BIOLOGIJA. 2011. Vol. 57. No. 4. P. 141–147

Studies on biodegradation of LDPE film in the presence of potential bacterial consortia enriched soil

Harshita Negi[1],

Sanjay Gupta[3],

M. G. H. Zaidi[2],

Reeta Goel[1]*

[1] *Department of Microbiology, G. B. Pant Univ. of Ag. & Tech., Pantnagar-263145, Uttarakhand, India*

[2] *Department of Chemistry, G. B. Pant Univ. of Ag. & Tech., Pantnagar-263145, Uttarakhand, India*

[3] *Department of Biotechnology, S. B. S. P. G. I., Balawala, Dehradun, Uttarakhand, India*

Low density polyethylene (LDPE) is an important commodity plastic and has a wide applicability in the modern era. In virtue of their wide applicability, the generation of the huge plastic waste became a conundrum for environment and public health. Consequently, the present study was conducted for the microbial degradation of LDPE film in natural conditions using mixture of potential polymer degrading consortia. For this purpose, the talc based formulation of bacterial consortia was inoculated into soil with LDPE film for the period of three months. Fourier transform infrared spectroscopy (FT–IR) in combination with SEM revealed that the consortia incurred significant surfacial degradation of LDPE film, introduction of hydroxyl (–OH) functionality and significant shifts in fingerprint region, respectively. The potential of the consortia towards degradation of LDPE has further been ascertained through change in bulk structural characteristics using differential scanning calorimetry (DSC). Moreover, the comparative *in situ* biodegradation study of LDPE film in laboratory and natural conditions indicates that environmental factors like sun-light, temperature and rainfall may enhance the rate of biodegradation of the polymer in nature.

Key words: bacterial consortia, LDPE, *in situ* biodegradation, DSC, FT-IR, SEM

INTRODUCTION

Low density polyethylene (LDPE) is a hydrophobic synthetic polymer of high molecular weight. It is characterized by good toughness, resistance to chemicals, flexibility and clarity. These properties make LDPE an important plastic grade widely used for manufacturing various laboratory containers, dispensing bottles, wash bottles, tubing, plastic bags, food containers and corrosion-resistant work surfaces, etc. Despite their wide applicability, the waste management is emerging as a parallel industry which adversely affects the environment as mentioned by Shah et al. [1]. Polyethylene wastes are normally discarded as landfill or thrown in water bodies as garbage material to decompose / degrade. To deal with this conundrum, biodegradation on the disposal site appears to be the best way as an alternative when approaches, recycling, land filling and incineration has various environmental constraints as described by several authors [2–4].

However, microbial degradation of LDPE blends with cellulose [5] and starch [6–8] has been reported earlier. Moreover, surface degradation of pure LDPE was also observed *in vitro* [9] and *in situ* conditions [10, 11] using indigenously developed bacterial consortia. The advantages of employing mixed cultures in the form of consortia unlikely to pure cultures in biodegradation have been previously demonstrated by Satlewal et al. [9]. It could be attributed to the effects of synergistic interactions among members of the association. Thus, the utilization of microbial consortia offers considerable advantages in the degradation of recalcitrant compounds [12]. Considering that, Soni et al. [10] and Kapri et al. [13] achieved the degradation of LDPE powder

* Corresponding author. E-mail: rg55@rediffmail.com

using bacterial consortia after six days of incubation. Further, Kapri et al. [11] descries the *in situ* biodegradation studies of LDPE coupons buried in the soil-bed in a beaker with the bacterial consortia proclaim the reproducibility and polyethylene degradation efficacy of the developed consortia. Therefore, the test of the potentiality of the used bacterial consortia at original disposal sites was designed by using carrier based formulations of the bacterial consortia. Moreover, the viability and reproducibility of the bacterial strains were maintained in talc based formulation during storage at room temperature as described by Goel et al. [14]. Nevertheless, this study also deals with the comparative *in situ* biodegradation efficacy of the LDPE-degrading bacterial consortia under laboratory and natural conditions. Moreover, the literature survey reveals that this may be the first report to evaluate the eco-friendly biodegradation of LDPE film in consortia enriched natural soil-bed.

