International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com

Research Article

Polyethylene degradation by *Pseudomonas putida* S3A

Majid H. Al-Jailawi[1], Rasha S. Ameen[2] and Ali A. Al-Saraf[3]

[1, 3] Department of Biotechnology, College of Science, Al-Nahrain University.
[2] Forensic DNA Research & Training Center, Al-Nahrain University.
*Corresponding author: : *majed_algelawi@hotmail.com*

Abstract

Pseudomonas putida S3A that has ability to degrade nylon6 film, crude nylon 6 and nylon 66 as sole source of nitrogen and carbon, isolated from soil contaminated with plastic waste, was included. This study was aimed to determine the ability of this isolate to degrade polyethylene as sole source of carbon. Some optimum conditions for degradation of polyethylene by this bacterium were studied. It was found that these conditions are growing *P. putida* S3A in mineral salt medium (pH 6.5) containing 0.5% of polyethylene and incubated with shaking (180 rpm) at 37°C for seven days. In addition, it has been found that this bacterium was able to survive with up to 0.9% of polyethylene. In order to insure that this bacterium was capable to degrade polyethylene, the Fourier Transformer Infrared Red Spectroscopy (FTIR) was used. Results indicated that polyethylene was degraded by *P. putida* S3A, which used the (O-H, C-O and C-H) groups as carbon source.

Keywords: Biodegradation, plastic, polyethylene, *Pseudomonas putida*, FTIR.

Introduction

Rapid developments in the chemical industry have lead to the distribution of a wide variety of synthetic compounds into the environment (Negoro, 2002). Approximately 140 million tones of synthetic polymers are produced worldwide every year. Since polymers are extremely stable, their degradation cycles in biosphere are limited. Environmental pollution by synthetic polymers, such as waste of plastic and water-soluble synthetic polymers in wastewater has been recognized as a major problem (Premraj and Doble, 2005).

Waste plastics lay enormous burden on the environment, because their recalcitrance to degradation accelerates the accumulation in nature. Waste plastics buried in soil cause the water clogging phenomena and devastate soil for agricultural cultivation. Many animals die of waste plastics either by being caught in the waste plastics trap or by swallowing the waste plastics debris to exert ruinous effects on the ecosystem (Usha *et al*., 2011).

The biodegradation of synthetic polymers in natural habitats is of considerable importance because of the vast quantities used (Mohan and Srivastava, 2010). The accumulation of many of the polymers used for packaging in recreational areas, adjacent to highways, and in sanitary landfills is readily apparent even to the casual observer. One of the major classes of synthetic polymers is the polyethylene group (Huang *et al*., 2005).

Polyethylene is a polymer made of long chain of monomers of ethylene. Polyethylene is highly hydrophobic and chemically inert, and microbes on the earth surface have not yet been fully evolved to digest the artificially made plastics. A lot of research has been carried out to alleviate the environmental burden by improving degradability of the waste polyethylene (Kavitha *et al*., 2014). Abiotic pretreatment such as weathering, UV irradiation and thermal treatment was employed to raise the hydrophobicity of polyethylene by introducing polar groups such as carbonyl groups to the polyethylene backbone chain and thus facilitates the microbes to

metabolize the unwieldy plastics (Albertsson *et al*., 1987; Groning *et al*., 2004). A few microbes capable of degradation of the pretreated polyethylene have so far been isolated from soil, seawater, and compost and activated sludge (Yoon *et al*., 2012).

The biodegradable polymers are designed to degrade it fast using microbes since microorganisms are capable degrading most of the organic and inorganic materials, including lignin, starch, cellulose and hemicelluloses (Sadocco *et al*., 1997), there is lot of interest in the microbial degradation of polyethylene waste material (Kambe *et al*., 1999). Biodegradation resulting from the utilization of polyethylene as nutrient may be more efficient if the degrading microorganism forms a biofilm on the polyethylene surface (Shah *et al*., 2009). The microbial species are associated with the degrading materials were identified as bacteria (*Pseudomonas, Streptococcus, Staphylococcus, Micrococcus, Moraxella*), fungi (*Aspergillus niger, Aspergillus glaucus*), *Actinomycetes* sp. and *Saccharomonospora* genus (Swift, 1997; chee *et al*., 2010). Specifically some white rod fungi also can degrade toxic compounds by the secretion of extracellular enzymes. Microbial degradation of plastic caused by oxidation or hydrolysis using microbial enzymes that lead to chain cleavage of the high molecular weight polymer into low molecular weight oligomer and monomer by aerobic or anaerobic metabolism (Kumar *et al*., 2013).

