BREAKING DOWN OF POLYETHYLENE BY PSEUDOMONAS SPECIES

Preeti Agrawal, Rukendra Kumar singh

Abstract— Exhausted studies on the degradation of polyethylene has been carried out in order to overcome the environmental problems associated with synthetic plastic waste. Recent work has include the isolation of most efficient microorganism using different soil samples taken from three waste disposal sites such as industrial plastic waste dump area, leather industry waste and domestic waste dump area. In this study, various microorganism were isolated from the soil samples, grown in an inorganic media (M9 media). There are some microorganisms that have the capacity to degrade plastic waste up to 51.5%. This result is achieved due to addition of starch as additive in M9 media. This study reveals that pseudomonas sp. posses greater potential to degrade polyethylene.

Index Terms— M9 media, pseudomonas sp., Biodegradation.

1 INTRODUCTION

Low density polyethylene is one of the major sources of environmental pollution. Polyethylene is a polymer made of long chain monomers of ethylene. The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 tonnes of synthetic polyethylene are produced worldwide each year[1]. With such huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue. It takes thousand years for their efficient degradation. Environmental pollution by synthetic polymer, such as waste plastic and water-soluble synthetic polymer in wastewater has been recognised as a major problem. In view of this, energetic, chemical and biological polymer- degrading techniques has been studied extensively during the last three decades[2]. Biological degradation is most economical method of degradation. Some microorganism and enzymes are capable to degrade polymers such as plastic (low density polyethylene). The biodegradability is depending upon moleculer weight, molecular form and crystallinity[3]. It decrease with increase in molecular weight, while monomers, dimers and repeating units degrade easily.

Polyethylene of low density are used widely as films in packaging industry. They pose serious problem with biodegradability because of their slow rate of degradation under natural conditions. They also pose problem to the environment, fresh water and animals. Starch blend polythene has a continuous starch phase that makes the material hydrophilic and therefore, catalysed by amylase enzymes. Microorganism can easily access, attach and remove this part[4]. Thus the hydrophilic polythene with matrix continuous to be hydro- Biodegraded. Yamada-Onodaral et al., 2001). EI-Shafei et al. (1998) investigated the ability of fungi and Streptomyces strains to attach degradable polythene consisting of disposed polythene bags containing 6% starch.

Two approaches exist in principle for biodegradation experiments. The first utilizing natural complex media, with established mixed microbial communities with a broad range of microbial strains and activities, enable to mimic biodegradation in situ, like in soil or compost. The second working with defined microbial strains in a synthetic medium where the experiments can be controlled and reproduced precisely, giving the possibility to compare experiments from different laboratories and to deduce information concerning the mechanism of biodegradation.

R. Usha, T. Sangeetha and **M. Palaniswamy** (2011), report that the heterotrophic population of microbes in polythene and plastics, bacterial counts was recorded up to 62.71x104 and 56.52x104, the fungal count ranged from 44.32x102 and 35.62x102 and actinomycetes count ranges from 72.54x104 and 64.75x104. The microbial species associated with the polythene materials were identified as Pseudomonas sp, Bacillus sp, Staphylococcus sp, Aspergillus nidulans, Aspergillus flavus and Streptomyces sp.

The purpose of present study was to isolate microorganism from different waste dump area soil: plastic making industry dump area, leather industry dump area and sewage area and screening of polyethylene degrading microorganism and find the efficiency of microorganism with normal plastic and starch blend plastic.

2 MATERIALS AND METHODS

2.1 Material

Low density polyethylene powder (LDPE) was obtained from plastic power industry.

2.2 Sample Collection

Plastic industry waste soil samples (waste disposable site dumped with polyethylene waste from industry) were collected from Mathura. The soil samples were collected at a depth of 3-5cm, in a sterile container and then air dried at room temperature.

Leather soil samples (waste disposable site where waste lather are dumped) were collected from leather industry Agra.

Pond soil samples (pound disposable site dumped with polythene bag) were collected from near pound HCST Mathura.

2.3 Isolation of Polyethylene Degrading Microorganisms

One gram of soil sample was transferred into a conical flask containing 99 ml of sterile distilled water. This content was shaken and serially diluted. To isolate microorganisms associated with materials by pour plate method was adopted using the nutrient agar for bacteria. For each dilution, three replicates were made. The plates were then incubated at 30°C for 2-7 days. The developed colonies were isolated and sub cultured repeatedly to get pure colonies and then preserved in glycerol stock at -4°C.

