WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 17, 490-501. Review Article ISSN 2277– 7105

BIODEGRADATION OF PLASTICS BY MICROORGANISMS AS PLASTIC DEGRADATION AGENTS

Pratibha Singh*[1], Iqbal Hussain Mir[1] and Dr. Mukesh K. Sharma[1]

Department of Biotechnology, Maharaj Vinayak Global University, Jaipur Rajasthan (India).

Article Received on
30 July 2018,
Revised on 20 August 2018,
Accepted on 10 Sept. 2018
DOI: 10.20959/wjpr201817-13388

***Corresponding Author**
Pratibha Singh
Department of Biotechnology, Maharaj Vinayak Global University, Jaipur Rajasthan (India).

ABSTRACT

Biodegradation of LDPEs sheet and film was analyzed after 30 days of incubation in garden soli of MVGU, Campus. The biodegradation of LDPEs film was significantly higher (up to 2.46% in 30 days) than that of LDPEs sheet (up to 1.53% in 30 days). The microbial species discovered related with the degrading materials were identified as five Gram positive, two Gram negative bacteria, and eight fungal species of *Aspergillus*. The Gram positive species that were predominant were *Streptococcus, Staphylococcus* and *Bacillus* while as Gram negative species include *Moraxella,* and *Pseudomonas* and species of fungi (*Aspergillus niger*). Effectiveness of the microbial species in degradation of plastics and polythene was analyzed in shaker cultures. Among the microbes *Aspergillus niger* species degraded higher amount of LDPEs film and sheet in one-month time span. This work reveals that the mangrove soil is a good source of microorganisms capable of degrading LDPEs sheet and film.

KEYWORDS: Biodegradation, LDPE, *Bacillus subtilis, Staphylococcus aureus, Aspergillus niger.*

INTRODUCTION

Polythene plays a very important role in packaging of food materials, goods, medicine and garbage bags and many more, but in today scenario its degradation is becoming a big threat and vital cause of environmental pollution. There are various polythene degradation methods available but the best eco-friendly and most acceptable method is by using microorganisms. As we all know that the accumulation of the plastic plays a most vital and unique role in the disturbance of environment because it causes pollution at large scale. Plastic is not easily degradable material if we try to degrade it by burning it, it causes pollution by increasing the

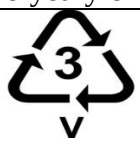
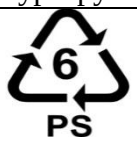
harmful gases in environment, that is why, degradation by microorganism is most acceptable. The composition of the plastics consists of carbon, hydrogen, oxygen, silicon, chloride and nitrogen. Because of the stability and durability, plastics are widely used. Mostly used plastics are polyethylene (LDPE, MDPE, HDPE and LLDPE), poly (ethylene terephthalate) (PET), poly-butylene terephthalate (PBT), nylons, poly-propylene (PP), polystyrene (PS), polyvinylchloride (PVC) and polyurethane (PUR).[1] These are the synthetic polymers which accumulate in the environment due to the absence of efficient methods for safe disposal and posing an ever increasing ecological threat to flora and fauna.

About 140 million tons of synthetic polymers are produced worldwide annually with their utility escalating at a rate of 12% per annum.[2] Each year, it is estimated that, 500 billion to 1 trillion plastic bags are consumed worldwide.[3] After their usage these packing materials are dumped in landfills leading to pollution since they are non-biodegradable under natural environmental conditions.[4] LDPEs are a thermoplastic polymer with significant short branches, usually made by copolymerization of ethylene with longer chain olefins.

Classification and Microbial Biodegradation of plastics

Fundamentally, plastics are typically long chains of carbon and hydrogen molecules. The catalysts found in living things can perform numerous concoction responses, however they by and large endeavor a type of lopsidedness of electric charge inside a particle to carry out their activity. A long chain of carbons and hydrogens contains extremely adjusted charges along its length, influencing the particle to steady and hard to change with catalysts. Most biodegradable substances contain some blend of carbon and iotas like oxygen, nitrogen, sulfur, and phosphorus, which make charge awkward nature that proteins can misuse. There are a few microorganisms that can separate plastics. These microbes ordinarily contain proteins called oxygenases, which can add oxygen to a long carbon chain. This destabilizes the neighborhood electric charge, and the plastic would then be able to be separated. The oxygenase proteins over and over again are not found, in any case, since they can without much of a stretch wreck the particles in the microbes that convey them.[5]

Table 1: Plastics Classification.

