CSIRO PUBLISHING The South Pacific Journal of Natural Science, **27**, 66-70, 2009
www.publish.csiro.au/journals/spjns 10.1071/SP09012

Microbial degradation of Polyethylene (PE)

Kiran Kumari, R. C. Aanad and Neeru Narula

Department of Microbiology ,CCS Haryana Agricultural University, Hisar,India.

Abstract

Thirty two bacterial isolates were obtained from soil by soil burial method followed by enrichment culture technique in film culturing (FC) media. Bacterial isolates differing in morphology were selected, purified and maintained at 4^0C. Thirty % of these isolates were found to be Gram negative and 50% showed positive starch hydrolysis test and were screened for their ability to degrade Low Density Polyethylene (untreated, UV and heat strips) in film culturing media and percent weight loss of polyethylene after 4^{th} week was determined. Among various isolates, highest degradation was by Is 3, Is 22 and Is 31 to the range of 25- 27%, of UV treated polyethylene strips. High temperature (40^0C), was found to enhance degradation rate of polyethylene more effectively by 24-28% compared to low temperature at 30^0C (18-21%). Degradation of treated polyethylene strips (UV, heat steam) was up to 4% by compost treatment as studied by using CO_2 evolution, an estimation tool to analyze % degradation. Bacterial activity was also affected by various environmental factors like sunlight, temperature, oxygen etc.

Key words: *LDPE (low density polyethylene), film culturing media, degradation, % (percent) weight loss, Enrichment and treatment*

1. Introduction

Polyethylene is a synthetic polymer from a hydrocarbon source and is a primary building block of polyethylene resin. It is made up of a large number of small molecular units that are engineered, manipulated and then processed for the purpose of bonding together into long polymer chains (Szabo, 2005). About 1/3rd of this plastic material is used in the manufacture of disposal items such as wraps, bags and other packaging materials such as cups, trays, fast food items and film for agriculture use (Yabannavar and Bartha, 1994). Recalcitrant plastic accumulates in the environment at the rate of 25 million tonnes per year and modulation of plastic is increasing per year throughout the world (Lee *et al.*, 1991). Being a xenobiotic, "man-made" compound, plastics are recalcitrant, or resistant, to microbial degradation because bacteria have not been exposed to them through the course of evolution.

In recent years, there are many negative reports on plastic bags and tremendous effect on environment which has created public interest to solve the problem of plastic waste. 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. About 3 % of plastic material is recycled while remaining remains as litter or land filler.

Most important disposable films are polyethylene (PE), polypropylene (PP) and polyvinyl chloride (PVC). Polyethylene (PE) hence becoming a serious problem in waste disposable as these are synthesized keeping in mind its resistance to degradation.

In the 21st century, scientists and companies need to play an active role in protecting resources and to become more aware of the damages that their action and activities can have on the environment. Various methods employed for estimation of degradation rate are: measuring carbon dioxide evolution, residual weight analysis and measurement of tensile strength (Johnson *et al.*, 1993). All these methods are laborious and slow, therefore there is an urgent need to have a rapid biological method for the degradation of polyethylene to avoid environmental pollution. Keeping above facts into consideration, the present investigation was carried out with a view to isolate, screen and identify bacteria degrading polyethylene (PE) and also optimization of conditions of polyethylene degradation by using and comparing two methods viz; % weight loss and CO_2 evolution.

2. Material and Methods

Soil samples and compost as raw material were taken from CCS Haryana Agricultural University, Hisar. Low density polyethylene (LDPE without starch) (Newtone, Vikrant extrusions) was used as substrate during the present studies and was bought from the local market.

