WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES

SJIF Impact Factor 6.647

Volume 6, Issue 8, 911-918 Research Article ISSN 2278 – 4357

INVESTIGATION OF GARBAGE SOIL FOR POLYETHYLENE DEGRADING MICROORGANISMS AND STUDY THEIR GROWTH

K. Shrikant*, T. Abha and Chauhan U. K.

Research Scholar, Awadesh Pratap Singh University, Rewa (486001).

Article Received on 29 May 2017,

Revised on 19 June 2017,
Accepted on 10 July 2017

DOI: 10.20959/wjpps20178-9597

***Corresponding Author**

K. Shrikant

Research Scholar, Awadesh Pratap Singh University, Rewa (486001).

ABSTRACT

Plastic and polythene waste amassing in the environment are posing an ever increasing ecological threat. Biodegradable plastics are environment friendly; they have an increasing range of potential application and are determined by the growing use of plastics as wrapper. In this study, biodegradation of polythene bag and plastic cup were analyzed 2, 4 and 6 month of incubation in liquid culture method. The heterotrophic population of microbes in polythene and plastics, bacterial counts was recorded. The microbial species associated with the polythene materials were identified as *Pseudomonas* sp, *Bacillus* sp, *Staphylococcus* sp, *Aspergillus nidulans*, *Aspergillus flavus* and *Streptomyces* sp. Efficacy of the microbes in degradation of polythene and plastics were analyzed in liquid (shaker) culture method, among the bacteria *Pseudomonas* sp degrade 47.09% of polythene and 38.42% of plastics in 6 month period. Among the fungal species 20.96% of polythene and 16.84% of plastics and *Streptomyces* species 46.16% of polythene and 35.78% of plastics.

KEYWORDS: Biodegradation, Garbage soil, Polythene, Plastics, *Streptomyces* sp.

INTRODUCTION

Our earth is so beautiful and full of resources on which the humanism depends but due to the excess use of them, generates the disturbance and its foolishness generates pollution problems.[22] 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 million tons of synthetic polymers are produced worldwide each year. The physical and chemical properties of polymers depend on the nature, arrangement of chemical groups of their composition and the magnitude of intra or intermolecular forces i.e primary and secondary valence bonds present in the polymer. Degradation process occursdue to the influence of thermal, chemical, mechanical, radiative and biochemical factors occurring over a period of time resulting in deterioration of mechanical properties and colour of polymers. The degradation occurs due to changes accompanying with the main backbone or side groups of the polymer. Degradation is a chemical process which affects not only the chemical composition of the polymer but also the physical parameters such as colour of the polymer, chain conformation, molecular weight, molecular weight distribution, crystallinity, chain flexibility, cross-linking and branching. The nature of weak links and end groups in the polymers contribute to stability of polymers. The degradation process is initiated at the terminal units with subsequent depolymerization.[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.

Bio degradable polymers are designed to degrade upon disposal by the action of living organisms. Biodegradable polymers generally decompose in various medium in our environment. The depolymerisation results due to various physical and biological forces. The physical forces such as temperature, moisture, pressure etc, deal with causing mechanical damage to the polymer. The microbial biodegradation is widely accepted and is still underway for its enhanced efficiency. Recently several microorganisms have been reported to produce polyester degrading enzymes. The microbial species are associated with the degrading materials were identified as bacteria (*Pseudomonas*, *Streptococcus*, *Staphylcoccus*, *Micrococcus and Moraxella), fungi (Aspergillus niger*, *Aspergillus glaucus*), *Actinomycetes* sp. and *Saccharomonospora* genus.[2]

Poly (ethylene terephthalate) known by the trade names Mylar, Decron, terylene, Recron, has high crystalline melting temperature (260°C), and the stiff polymer chains in the PET polymer imparts high mechanical strength, toughness and fatigue resistance up to 150- 175°C as well as good chemical, hydrolytic and solvent resistance. Poly (ethylene terephthalate) fiber has a very outstanding crease resistance, good abrasion resistance and can be treated with cross-linking resin to impart permanent wash and wear properties.[2-4] The fiber can be blended with cotton and other cellulosic fibers to give better feel and moisture permeation. Thus the fiber is used for applications such as wearing apparel, curtain, upholstery, thread, tire cord filaments, industrial fibers and fabric for industrial filtration.[19-21]

Microbial degradation of plastics is caused by certain enzymatic activities that lead to a chain cleavage of the polymer into oligomers and monomers. These water soluble enzymatically cleaved products are further absorbed by the microbial cells where they are metabolized. Aerobic metabolism results in carbon dioxide and water,[3] whereas anaerobic metabolism results in carbon dioxide, water and methane as the end products, respectively.[4] The degradation leads to breaking down of polymers to monomers creating an ease of accumulation by the microbial cells for further degradation.

MATERIALS AND METHODS

Material: Low density polyethylene powder (LDPE) was achieved from Sigma Aldrich Chemical Co. (Germany).