This research was aimed to study the ability and some optimum conditions of polyethylene degradation by *Pseudomonas putida* S3A as carbon and nitrogen source, In addition to use FTIR analysis to confirm this ability.

Materials and Methods

Polyethylene (PE)

The low-density (LDPE) granule pellets used in this study were obtained from local plastic bags factory. LDPE were cut into small pieces of about 0.2 - 0.5 Cm in diameter, washed with 70% ethanol for 30 min, then washed with distilled water, and air dried for 15 minutes in Laminar air flow chamber and was added to the medium.

Bacterial isolate

The *Pseudomonas putida* S3A isolate, which has the ability to degrade nylon 6 film, crude nylon 6 and nylon 66 as a sole source of carbon and nitrogen, was used in this study. This bacterium was isolated from soil with a history of plastic waste pollution in Iraq (Al-Saraf and Al-Jailawi, 2013).

Biodegradation assay of Polyethylene by *Pseudomonas putida* S3A

To determine the ability of the tested isolate to degrade polyethylene, 20 milliliter of mineral salt medium (EM) (Negoro *et al.*, 1980) were dispensed in Erlenmyer flasks (100ml) and sterilized by autoclaving. After sterilization, the flasks were supplemented with 1g/L of polyethylene (disinfected 30 min in 70 % ethanol and air dried for 15 minutes in Laminar air flow chamber), inoculated with 1% of fresh culture (18hrs. old) of *P. putida* S3A and incubated in shaker incubator (180rpm) at 30 °C for 3 and 7 days. Control was made by inoculating flasks with bacterial isolate, these flasks containing the same mineral salt medium (EM) but without any source of carbon (polyethylene). Biodegradation test was performed in triplicates. The degradation ability of this bacterium was determined by monitoring the growth density of the liquid culture in spectrophotometer at 600nm.

Optimization of polyethylene biodegradation

Effect of pH

The effect of pH on the ability of *P. putida* to utilize polyethylene as a sole source of carbon and nitrogen was determined by supplemented Mineral salt medium (EM) with 0.1 % of polyethylene at different pH values (6, 6.5, 7, 7.5), in an attempt to determine the suitable pH value, then cultures were incubated in a shaker incubator (180 rpm) at 30 °C for three and seven days. The optimum pH value was employed in the subsequent experiment.

Effect of Temperature

To determine the effect of temperature on the ability of *P. putida* to degrade polyethylene, Mineral salt medium (EM) (pH 6.5) supplemented with 0.1 % of Polyethylene film was inoculated and incubated in shaker incubator (180 rpm) at different temperatures 30, 37 and 45 °C for three and seven days. Optimal temperature was subsequently employed, depending on the growth density measurement.

Effect of polyethylene concentration

In order to determine the optimum concentration of polyethylene film that can be degraded by *P. putida*, this film was added at different concentrations (0.05%, 0.06%, 0.07%, 0.08%, 0.09%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9% and 1%), pH was adjusted to 6.5, and then incubated in a shaker incubator (180 rpm) at 37 °C for three and seven days. Bacterial growth was measured and the optimal concentration was employed later on.

Characterization of polyethylene

In order to characterize the chemical nature and structure of polyethylene, and analyze the change in polyethylene structure after incubation with bacterial isolate, Fourier transformed infrared spectroscopy (FTIR) (Shimadzu) analysis was done at AL-Nahrain University/ College of Science / Department of Chemistry. *P. putida* S3A was grown on mineral salt medium (EM) pH 6.5 containing 0.5% of polyethylene with shaking (180 rpm) at 37 °C for seven days. After incubation, samples of polyethylene were taken and subjected to FTIR analysis.

Results and Discussion

Biodegradation assay of *P. putida* S3A

In order to test the ability of *P. putida* S3A to utilize polyethylene as a sole source of carbon, low density polyethylene was used and the growth experiment were performed by inoculating the tested bacterial isolate in EM media and incubated in shaker incubator (180 rpm) at 30 °C for 3 and 7 days. These experiments revealed that *P. putida* S3A was able to grow with polyethylene yielding optical density of (0.152) after 3 days and (0.193) after 7 days of incubation. This observation indicated that this bacterial isolate utilized polyethylene as a sole of carbon source resulting in partial degradation of plastics.