MATERIALS AND METHODS

Bacterial culture and talc based formulations

A total of six bacterial cultures were retrieved from the departmental culture collection of Microbiology, CBSH, G. B. Pant University of Agriculture and Technology, Pantnagar, India (Table 1). The cultures were revived by inoculating into 10 mL of nutrient broth (HiMedia, India) and maintained on nutrient agar (HiMedia, India) at optimum pH (7.0 ± 0.2) and temperature (37 °C). A single colony from each culture was inoculated into 20 mL flask containing 10 mL of nutrient broth (pH 7.0 ± 0.02) and active cultures were prepared by incubating the flask at 37 °C for 16 h with continuous shaking at 120 rpm. The calculated amount (CFU mL-1) of each strain was mixed for the development of two consortia as described by Goel et al. [14]. For the preparation of talc based formulation, the active consortium (200 mL) was divided into four parts, 50 mL each in centrifuge tubes and spun at 5 000 rpm for 10 min to remove the cells. Later, the supernatant was partially decanted and the tubes were vortexed for 15 min. Then, 2.5 gm talc was weighed and added to each tube with pellets under sterile conditions. The tubes were vortexed again for homogenous mixing of talc with the bacterial suspension. With a sterile spatula, the mixture was then emptied into glass petri-plates and these were kept at room temperature (28 ± 1 °C) aseptically for drying the mixture.

LDPE film

Commercially available branched low-density (0.92 g/cm^3) polyethylene film was used in this study. It contained different additives in the form of a Masterbatch (trade name), a mixture that contains corn starch, linear low-density polyethylene (LLDPE), styrene butadiene copolymer (SBS), and manganese stearate.

In situ biodegradation assay and recovery of degraded film

Top-soil was dug from a barren land at Pantnagar, India and filled into 45 × 34 cm^2 sinks. LDPE film coupons of dimensions 2.54 × 2.54 cm^2 were surface sterilized with 70% ethanol for 10 min and placed at various depths below the soil surface. Talc based formulation of consortia was added to the soil of the respective treatments in sinks which were incubated in natural condition. Further, autoclaved distilled water was sprinkled at regular intervals of 2 weeks to maintain the moisture content of the soil. Aeration conditions were maintained by shoveling the soil at regular intervals. The LDPE film coupons incubated in uninoculated soil was taken as treatment to compare the changes in LDPE film in presence or absence of the consortia. Further, the treated LDPE film samples were carefully recovered from the soil after a period of 3 months. The samples were surface sterilized with 70% ethanol for 10 min and dried in a desiccator for 24 h under vacuum.

Characterization and analysis

The dried samples were characterized for their spectra, morphology and thermal behavior with reference to the untreated LDPE film as control. The chemical changes of the samples were studied through FT-IR spectra using Perkin Elmer FT-IR spectrophotometer through KBr disc method. The morphology of the samples was examined through scanning electron microscopy after samples were metalized with gold particles (3 discharges of 40 mA/50s in argon atmosphere) in a high vacuum metalizator (Bal-Tec SCD 005) and analyzed by SEM (Leo, 435VF, U. K.) at 15.00 kV EHT under three successive magnifications (1.5, 3.0 and 5.0 KX). The thermal characteristics of treated and untreated LDPE samples were evaluated through DSC over Universal V4.5A Thermal Analyzer at temperature ranging 25 to 400 °C with heating rate of 10 °C min^{-1} under nitrogen atmosphere (200 mL min^{-1}).

Table 1. **Bacterial strains used in the study**

Consortium	Bacterial strains
C1	*Microbacterium* sp. strain MK3 (DQ318884), *Pseudomonas putida* strain MK4 (DQ318885), *Bacterium* Te68R strain PN12 (DQ423487)
C2	*Pseudomonas aeruginosa* strain PS1 (EU741797), *P. putida* strain PW1 (EU741798), *P. aeruginosa* strain C1 (EU753182)

RESULTS AND DISCUSSION

Bacterial consortia

The bacterial strains used in this study were originally isolated from different plastic waste disposal sites and artificial soil as mentioned by several authors [9, 11, 13] and then consortium was developed in different combinations as reported by Satlewal et al. [9] and Kapri et al. [15] (Table 1). The used two bacterial consortia were selected on the basis of their pre-identified potential to degrade a variety of polymers like HDPE [9], LDPE [10, 11, 13, 15, 16], epoxy, and epoxy silicone blends [17]. Thus, the mixture of talc based formulations of these two potential bacterial consortia was used to enrich the soil for biodegradation of LDPE film in natural conditions.

