Review Article

Biodegradation of Low Density Polythene Materials Using Microbial Consortium – An Overview

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ABSTRACT

Low density polyethylene play a vital role in our day to day life, simultaneously it produces drastic environmental problems. They are recalcitrant and hence inert to degradation. Some microorganisms have the ability to degrade the Low Density Polythene (LDP) materials and some plastics in natural environment. The aim of this review is to summarize and to highlight the recent studies on low density polyethylene degradation and enrichment methods for effective degradation.

Keywords: LDP, Microorganism, SEM, FTIR, Enriched medium.

INTRODUCTION

Polythene is defined as the polymers which on heating become mobile and can be cast into moulds. They are non metallic compounds and the materials that are made from them can be pushed into any desired shape and sizes. They are classified into different categories such as LDPE (Low Density Polyethylene), LLDPE (Linear Low Density Polyethylene), HDPE (High Density Polyethylene). Among these LDPE play a vital role and it is a thermoplastic made from the monomer ethylene. It was the first grade of polyethylene, produced in 1933 by Imperial Chemical Industries (ICI) using a high pressure process via free radical polymerization.

During the past 3 decades, polythene materials have been increasingly used in food clothing, shelter, transportation, construction, medicals, and recreation industries. Polythene are advantageous as they are strong, light weighted and durable (Kathiresan, 2003). Polyethylene remains in the environment for a long period of time as it lacks functional groups required for the microbial degradation. Thus, polyethylene based plastic materials are accumulating in the environment at an alarmingly high rate of approximately 25 million tons per year (Soni *et al*., 2009; Zahra *et al*., 2010; Prosun Tribedi and Alok Sil, 2012) Due to the short time on earth, nature has not been able to design new enzymatic structures that can attack these synthetic polymers. This has brought concern about how to degrade them. There are some mechanisms like photo degradation, thermal degradation, environment erosion and biodegradation. Biodegradation is a process in which organic substances are degraded by living organisms (Aracil Loredo *et al*., 2011 and Shah *et al*., 2008). These compounds may be degraded by some physico-chemical and biological reaction. Diverse metabolic capabilities of microorganism have been exploited by men in diverse ways in the biodegradation of waste materials (Meerina Paul Das and Santosh Kumar, 2013; Okpokwasili 2005).

There are enormous data on the development of biodegradable polythene as well as degradation of existing polythene by microorganisms since they are capable of depredating most of the organic and inorganic materials, including lignin, starch, cellulose and hemicelluloses. Researchers are looking a new dimension on microbial degradation of polythene and polymer waste material (Sharma *et al*., 2013).

Table 1: List of microorganisms reported for degradation of different types of plastic and polyethylene materials

Synthetic plastics	Microorganisms	References
Polyethylene	*Brevibacillus borstelensis* *Rhadococcus rubber* *Pencillium simplicissimum YK* *Comamonas acidovorans TB – 35* *Curvularia senegalensis* *Fusarium solani* *Aureobasidium pullulans* *Cladosporium* sp. *Pseudomonas chlororaphis*	Hadad *et al*., (2005) Sivan *et al*., (2006); Gilan *et al.,*(2004) Yamada Onodera *et al*., (2001) Akutsu *et al*., (1998) Howard , (2002) Zheng *et al*., (2005)
Polyvinyl chloride	*Pseudomonas putida AJ* *Ochrobacterium TD* *Pseudomonas fluorescens B-22* *Aspergillus niger* *Van Tieghem F-1119*	Anthony *et al*., (2004) Mogil Nitskii *et al*., (1987)
Plasticized polyvinyl chloride	*Aureobasidium pullulans*	Webb *et al*., (2000)
BTA -copolyester	*Thermomonspora fusca*	Kleeberg *et al*., (1998)
Natural plastics		
Poly (3- hydroxybutyrate-co-3-merccaptopropionate)	*Schlegella thermodepolymerans*	Elbanna *et al*., (2004)
Poly (3-hydroxybutyrate)	*Pseudomonas lemoignei*	Jendrossek *et al*., (1995)
Poly(3-hydroxybutyrate-co-3-mercaptoproponate)	*Pseudomonas indica K2*	Elbanna *et al*., (2004)
Poly(3-hydroxybutyrate-co-3-hydroxypropionate)	*Ralstonia pikettii T1*	Mabrouk and Sabry, (2001)
	Acidovorax sp. *TP4*	Wang *et al*., (2002)
Poly (3-hydroxybutyrate)	*Alcaligenes faecalis*	Kasuya *et al*.., (1999)
Poly(3-hydroxy butyrate)	*Schlegelella thermodepolymerans*	Romen *et al*., (2004)
Poly (3-hydroxybutyrate - co-3-Hydroxyvalerate)	*Clostridium botulinum* *Clostridium acetobutylium*	Abou-Zeid *et al*., (2001)
Polylactic acid	*Bacillus Brevis* *Rhizopus delemer* *Fusarium* spp.	Tomita *et al*., (1996) Fukuzaki *et al*., (1989) Torres *et al*., (1996)
Polymer blends		
Starch/polyethylene	*Aspergillus niger* *Pencillium funiculosm* *Phanerochaete chrysosporium*	Lee *et al*., (1991)
Starch/ polyester	*Streptomyces* *Phanerochaete chyrososporium*	
Low density polyethylene		
LDP	*Aspergillus niger* *Aspergillus japonicas* *A.Terreus* *Bacillus amyloliquefaciens* *Staphylococcus* *A.fumigatus* *Pencillium* spp.	Webb *et al*., (2000) Kleeberg *et al*., (1998) Raaman *et al*., (2012) Merina Paul Das and Santosh Kumar (2015) Vatseldutt and Anbuselvi (2014) Vishnu sigh *et al*., (2002)