Such results are similar to those obtained by Okoh and Atuanya (2014) who revealed that the *Pseudomonas* sp. possesses greater potential to degrade polyethylene compared to other bacteria and fungi. Muckschel *et al*., (2012) also investigated the potential of the *Pseudomonas putida* strains KT2440 and JM37 for the microbial oxidation of ethylene glycol into the more valuable product glyoxylic acid. Their results revealed that, In contrast to strain KT2440, strain JM37 showed rapid growth with ethylene glycol, glycolic acid, or glyoxylic acid as the sole source of carbon and energy.

In the study of Kathiresan (2005) it has been mentioned that the microbial species found associated with the degrading of plastics and polythene were identified as five Gram positive and two Gram negative bacteria, and eight fungal species of *Aspergillus*. The species that were predominant were *Streptococcus*, *Staphylococcus*, *Micrococcus* (Gram +ve), *Moraxella*, and *Pseudomonas* (Gram -ve) and two species of fungi (*Aspergillus glaucus* and *A. niger*), and the efficacy of the microbial species in degradation of plastics and polythene was analyzed in shaker cultures, Among the bacteria, *Pseudomonas* species degraded 20.54% of polythene and 8.16% of plastics in one month period. Among the fungal species, *Aspergillus glaucus* degraded 28.80% of polythene and 7.26% of plastics in one-month period.

Prabhat *et al.,* (2013**)** were isolated and identified plastic degrading microorganisms from soil. The microorganisms produces different types of changes during biochemical analysis. The bacteria are identified to be *Pseudomonas* spp,

Streptococcus spp, *Staphylococcus* spp, *Micrococcus* spp and *Moraxella* spp *Bacillus subtilis*, *Bacillus amylolyticus* and *Arthobacter defluvii. Bacillus amylolyticus* is more useful than other bacteria. *Bacillus subtilis* has less capacity to degrade plastic as compared to other bacteria.

Sharma and Sharma (2004) study the extend of degradation of low-density polythene (LDP) and polythene (PP) using *Pseudomonas stutzeri* under laboratory test condition. Throughout the investigation, both the plastic types are found to undergo qualitative and quantitative changes by bacteria but PP is found to be more biodegradable as compared to LDP.

The ability of a microorganism to utilize any substrate depends on its growth and adherence to that substrate. Bacterial adhesion to either a hydrophilic or a hydrophobic substrate is governed by many factors, including the forces by which the bacterium attaches to the surface and the properties of the substrate and micro-organism. Generally, a hydrophobic bacterium prefers a hydrophobic surface for attachment, whereas the opposite is true for bacteria with hydrophilic properties. As the polyethylene surface is hydrophobic

in nature, it has been suggested that the more hydrophobic the bacterial cell surface, the higher the interaction with polyethylene (Gilan *et al*., 2004). 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), dimmers or monomers. The degradation is due to extra cellular enzyme secreted by the organism (Usha *et al*., 2011).

Optimization of polyethylene degradation by *P. putida* S3A

Effect of pH

Mineral salt medium was prepared at different pH values (6, 6.5, 7 and 7.5) in an attempt to determine the optimum pH required for growth of *P. putida* S3A on Polyethylene. The obtained results as shown in Figure (1) elucidated that an optimum growth was occurred at pH 6.5; the optical density for bacterial growth was reached 0.22 and 0.30 after three days and seven days respectively.

While bacterial growth was decreased at other pH values compared with growth at pH 6.5, Within the pH 6 the optical density for bacterial growth was 0.15 and 0.135 after three and seven days respectively. while within pH 7 and pH 7.5 there was a dramatic increase and decrease in the growth, with pH 7 the optical density for bacterial growth was 0.155 after three and 0.199 after seven days, with pH 7.5 the optical density for bacterial growth was 0.135 and 0.13 after three and seven days respectively.

The same result was found by Al-Saraf and Al-Jailawi (2013) who pointed that the optimal pH value for *P. putida* S3A to degrade nylon6 was 6.5. Also in the study of Muckschel *et al*. (2012) they mentioned that the pH of the medium used to metabolize ethylene glycol by *Pseudomonas putida* was adjusted to 6.7.