2.4 Screening of Polyethylene Degrading Microorganisms by Clear Zone Method

Polyethylene powder was added in mineral salt medium at a final concentration of 0.1% (w/v) respectively and the mixture was shacked for 1hour at 120 rpm. After shaking the medium was sterilized at 121°C and pressure for 15 lbs/inch2 for 20 minutes. About 15 ml sterilized medium was poured before cooling in each plate. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 25-30°C for 2-4 weeks. The organisms, producing zone of clearance around their colonies were selected for further analysis.

2.5 Identification Polyethylene Degrading Microorganisms

The identification of bacteria was performed on the basis of degradation of microscopic examination. 0.5% (w/v) of serialized plastic powder is added in 10 ml of M9 media (inorganic media having no carbon content) then isolated organisms were incubated at 30-35°C for 2-3 weeks. The microorganism can be growing and turbidity will be appeared on comparison with control.

2.6 Microbial Degradation of Polythene and Plastics under Laboratory Conditions

Five grams of polyethylene powder was aseptically transferred into the conical flask containing 100 ml of M9 medium and then inoculated with identified polythene degrading microorganisms. Control was maintained with polyethylene powder in the microbe free medium and left in a shaker at 37°C, 150 rpm for 2, 4 and 6 month period. After the period of shaking the polyethylene powder were collected, washed thoroughly using distilled water, shade dried and then weighted to check the final weight. Finally the weight loss of the polyethylene powder were calculated and compared with control.

3 Result and Conclusion

3.1 Microorganisms Associated with Different Soil Samples

The number of heterotrophic microbes was isolated from different soil sample and the bacterial population ranged from 62.71x104 to 50.42 x104 in the case of Plastics. The total number of microbes associated with polythene and plastics showed some variation. Kathiresan, (2001) reported that the plastic materials in mangrove soil have shown rich total heterotrophic bacterial counts of up to 79.67 x 104 and the plastic materials have been colonized commonly by five species of bacteria. Abundance of polymer degrading microorganisms in a seabed solid waste disposal site has been reported by Ishigaka et al [12]. Imam et al observed that significant biodegradation occurred only after colonization of the plastic, a parameter that was dependent on the resident microbial populations. Therefore, it can be reasonably inferred that an increase in the bacterial load has correlation with degradation of the polymer [13].

3.2 Screening and Identification of Polyethylengrading Microorganisms

The polyethylene containing mineral salt agar plates were inoculated with the isolated bacteria, fungi and actinomycetes. All isolates were screened for their degradation activity. Clear zone was observed after 10 days of incubation at 25-30°C around the colony. On this screening 14 species which were named as P1, P2, PS1, LL1, LL2, LL3, LL5, L5, L5, L4, R, S1, and SS1 howed among these LL1, R and L4 show high degradation activity. Similar type of organisms were reported earlier which associated with the polythene. Further these soil microorganisms were reported to have the ability for degrading plastics. However the strains were with high degradation activity were selected for fermentation. Augusta et al., reported that the extracellular hydrolyzing enzymes secreted by the target organism hydrolyze the suspended polyesters in the turbid agar medium into water soluble products thereby producing zones of clearance around the colony [15]. Kambe et al. isolated and characterized a bacterium from soil which utilizes polyester polyurethane as a sole carbon and nitrogen source. Two strains with good polyurethane degrading activity were isolated and identified as Comamonas acidovorans [16]. Oda et al. [17] studied the Polycaprolactone depolymerase produced by the bacterium Alcaligenes faecalis. He isolated several bacteria capable of degrading polycaprolactone (PCL) from soil and activated sludge. Webb et al. [18] studied the fungal colonization and biodeterioration of plasticized polyvinyl chloride in in situ and ex situ conditions. These results suggest that microbial succession may occur during the long periods of exposure in situ. They have identified Aureobasidium pullulans was the principal colonizing fungus and a group of yeasts and yeast-like fungi, including
Rhodotorula aurantiaca and Kluyveromyces spp. Incidence of marine and mangrove bacteria accumulating polyhydroxy alkanoates on the mid-west coast of India has been reported by Rawte et al. [19].