FT-IR spectra

The effect of incubation and bacterial action on LDPE film was preliminary analyzed through FT-IR spectra (Fig. 1). FT-IR spectra revealed remarkable shift in the group frequencies in terms of wavenumber (cm^{-1}) of the LDPE treated in presence and absence of consortia over untreated LDPE as control.

Untreated LDPE film rendered wavenumber (cm^{-1}) corresponding to ν (stretching) O–H (3,435.6), ν_{as} CH_2 (assymetrical, 2,930.5), CH_2 deformation (1,594.2), δ (bending) CH_2 (1,461.5), δ CH_3 (symmetrical, 1,351.6) and ρr CH_2 (768.4), respectively (Fig. 1a) as described by Grisa and Zeni [18]. The LDPE film sample buried in soil, devoid of consortia had reported significant shifts in CH/CH_2 stretching, bending and deformation. Further, the introduction of ν_{as} C–O (1,218.6) and ν_s C–O (1,031.6) frequencies may be due to the effect of incubation in soil (Fig. 1b). Similarly, the consortia treated samples had reported disappearance of CH_3 bending and CH_2 deformation. Moreover, a change in the fingerprint region of the IR spectrum between 1,300 cm^{-1} and 950 cm^{-1} was observed. Additionally, the consortium has introduced simultaneous shift in