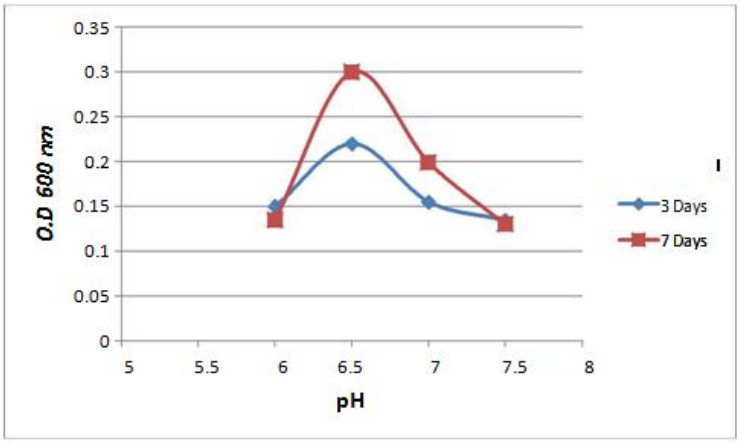

Figure (1): Effect of pH on polyethylene degradation by *P. putida* S3A grown in mineral salt medium containing 0.1 % of Polyethylene in shaker incubator (180rpm, 30 °C) for 3 and 7 days.

Effect of temperature

Pseudomonas putida S3A was grown and incubated at different temperatures (30, 37 and 45 °C). Results shown in Figure (2) pointed out that the optical density of bacterial growth at 37 °C was 0.3 and 0.36 after three and seven days of incubation respectively, which was suggested as the optimum temperature for bacterial growth. Relative result of bacterial growth was recorded at 30 °C. Whereas, at 45 °C, bacterial growth was lower than at other incubation temperatures. It was revealed that the optimum temperature for nylon6 degradation by *P. putida* S3A was 37°C (Al-Saraf and Al-Jailawi, 2013). Yoon *et al.* (2012) were isolated *Pseudomonas* sp. active for the low MW polyethylene biodegradation with appreciable the controlled conditions.

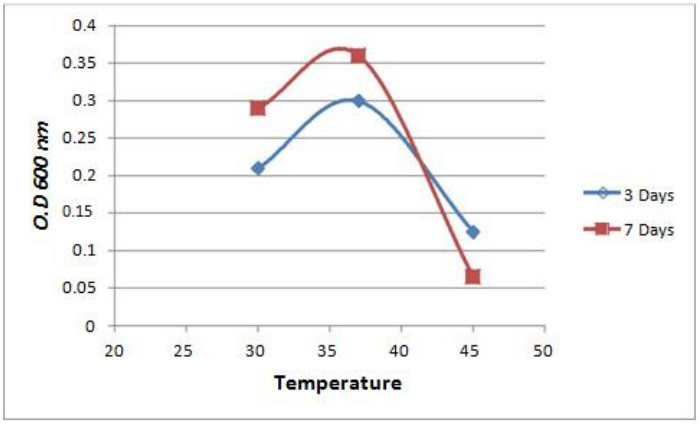

Figure (2): Effect of temperature on polyethylene degradation by *P. putida* S3A grown in mineral salt medium (pH 6.5) Containing 0.1% of polyethylene in shaker incubator (180rpm) for 3 and 7 days.

Effect of polyethylene concentration

Different concentrations (between 0.05% and 1%) of polyethylene were used to grow Pseudomonas putida S3A in order to determine the optimum concentration. Results in figure (3) indicated that the optimum concentration for bacterial growth was 0.5%, in which the optical densities of bacterial growth, after three and seven days of incubation were 0.4 and 0.43 respectively. Figure (3) showed also that gradual increasing of Polyethylene concentration accompanied with increasing of bacterial growth, and then the growth reached to its optimum at a concentration of 0.5%, while Polyethylene concentrations higher than 0.5% showed decrease in bacterial growth. This bacterium was able to survive with up to 0.9 % of polyethylene.

It was found that the optimum concentration of nylon 6 which degraded by P. putida S3A was 0.1 % and this bacterium was able to survive with up to 0.7 % of nylon 6 (Al-Saraf and Al-Jailawi, 2013). It has been proved that *P. putida* cause degradation of all plastic samples used such as Plastic cup, Polythene bag, Plastic bag and Milk cover within a month (Saminathan *et al*., 2014).