Sample Collection: Garbage soil samples (waste disposable site dumped with polythene bag and plastic cup) were collected from sidco, Coimbatore, Tamil nadu. The soil samples were collected at a depth of 3-5cm, in a sterile container and then air dried at room temperature.

Isolation of Polyethylene Degrading Microorganisms: One gram of soil sample was transferred into a conical flask containing 99ml of sterile distilled water. This content was shaken and serially diluted. To isolate microorganisms associated with materials (polythene bag and plastic cup) by pour plate method was adopted using the starch casein agar for actinomycetes, nutrient agar for bacteria and sabouraud dextrose agar for fungi. 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 slant at 4°C.

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 sonicated for 1hour at 120 rpm in shaker. After sonication the medium was sterilized at 121°C and pressure for 15 lbs/inch^2 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.

Identification Polyethylene Degrading Microorganisms: The identification of bacteria was performed on the basis of macroscopic and microscopic examination and biochemical test according to Bergey's manual of determinative bacteriology.[6] The fungus was identified after staining them with cotton blue by following the keys Raper and Fennell.[7] The phenotypic and chemotaxonomic characteristics of the actinomycetes were determined by the method described by Shirling and Gottlieb.[8]

Microbial Degradation of Polythene and Plastics under Laboratory Conditions Liquid Culture Method:[9] The pre weighted strip of 2cm diameter prepared from polythene bags and plastic cups were aseptically transferred into the conical flask containing 100 ml of mineral salt medium and then inoculated with identified polythene degrading microorganisms. Control was maintained with polythene and plastic strips in the microbe free medium and left in a shaker at 30°C, 150 rpm for 2, 4 and 6 month period. After the period of shaking the strips were collected, washed thoroughly using distilled water, shade dried and then weighted to check the final weight. Finally the weight loss of the polythene bags and plastics were calculated and compared with control.

RESULTS AND DISCUSSION

Microorganisms Associated with Garbage Soil Samples: The number of heterotrophic microbes was isolated from garbage soil sample and the bacterial population ranged from 62.71×10^4 polythene bag and 50.42×10^4 in the case of plastics. While the fungi count ranged from 36.24×10^2 polythene and 28.16×10^2 in case of plastics. The actinomycetes count ranged from 68.54×10^4polythene and 56.22×10^4 in case of plastics. The total number of microbes associated with polythene and plastics showed some variation. Vijaya, reported that average number of heterotrophic bacteria and fungi found in association with polythene film and plastic cups were 37.08×10^4 and 38.04×10^4, 26.94×10^2 and 35.13×10^2 respectively.[10] Kathiresan, (2001) reported that the plastic materials in mangrove soil have shown rich total heterotrophic bacterial counts of up to 79.67×10^4 and fungal counts of up to 55.33×10^2 and the plastic materials have been colonized commonly by five species of bacteria and two species of fungi.[11] 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]

Screening and Identification of Polyethylene Degrading 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 5 of *Streptomyces* sp, one of *Psudomonas* sp, *Bacillus* and *Staphylococcus* sp two of *Aspergillus* sp showed high degradation activity. Similar type of organisms were reported earlier which associated with the polythene bag and plastic films in the soil.[14] 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 situ* an *ex situ* conditions. These results suggest that microbial succession may occur during the long periods of exposure in *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]

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, fungi and *actinomycetes* separately allowed degrading the polythene and plastic under shaking condition for period of 2, 4 and 6 months. After the period of shaking the strips were collected, washed thoroughly using distilled water, shade dried and then weighted to check the final weight. These microorganisms utilize polythene film as a sole source of carbon resulting in partial degradation of plastics. They colonize on the surface of the polyethylene films or plastic cups 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]

Table 1: Comparative analysis of polythene and plastics weight loss with different microbial species in shaker cultures under laboratory conditions.

Name of microbes	Microbial degradation (%weight loss)					
	2nd month		4th month		6th month	
	Polythene	Plastics	Polythene	Plastics	Polythene	Plastics
Bacteria						
Pseudomonas sp	7.67±0.14	5.45±0.16	24.80±0.02	20.00±0.10	47.09±0.01	38.42±0.11
Bacillus sp	4.22±0.31	3.15±0.10	11.29±0.10	18.5±0.05	25.64±0.08	24.21±0.02
Staphylococcus sp	2.71±0.01	2.31±0.12	4.31±0.08	4.83±0.08	15.89±0.06	16.12±0.11
Fungi						
A.nidulans	4.51±0.10	3.22±0.02	6.45±0.27	8.42±0.06	12.63±0.05	11.29±0.05
A.flavus	6.06±0.14	5.36±0.20	16.12±0.05	11.57±0.19	20.96±0.15	16.84±0.12

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.