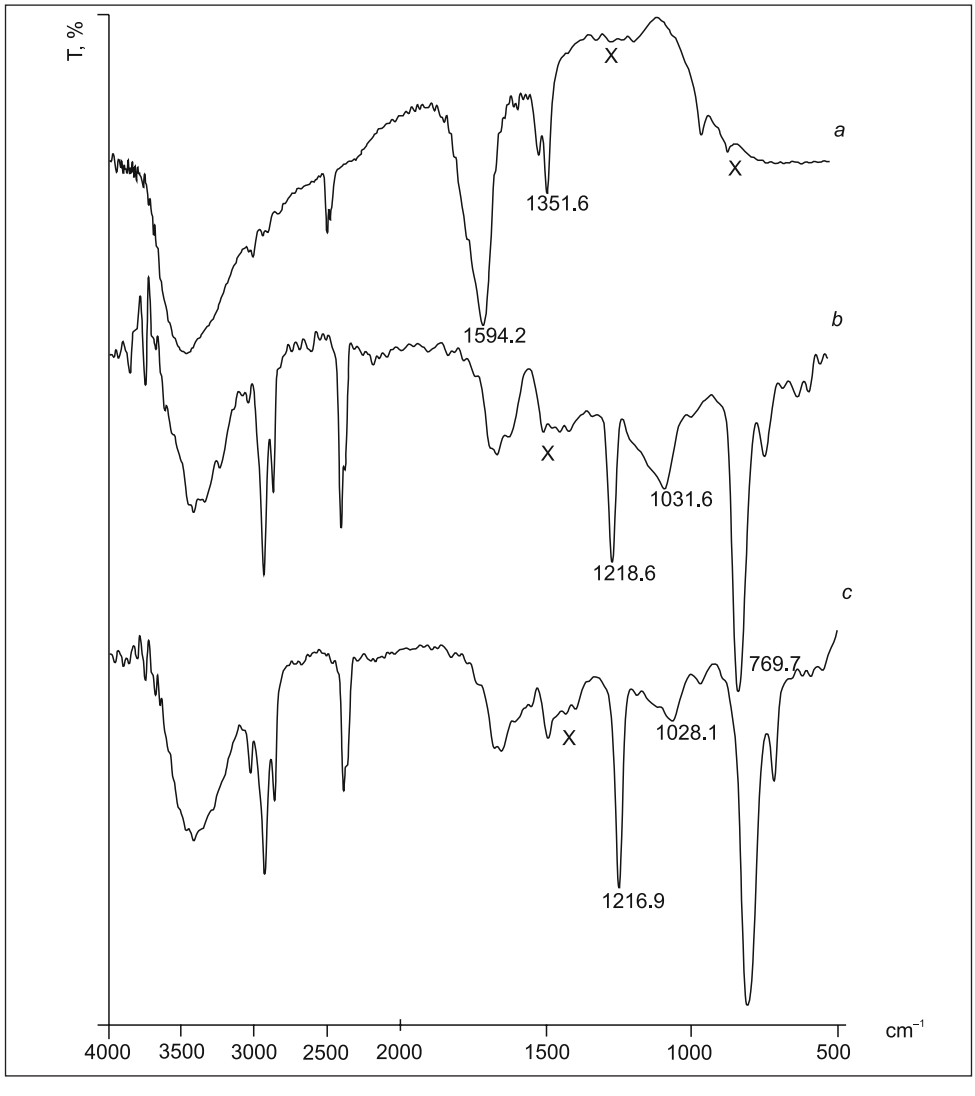

Fig. 1. FT-IR spectra of untreated LDPE (a), treated LDPE in absence (b) and in presence of consortia (c) after 3 months of incubation in soil

the wavenumber to lower values elucidate the weakening of the bonds in hydrocarbon chain (Fig. 1c). The wavenumber corresponding to the bending frequency of the water has appeared at 1628.4–1628.7 for LDPE films treated in the presence and absence of consortia. These spectral observations indicate the changes in the macromolecular segments of the LDPE due to consortia under sunlight, moisture and temperature inside the soil. Further, the action of consortia may induce bioactive hydrolysis in polymer film after 3 months of incubation in soil as reported by Kapri et al. [11]. In order to have further insight into the effect of consortia comparative accounts of the changes in the morphology and thermal characteristics of treated LDPE film were observed through SEM and DSC as described by Raghavan et al. [19] and Arutchelvi et al. [20] analysis, respectively.

Microscopy

The comparative account of the effect of presence and absence of bacterial consortia on LDPE film during incubation period of 3 months has been analyzed through SEM at three respective magnifications (Fig. 2). The pure LDPE film taken as reference revealed smooth and homogenous morphology (Fig. 2a, b). However, the morphology of film incubated in the absence of consortia has been relatively changed after 3 months of incubation (Fig. 2c, d), as the inhabitant microbes of the soil take more than 32 years to gradually degrade the LDPE films buried in soil as noted by Otake et al. [21]. The physical forces, such as heating or cooling, wetting or drying under the soil bed can cause mechanical damage such as the cracking of polymeric materials as noted by Santhoskumar et al. [22] and Rudnik et al. [23]. Thus, the well resolved worn-out areas with randomly distributed cracks and fissures may be either due to burial pressure of soil or the action of inhabitant microbes on film. However, Mehdi et al. [8] mentioned that LDPE is a hydrophobic polymer with a high molecular weight and thus takes a long time to degrade in nature. Further, the occurrence of fissures, heterogeneous morphology and the surface dissolution was found to remarkably increase in the film sample treated in the consortia enriched soil (Fig. 2e, f). Moreover, the *in situ* biodegradation of LDPE conducted in lab conditions by Kapri et al. [11], using consortium C1, illustrated the efficacy of the candidate organisms. However, the extent of surface-degradation of LDPE film using combination of consortium C1 and C2 was relatively higher in natural conditions and may be due to the synergistic activities of consortia and effect of environmental factors. The environmental factors like rain, temperature, sunlight, etc may accelerate the degradation. As a result of the degradation, the condition of LDPE encourages attack by microbes, i. e. an affinity of film to microbes becomes increased and resulting in enhanced biodegradation as noted by Ohtake et al. [24]. Moreover, the polymer samples probably had a chance to be entirely exposed to sunlight for a long time during the burial period in soil, and thus they were photodegraded. Furthermore, Kapanen et al. [25] described that the photo-degradation made the surfaces hydrophilic and these surfaces enhanced their affinity to microbes. Similarly, an outdoor soil burial test was carried out by Mumtaz et al. [26, 27] to evaluate the degradation of commercially available LDPE carrier bags in soil through the inhabitant microbes.