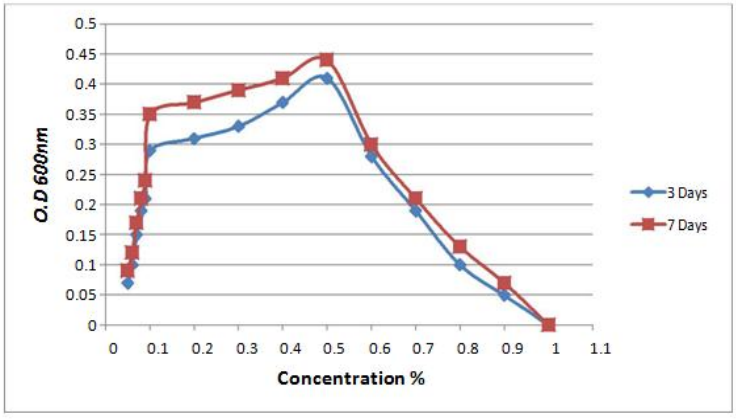

Figure (3): Effect of Polyethylene on *P. putida* S3A grown in mineral salt medium (pH 6.5) in shaker incubator (180rpm, 37°C) for 3 and 7 days.

Characterization of polyethylene

In order to confirm the ability of bacteria to degrade polyethylene, *Pseudomonas putida* S3A was grown on mineral salt medium (pH 6.5) containing 0.5% of polyethylene at 37 °C for seven days, any changes in the polyethylene structure with bacterial growth were analyzed by Fourier Transform Infrared Spectroscopy spectra in the frequency range of 4000 –500 cm-1. The polyethylene was subjected to FTIR to detect its structure (Figure 4). FTIR spectrum, showed the following bands: - band for O-H stretching at wavelength 3442.7 and C-O stretching of alcohol group of 1112.9. Bending of O-H give band at 1643.2. The spectrum also shows bands at 2858.3 and 1460.0 for C-H stretches and C-H banding respectively.

The FTIR spectrum for the sample of polyethylene after bacterial growth showed decrease in wavelength number for O-H stretching and shift the band to 3382.9 and disappearance of C-H at 28580.1 stretching band, which could be attributed to consume the polyethylene by the action of bacteria (Figure 5).

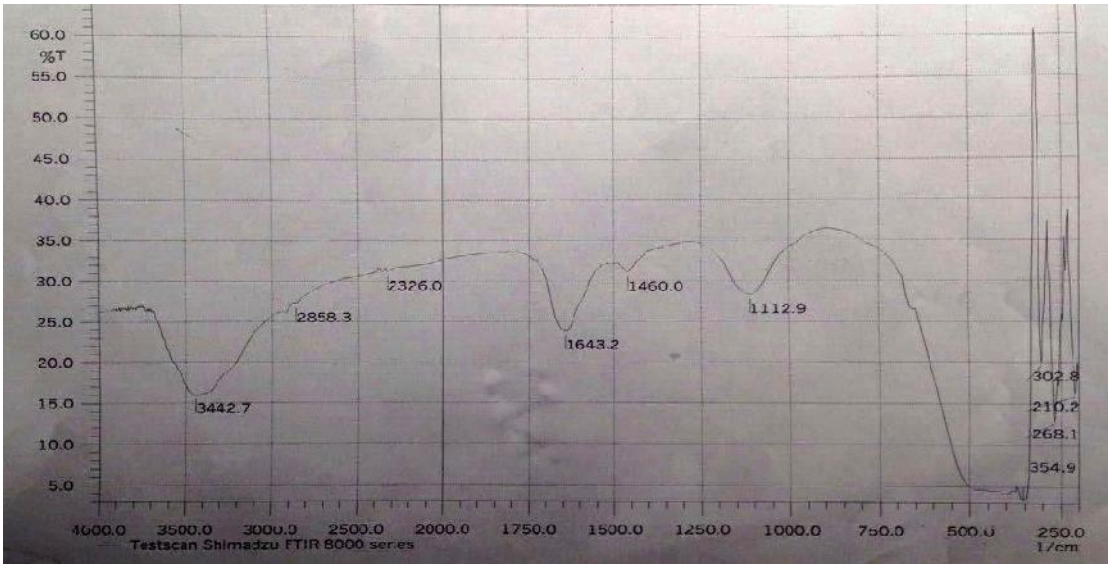

Figure (4): FTIR spectroscopy of polyethylene (control).