Types of plastics	Properties	Uses
 PET Polyethylene Terephthalate	➢ High heat resistance ➢ Good gas barrier and chemical resistance	➢ Water and soft drink bottles ➢ Shampoo bottles ➢ Flexible food packaging ➢ Used in tape applications
 HDPE High Density Polyethylene	➢ High strength to density ratio ➢ Moisture resistance ➢ Good chemical resistance	➢ Production of plastic bottles ➢ Corrosion-resistant piping, ➢ Geomembranes
 V Polyvinyl Chloride	➢ Very dense compared to most plastics ➢ Readily available and cheap ➢ Good tensile strength	➢ Pipes ➢ Electric cables ➢ Constructions
 LDPE Low Density Polyethylene	➢ Soft and flexible ➢ Good transparency ➢ Low melting point	➢ Garbage bags ➢ Films ➢ Disposable gloves ➢ Cookware
 PP Polypropylene	➢ High melting point ➢ Heat resistance ➢ Chemical inertness	➢ Container ➢ Appliances ➢ Household goods
 PS Polystyrene	➢ High tensile strength ➢ Rigid ➢ Insoluble in water	➢ Video cases ➢ Containers ➢ Fast food trays
 OTHER	➢ ABS is robust ➢ Flexible ➢ Highly processable	➢ CD's ➢ Beverage bottles ➢ Children's toys

According to the worldwide problem of plastics, it's not possible to degrade on large scale, which accumulated or released into the environment. Plastics are a big threat of environment because after burning, it release harmful gas like CO_2 and this plays a key role in ecosystem disturbance. However, biodegradation plays a important role in reducing the amount of accumulated polymer by naturally occurring microbes like bacteria, fungi etc. isolated from different environments.[6] There are four degradation methods of plastics in the environment: photo-degradation, thermo-oxidative degradation, hydrolytic degradation and biodegradation by microorganism also known as microbial biodegradation. Above four the most acceptable method is biodegradation which is environment friendly and followed by microbes.

As of late, the biodegradation of plastic waste and the utilization of microorganisms to debase the polymers have increased outstanding significance in light of the wastefulness of the synthetic and physical transfer strategies utilized for the toxins, as they causes numerous natural hitches. Microorganisms assume a significant part in the natural decay of material, parasite likewise have ability to debase LDPEs.

Microorganisms reported for degradation of LDPEs.

Bacteria	Fungus
Bacillus subtilis	*Aspergillus niger*
Staphylococcus aureus	

MATERIALS AND METHODS

Materials

Low density polyethylene (LDPE) molded sheet with thickness of 1mm and different densities were purchased from Reliance Polymer, Gujarat (India).

Sample collection

Soil sample was collected ascetically from the garden soil of MVGU, campus, Jaipur and brought to the laboratory, preserved under laboratory conditions for further use.

Isolation and identification of microorganism

Serial weakening strategy Soil test of 1.0 gm. was moved into a cone shaped carafe which has 10ml of refined water. The blend was shaken and serial weakened precisely [7]. The supplement agar media for microorganisms was readied (w/v) and detachment was performed by spread plate technique. For each example, five copies were kept up and kept for brooding for 24-48 hrs. at 37^0C for microscopic organisms. Arranged spreading plates (1ml for 1 plate) and brooding for 24 hrs. at 37^0C. The segregated provinces created were sub refined in agar plates and protected under refrigeration temperature.

Screening of LDPE degrading microorganism

The screening of LDPE debasing microscopic organisms conveyed by zone of leeway strategy. PEG (Poly-ethylene Glycol) was added to mineral salt medium at 0.5 fixations (w/v) and sonicated for 1 hr at 120rpm. After sonication agar was included in this media and autoclaved at 15lbs weight and at 120^0C temperature for 15 min. Sanitized media was cooled at 45^0C and filled sterile petriplates. After the hardening, segregated bacterial states (zone of

leeway around the provinces) were vaccinated and hatched at 30-35^{0}C for 15-30 days. The microorganism delivering zones of freedom were chosen for encourage investigation.[8]

Characterization and identification of microorganism

After screening of isolates, microorganism was characterized by various morphological and biochemical tests with the help of Bergry's Manual of Determinative Bacteriology.[9]