2.1 Isolation of Polyethylene (PE) Degrading Bacteria

Film culturing media was used in the present investigation. Composition of Film culturing (FC) media in gl^{-1} (Lee *et al.*, 1991): Na_2HPO_4 5.03, KH_2PO_4 1.98, $MgSO_4$ 0.2, NaCl 0.2, $CaCl_2$ 0.05, Agar-agar 15, Yeast Extract 6.0 and Trace-elements (Hoagland complete solution) -1ml and distilled water 1000 ml

Ingredients of Hoagland complete solution (Hoagland and Arnon, 1950): Major salts

Ca $(NO_3)_2$ 364.0, KNO_3 221.3, $MgSO_4$ 217.6, $KHPO_4$ 62.1 and Micro nutrients $ZnSO_4$ 0.097, H_3BO_3 1.269, Na_2MoO_4 0.4,$CaSO_4$ 0.035, $MnSO_4$ 0.609, Tartaric acid 2.0 and $FeSO_4$ 2.5 (gl^{-1})

Film culturing (FC) broth: Same as above without agar-agar.

2.2 Isolation of Bacterial Culture by Enrichment Culture Technique

Isolation of polyethylene degrading bacteria (PDB) was done by preparation of three types of enrichment under pot house conditions by soil burial method (Gosh, 2004) followed by enrichment culture technique. Enrichments were prepared in three medium sized properly cleaned earthen pots (4 kg capacity) in which dried and preweighed 20 g polyethylene strips were kept at the bottom of the pot. E:1 Enrichment covered with well dried and sieved soil sample of about 2 kg + PE, E:2 - PE+ 2 kg of compost, E: 3- PE + soil and compost were mixed together in 1:1 ratio. Water holding capacity (WHC) per day was maintained at 40%.

2.3 Enrichment under Laboratory Conditions

Ten g of sample from each of above enrichment was added in film culturing broth of 90 ml along with 1 g of polyethylene. These flasks were incubated at 30 ^{o}C, 40 ^{o}C and 50 ^{o}C for ten days respectively and was transferred to

fresh film culturing broth twice. Serial dilutions from each enrichment sample was plated on the above media plates and incubated at various temperatures.

Bacterial cultures were purified and maintained on FC media slants for further studies. For morphological studies: size, shape and color of colonies was observed. Gram staining and starch hydrolysis were done for each isolate according to Bergy's Manual of Systematic Bacteriology (Staley *et al.,* 2001) for identification.

2.4 Preparation of Inoculum

Inoculum was prepared in 100 ml of sterilized media broth (FC), inoculated with various bacterial cultures separately and incubated at 30 ^{o}C for 5 days.

2.5 Screening of Polyethylene Degrading Bacterial Cultures

Polyethylene degrading ability of isolates was studied by inoculating 1% inoculum into 100 ml broth (film culturing media) along with 1g of polyethylene strips as substrate and incubated at 30 ^{o}C and 40 ^{o}C, respectively. Three types of treated polyethylene strips, viz., UV, heat and steam along with untreated as control were used and were incubated for 4 weeks. Two hundred mg (10 strips) of polyethylene strips were retrieved every week and % weight loss of polyethylene was determined till one month.

2.6 Pretreatment of Polyethylene Strips

Pretreatment (UV, heat and steam treatment) of polyethylene bags was done by method of Lee *et al.,* 1991. Treated plastic bags were cut into small strips of 2 cm×4cm of 20 mg weight each.

Chemical disinfectant treatment**:** All treated and untreated polyethylene strips were given the following disinfectant treatment: 7 ml of tween 80, 10 ml of bleaching powder and 983 ml sterilized water. A single strip was treated with universal disinfectant and was kept on nutrient agar media plates and incubated for 5 days to check the sterility.

Drying: Chemically disinfected polyethylene strips after treatment were kept on filter paper and incubated at 50^{0}C over night.

Weighing: Dried polyethylene strips were preweighed on electronic balance and added into the sterilized broth under sterilized conditions.

One gram of polyethylene was added into 100 ml film culturing broth inoculated with bacterial culture and incubated at 30^{0}C and 40^{0}C, respectively for 1month. After every week, 200 mg of PE strips was retrieved every week and % weight loss was determined.

2.7 Harvesting of Polyethylene (PE) Strips

Harvesting of polyethylene was done under sterilized conditions with the help of sterilized forceps followed by washing, drying and weighing as described Lee *et al* 1991.