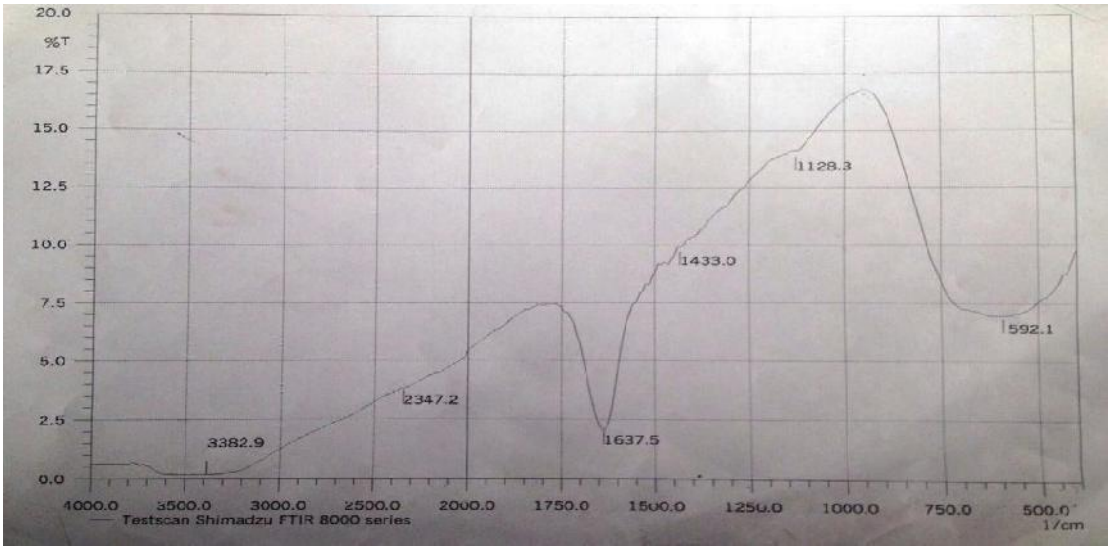

Figure (5): FTIR spectroscopy of polyethylene after grown of *P. putida* S3A in mineral salt medium (pH 6.5) containing 0.5 % of polyethylene in shaker incubator (180rpm, 37 °C) for seven days.

Conclusion

In the present study, it was concluded that the bacterial isolate *P. putida* S3A was able to grow in medium containing polyethylene as a sole source of carbon. The optimum conditions for the growth are growing this isolate in the mineral salt media (EM) (pH 6.5) containing 0.5% of polyethylene and incubated with shaking (180rpm) at 37 °C for seven days. In addition, FTIR analysis confirmed the degradation ability of *P. putida*, it was indicated that this bacterium use the (O-H, C-O, C-H) group as carbon source. Thus, further molecular study is needed to determine the catabolic genes resident in *P. putida* S3A that were responsible for the polyethylene-utilizing ability. Also it is recommended to understand the mechanism responsible for the biodegradation of polyethylene.

References

Albertsson, A. C., Andersson, S. O. and Karlsson, S. (1987). The mechanism of biodegradation of polyethylene. Polym Degrad Stab 18: 73-87.

Alsaraf, A. A. and Al-Jailawi, M. H. (2013). Isolation and identification of nylon 6 degrading bacteria and study the optimum conditions for degradation. J. Biotechnology Research (JBR) 13: 73-86.

Chee, J. Y.; Yoga, S. S., Lau, N. S., Ling, S. C., Abed, R. M. M. and Sudesh, K. L.(2010). Bacterially Produced Polyhydroxyalkanoate (PHA): Converting Renewable Resources into Bioplastics. *Appl Microbiol & Microbiol Biotech* A Mendez Vilas (Ed).

Gilan, O., Hadar, Y. and Sivan, A. (2004). Colonization, biofilm formation and biodegradation of polyethylene by a strain of *Rhodococcus ruber*. Appl.Microbiol.Biotechnol, 65: 97–104.

Groning, M., Hakkarainen, M. and Albertsson, A. C. (2004). Recycling of glass fiber reinforced phenolic prepreg waste. Part 1: Recovery and reuse of glass fibers in PP and PA6. Polym Polym Compos 12: 491-500.

Huang, Y., Deng, X., Lu Ying-Hua, Liao X., Hong, M. and Wang, Y. (2005). Aerobic and anaerobic biodegradation of polyethylene glycols using sludge microbes. Process Biochemistry 40: 207–211.