MORPHOLOGICAL AND BIOCHEMICAL

Gram Staining Method

A perfect oil free slide was taken and a spread of the bacterial culture was made on it with a sterile circle. The spread was air-dried and afterward warm settled. At that point it was subjected to the accompanying recoloring reagents:

1. Overflowed with Precious stone violet for 1 min. taken after by washing with running tap water.
2. Once more, overflowed with Gram's Iodine for 1 min. taken after by washing with running tap water.
3. At that point the slide was overflowed with Gram's Decolourizer for 30 seconds.
4. After that the slide was counter recolored with Safranin for 30 seconds, trailed by washing with running tap water.
5. The slide was air dried and cell morphology was checked under magnifying lens.
6. Same for fungus, staining by cotton blue.

Colony Morphology

This was done to decide the morphology of chose strains based on shape, size and colour.

Pre-treatment of LDPE

The LDPE formed sheet was cut into little and equivalent size pieces and moved into a new arrangement having 70 ml Tween 80, and 930ml distilled water and blending for 30 -60 minutes.[10] From that point onward, shaped bits of LDPE were moved into a recepticle with refined water and mixed for 1 hr. Further, they were put in 70% v/v ethanol for 30 minutes. At last, the LDPE formed sheets were exchanged to a petri dish and used to clean the polyethylene.

Biodegradation of LDPE

The weighed formed sheet of LDPE of same size was included into a cone shaped jar, containing 50 ml of supplement juices medium and vaccinated with microorganisms (LDPE corrupting). Control has been kept up for additionally reference and to affirm the decrease of sub-atomic weight of the LDPE formed sheet. After a timeframe, the LDPE sheets were washed with 70% ethanol, air dried and weighed to check the last weight by utilizing underneath recipe.[8,11]

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

RESULTS

Table 2: Morphological and biochemical characterization of recovered isolates.

S.NO.	Characteristics	SP1	SP2
1.	Gram staining	Gram +ve	Gram +ve
2.	Shape	Rods	Cocci
3.	Carbohydrate ferm. (Sucrose)	+	+
4.	Carbohydrate ferm. (Lactose)	+	+
5.	Nitrate reduction	+	+
6.	Indole	+	+
7.	Urease activity	-	-
8.	H2S production	-	-
9.	Citrate utilization	-	-
10.	Voges proskauer	+/-	+/-
11.	Methyl Test	-	-
12.	Oxidase	-	-
13.	Catalase	+	+
	Identified isolates	Bacillus sp.	Staphylococcus sp.

Table 3: Comparative study of LDPEs weight loss with different microbial species under laboratory conditions.

S.No.	Microorganism	% degradation 10 days	% degradation 20 days	% degradation 30 days
1.LDPE1 Sky Blue	*Staphylococcus aureus*	0.00	0.00	0.00
	Bacillus subtilis	0.00	0.00	0.00
	Aspergilllus niger	0.40	0.80	1.39

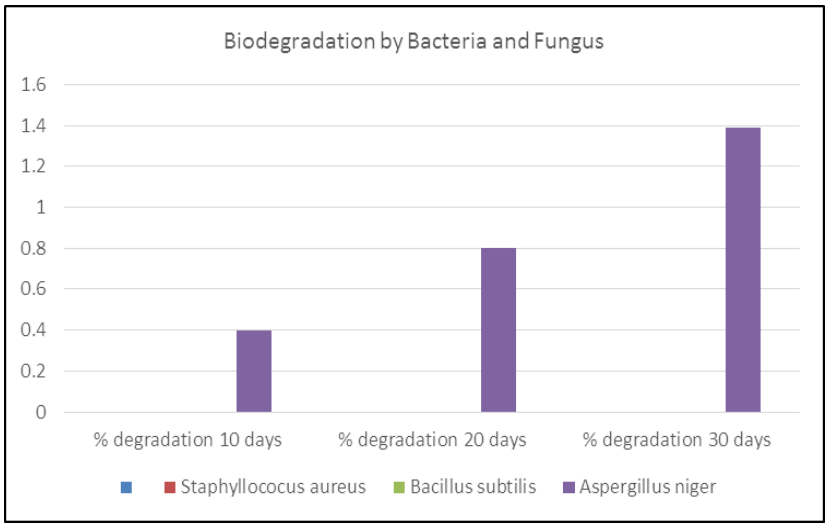


Figure-1. Percent loss in weight of LDPE (sky blue) by *Staphylococcus aureus, Bacillus subtilis, Aspergillus niger.*

Table 4: Comparative study of LDPEs weight loss with different microbial species under laboratory conditions.