CO_2 Compost treatment: Weight loss of polyethylene was studied on the basis of CO_2 evolved during incubation. The correlation between CO_2 evolution and percent degradation was analyzed. Soil was replaced by sterilized compost (100g) and inoculated with selected isolates @ 1% (v/v) along with treated and untreated polyethylene strips (1g) in 500 ml flask and incubated at room temperature for one month. Control with inoculated bacterial culture along with compost without polyethylene was also kept. Estimation of CO_2 evolved was analyzed by method of Pramer and Schmidth (1964). Ten ml of 0.5N NaOH was kept in 25 ml capacity small sterilized tube and was kept in each flask. Flasks were stoppered with rubber cork, made air tight with wax sealing. Every week, NaOH from small tubes was transferred to 150 ml Erlenmeyer flask under sterilized conditions. One ml of saturated barium chloride and phenolphthalein (as indicator in 1 % (v/v) ethanol solution) was used in the sample for titration with HCl. The amount of CO_2 evolved and % degradation of PE was calculated from the following formulae:

(Blank –X) x 11 {where X= amount of HCl used)}

Y x12/44{Y = CO_2 mg /100 g compost}

Z-C {Z = Carbon dioxide in mg / 100 g of compost and C = accumulative carbon (in form of CO_2) evolved from control}

= b x 100/1000 (b = carbon in mg g^{-1} of PE)

= K (Percent of carbon in mg g^{-1} of PE)

3. Results

Forty bacterial isolates were isolated from different enrichment samples (E1-E3) at three different temperatures viz; 30 ^{o}C (1-8 and 23-40), 40 ^{o}C (12-22) and 50 ^{o}C (9-11).The morphological characteristics of each isolate in terms of colony shape, size, color and their biochemical tests like Gram's reaction and starch hydrolysis test are given in table 1. Most of the colonies were round (33 bacterial isolates) and few were irregular in shape (7 bacterial isolates). Size of isolates varied from small to large. Some isolates were large, pale-white to fluidy-transparent in appearance. Most of them were small in size and pale yellow to pale green in color and few were medium in size and dull yellow to dull white in color (Table 1). 70 % of the isolates were Gram positive and rest were Gram negative while 50 % of these isolates were starch hydrolysis positive (Table-1).

The screening of bacterial isolates for degradation of polyethylene (PE) by % weight loss at 30 ^{o}C and 40 ^{o}C was accessed by using three types of pretreated UV, heat and untreated PE strips respectively. About 1g of PE was added in the medium along with culture and percent weight loss of polyethylene (PE) was estimated every week by extracting 200 mg of polyethylene till one month. Increase in polyethylene (PE) degradation by isolate Is 3 at 30 ^{o}C from 1st to 4th week for untreated, UV and heat treated strips in terms of % weight loss was 14.50, 20.30 and 20.00 respectively, (Tables:1 & 2). Similarly at 40 ^{o}C it was 18.00, 25.00 & 24.45, respectively (Tables1 & 2). Increase in percent weight loss with Is 22 using untreated, UV and heat treated PE strips from 1st to 4th week at 30 ^{o}C was 14.95, 20.25 & 18.80 respectively, (Tables 1 & 2). At 40 ^{o}C from 1st to 4th week, it was 19.95, 27.55 & 24.90 (Tables1 & 2). Similarly Is 31 showed increase in the degradation in terms of % weight loss of polyethylene from 1st to 4th week at 30 ^{o}C ; as 14.85, 21.50 & 17.00 (Tables1 & 2) and at 40 ^{o}C it was 18.45, 26.75 & 23.40 for untreated, UV and heat respectively (Tables1&2).

Estimation of CO_2 evolved by respiratory activity of selected cultures was done till one month. The correlation between CO_2 evolved per gram polyethylene and % weight loss of polyethylene (PE) strips was studied. Carbon dioxide evolved in presence of untreated, UV, heat and

steam with Is 3 was 148, 120.4, 130.4 and 147, with Is 22 was 152.1, 117.9, 136.3 and 147 followed by Is 31 as 155.1, 119.1, 142.6 and 149 after 4th week respectively as compared to control (only bacterial culture) of Is 3, Is 22 and Is 31 respectively. Percent degradation of PE/1g PE using above method after 4th week with Is 3 was 4.00, 0.67, 1.68 and 4.34, followed by Is 22 it was 3.83, 0.41, 2.25 and 4.50 and Is 31 as 0.45, 0.91, 3.25 and 3.89 for untreated, UV, heat and steam treated PE strips respectively and no % degradation was observed in control treatment (Table 3).