The present review article focuses on degradation of low density polyethylene by using microorganisms and enriched its activity by suitable media with reference to its surface changes through SEM and FTIR studies.

MODE OF DEGRADATION

Polymeric materials are potential source of carbon and energy for heterotrophic microorganisms including bacteria and fungi in several ways. The action of microorganisms on polymer is influenced by two different processes:

1. Direct action: The deterioration of polythene which serve as a nutritive substance for the growth of microorganisms and
2. Indirect action: The influence of metabolic products of the microorganisms regarding discoloration or further deterioration (Krishna Mohan and Tanu Srivastava, 2010)

Microorganisms secrete variety of enzymes into the soil, which begin the breakdown of the polymer. Two types of enzymes are involved in the process, namely intracellular and extracellular de polymerases. Exo-enzymes from the microorganism's first breakdown the complex polymer giving short chains or monomers that are small enough to permeate through the cells to be utilized as carbon and energy sources this process is known as de-polymerization. When the end product is carbon dioxide, water or methane, then the process is known as mineralization (Uttiya Dey *et al.*, 2012).

The environment includes the biological agents such as bacteria, fungi and their enzymes responsible for the deterioration of polymeric substances. They consume a substance as a food source so that its original form disappears.

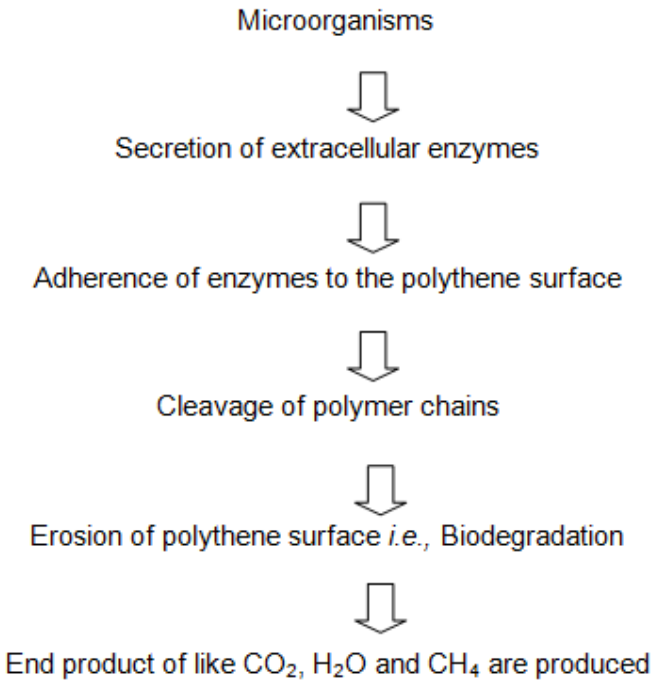

Fig. 1: Mechanism of enzymatic biodegradation of Polythene (Shah and Fariha., 2008)

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ISOLATION OF LDPE 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 by pour plate method was adopted using the starch casein agar for actinomycetes, nutrient agar for bacteria and potato 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 then preserved in slant at 4°C.