S.No.	Microorganisms	% degradation 10 days	% degradation 20 days	% degradation 30 days
2.LDPE 2 yellow	*Stayphyllococus aureus*	0.00	0.00	0.00
	Bacillus subtilis	0.00	0.00	0.00
	Aspergillus niger	0.56	0.94	1.32

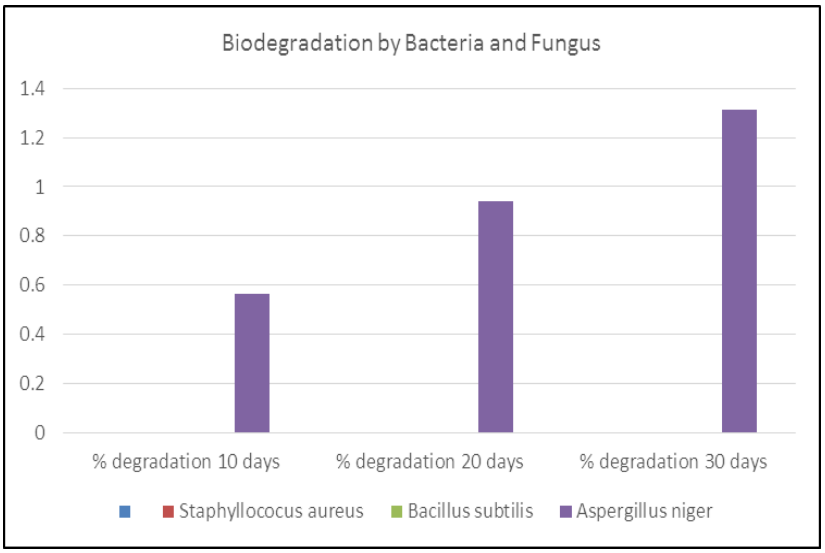


Figure 2. Percent loss in weight of LDPE (Yellow) by *Stayphyllococus aureus, Bacillus subtilis, Aspergillus niger.*

Table 5: Comparative study of LDPEs weight loss with different microbial species under laboratory conditions.

S.No.	Microorganisms	% degradation 10 days	% degradation 20 days	% degradation 30 days
3.LDPE 3 Violet	*Staphylococcus aureus*	0.00	0.00	0.00
	Bacillus subtilis	0.00	0.00	0.00
	Aspergillus niger	0.34	0.85	1.53

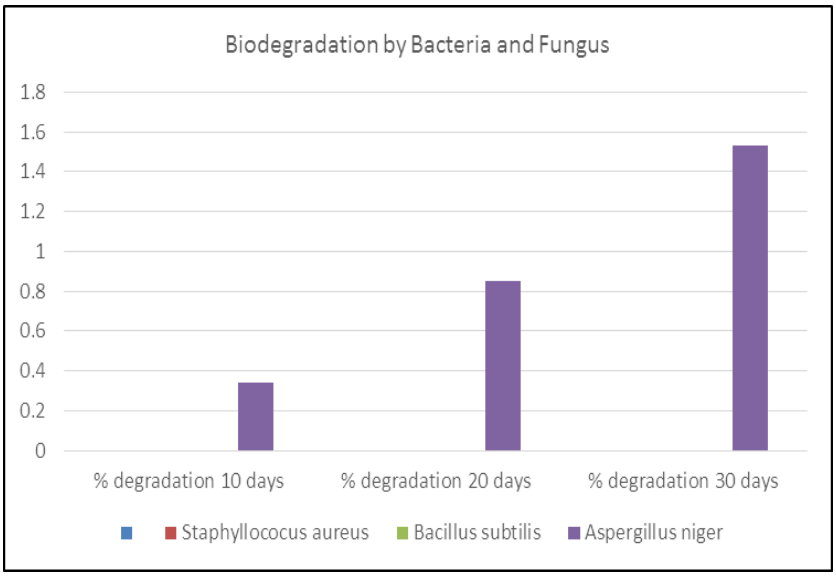


Figure-3. Percent loss in weight of LDPE (Violet) by *Staphylococcus aureus, Bacillus subtilis, Aspergillus niger.*

Table 6: Comparative study of LDPEs weight loss with different microbial species under laboratory conditions.

