

ORIGINAL RESEARCH ARTICLE

Analysis of Polyethylene Degrading Potentials of Microorganisms Isolated From Compost Soil

V.Mahalakshmi¹, Abubakker Siddiq², S.Niren Andrew*¹

Department of Microbiology, Madras Christian College, East Tambaram, Chennai-600059, Tamilnadu, India
Research and Development Centre, Bharathiar University, Coimbatore, Tamilnadu, India

Received 16 May 2012; Revised 11 Oct 2012; Accepted 21 Oct 2012

ABSTRACT

Plastic play important role for many “short live” applications such as packaging, disposable gloves, garbage bags etc and these represent the major part of plastic waste. Because of their persistence in our environment, improperly disposed plastic materials are significant source of environment pollution, potentially harming life. Among the synthetic plastics, one of the most problematic plastics in this regard is polyethylene (PE). In the absence of appropriate disposal methods polyethylene waste is usually burned, causing grave air pollution. Polyethylene-considered to be inert-can be biodegraded if the right microbial strains are used. In the present study microorganisms able to degrade polyethylene were isolated from compost soil and characterized. Physicochemical analysis of PE was done by Scanning electron Microscopy (SEM) & Fourier Infrared Spectroscopy (FTIR). The degraded products were analyzed by Gas Chromatography-Mass-Spectrometer (GC-MS).

Key words: polythene degrading microbes, environment pollution, polyethylene, Scanning electron Microscopy, Fourier Infrared Spectroscopy.

INTRODUCTION

Polyethylene is one of the synthetic polymers of high hydrophobic level and high molecular weight. In natural form it is not biodegradable. Thus their use in the production of disposal or packing materials causes dangerous environmental problems (Potts, 1978).

Biodegradation of polyethylene is known to occur by two mechanisms: Hydro-biodegradation and oxo-biodegradation (Bonhomme *et al.*, 2003). These two mechanisms agree with the modification due to the two additives, starch and pro – oxidant, used in the synthesis of biodegradable polyethylene. Starch blend polyethylene has a continuous starch phase that makes the material hydrophilic and therefore, catalyzed by amylase enzymes. Microorganisms can easily access, attack and remove this part. Thus the hydrophilic polyethylene matrix continues to be hydro-biodegraded. In case of pro-oxidant additive, biodegradation occur following photo degradation and chemical degradation. If the pro-oxidant is a metal combination, after transition, metal catalyzed thermal per oxidation, biodegradation of low molecular weight oxidation products occurs sequentially (Bonhomme *et al.*,

2003; El-Shafei *et al.*, 1998; Yamada-Onodera *et al.*, 2001).

El-Shafei *et al* (1998) investigated the ability of fungi and Streptomyces strains to attack degradable polyethylene consisting of disposed polyethylene bags containing 6% starch. He has isolated 8 different strains of Streptomyces and fungi Mucor rouxii NRRL 1835 and Aspergillus flavus.

The evaluation of visible changes in plastics can be performed in almost all tests. Effects used to describe degradation include roughening of the surface, formation of holes or cracks, de-fragmentation, changes in color, or formation of bio-films on the surface. These changes do not prove the presence of a biodegradation process in terms of metabolism, but the parameter of visual changes can be used as a first indication of any microbial attack.

To obtain information about the degradation mechanism, more sophisticated observations can be made using either scanning electron microscopy(SEM) or atomic force microscopy (AFM) (Ikada,1999).

FT-IR spectra obtained by the films of four different Low density polyethylene (LDPE) samples. It was found that some new peaks arose after the period of biodegradation (Sudesh *et al.*, 2007). In the present study Polyethylene degradation by microbes determined by SEM-EDAX, FTIR GC-MS analysis.

MATERIALS AND METHODS

Plastic films

High Density Polyethylene (HDPE) and Low Density Polyethylene (LDPE) which are widely used to manufacture carry bags, milk and oil pouches are used in the study.

Area of Study

Soil samples were collected from dumpsite in Madras Christian College campus, Tambaram, Chennai, Tamilnadu during the month of July 2011.