PURE CULTURE METHOD

In pure culture method, specific bacteria and fungi can be applied for degradation of polymers. In laboratory conditions, isolated microorganisms strain has been allowed for

sufficient growth in different media. In pure culture method, pre-weighed disinfected films are aseptically added to sterilized culture medium and films in culture medium are incubated with shaking for 24 h before inoculation to ensure asepsis. Culture medium is inoculated with spore from a specific microorganism and is incubated with shaking 125 rpm for 4 weeks at optimal growth temperature for the selected microorganisms. Four replicates are prepared for each different pretreated film. The sample is weighed after washing with 70% ethanol and drying at 45ºC until equilibrates. Each of the different films is then compared with corresponding .uncultured material. The presence of microbes can be confirmed by using SEM (Singh and Sharma, 2008)

SOIL BURIAL METHOD

To ensure the biodegradation of the polymer in natural environment condition polymer strips were buried in the soil up to a depth of one meter. Strips were removed from the soil at regular intervals and thoroughly rinsed with distilled water to get clear and dried. They were allowed to equilibrate to ambient temperature and humidity for at least 24 h before measurement (Goheen and wool, 2003; Thakore, *et al*., 2001 and Vigneswari *et al*., 2009).

STANDARD TESTING METHOD

CO_2 EVOLUTION TEST

The self- modified simple apparatus was designed which consists of control and test vessels and sterile air was supplied to the system for aeration. Here, the polymer incubated with microbes served as the test and polymer without microbes served as control. After incubation, both the metabolic and atmospheric CO_2 from the test vessel and atmospheric CO_2 from the control vessel were trapped and assessed using “Strum test” (Strum 1973) for each isolate (Merina Paul Das and Santosh Kumar, 2015)

STRUM TEST

The pieces of polymer were added to culture bottles containing mineral salt medium (MSM) without any carbon sources. Spore suspension was used as inoculums in test and control bottles. Sterilized air was supplied to keep conditions aerobic and reaction bottles were stirred continuously by placing them on magnetic stirrer. After 30 days gravimetric analysis of CO_2 production was done by trapping the gas in adsorption bottle containing KOH. The precipitates formed after titration with barium chloride solution of test and control were filtered, weighed and calculated for CO_2 produced per liter.

In another study by Emmanuel *et al.,* (1993) the ability of a complex enzyme to degrade polythene such as Sesbania gum and Guar gum was investigated. The study was based on an assumption that the polymer compounds dissolved in water world increase viscosity and be able to plug an artificial rock samples placed in pressured chamber. The study was also based on an assumption that complex would slowly degrade the polymer compounds thereby reducing their viscosity and hence forth unplugs the artificial rock. The polymer compounds were not auto degradable at room temperature though showed a decreasing viscosity after one hour

UV light treatment was done by Kenneth *et al*, (1993) to evaluate the photosensitivity of each film of a plastic bag. A UV lamp with long wave was used. Plastic film were cut in machine direction and placed into UV box at a distance of 17.78 cm from the lamp for 8 weeks. The plastic film was turned twice a week to ensure even exposure to the light. Samples were removed after 1,2,3,4, and 8 weeks. Film mechanical properties and polyethylene molecular weight distribution were determined (Gnanavel *et al*., 2012).

CLEAR- ZONE FORMATION

A very simple semi-quantitative method is clear-zone test. This is an agar plate test in which the polymer is dispersed as very fine particles within the synthetic medium agar, this result in the agar having opaque appearance. After inoculation with microorganisms, the formation of a clear halo around the colony indicates that the organisms are at least able to de polymerize the polymer, which is the first step of biodegradation. This method is usually applied to screen organisms that can degrade a certain polymer (Nishida and Tokiwa, 1993; Abou-Zeid, 2001), but it can also used to obtain semi- quantitative results by analyzing the growth of clear zones (Augusta *et al*., 1993).

16S r RNA GENE SEQUENCING

Genomic DNA was extracted by boiling–lysis method and stored at -80^{0}C until PCR assay. Amplification of 16S rRNA was carried out using previous described primers (Weisburg *et al*., 1991).