CONCLUSION

The isolated microbes were native to the site of polyethylene disposal and might show some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media. This gives some suggestion that these microbes can be used in both natural and artificial conditions for the purpose of degradation of polymers. Our knowledge, microbes cause greatest degradation of polythene and plastics.

Among the microbes, viz S*treptomyces* sp, A. *nidulance* and *Pseudomonas* sp, the S*treptomyces* sp having greater degradation ability when compared with fungi and other bacteria. Hence, the further attention is required from microbiologists for commercial degradation and eco-friendly polyethylene with *Streptomyces* sp.

REFERENCES

1. Shimao, M., Biodegradation of plastics. Curr. Opin. Biotechnol., 2001; 12: 242-247.
2. Swift, G., Non-medical biodegradable polymers: environmentally degradable polymers Handbook of biodegradable polymers. Hardwood Acedemic, Amsterdam, 1997; 473-511.
3. Starnecker, A. and M. Menner, Assessment of biodegradability of plastics under stimulated composting conditions in a laboratory test system. International bio-deterioration and Biodegradation, 1996; 85-92.
4. Gu, J.D., T.E. Ford, D.B. Mitton and R. Mitchell, Microbial corrosion of metals. The uhling corrosion hand book. 2nd Edition. Whiley, New York, USA, 2000; 915-927.
5. Augusta, J., R.J. Muller and H. Widdecke, A. rapid evaluation plate-test for the biodegradability of plastics. Appl. Microbiol. Biotechnol., 1993; 39: 673-678. and S.T. Williams, Gram-Positive Cocci. In: Bergey's Manual of Determinative Microbiology, Hensyl, W.R. (Ed.). 9th Edn., Williams and Wilkins, Baltimore, USA., 1994; 527-558.
6. Raper, K.B. and D.I. Fennell, The genus *Aspergillus.* R.E. Krieger(ed). Huntington, New York, 1987; 686.
7. Shirling, E.B. and D. Gottlieb, Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol., 1966; 16: 313-340.
8. Orhan, Y., J. Hrenovic and H. Buyukgungor, Biodegradation of plastic compost bags under controlled soil conditions. Acta Chim. Slov., 2004; 51: 579-588.
9. Ch Vijaya. and R. Mallikarjuna Reddy, Impact of soil composting using municipal solid waste on biodegradation of plastics. Indian J. Biotechnol., 2008; 7: 235-239.
10. Kathiresan, K. and B.L. Bingham, Biology of mangroves and mangrove ecosystems. Advances Mar. Biol., 2001; 40: 81-251.
11. Ishigaka, T., W. Sugano, M. Ike, Y. Kawagoshi and M. Fujita, Abundance of polymers degrading microorganisms in sea based solid waste disposal site. J. Basic Microbiol., 2000; 40: 177-186.

12. Imam, S.H., S.H. Gordon, R.L. Shogren, T.R. Tosteson, N.S. Govind and R.V. Greene, Degradation of starch-poly (b-Hydroxybutyrate-Cob-Hydroxyvalerate) bioplastic in tropical coastal waters. Appl. Environ. Microbiol., 1999; 65: 431-437.
13. Kathiresan, K., Polythene and plastics degrading microbes form mangrove soil. Rev. Biol. Trop., 2003; 51: 629-634.
14. Augusta, J., Müller, R.J. and H. Widdecke, A. rapid evaluation plate-test for the biodegradability of plastics. Appl. Microbiol. Biotechnol., 1993; 39: 673-678.
15. Nakajima-Kambe, T., F. Onuma, N. Kimpara, T. Nakahara, Isolation and chareacterization of a bacterium which utilizes polyester polyurethane as a sole carbon and nitrogen source. FEMS Microbiology Letters., 1995; 129: 39-42.
16. Oda, Y., N. Oida, T. Urakami and K. Tonomura, Polycaprolactone depolymerase produced by the bacteriaum *Alcaligenes faecalis*. FEMS Micobiol. Lett., 1998; 150: 339-343.
17. Webb, J.S., M. Nixon, I.M. Eastwood, M. Greenhalgh, G.D. Robson and P.S. Handley, Fungal colonization and biodeterioration of plasticized polyvinyl chloride Appl. Environ, 2000.
18. Principles of polymerization IV edition, Gerge Odion, John Wiley Interscience, 2004; 92-97.
19. Reese G “Polyester Fibers” in “Encyclopedia of Polymer science and Technology 3rd Ed.: Wiley: New York, 2003; 3: 652.
20. East A.J. In synthetic Fibers, Nylon, Polesters, acrylic, polyolefine: McIntyreJE Ed., The Textile Institute, Wood Head Publishing Limited, Cambridge, 2005; 95-167.
21. Shrikant Kol, Rishabh D Saket, U.K. Chauhan; Bioremedial Potential Of Microbial Culture; Isolated From The Contaminated Soil With Crude Oil. International Journal of Research in Biotechnology and Biochemistry, 2014; 4(1): 12-15.