Sample Preparation

A total of 1 gram of the soil sample was suspended in 10 ml of sterile 'Milli- q water' and vortexed for 15 min.

Enrichment of polyethylene degrading bacteria

Nearly 1 ml of suspension was added to Erlenmeyer flasks containing 100 ml of mineral salt medium, 1 gram of untreated polyethylene films (cut into small strips) was added as the sole source of carbon and energy (S.H.Imam *et al.*, 1999).

IDENTIFICATION OF THE SELECTED ISOLATES

Fungal Isolates

The isolated fungal strains were named as Fungal strain 1 (FS1) and Fungal strain 2 (FS2) .The fungal strains were identified by both macroscopic and microscopic examinations. Macroscopic identification was done by visualizing surface pigment on Sabouraud Dextrose Agar and Microscopic characterization includes shape,color and structure of conidia and hyphae.

Bacterial isolates

The isolated bacterial strains were named as Bacterial strain1 (BS1) and Bacterial strain 2 (BS2). The bacterial strains were identified macroscopically by examining colony morphology, surface pigment, shape and size on Nutrient Agar plates. Microscopic examination including Gram's staining to study the staining behavior, shape and cell arrangement. Motility test was also performed.

Further characterization was done performing the following biochemical tests such as urease,

IMViC, TSI, oxidase and catalase and following the procedures described in Bergey's manual and Murray *et al.*

POLYETHYLENE DEGRADATION STUDIES

Physical analysis

SEM-EDAX:

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 17000x, 28000x, 40000x, 50000x and 60000x (Ikada, 1999).

Chemical analysis of the polymer surface was performed by measuring the wavelength and intensity distribution of X-ray signal generated by a focused electron beam on the specimen with the EDAX. (Artham.T and Doble. 2008).

Chemical analysis

FT-IR Spectroscopy Analysis

Fourier Transform Infrared Spectroscopy analysis was used for detecting the formation of new functional groups or changes in the amount of existing functional groups (Milstein *et al.*, 1994).

Analysis of Degraded Products by Gas Chromatography

After 2 months of incubation period, the mycelia pellet (in case of fungal culture) or the bacterial pellet (in case of bacterial culture) was removed by filtration, and the filtrates were extracted with distilled ether. The degraded products of PE were determined by Gas chromatography-mass spectrometer(JEOL GCMATE II GC-MASS SPECTROMETER, Indian institute of technology, Chennai.) using HP5 column, helium gas, was programmed to raise the oven temperature from 70°C to 200°C(maximum temperature-250°C at 15°C/min, Injection liquid 1microliter). Mass spectrometer consists of tungsten filament as electron source which works with 70eV, a double focusing analyzer and photo multiplier tube as detector with resolution of maximum 5000. Using PerFluoro Kerosene (PFK) as standard, mass spectrometer was calibrated (Wen chai, *et al.* 2008).