4. Discussion

Morphologically different forty bacterial isolates from three enrichments (E1, E2 and E3) were obtained. Out of which 17 isolates at 30 ^{0}C, 12 at 40 ^{0}C and 3 at 50 ^{0}C were obtained and purified isolates were maintained on the film culturing media slants. Most of the studies carried out by different researchers (Lee *et al.*,1991, Onoedra *et al.*, 2002, Scherer *et al.*,1999, Labuzek *et al.*, 2004, Raghavan and Torma, 1992) who isolated fungal cultures from various enrichments for degradation of polyethylene. During our studies we have not got any fungal isolates from these enrichments and bacterial isolates dominated over fungal cultures for PE degradation. This fact is supported by the various workers (Booth *et al.*, 1968, Lee *et al.*, 1991, Schink *et al.*, 1992, Agamuthu and Faizura, 2005, Albertsson *et al.*, 1993, Satlewal *et al.*,, 2008, Hadad *et al.*, 2005 and Kounty *et al.*, 2006).

Seventy % of the isolates were Gram positive and rest were Gram negative while 50 % of these isolates were starch hydrolysis positive as shown in Table 1(a). Studies were supported by Burd, 2008 who also reported that most of the PE degrading isolates were Gram negative and belong to genus *Pseudomonas* and *Sphingomonas*,respectively on the basis of their phenotypic characteristics. Gilan *et al.* (2004) studied degradation of unmodified PE strips by *Rhodococcus ruber*, a thermophilic strain incubated twice for 30 days each and was able to utilize carbonyl residue up to 14 % and 21 %, respectively and showed efficient utilization of PE strips. Similarly *Pseudomonas spp* and *Brevibacterium spp* were recognized as effective degrader of polymer (PVC) of plastic upto 20 % after 2 weeks under laboratory conditions (Booth *et al.*, 1968).

4.1 Analysis of Degradation of Low Density Polyethylene (LDPE) by % Weight Loss Method

Degradation of polyethylene (LDPE) using untreated, UV and heat treated strips at 30 ^{0}C and 40 ^{0}C in film culturing media was studied. Is 22 (27 %), Is 31(26.5 %) & Is 3(25 %), showed maximum % weight loss for UV treated PE strips (Table -1(b)). Selected isolates with UV treated PE showed higher weight loss as compared to heat and untreated PE strips (Table-1(b)). Polyethylene at higher temperature (40 ^{o}C) showed more weight loss as compared to % weight loss at 30 ^{o}C (Table-2). Similar studies were carried out by Sivan *et al.* (2006) where *Rhodococcus rubber* C-208 strain colonized more effectively on UV irradiated PE strips and was also capable of degrading PE at 50 ^{0}C and 60 ^{0}C. During our studies, we observed that high temperature effectively influenced degradation of PE. Our work is supported by Burd, (2008) where strains showed weight loss of PE 4% higher at 37 ^{o}C as compared to 30 ^{o}C. Rabb *et al.* (2003) which revealed that high temperature resulted in reduction of HMWPE to LMWPE and lowers the tensile strength of PE films. While similar effect of UV irradiated PE strips have much influence on breakage of bond cleavage as compared to heat and steam treated PE due to its penetrating potential of radiations. Effect of high temperature on degradation of UV irradiated PE strips might be showing maximum penetration affect by generation of free radical ions as compared to heat and steam treated and which led to weakening of bond and results in void formation in polymer matrix. It seems that UV and heat treated PE strips led to generation of oxidized PE product which showed direct effect on biodegradability of PE. Importance of pre-treatment was also confirmed by Volterra *et al.* (1996) before incubation of bacterial cultures. Increase in % weight loss of heat treated PE strips supported by Chiellini *et al.* (2008) and Karrlson *et al.*(1977), observed reduction in molecular weight of PE from 148000 to 5000.