Forward - CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG,

Reverse - CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC

PCR was carried out in a 50µl reaction volume which contain 5µl of the template, 10pM of each primer (Sigma Aldrich), 0.25mM of each dNTP (Bangalore Genei, India), 2U of *Taq* polymerase (Bangalore Genei, India). The thermo-cyclic condition included, an initial denaturation step at 94^0C, 30 s, annealing at 65^0C, 30 s and extension at 72^0C, 7 min.

The amplified PCR product was resolved in 1% Agarose gel in TBE. The amplicons was carried out (BioRad, Hercules, CA). DNA sequencing was performed to identify the amplified PCR products using applied bio system (ABI) 3130 Genetic Analyzer with ABI PRISM Big Dye Terminators V 3.1. The gene sequences were compared with sequences in the Gen Bank database by using BLAST program. The sequences were deposited in the Gen Bank database (Pramila *et al*., 2012).

ENRICHMENT MEDIUM

The enrichment procedure was performed to isolate microorganisms that utilize LDP as the sole source of carbon. After the enriched with suitable medium, the microorganism grow well and degrade the polythene very effectively with in short time incubation. They are many techniques used for the enrichment of microorganisms.

ENRICHMENT TECHNIQUES

Nutrient and Potato Dextrose Broth:

It especially enriches the growth of bacteria and fungi very effectively; they utilize the carbon source and grow well for effective degradation of LDPE.

Sugar cane molasses:

Instead of Nutrient and Potato dextrose broth, sugar cane molasses also can be used as carbon sources, however it is cheap when compare with potato dextrose broth and nutrient broth. The microorganisms which grow well in this media and have the ability to degrade polythene effectively.

MICROBIAL DEGRADATION OF LDPE UNDER LABORATORY CONDITIONS

LDP film of 2cm diameter prepared from polythene bags were aseptically transferred into the conical flask containing 100ml of mineral salt medium and then inoculated with identified LDP degrading microorganisms. Control was maintained with polythene and strips in the microbe free medium and left in a shaker at 30^0C, 150 rpm for 2,4 and 6 month period. After the period of shaking 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.

VISUAL OBSERVATION

The evaluation of visible changes in polythene can be performed in almost all tests. Effect used to describe degradation includes roughening of the surface, formation of holes or cracks, defragmentation, changes in color, or formation of bio film on the surface. These changes do not prove the presence of a biodegradation process in terms of metabolism, but the parameter of visual changes can be used as a first indication of any microbial attack. To obtain information about the degradation mechanisms, more sophisticated observation can be made using either scanning electron microscope (SEM) or atomic force microscope (AFM) (Ikada, 1999). After an initial degradation, crystalline spherolites appear on the surface; that can be explained by a preferential degradation of the amorphous polymer fraction, etching the slower degrading crystalline parts out of the material. In another investigation (Kikkawa *et al*., 2002) used AFM micrographs of enzymatically degraded Polymer film to investigate the mechanism of surface erosion. A number of other techniques can also be used to assess the biodegradability of polymeric material. These include; Fourier transform infrared spectroscopy (FTIR), differential scanning colorimeter (DSC), nuclear maganetic resonance spectroscopy (NMR), x-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD) (Shah *et al*., 2008). This techniques is generally analyzed for visualizing the surface changes in LDP.

CONCLUSION

Polythene is one of the major threats to the environment. Research has been initiated to find out the solution for effective degradation LDPE and some plastics. It is now really a mess for mankind.

The microorganisms associated with the LDP were identified through clear zone test which revealed the presence of both bacteria and fungi in large number. These microorganisms were used for future degradation process.

The results are sensitive and also affect various factors, particularly the consortia of microorganisms used. After the inoculation of LDP in microbial consortium the film has turned from smooth to rough with cracking. This may be due to the compounds secreted extracellular by the microbes that may break the complex molecular structure of LDP.

These degradations can further be enhanced by the addition of enrichment medium for effective degradation. It is also concluded from this discussion that LDP degradation could be enhanced by its enrichment techniques and the results can be predicted through FTIR and SEM analysis.

This review thus stresses for the need of microbial consortium and its enrichment for effective degradation of low density polyethylene, inside the laboratory (under controlled condition) and outside the laboratory (under natural condition) by the help of microbial tools.

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