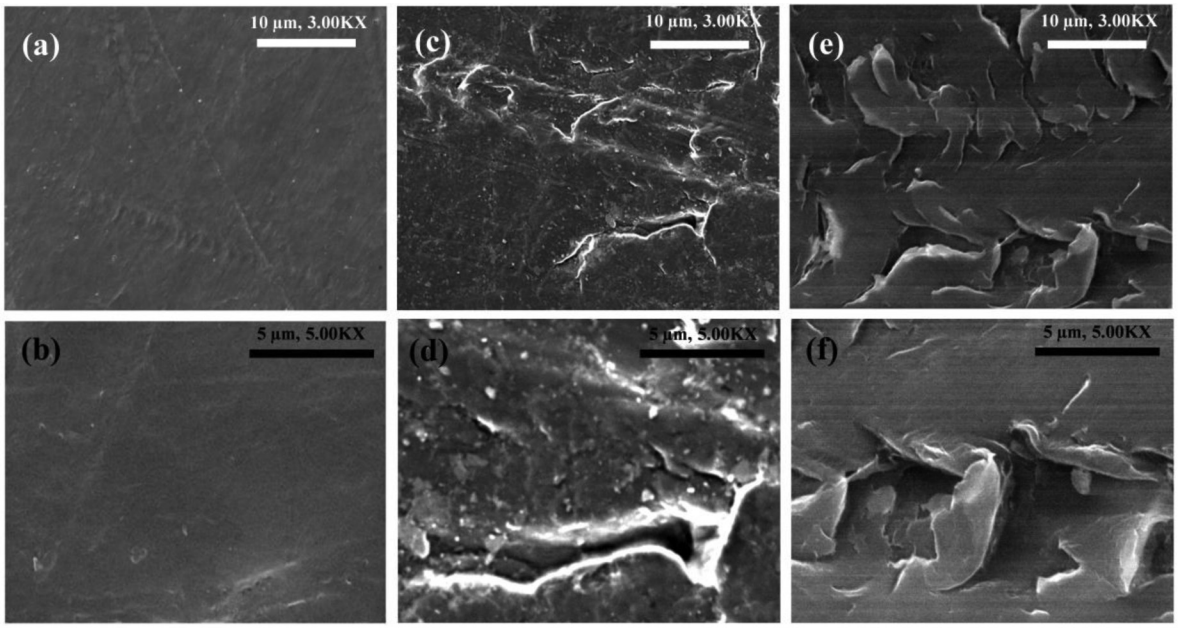

Fig. 2. Effect of incubation on the morphology of the LDPE film in the absence (c, d) and presence of consortia (e, f) in reference to untreated LDPE film (a, b). Scale bars = 10, 5 µm; Magnification = 3.00, 5.00 KX, respectively

Table 2. **Bulk structural characteristics of LDPE sample**

Sample	Heating mode		Cooling mode	
	T_0/ T_E	ΔH_f (T_m °C)	T_0/ T_E	ΔH_c (Tc °C)
Untreated LDPE	41.1 / 137.0	115.2 (113.06)	111.50 / 33.0	113.1 (102.85)
Treated LDPE in absence of consortia	67.9 / 135.36	79.08 (114.36)	112.67 / 37.0	111.3 (102.73)
Treated LDPE in presence of consortia	46.4 /131.10	114.5 (112.10)	111.54 / 36.0	111.0 (103.80)

T_0; Onset temperature (°C), T_E; Endset temperature (°C), ΔH_f; Heat of fusion (J g^{-1}), ΔH_c; Heat of crystallization (J g^{-1}), Tc; Crystallization temperature, T_m; Melting temperature