Kambe, T. N., Akutsu, S. Y., Nomura, N., Onuma, F. and Nakahara, T. (1999). Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes. *Appl. Microbiol.* *Biotechnol.* 51: 134 -140.

Kathiresan, K. (2003). Polythene and plastic-degrading microbes in an Indian mangrove soil. Rev Biol Trop. 51(3-4):629-33.

Kavitha, R., Mohanan, A. k. and Bhuvaneswari, V. (2014). Biodegradation of low density polyethylene by bacteria isolated from oil contaminated soil. *International Journal of Plant, Animal and Environmental Sciences*. 4(3): *601*- 610.

Kumar, S., Das, P. M., Rebecca, L. J. and Sharmila, S. (2013). Isolation and identification of LDPE degrading fungi from municipal solid waste. Journal of Chemical and Pharmaceutical Research. 5(3):78-81.

Mohan, S. K. and Srivastava, T. 2010. Microbial deterioration and degradation of polymeric materials. J Biochem Tech. 2(4):210-215.

Muckschel, B., Simon, O., Klebensberger, J., Graf, N., Rosche, B., Altenbuchner, J., Pfannstiel, J., Huber, A. and Hauera, B. (2012). Ethylene Glycol Metabolism by *Pseudomonas putida*. Applied and Environmental Microbiology. 78 (24): 8531– 8539.

Negoro, S., Shinagawa, H., Nakata, A., Kinoshita, S., Hotozaki, T. and Okada, H. (1980). Plasmid control of 6-aminohexanoic acid cyclic dimer degradation enzymes of Flavobacterium sp. K172. J. Bacteriol. 143: 328-245.

Negoro, S. (2002). Biodegradation of nylon and other synthetic polymamides. Biopolymers. 9:395-415**.**

Okoh, E. B. and Atuanya, E. I. (2014) Impacts of soil composting and poultry manure on biodegradation of polyethylene. International J. Appl. Micro. Biotech. Res. (IJAMBR) 2: 18-29 ISSN 2053-1818.

Prabhat, S., Bhattacharyya, S., Vishal1, V., Kalyan, R. K., Vijai, K., Pandey, K. N. and Singh, M. (2013). Studies on Isolation and Identification of Active Microorganisms during Degradation of Polyethylene / Starch Film. *Int. Res. J. Environment Sci.* 2(9), 83-85.

Premraj, R. and Mukesh, C.D. (2005). Biodegradation of polymer. Indian. J. of Biotech. 4: 186-193.

Sadocco, P., Nocerino, S., Dubini-Paglia, E., Seves, A. and Elegir, G. (1997). Characterization of a poly (3-hydroxybutyrate) depolymerase from Aureobacterium saperdae: Active site and kinetics of hydrolysis studies. Journal of Environmental Polymer Degradation 5: 57-65.

Saminathan, P., Sripriya, A., Nalini, K., Sivakumar, T. and Thangapandian, V. (2014). Biodegradation of Plastics by Pseudomonas putida isolated from

Garden Soil Samples. J. of Advanced Botany and Zoology, V1I3. DOI: 10.15297/JABZ.V1I3.06.

Shah, A. A., Hasan, F., Hameed, A., Akhter, J. I. (2009). Isolation of *Fusarium* sp. AF4 from sewage sludge, with the ability to adhere the surface of polyethylene. *Afr. J. Microbiol. Res*. 3: 658-663.

Sharma, A. and Sharma, A. (2004). Degradation assessment of low-density polyethylene (LDP) and polyethylene by an indigenous isolate of *Pseudomonas stutzeri*. Journal of scientific and industrial research. 63: 293-296.

Swift, G. (1997). *Non-medical biodegradable polymers: environmentally degradable polymers*. Handbook of biodegradable polymers. Hardwood Acedemic, Amsterdam. 473-511.

Usha, R. Sangeetha, T. and Palaniswamy, M. (2011). Screening of Polyethylene degrading Microorganisms from Garbage Soil. *Libyan Agric. Res. Cen. J. Intl.* 2 (4): 200-204.

Yoon, M. G., Jeon, H. J. and Kim, M. N. (2012). Biodegradation of Polyethylene by a Soil Bacterium and AlkB Cloned Recombinant Cell. J Bioremed Biodegrad. 3(4): 1-8. doi:10.4172/2155-6199.1000145.