S.No.	Microorganism	% degradation 10 days	% degradation 20 days	% degradation 30 days
4.Very LDPE	*Stayphyllococus aureus*	0.30	1.19	1.79
	Bacillus subtilis	0.67	1.01	1.68
	Aspergillus niger	1.06	1.76	2.46

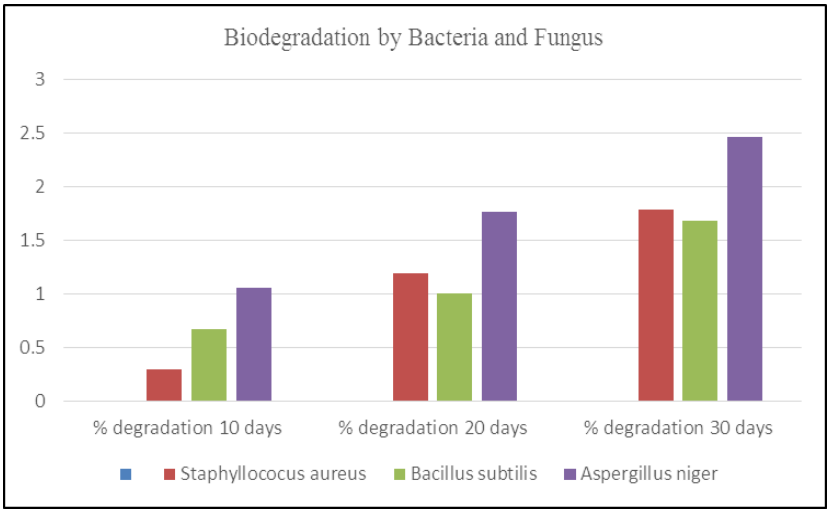


Figure-4. Percent loss in weight of very LDPE by *Stayphyllococus aureus, Bacillus subtilis, Aspergillus niger.*

Figure-5. Before the Degradation of LDPEs sample.

Figure 6. After the degradation of LDPEs Sample.

DISCUSSION

The present investigation manages the isolation, identification and degradative capacity of plastic debasing microorganisms from soil. Diverse kinds of changes are delivered by the microorganism amid morphological and biochemical examination. Manufactured plastic example was gathered from the dumped soil of college garden area was utilized as a part of this study. The plastic was utilized to think about their biodegradation by microorganisms detached from them. Microbial corruption of a strong polymer like LDPE requires the arrangement of a biofilm on the polymer surface to empower the microorganisms to productively use the non-solvent substrates by enzymatic debasement exercises. Advancement of multicellular microbial networks known as biofilm, joined to the surface of manufactured squanders has been observed to be intense debasing specialists in nature. When the total biodegradation process of any organic substrate is considered, the formation of microbial colony is critical to the initiation of biodegradation. Thus, the duration of the microbial colonization is an important factor that effects total degradation period.

In the present study, pieces of plastics were inoculated in the liquid culture medium containing bacterial isolates and kept for 1 month to observe the percentage of weight loss by bacteria. The result shows the degradative ability of the microorganisms after one month of incubation. The percentage of weight loss due to degradation was found more by *Bacillus subtilis, Stayphylococcus aureus* and *Aspergillus niger*. This shows the greater potential of degradation compared to other.

The bacteria which are identified from the above biochemical tests are *Bacillus Subtilis*, *Stayphylococcus* and *Aspergillus niger* these bacterial and fungal species were found on the basis of common morphological characteristics.

Microorganisms assume an essential part in organic deterioration of manufactured polymers in indigenous habitats. During the time spent depolymerization, two sorts of catalysts are included i.e. extracellular and intracellular depolymerases.[12] Amid the procedure of debasement, exo-chemical from microorganisms separate complex polymers of long chains into littler particles of short chains, eg., oligomers, dimers, and monomers and are littler that can pass by means of semi-porous external films of the organisms, and afterward used as carbon and vitality sources.[13]

In the present study, two kinds of LDPEs were utilized for the perception of corruption rate and those were shaped sheet and drain polythene. The past investigation indicates High-thickness and Low-thickness polythenes are the most normally utilized manufactured plastics and they corrupt gradually in regular habitat, causing genuine natural issues.[14]