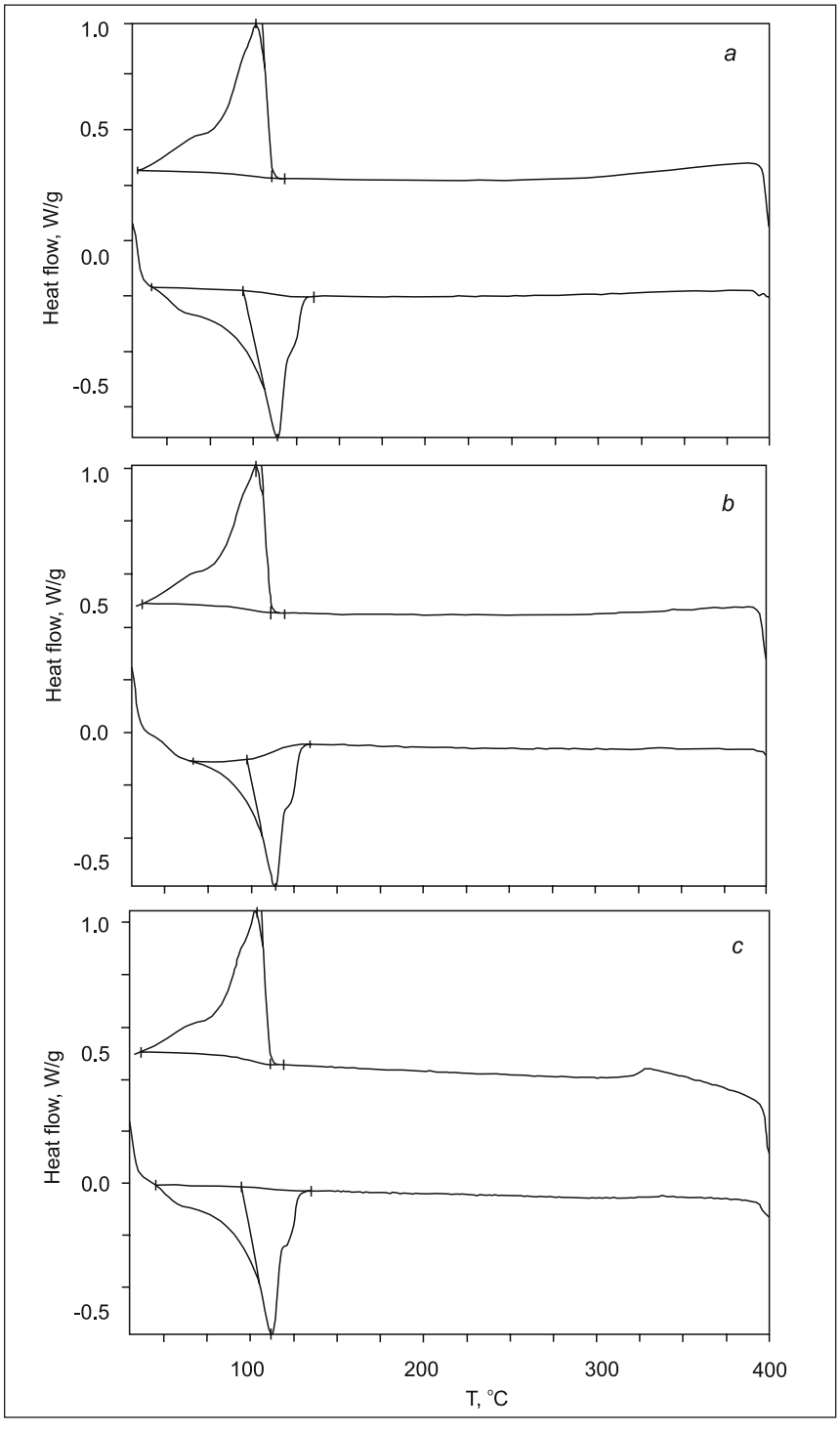

Fig. 3. Effect of incubation on the bulk structural characteristics of LDPE film in the absence (b) and presence of consortia (c) in reference to untreated LDPE film (a)

