7500 and x10000 respectively; (b), (d), (f) and (h) – low density polyethylene after 30 days incubation with Bacterial isolate 2 at x2500, x5000, x7500 and x10000 respectively.

DISCUSSION

Exhausting natural resources (raw materials) and generating a large amount of waste are the important environmental problems. Plastic packaging is a symbol of these problems, also regarding its high weight to volume relationship. Production of plastic packaging, which is quite difficult for degradation or biodegradation results in the exhaustion of non renewable resources. Moreover, the small energetic and material recovery of polymers causes that more and more areas are occupied for landfill sites [17]. Degradation of LDPE by microorganism had been known for several years. the degradation capacity of bacterial and fungal consortium under natural conditions was reported previously by several authors.

In the present study two bacterial strains were isolated from chronically oil contaminated soil since plastics are mostly made from fossil resources such as petroleum, coal and natural gas. It has been observed that the soils and sediments contaminated with hydrocarbon are teemed with hydrocarbon- degrading microorganism [5]. An enhanced number of hydrocarbon degraders were reported in hydrocarbon contaminated soils from Scott Base, Marble Point and Wright Valley in Antarctica and in oil-polluted Antarctic seawater [2]. In the present study reports that the low density polythene degrading ability of the isolated bacterial strains were assessed *in vitro* in the synthetic medium containing untreated LDPE film as the sole carbon source for 30 days. After 30 days of incubation period, the biodegradation of the LDPE film was measured in terms of weight loss. Changes in the chemical and physical properties of LDPE film was assessed by FT- IR spectroscopy and SEM respectively. To measure the physical changes of the polythene after the microbial attack various parameters are usually used to determine the weight loss, percentage of elongation and change in tensile strength [20].

The growth profile of the bacterial isolates showed that both isolates were able to grow in liquid synthetic medium containing untreated polyethylene as sole carbon source. A sudden decrease in transmittance that is increase in absorbance can be observed during day 0 to day 7 which coincides with the logarithmic increase in the number of bacterial cells during the same period. This serves as an indication that more LDPE was utilized due to increase in the number of cells. Similar results were reported by Rajandas [21] and Huang [12]. To measure the physical changes of the polythene after the microbial attack various parameters are usually used to determine the weight loss, percentage of elongation and change in tensile strength were measured [20]. After 30 days of incubation period, Low density polyethylene films incubated with Isolate 1and isolate 2 showed weight loss of 1.29% and 1.31% respectively. This biodegradation level is in agreement with the earlier reports ranging from 3.5% to 8.4% for polyethylene incubated in soil for 10 years (3). Weight reduction in low density polyethylene films after 30 days incubation with marine bacteria was 1 ± 0.033%, 1.5 ± 0.038% and 1.75 ± .06% for K. palustris M16, B. pumilus M27 and B. Subtilis H1584, respectively [11].

The ability of a microorganism to utilize any substrate depends on its growth and adherence to that substrate. Bacterial adhesion to either a hydrophilic or hydrophobic substrate is governed by many factors, including the forces by which the bacterium attaches to the surface and the properties of the substrate and micro- organism. Generally, a hydrophobic bacterium prefers a hydrophobic surface for attachment, whereas the opposite is true for bacteria with hydrophilic properties. As the polyethylene surface is hydrophobic in nature, it has been suggested that the more hydrophobic the bacterial cell surface, the higher the interaction with polyethylene [9]. The BATH assay clearly shows the hydrophobic nature of the bacterial isolates even at low concentration of hexadecane (0.04%). The micro destruction of the small samples is widely analyzed by an important tool such as Fourier Transform Infrared spectroscopy (FT-IR), and due to the recent up-gradation of this instrument the map of the identified compounds on the surface of the sample can be documented via collection of large number of FT-IR spectra (19). In the present study the changes in the polyethylene structure with subsequent bacterial inoculation were analyzed by Fourier Transform Infrared Spectroscopy (Shimadzu) spectra in the frequency range of 4000 – 800 cm^{-1}. (Figure 3, 4 and 5). Monitoring the formation and disappearance of carbonyl and double bond bands using FT-IR is necessary to elucidate the mechanism of the biodegradation process. The carbonyl absorption bands can be observed in the range of 1,710−1,750 cm^{-1} because of the formation of ketone or aldehyde C = O groups by the action of the selected microorganisms [6]. In the present study the FT-IR spectra showed the band in the range of 1,710-1,750 cm^{-1} which indicates the formation of ketone or aldehyde groups by the action of bacterial isolates 1 and 2. The formation of new C-O stretching frequency at 1,710−1,750 $cm^{-1}$and a broad absorption peak assigned to stretching vibration of –OH also indicated polymer biodegradation. (1). Additionally, new absorption bands between 3800 - 3100 cm^{-1} and 1900 - 1500 cm^{-1} of the spectra were observed in the bacterial isolates treated low density polyethylene and this is possibly due to the formation of hydroxylated compounds and carboxylated compounds respectively. In addition, the bands at 1500-1600 cm^{-1}also appeared which can be ascribed to unsaturated hydrocarbons. In both the treated LDPE film the new band at 948^{cm-1} appeared, this indicated the formation of new vinyl groups. Also disappearance of peaks was observed at 1041 cm^{-1} for both the samples which indicated effective biodegradation. Similar results were observed in the bands at 1500-1600 cm^{-1} after biodegradation of modified LDPE in different soils under laboratory conditions [15].