CONCLUSION

The worldwide usage of plastics is undeniable and its impact started to worry the environment. It takes thousands of years to degrade plastics. During this period of time it causes various types of pollution like- soil pollution, water pollution etc. Some method to get rid of plastics is by burning of it. But burning plastics usually produced some noxious gases like- dioxins and furans, some greenhouse gases which cause ozone depletion and also effect human health. Environment friendly method should be practiced. Plastic is a long hydrocarbon chain polymer and for the breakdown into simple hydrocarbon, considered the process which can be done with the production of enzymes by some microorganism that can degrade long hydrocarbon chain into simple hydrocarbon. The process is known as microbial degradation process. Some bacteria and fungus like – *Staphylococcus aureus*, *Bacillus subtilis* and *Aspergillus niger*, they have a good potential of producing metabolites that degrade LDPE. There is need for massive isolation and screening of the microorganisms for production of metabolites that are capable of degrading polythene. The method used in the set of experiments is cost effective, easy to perform, and environment friendly.

ACKNOWLEDGEMENTS

The author is highly thankful to HOD and Authorities of Maharaj Vinayak Global University for providing laboratory facility and Mr. Raj Kamal Mishra for his help.

REFERENCES

1. KumariNA, Kumari P, Murthy NS, A Novel Mathematical Approach for Optimization of Plastic Degradation, Int. J. Engg. Trends and Tech., 2013; 4(8): 3539- 3542.
2. Shimao M. Biodegradation of plastics. Current opinion in Biotechnology, 12: 242-247.
3. Roy PK, Titus S, Surekha P, Tulsi E, Deshmukh C, Rajagopal C (2008) Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. Polymer Degradation and Stability, 2001; 93: 1917-1922. http://dx.doi.org/10.1016/j.polymdegradstab.2008.07.016.
4. Burd D (2008) Plastic not fantastic. Available on http://wwsef.uwaterloo.ca/archives/2008/08BurdReport.pdf. Accessed on 20 April 2008.

5. Ghosh S.K., S. Pal and S. Ray, 2013. Study of microbes having potentiality for biodegradation of plastics. Environ Sci Pollut Res DOI 10.1007/s11356-013-1706-x.
6. Gu JD (2003) Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. International Biodeterioration & Biodegradation, 52: 69-91.
7. Cappuccino J.G., Sherman N., Microbiology- a laboratory manual, 1996; 159- 201.
8. Usha R., T. Sangeetha and M. Palaniswamy, Screening of Polyethylene Degrading Microorganisms from Garbage Soil, Libyan Agriculture Research Center Journal International, 2011; 2(4): 200-204.
9. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, (1994), Gram-Positive Cocci. In:Bergey's Manual of Determinative Microbiology, Hensyl, W.R. (Ed.). 9th Edn., Williams and Wilkins.
10. El- Shafei, HA; El-Nasser, NHA; Kansoh, AL; Ali, AM. Biodegradation of disposable of disposable polyethylene by fungi and Streptomyces species. Polymer Degradation and Stability, 1998; 62: 361-365.
11. Usha R., T. Sangeetha and M. Palaniswamy, Screening of Polyethylene Degrading Microorganisms from Garbage Soil, Libyan Agriculture Research Center Journal International, 2011; 2(4): 200-204.
12. Gu, J.D., Ford, T.E., Mitton, D.B. and Mitchell, R., Microbial degradation and deterioration of polymeric materials, W. Revie (Ed.), The Uhlig Corrosion Handbook (2nd Edition), Wiley, New York, 2000; 439–460.
13. Frazer AC, O-methylation and other transformations of aromatic compounds byacetogenic bacteria. In: Drake HL, editor. Acetogenesis. New York: Chapman & Hall, 1994; 445-83. Hamilton, J.D., Reinert, K.H., Hogan, J.V. and Lord, W.V., Polymers as solid waste in municipal landfills. J Air Waste Manage Assoc, 1995; 43: 247–251.
14. Lee, B., Pometto, A.L., Fratzke, A. and Bailey Jr., T.B. Biodegradation of degradable plastic polyethylene by Phanerochaete and Streptomyces species. Appl. Environ. Microbiol, 1991; 57: 678-685. Gu, J.D., Microbiological deterioration and degradation of synthetic polymeric materials. Res.Adv. Int. Biodeterior. Biodegrad, 2003; 52: 69-91.