DSC analysis

The changes in the thermal properties of the treated LDPE film were analyzed through determination of bulk structural characteristics with reference to untreated LDPE film as control (Table 2, Fig. 3). The phase transformation of untreated LDPE has been initiated at 41.1 °C and shown melting temperature (T_m) at 113.06 °C with heat of fusion (ΔH_f) 115.2 J g^{-1} as described by Tajeddin [28]. Further, the molten LDPE has shown flow up to 137.0 °C followed by heating up to 400 °C, after which the DSC cooling curve has been drawn. The cooling curve has been initiated at 111.50 and shown crystallization temperature (T_c) at 102.85 °C (Fig. 3a). Relatively higher onset temperature (T_0) observed for melting and cooling of the LDPE film buried in soil (Fig. 3b, c) over untreated film may be ascribed to soil contamination. However, the comparative account of the thermal data has shown that the treated LDPE in the presence of consortia exhibited relatively lower T_0 for melting (46.4 °C), cooling (111.54 °C) as well as decreased T_m (112.10 °C) and ΔH_f (114.5) (Fig. 3c) in comparison to the film treated, devoid of consortia (Fig. 3b). The changes in bulk structural characteristics clearly do not indicate the degradation of the polymer, albeit attributed to the changes in thermal properties of the polymer due to incubation in soil with potential bacterial strains.

In conclusion, the present study includes the biodegradation of LDPE film under natural conditions in soil bed. Furthermore, the study demonstrates that the use of the combination of potential bacterial strains in the form of consortia accelerated the degradation of polymer film, buried in soil in landfills, as revealed by the structural, morphological and thermal changes in the treated LDPE. The present investigation may be a step towards large scale application of used LDPE degrading consortia in carrier based formulations. Further, several questions remain to be answered including the rate and completeness of biodegradation and cumulative time for biodegradation under different environmental conditions.

ACKNOWLEDGEMENTS

This work is supported by a DBT grant to R. G. The senior author (H. N.) acknowledges the DBT for financial assistance in terms of JRF. We are also thankful to CDRI (SAIF) Lucknow; College of Veterinary Sciences and Animal Husbandry, G. B. P. U. A. T. Pantnagar and DMSRDE, Kanpur for FT-IR, SEM and DSC, respectively.

Received 12 September 2011
Accepted 9 December 2011

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MAŽO TANKIO POLIETILENO (LDPE) PLĖVELĖS BIODEGRADACIJA PANAUDOJANT POTENCIALIU BAKTERIJŲ MIŠINIU PAGERINTĄ DIRVOŽEMĮ

Santrauka

Mažo tankio polietileno (LDPE) plėvelė yra svarbus plastiko produktas, plačiai naudojamas šiuolaikiniame pasaulyje, tačiau dėl milžiniško plastiko atliekų kiekio kyla problemos aplinkai ir žmogaus sveikatai. Mes tyrėme LDPE plėvelės mikrobiologinę degradaciją gamtinėmis sąlygomis panaudodami potencialų bakterijų mišinį. Tam tikslui talko pagrindu sukurtas bakterijų mišinys trims mėnesiams buvo įterptas į dirvožemį su LDPE plėvele. Infraraudonųjų spindulių spektroskopija ir nuskaitančia elektronine mikroskopija nustatyta, kad bakterijų mišinys sukelia patikimą LDPE plėvelės paviršiaus degradaciją, paskatina hydroxylo (-OH) veikimo pradžią ir patikimus savybių pokyčius. Bakterijų mišinio mikroorganizmai, galintys suardyti LDPE plėvelę, vėliau buvo apibūdinti pagal pagrindinių požymių pokyčius panaudojant diferencinę nuskaitančią kalorimetriją. Be to, palyginamieji LDPE plėvelės biodegradacijos tyrimai *in situ* laboratorijoje ir gamtinėmis sąlygomis rodo, kad tokie aplinkos veiksniai, kaip saulės šviesa, temperatūra ir lietus, gali paspartinti polimerų biodegradaciją gamtoje.

Raktažodžiai: bakterijų mišinys, mažo tankio polietilenas, *in situ* biodegradacija