4.2 Analysis of Degradation of Low Density Polyethylene by Compost Treatment Method

Effect of compost treatment via CO_2 evolution by three bacterial isolates was after 4th week and CO_2 evolution was maximum in Is 31 (44.7) followed by Is 22 (44.6) and Is 3 (40.6). Percent degradation (weight loss) of PE sample was maximum with Is 22 (4.50 %) (Table 3). This method was compared to % weight loss method which is more simple, non destructive and ultimate biodegradation analytical tool to assay the degradation rate of polymers. Our work is also

Table 1. (a) Morphological studies of selected isolates, (b) Effect of pretreatment of polyethylene strips on bacterial degradation.

(a)						(b)		
Isolate number (Is)	Size	Size	Shape	Gram's reaction	Starch hydrol-ysis	Untreated % wt loss of PE (after 4th week)	UV treated % wt loss of PE (after 4th week)	Heat treated % wt loss of PE (after 4th week)
Is 3	very small	creamy yellow	circular or round	-	-	18.00	25.00	24.45
Is 22	very small	green	circular or round	-	+	19.95	27.75	24.90
Is 31	small	transparent	circular or round	-	+	18.85	26.25	23.40
CD @ 5%						0.048	0.055	0.096

Table:2. Effect of temperature on degradation of pretreated polyethylene by selected bacterial isolates.

Isolate number (Is)	% wt loss of Untreated PE from 1st to 4th week		% wt loss of UV treated PE from 1st to 4th week		% wt loss of Heat treated PE from 1st to 4th week	
	30^0C	40^0C	30^0C	40^0C	30^0C	40^0C
Is 3	2.80-14.50	3.70-18.00	7.95-20.30	13.90-25.00	5.05-20.00	10.40-24.45
Is 22	2.95-14.95	3.60-19.95	11.15-20.25	13.65-27.55	7.30-18.80	9.70-24.90
Is 31	2.60-14.85	3.70-18.45	8.95-21.50	13.05-26.25	6.80-17.00	10.10-23.40
C.D @ 5%	0.024	0.034	0.028	0.040	0.048	0.069

Table 3. Effect of compost treatment (CO_2) evolved by selected isolates on degradation of pretreated polyethylene.

Isolate number (Is)	Types of treatment of PE strips	CO_2 evolved (mg) /100g compost 1st week	2nd week	3rd week	4th week	Carbon eveloved mg/100 g of PE	% degradation of 1g of PE
Is 3	Untreated PE	42.333 (42.3)	28.533 (28.5)	26.433 (26.4)	24.000 (24.0)	34.4	3.40
	UV treated PE	36.633 (36.6)	22.433 (22.4)	29.100 (29.1)	19.200 (19.2)	6.70	0.67
	Heat treated PE	46.600 (46.6)	39.600 (39.6)	31.467 (31.5)	23.767 (23.8)	16.8	1.68
	Steam treated PE	44.567 (44.6)	39.900 (40.0)	34.767 (34.8)	29.100 (29.1)	43.4	4.34
	Control (without PE)	40.200 (40.2)	32.600 (32.6)	22.767 (22.8)	15.000 (15.0)	—	—
Is 22	Un treated PE	43.233 (43.2)	40.033 (40.0)	37.233 (37.2)	31.533 (31.5)	38.3	3.83
	UV treated PE	26.933 (26.9)	22.433 (22.4)	21.233 (21.2)	20.567 (20.6)	4.10	0.41
	Heat treated PE	44.633 (44.6)	38.000 (38.0)	24.433 (24.4)	22.867 (22.9)	22.5	2.25
	Steam treated PE	46.033 (46.3)	39.433 (39.4)	34.533 (34.5)	27.867 (27.9)	45.0	4.50
	Control (without PE)	42.633 (42.7)	34.200 (34.2)	23.667 (23.5)	16.200 (16.2)	—	—
Is 31	Untreated PE	43.767 (43.8)	41.433 (41.4)	27.833 (27.8)	26.467 (26.5)	4.50	0.45
	UVtreated PE	39.000 (39)	32.667 (32.7)	26.867 (26.9)	23.333 (23.3)	9.10	0.91
	Heat- treated PE	44.167 (44.2)	27.467 (27.5)	26.533 (26.5)	25.433 (25.4)	32.5	3.25
	Steam treated PE	44.700 (44.7)	29.767 (29.8)	24.467 (24.5)	21.000 (21.0)	38.9	3.89
	Control (without PE)	43.000 (43.0)	34.767 (34.8)	25.533 (25.5)	19.233 (19.2)	—	—