3.3 Microbial Degradation of Polythene and Plastics in Laboratory Condition Determination of Weight Loss

Selected microorganisms were further tested in the laboratory condition to check the ability of degrading polythene and plastics. The bacteria allowed to degrade the polythene powder and plastic under shaking condition for period of 2, 4 and 6 months. After the period of shaking the powder were collected, washed thoroughly using distilled water, shade dried and

then weighted to check the final weight (Table1). These micro-organisms utilize polythene film as a sole source of carbon resulting in partial degradation of plastics. They colonize on the surface of the polyethylene powder forming a biofilm. Cell surface hydrophobicity of these organisms was found to be an important factor in the formation of biofilm on the polythene surface, which consequently enhanced biodegradation of the polymers.

Kathiresan and Bingham, [11] reported that bacteria caused the biodegradation ranging from 2.19 to 20.54% for polythene and 0.56 to 8.16% for plastics. Among all the species, Aspergillus glaucus was more active than A. Niger in degrading 28.8% of polythene and 7.26% of plastics within a month. This may be attributed to the thickness of the polythene that is 5-times thinner than the plastics. Once the organisms get attached to the surface, it starts growing by using the polymer as the carbon source. In the primary degradation, the main chain cleaves leading to the formation of low-molecular weight fragments (oligomers), dimers or monomers [20]. The degradation is due to the extra cellular enzyme secreted by the organism. These low molecular weight compounds are further utilized by the microbes as carbon and energy sources. The resultant breakdown fragments must be completely used by the microorganisms, otherwise there is the potential for environmental and health consequences [21]. Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil [22]. Many of them are known to have the capacity to degrade plastic materials and synthesis bioactive secondary metabolites which include enzymes, herbicides, pesticides and antibiotics.

Species	Type of plastic	% of degradation rate	Days
LL2	TREATRED PLASTIC (LD)	51.02	90
R	TREATRED PLASTIC (LD)	39	90
L4	TREATRED PLASTIC (LD)	38.5	90
P2	NORMAL PLASTIC (LD)	31.5	90
PS1	NORMAL PLASTIC (LD)	29.5	90
LL3	NORMAL PLASTIC (LD)	16.5	90
LL2	TREATRED PLASTIC (LD)	30	68
R	TREATRED PLASTIC (LD)	23.7	68
L4	TREARTED PLASTIC (LD)	26.5	68
LL2	TREARTED PLASTIC (HD)	3	52
R	TREATED PLASTIC (HD)	1	52
L4	TREATED PLASTIC (HD)1	2	52

Fig No1:- Table having information about degradation rate of different plastic samples.. Treated plastic (LD) have maximum degradation rate in 90 days.

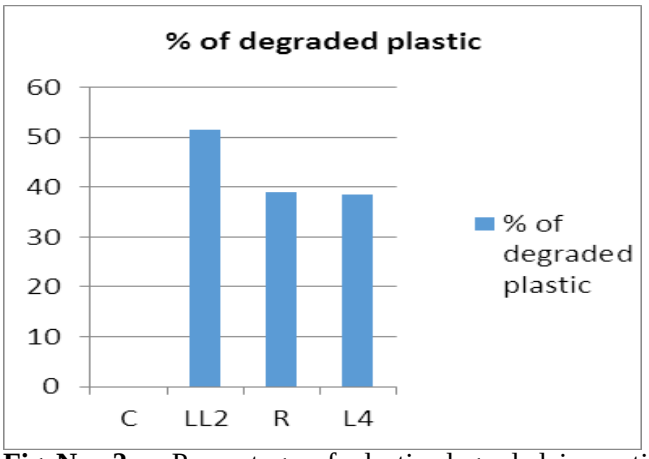

Fig No. 2 :- Percentage of plastic degraded in perticuler time interval (maximum time 60 days) with specific micro-organism(C:control , LL2, R , L4)

CONCLUSION :

Many studies on the degradation of plastics have been carried out in order to overcome the environmental problems associated with synthetic plastic waste. Recent work has included studies of the distribution of synthetic polymer–degrading microorganism in the environment, the isolation of new microorganisms for biodegradation and the discovery of new degrading enzymes. Biodegradation of polyethylene is possible mechanism by some alteration in polyethylene structure and by treating with starch solution , provide continuous starch phase for microbial attack. On culturing isolated microbes with plastic give 51% efficiency rate of degradation within approximately 90 days. Some microorganism having capacity to degrade polyethylene like , Pseudomonas putida strain ATCC 11172 16S ribosomal RNA gene , *Pseudomonas aeruginosa* strain E1. 16s rDNA gene , *Pseudomonas stutzeri*. 16s rDNA .