RESULTS

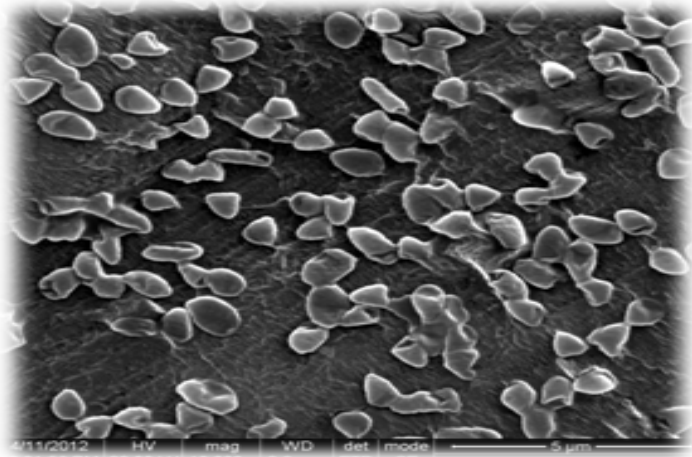
Physical Analysis

SEM-EDAX of Polyethylene

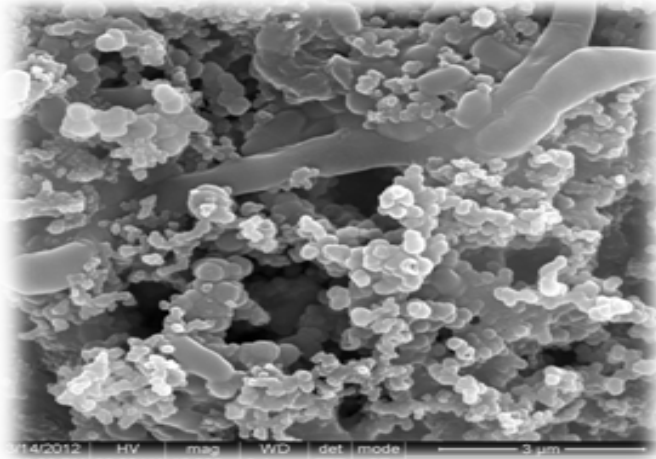
Structural changes and erosions on the surface of the PE films were observed. Cavities were also observed on the polyethylene surface.