*Data in parenthesis '()' represent observation from which % weight loss calculated
C.D @ 5 %
F(A) = 0.069, F(B) = 0.079, F(C) = 0.079,
F(A×B) = 0.138, F(A×C) = 0.138, F(B×C)= 0.159 and F(A×B×C) = 0.275
F(A) = bacterial isolates, F(B) = treatment and F(C) = time period

supported by Dave *et al.* (1997) who also used soil burial compost treatment with 30 % starch polyethylene strips. Although % degradation was much less in both the methods used as compared to other reports. The reason of this low degradation of polyethylene might be use of starch free PE bags while other researchers used starch based green polyethylene. Also many other factors which control the degradability of PE strips are position, oxygen, sunlight etc. Firstly position of PE in compost environment like exteriorly placed PE strips in compost treatment was degraded at much higher rate as compared to interiorly placed PE strips. Secondly oxygen tension is limiting factor which negatively affects degradation (Pometto-III *et al.*, 1993) in compost environment is also confirmed by Johnson *et al.* (1993).

5. Conclusions

Selected bacterial isolates obtained from enrichment culture technique were found to be effective in degradation of low density polyethylene (LDPE) using pretreated strips. During our studies we observed that high temperature influenced degradation of PE. UV treated PE

strips with bacterial isolates showed maximum degradation as compared to untreated and heat treated polyethylene. Pretreatment of polyethylene strips like UV, heat, steam led to the oxidation of substrate and become more accessible to microbes and hence influence the degradation. Isolates in compost treatment incubation showed degradation of polyethylene upto 4% with treated polyethylene strips. The degradability of PE strips in compost environment was also affected by position and reduction in oxygen tension. Before recommending this on large scale, many more optimized laboratory studies with large numbers of polyethylene degrading microbes are needed to explore.

References

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

Albertsson, A.C., Sares, C. and Karlsson, S. 1993. Increased degradation of LDPE with nonionic surfactants. *Acta Polymerica*, **44**, 243–246.

Booth, G. H., Cooper, A.W. and Robb, J.B. 1968. Bacterial degradation of plasticized PVC *Applied Microbiology*, **31,** 305-310.

Burd, D. 2008. Plastic is not fantastics. *VSF-FSJ Canadian Press* 1-5.

Chiellini, E., Corti, A and D-Antone, S. and Baciu, R. 2008. Oxo–biodegradable full carbon backbone polymers biodegradation behavior of thermally oxidized PE in aqueous medium. *Polymer of degradation and Stability*, **92**, 378-1383.

Dave, H., Rao, P. V. C. and Desai, J. D. 1997. Biodegradation of starch polyethylene films in soil and by microbial culture. *World Journal of Microbiology and Biotechnology*, **13**, 655-658.

Gilan, (Orr) I., Hadar, Y. and Sivan, A. 2004. Colonization, biofilms formation and degradation of polyethylene by strain of *Rodococcus ruber Applied Microbiology and Biotechnology*, **65,** 97-104.

Gosh, R. N. 2004. E-News letter on the printed polyethylene package degradable in soil. Tech. paper in Pub. of Cygenus Bussiness media. 1-5.

Hadad, D., Geresh, S. and Sivan, A. 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus brostelensis. Applied Microbiology*, **98**, 1093–1100.

Hoagland, D. R. and Arnon, D. I. 1950. The water culture method for growing plants without soil. Calfornia Agricultural Experimental Station, Berkeley.