In the present study, any changes or either new peak formation or disappearance of a peak or else change in the peak range was accounted as monitoring parameter and regarded as the change occurred on the surface of polyethylene due to action of bacterial isolate. The native band at 2846cm^{-1} was increased to 2854 in the FT-IR spectra of LDPE film inoculated with bacterial isolate 2. This was in accordance with the Das et al [7]. Also, the FT-IR spectra of LDPE film inoculated with bacterial isolate 2 showed broadening of the band were observed in the range of 2916 $cm^{-1}$and 2854 cm^{-1}which indicates that the presence of more than one oxidation products [24].

Formation of bands at 1620 – 1640 cm-1 and 840-880 cm-1 was attributed to oxidation of polyethylene [26]. A band was formed at 856 cm^{-1} in the FT-IR spectra of LDPE film inoculated with bacterial Isolate 2. Thus, the FTIR spectra of LDPE biodegraded by Isolate 1 and 2 gave conclusive evidence of the oxidation of polyethylene with the addition of carbonyl group and presence of more than one oxidation products in their spectra.

The level of polythene degradation can be determined by the various methods. At topographical level, the Scanning Electron Microscopy (SEM) is being used to see the level of scission and attachment of the microbes on the surface of the polythene before and after the microbial attack [23]. SEM micrograph of the LDPE film incubated with bacterial isolate 1 and 2 are shown in figure 6 and 7. The biodegradation of polyethylene was evidenced through formation of cavities on the surface of polyethylene and structural changes like erosion on the surface of LDPE film were observed. SEM micrograph of the LDPE film incubated with bacterial isolate 2 shows more degradation than the bacterial isolate 1.

CONCLUSION

In the present study, two bacterial strains were isolated from chronically oil contaminated soil since plastics are mostly made from fossil resources such as petroleum, coal and natural gas. Both the isolates were able to grow in a medium containing untreated LDPE as sole carbon source. The results of the BATH test demonstrated that both the isolates were hydrophobic and formed biofilm on the surface of untreated LDPE. It indicated that there is a great possibility of finding microorganisms from the oil contaminated soil also that can degrade synthetic plastic. Further studies are underway for the identification of the bacterial isolates and the mechanism responsible for the biodegradation of polyethylene.

REFERENCES

[1] Agamuthu, P., Faizura, N.P., 2005. Biodegradability of degradable plastics waste.Waste Manag and Res, 23, pp. 95-100.

[2] Aislabie, J., Fraser, R., Duncan, S., Farrell, R.L. 2001. Effects of oil spills on microbial heterotrophs in Antarctic soils. Polar Biol, 24, pp. 308-313.

[3] Albertsson, A.C., Karlsson, S. 1990. The influence of biotic and abiotic environments on the degradation of polyethylene. Progress in Polym. Science, 15, pp.177-192.

[4] Albertsson, A.C., Andersson, A.O., Karlsson, S. 1987. The mechanism of biodegradation of polyethylene. Polym. Degrad. Stab. 18, pp.73–87.

[5] Atlas, R.M. 1977. Stimulated petroleum biodegradation, CRC Crit. Rev. Microbiol, 5, pp. 371-386.

[6] Carol, D., Karpagam, S., Kingsley, S.J., Vincent, S. 2012. Synergistic effect of calcium stearate and photo treatment on the rate of biodegradation of low density polyethylene spent saline vials. Indian J Exp Biol, 50, pp. 497 – 501.