SEM images of degraded PE films

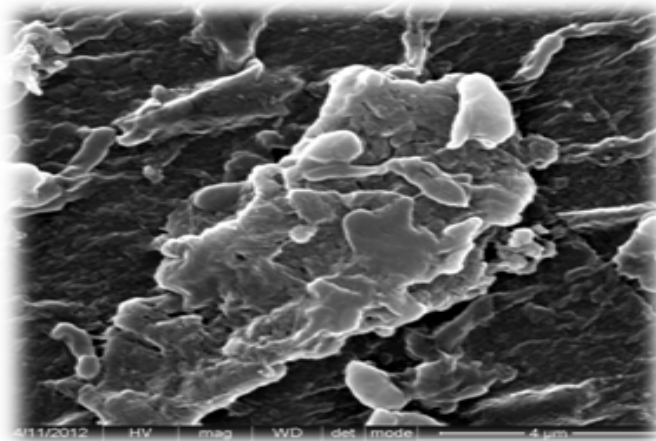
Bacillus



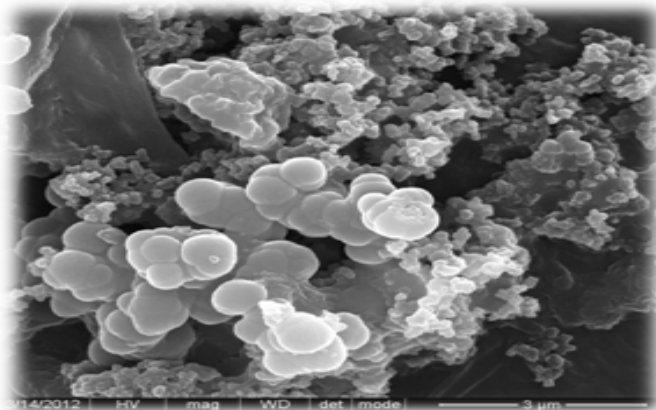
Aspergillus



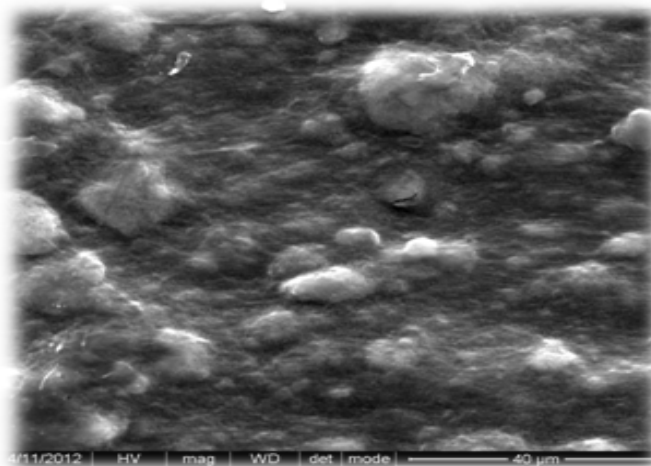
Pseudomonas



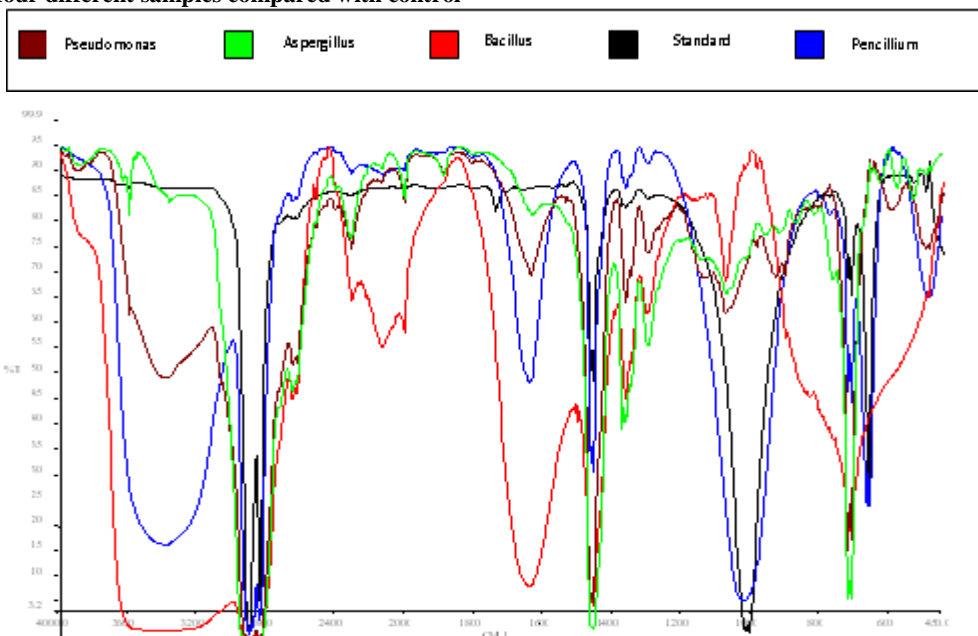
Penicillium



Control

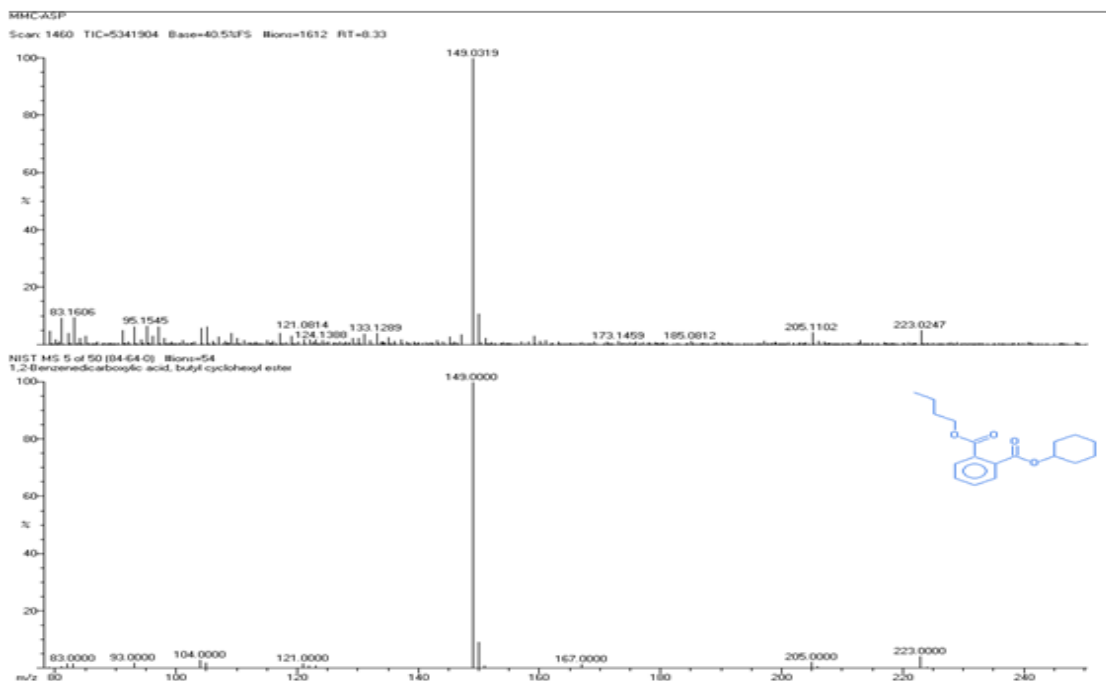


FTIR spectra of four different samples compared with control

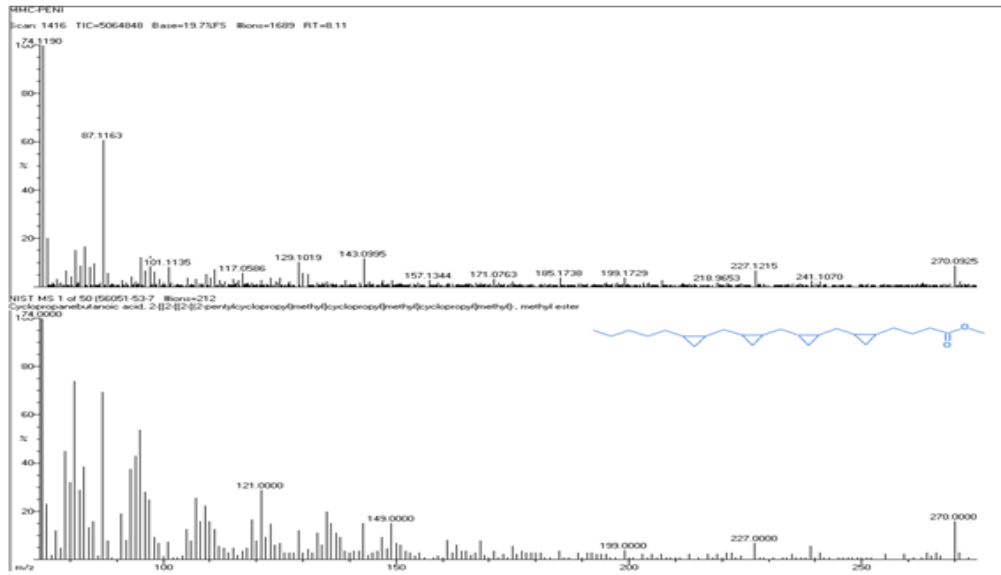


IJPBA, Sep - Oct, 2012, Vol. 3, Issue, 5

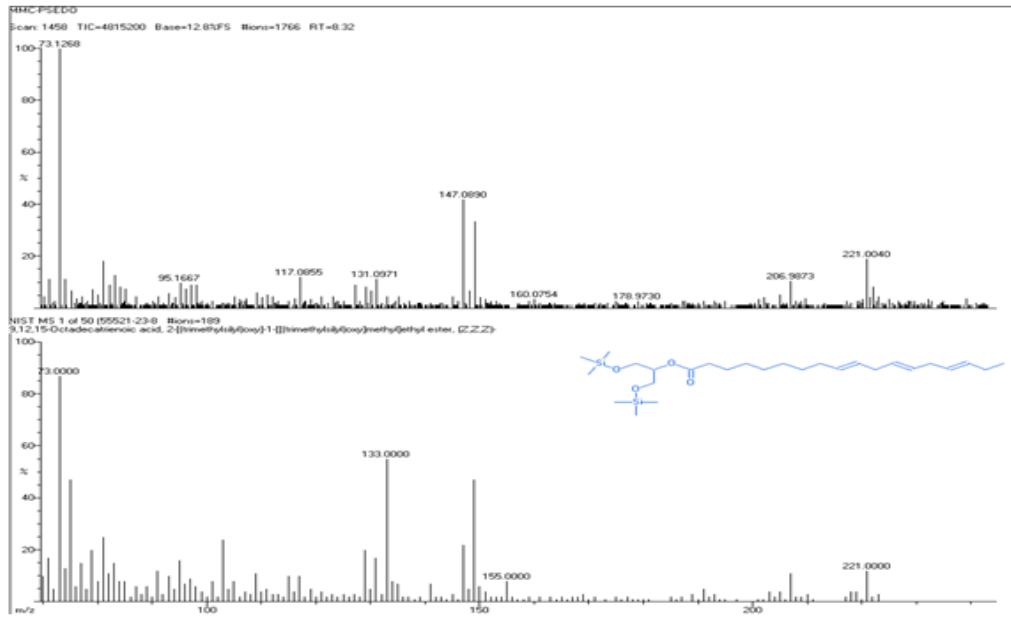
GC-MS RESULTS:
Bacillus



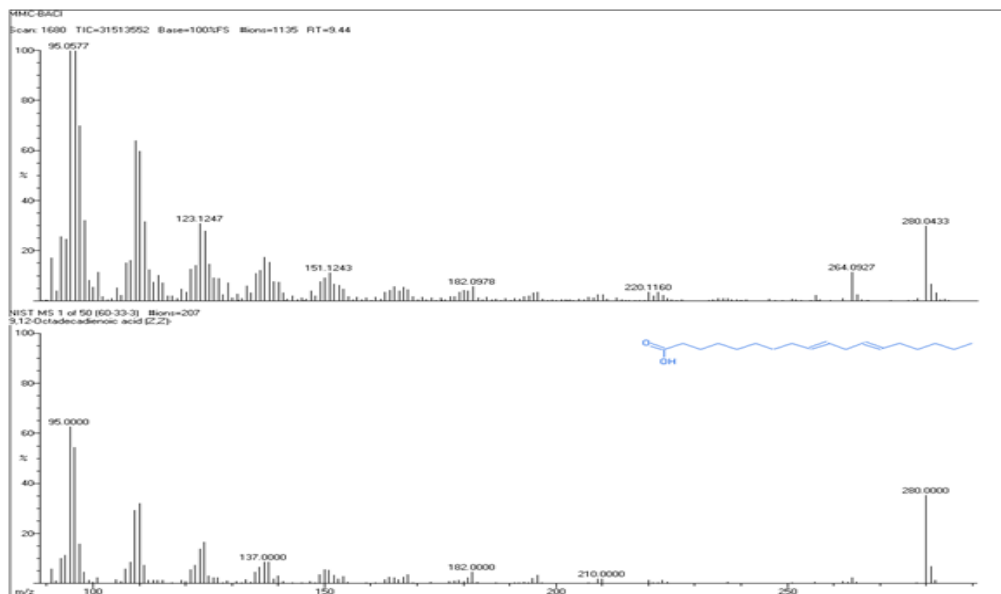
Pseudomonas



Aspergillus



Penicillium



IJPBA, Sep - Oct, 2012, Vol. 3, Issue, 5

DISCUSSION

Polyhydroxy butyrate (PHB) is incorporated into the mineral salt (minimal) broth media for the degradation studies (S.H.Imam *et al.*, 1999). In the present study, soil bacteria capable of degrading polyethylene isolated by plating on mineral salt broth medium with polyethylene film as a sole carbon source.

Electron microscopic examination showed that the hyphae of SF1 had adhered to Polycarbonate (PC), while SF2 penetrated the polymer matrix in the untreated samples after 12 months. The material shows clear crack initiation points, indicating that the polymer has become brittle in nature. Also, the microbial propagation has been initiated from these cracks. Such colonization and adhesion by microorganisms are a fundamental prerequisite for biodegradation of the polymer. Cavities were also observed on the polycarbonate surface (Artham and Doble. 2008). Similarly in the present study, the images of Scanning Electron Microscopy showed bacteria colonizing over the film. Also, cavities were observed in the film initiating biodegradation of the polymer.