Johnson, E.K., Pometto -III, A. L. and Nikolov, L. Z. 1993. Degradation of degradable starch polyethylene plastics in compost environment. *Appl Environ Microbiol*., **59**, 1155-1161.

Karlsson, S., Hakkarainen, M. and Albertsson, A. C. 1997. Dicaboxylic acid and ketoacid formed in degradable polyethylene by zip depolymerization through acyclic transition state. *Macromolecules*, **30**, 7721-7728

Koutny, M. Sancelme, M., Dabin, C., Pichon, N., Delort, A. M. and Lemaire, J. 2006. Acquired biodegradability of polyethylene containing pro-oxidant additives. *Polymer Degradation and Stability*, **91**, 1495-1503.

Labuzek, S., Nowak, B. and Pajak, J. 2004. The susceptibility of polyethylene modified with bionolle for degradation by filamentous fungi. *Polish Evironmental Studies*, **13,** 59-68.

Lee, B., Pometto-III, A. L., Fratzke, A. and Bailey, T. B. 1991. Biodegration of degradable plastic polyethyleneby *Phanerochaete* and *Streptomyces.* Appl and Environ Microbiol., **57**, 678-685.

Onodera, Y. K., Mukumoto, H., Katsuyama, Y. and Tani, Y. 2002. Biodegradation of long chain alkanes by a polyethylene degrading fungus *Penicillium simplicissimum YK. Enzyme and Microbial Technology*, **30,** 828-831.

Pometto-III, A. L., Johnson, K. E. and Kim, M. 1993. Pure culture and enzymatic assay for starch-polyethylene degradable plastics biodegradationwith *Streptomyces species*. *Polymers and the Environment*, ***1***, 213-221.

Pramer, C and Schmidt, A. 1964. Organic matter in methods of soil analysis. Part-III, C.A. Black (ed) AM SOC, Agron, Madism, Wis Consin, **6**, 1395-13976**.**

Raab, M., Kolarik, and Pospisi, J. 2003. The effect of ultraviolet light on the mechanical properties of polyethylene and polypropylene. *Journal of Applied Polymers Science*, **27**, 2457-2466.

Raghavan, D. and Torma, A. E. 1992. DSC and FTIR characterization of polyethylene.*Polymer Engineering and Science*, **32,** 438–442.

Satlewal, A., Soni, R., Zaidi, M., Shouche, Y. and Goel, R. (2008). Comparative biodegradation of HDPE and LDPE using an indigenously developed microbial consortium. *J Microbiol Biotechnol.,* **3,** 477-482.

Scherer, T. M., Fuller, R. C., Lens, R. W. and Goodwin, S. 1999. Hydrolyase activity of an extracellular depolymerase from *Aspergillus fumigatus* with bacterial and synthetic polymers. *Polym. Degrad. Stab,* **64,** 267.

Schink, B., Janssen, P. H. and Ferings, J. 1992. Microbial degradation of natural and new synthetic polymers. *FEMS Microbiology Letter*, **103**, 311-316.

Sivan, A., Szanto, M. and Pavlov, V. 2006. Biofilms development of the polyethylene degrading bacterium *Rodococcus ruber Appl Microbiol and Biotechnol.,* **72,** 346–3529.

Staley, J. T., Boone, D. R., Brenner, D. J. 2001. Castenholz, R.W., Garrity, G. M, Goodfellow, M., Kerig, N. R., Rainey, F. A. and Schleifer, K. Bergye's manual of systematic bacteriology. 2nd Edition. Springer Verleg, New York, Inc.,USA.

Szabo, T. L. 2005. Molecular structure and polyethylene manufacture. *Plastics*, **3,** 21-54.

Volterra, L. M., Gucci, P. M. B., Coccia, A. M. and Esposito, S. 1996. Behavior in soil of polyethylene films and polyethylene films additivated with starch. *Water, air and soil pollution*., **88**, 109-117.

Yabannavar, V. A. and Bartha, R. 1994. Methods for assessment of biodegradability of plastic films in soil. *Appl Environ Microbion.,* **60**, 3608-3614.

Correspondence to: N. Narula
(neerunarula@yahoo.com)