REFERENCES

[1] Kathiresan, K. (2003). Polythene and Plastics-degrading microbes from the mangrove soil, Rev. Biol. Trop. 51(3): 629-634

[2] Aamer, A. S., Hasan, F., Hameed, A., Ahmed, S. (2008). Biological degradation of plastics: A compreh rev. Biotech. Adv., 246–265.

[3] Okmoto. K, Izawa. M, AND Yanase. H, (2003), Isolation and Application of a Styrene-Degrading Strainof Pseudomonas putida to Biofiltration, Journal of Bioscience D Bioengineering Vol. 95, No. 6, 633-636.

[4] Zheng.Y, Bassi A. S, and Ernest K. Y., (2005), A Review of Plastic Waste Biodegradation, Critical Reviews in Biotechnology, 25:243–250, 2005,

[5] Usha. R, Sangeetha. T, and Palaniswamy. M, (2011), Screening of Polyethylene Degrading Microorganisms from Garbage Soil, Libyan Agriculture Research Center Journal International 2 (4): 200-204

[6] 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,Baltimore, USA., pp: 527-558.

[7] Raper, K.B. and D.I. Fennell, 1987. The genus Aspergillus. R.E. Krieger(ed). Huntington, New York, pp: 686.

[8] Shirling, E.B. and D. Gottlieb, 1966. Methods for characterization of Streptomyces species. Int. J. Syst. Bacteriol., 16: 313-340.

[9] Orhan, Y., J. Hrenovic and H. Buyukgungor, 2004. Biodegradation of plastic compost bags under controlled soil conditions. Acta Chim. Slov.51: 579-588.

[10] Ch Vijaya. and R. Mallikarjuna Reddy, 2008. Impact of soil composting using municipal solid waste on biodegradation of plastics. Indian J. Biotechnol., 7: 235-239.

[11] Kathiresan, K. and B.L. Bingham, 2001. Biology ofmangroves and mangrove ecosystems. Advances Mar. Biol., 40: 81-251.

[12] Ishigaka, T., W. Sugano, M. Ike, Y. Kawagoshi and M. Fujita, 2000. Abundance of polymers degrading microorganisms in sea based solid waste disposal site. J. Basic Microbiol., 40: 177-186.

[13] Imam, S.H., S.H. Gordon, R.L. Shogren,T.R. Tosteson, N.S. Govind and R.V. Greene, 1999. Degradation of starch-poly (b-Hydroxybutyrate-Cob Hydroxyvalerate) bioplastic in tropical coastal waters. Appl. Environ. Microbiol., 65: 431-437.

[14] Kathiresan, K., 2003. Polythene and plastics degrading microbes form mangrove soil. Rev. Biol. Trop., 51: 629-634.

[15] Augusta, J., Müller, R.J. and H. Widdecke, 1993. A. rapid evaluation plate-test for the biodegradability of plastics. Appl. Microbiol. Biotechnol., 39: 673-678.

[16] Nakajima-Kambe, T., F. Onuma, N. Kimpara, T. Nakahara, 1995. Isolation and chareacterization of a bacterium which utilizes polyester polyurethane as a sole carbon and nitrogen source. FEMS Microbiology Letters., 129: 39-42.

[17] Oda, Y., N. Oida, T. Urakami and K. Tonomura, 1998. Polycaprolactone depolymerase produced by the bacteriaum Alcaligenes faecalis. FEMS Micobiol. Lett., 150: 339-343.

[18] Webb, J.S., M. Nixon, I.M. Eastwood, M. Greenhalgh, G.D. Robson and P.S. Handley, 2000. Fungal colonization and biodeterioration of plasticized polyvinyl chloride Appl. Environ. Microbiol., 66: 3194 -3200.

[19] Rawte, T., M. Padte and S. Mavinkurve, 2002. Incidence of marine and mangrove bacteria accumulating polyhydroxyalkanoates on the mid-west coast of India. World J. Microbiol., 18: 655-659.

[20] Vasile, C., 1993. Degradation and decomposition, in Handbook of polyolefins synthesis andproperties, edited by C Vasile and R B Seymour (Marcel Dekker Inc, New York)., pp: 479-509.

[21] Narayan, R., 2006. Biobased and biodegradable polymer materials: rationale, drivers and technology exemplars. American Chemical Society Symposium Ser., 939(18): 282.

[22] Oskay, M., A.U. Tamer and C. Azeri, 2004. African J. Biotechnol., 3(9): 441- 446.