FT-IR spectra are obtained by the films of four different LDPE samples. It was found that some new peaks arose after the period of biodegradation. They can be assigned to specific peaks, such as dehydrated dimer of carbonyl group (1720 cm^{-1}), CH₃ deformation (1463 cm^{-1}) and C=C conjugation band (862 cm^{-1}). The FTIR spectra of pre-treated BPE10 shows, the introduction of ketocarbonyl functional group (1718 cm^{-1}) after 1 month of biodegradation and the intensity increases with irradiation period up to 3 months and at the same time a broadening of the band which indicates the presence of more than one oxidation product (Sudesh *et al.*, 2007). In the present study the results showed that in case of control, a peak at wavelength 1019 cm^{-1} increased to 1081 cm^{-1} in *Bacillus* & *Pseudomonas* sp, 1077 cm^{-1} in *Aspergillus*, and 1031 cm^{-1} in *Penicillium* due to the effective degradation of polyethylene film.

As previously reported by (Andersson *et al.*, 2002) a large number of different aldehydes, ketones and carboxylic acids were identified in smoke generated on film extrusion of LDPE in an extrusion coating process. In the present study, the degraded products in the culture supernatant extracted with distilled ether were determined by GC-MS analysis. Thus compounds like Octadecadienoic acid, Octadecatrienoic acid, benzene dicarboxylic acid, cyclopropanebutanoic

acid were found to be produced by the PE degrading cultures.

CONCLUSION

Thus the physicochemical analysis of PE degradation by microorganisms isolated from compost soil revealed clearly that the polymer is effectively degraded.

REFERENCES

1. Andersson, T., Wesslén, B., Sandström, J., *J. App. Polym. Sci*, 2002, 86, 1580-1586.
2. Artham T, Doble M. Biodegradation of Aliphatic and Aromatic Polycarbonates. *Macromol Biosci* 2008;8(1):14-24 January.
3. Bonhomme S, Cuer A, Delort AM, Lemaire J, Sancelme M, Scott C. Environmental biodegradation of polyethylene. *Polym Degrad Stab* 2003; 81: 441-52.
4. EI-Shafei H, EI-Nasser NHA, Kansoh AL, Ali AM. Biodegradation of disposable polyethylene by fungi *Streptomyces* species. *Polym Degrad Stab* 1998; 62:361-5.
5. Ikada E. Electron microscope observation of biodegradation of polymers. *J Environ Polym Degrad* 1999; 7:197-201.
6. Imam S.H, Gould JM (1990) Adhesion of an amyolytic *Anthrobacter* sp. To starch containing plastic films. *Appl. Environ. Microbiol*, 56:870-876.
7. Milstein, O., R. Gersonde, A. Huttermann, R. Frund, H. J. Feine, H. D. Ludermann, M. J. Chen, and J. J. Meister. 1994. Infrared and nuclear magnetic resonance evidence of degradation in thermoplastics based on forest products. *J. Environ. Polym. Degrad.* 2(2): 137-152.
8. Potts J E, Jelink H H G, Biodegradation Aspects of biodegradation & stabilization of Polymers. Elsevier, New York, (1978), 617-658.
9. Sudesh K, Abe H, Doi Y. Components characterization of LDPE blown film samples used in biodegradation. *Interesjournals.org*, (2007).
10. Wen Chai, Masako Suzuki, Yuichi Handa, Masahiko Murakami, Takamitsu Utsukihara, Biodegradation of Di-(2-ethylhexyl) phthalate by fungi (Rep. Nat'l. Food Res. Inst) No.72, 83-87 (2008)

11. Yamada-Onodera K, Mukumoto H, Katsuyaya Y, Saiganji A, Tani Y (2001). Degradation of polyethylene by a fungus, *Penicillium simplicissimum*. *YK.Poly.Degrad.Stab.*, 72:323-327.