[7] Das, M.P., Kumar, S. 2014. Microbial Deterioration of Low Density Polyethylene by *Aspergillus* and *Fusarium*sp. Int J. Chem. Tech Res, 6(1), pp. 299-305.

[8] Das, M.P., Kumar, S., Rebecca, J.L., Sharmila, S. 2013. Isolation and identification of LDPE degrading fungi from municipal solid waste. J. Chem. Pharm. Res, 5(3), pp.78-81.

[9] Gilan (Orr), Hadar, Y. and Sivan, A. 2004. Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. Appl.Microbiol.Biotechnol,65, pp.97–104.

[10] Hadad, D., Geresh, S., Sivan, A. 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillusborstelensis*. J ApplMicrob, 98, pp. 1093–1100.

[11] Harshvardhan, K., Jha, B. 2013. Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. Marine Pollution Bulletin, 77, pp.100–106.

[12] Huang, Y.L., Li, Q.B., Deng, X., Lu, Y.H., Liao, X.K., Hong, M.Y., Wang, Y. 2005. Aerobic and anaerobic biodegradation of polyethylene glycols using sludge microbes. Process Biochemistry, 40, pp. 207–211.

[13] Ikada, E. 1999. Electron microscope observation of biodegradation of polymers. J Environ Polym Degrad, 7, pp. 197–201.

[14] Kumar S., Hatha, A.A.M., Christi, K.S. 2007. Diversity and effectiveness of tropical mangrove soil micro flora on the degradation of polythene carry bags. Rev. Biol. Trop, 55(3-4), pp. 777-786.

[15] Nowak, B., Pajak, J., Drozd-Bratkowicz, M.,Rymarz, G. 2011. Microorgaisms participating in the biodegradation of modified polyethylene films in different soils under laboratory conditions. Int.Biodeterioration and biodegradation, 65, 757 – 767.

[16] Orhan, Y., Büyükgüngör, H., 2000. Enhancement of biodegradability of disposable polyethylene in controlled biological soil. Int. Biodeterior. Biodegrad. 45, pp. 49-55.

[17] Patkowska, S.P., Lesiuk, A. 2013. Evaluation of the commercial polyethylene packaging decomposition in the soil by FT-IR/PAS. Chemik, 67, 10, pp. 863–872.
[18] Pramila, R., Padmavathy, K., Ramesh, V.K., Mahalakshmi, K. 2012. *Brevibacillusparabrevis*, *Acinetobacterbaumannii* and *Pseudomonas citronellolis* -Potential candidates for biodegradation of low density polyethylene (LDPE). J. Bacteriol. Res, 4(1), pp. 9-14.
[19] Prati, S., Joseph, E., Sciutto, G., Mazzeo, R. 2010. New advances in the application of FTIR microscopy and spectroscopy for the characterization of artistic materials. AccChem Res, 43, pp. 792-801.
[20] Priyanka. N., Archana, T. 2011. Biodegradability of Polythene and Plastic by the Help of Microorganism: A Way for Brighter Future. J Environment Analytic Toxicol, 1, pp. 111.
[21] Rajandas, H., Parimannan, S., Sathasivam, K., Ravichandran, M., Yin, L.S. 2012. Novel FTIR-ATR spectroscopy based technique for the estimation of low- density polyethylene biodegradation. Polymer Testing, 31, pp. 1094–1099.
[22] Rosenberg, M., Gutnik, D., Rosenberg, E. 1980. Adherence of bacteria to Hydrocarbons: a simple method for measuring cell surface hydrophobicity. FEMS Microbiol. Lett, 9, pp. 29–33.
[23] Sivan, A. 2011. New perspectives in plastic biodegradation. Curr.Opin. Biotechnol, 22, pp. 422-426.
[24] Sudesh, K.,Abe, H., Doi, Y. 2007. Components characterization of LDPE blown film samples used in biodegradation. Interesjournals.org. pp. 28-35.
[25] Usha, R., Sangeetha, T. Palaniswamy, M. 2011. Screening of Polyethylene Degrading Microorganisms from Garbage Soil, Libyan Agric Res Center. J Internati, 2(4), pp. 200-204.
[26] Yamada Onodera, K., Mukumoto, H., Katsuyaya, Y., Saiganji, A. Tani, Y. 2001. Degradation of polyethylene by a fungus, *Penicilliumsimplicissimum*. YK. Poly. Degrad, Stab, 72, pp. 323-327.