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Biodegrdation of Polyethylene : A Review

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Abstract- **Ever increasing population, urbanization and modernization posing problem of plastic waste disposal and contamination of land as well surface and subsurface water. Plastic is disposed of ultimately into water bodies and get clogged due to which aquatic habitats get hampered and animals die by consuming plastic when disposed of in open garbage and remain uncollected. It has been observed that in many cities solid waste is not collected on regular intervals and remain littered. Hence treating it effectively so as to minimize pollution becomes the best solution. Polymers are synthetic substances produced by chemical reaction. Plastics are basically polymers that consist of monomers linked together by chemical bond. In the present review, an attempt has been made to put together all available literature on Biodegradation of Low Density Polyethylene (LDPE) with following objectives:**

1. Brief mechanism of Polyethylene Degradation
2. Sources and effects of polyethylene.
3. Potential of Specific microorganism in degrading Polyethylene.
4. Various techniques for the analysis of degradation.

Keywords— **Biodegradation, Low Density Polyethylene (LDPE), Microorganisms.**

I. INTRODUCTION

High and Low Density polyethylene is used widely because of its recalcitrant nature and effectiveness. Most of the application of high and low density polyethylene are plastic carry bags, wrappers, food packaging materials, plastic bottles, lab equipment's, pipes etc. Most of these materials after its use, either get piled up into landfill, garbage or into water bodies like nallas, rivers and oceans. Its disposal causes tremendous pollution in environment. According to the new study, around eight million metric tons of plastic ends up in our oceans every year. In India, 50-60 million tonnes of municipal solid waste is generated annually in urban areas. Estimated generation of Plastic waste is around 15,342 tonnes/day out of which 6000 tonnes remain uncollected and littered as per Central Pollution Control Board.

Plastic pollution in most of the countries are caused because of poor and improper recycling and waste management systems (Jayasiri et al, 2013) [3]. However, if prompt action is not taken, this figure will increase by ten times during the next ten years. LDPE wastes can easily be transported to long distances because of their low density. There are various technologies developed in recycling of Low density polyethylene but those which are disposed of in landfills, garbage and water bodies, remain there for many years damaging the ecosystem, environment and human health. Hence the biodegradation of low density polyethylene becomes simple, ecofriendly and viable treatment option to reduce pollution.

A. Sources and effects of plastic waste :

Plastic even restricts the flow of water resulting into ecological imbalance. Most of the aquatic life consume this low density polyethylene which is further carried away with the help of food chain by bioaccumulation and bio-magnification into human beings as well as other mammals. LDPE emits greenhouse gases causing potential source of global warming. Tourism activities are also affected by pollution.

Organisms can take up the plastic as a food source plastic waste and they can be transported with the help of food chain and is therefore hazardous affecting the complete ecosystem and environment (Sutherland et. al., 2010) [1] The plastic wastes, thrown in environment cause animal death, clogging of drainage system cause restriction of sewage flow, deterioration of beauty of environment and human health problems of cattle.

Besides, the intake of plastic items cause consequence such as reduction of yield of milk (Ramasamy and Sharma, 2011). [2]

International Coastal Clean-up says that the plastic wastes have become a serious threat to the aquatic life. The plastic bag waste holds second and third positions in India and South Africa respectively as one of the items in the marine debris (ICC, 2003) [4]. 75% of plastic are found in sediments of beaches with size of

1-20 mm which is affecting marine ecosystem in Mumbai, India. (Jayasiri, 2013) [3].

B. OBJECTIVES

The overall objective of this review is to find potential of specific microorganism in degrading Low Density Polyethylene. The specific objective include:

1. To brief about mechanism of Biodegradation of Polymer.
2. To find the analytical techniques involved in checking biodegradation of polyethylene.

II. MECHANISM OF BIODEGRDATION

Biodegradation of polymer consist of following steps:

1. Attachment of microbes to the surface of the polymer
2. Growth of microorganisms utilizing the polymer as a carbon source
3. Primary degradation of polymer
4. Ultimate degradation

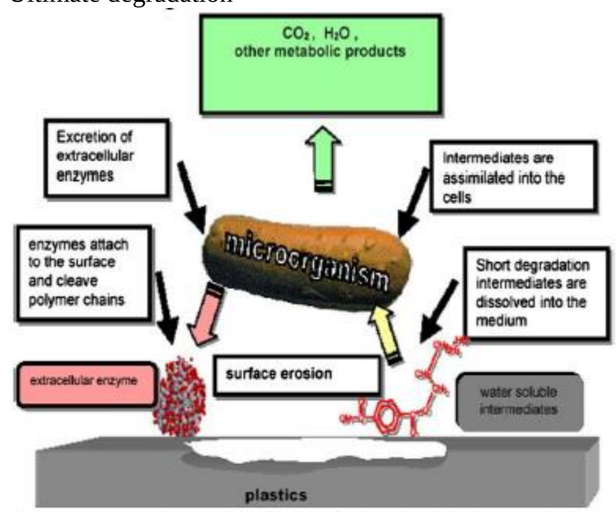

Fig 1. General mechanism of plastic biodegradation under aerobic conditions (Mueller, 2003).

Microbes can attach to the surface, if the polymer surface is hydrophilic. Since polyethylene have only CH2 groups, the surfaces are hydrophobic. Initial physical or chemical degradation leads to insertion of hydrophilic groups on polymer surface making it more hydrophilic. Once the organisms get attach to the surface, it start growing by using polymer as a carbon source. Physical forces, such as heating/cooling, freezing/thawing, or wetting/drying, can cause mechanical damage such as the cracking of polymeric materials (Kamal and Huang, 1992, Woodyard, 2003). In primary degradation main chain cleaves, leading to the formation of low molecular weight fragments (oligomers), dimers or monomers. The degradation is due to extracellular enzymes secreted by the organism. These low molecular weight compounds are further utilized by the microbes as carbon and energy sources. During degradation process when polymer gets converted into monomer mineralization take place. Small oligomers may also diffuse into organisms and get assimilated. The ultimate products of degradation are CO2, H2O and biomass under aerobic condition. The environmental conditions decide the group of microorganisms and the degradative pathway involved (Mueller, 2003) [5].

III. TECHNIQUES USED TO DETERMINE BIODEGRADATION OF POLYETHYLENE

1. Weight Loss Measurement:

Mass of the polymer material gets reduced as microorganisms take carbon from LDPE. Weight loss measurement can be determined by formula:

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

(Gauri singh, et al. 2016

The weight loss measurement is used often for the estimation of biodegradation of polymer or plastic. This method is standardised for in situ biodegradation tests (NF EN ISO 13432, ISO 14852; Krzan et al., 2006, ISO 14855 Krzan et al., 2006) [8]

2. Scanning electron microscope:

At topographical level, the Scanning Electron Microscope (SEM) are being used to see the level of scission and attachment of microbes on the surface of polythene before and after microbial attack (Manisha k Sangale, et al. 2012) [9]. The structural changes in the form of pits and erosion observed through scanning electron microscopy indicated surface damage of PE incubated with Fusarium sp.AF4 were able to adhere to surface of LDPE and can cause surface damage (Sudip Kumar Sen, et al. 2015). In the study by (Jose Maria Rodrigues da Luz, et al. 2015) [10], Physical and Chemical alterations of Green polyethylene Plastic bags were observed with incubated for 30 days with exposure to Pleurotus Ostreatus. Before 30days of incubation, Green polyethylene was exposed to sunlight for 120 days but after this period, no pits, cracks or new functional groups in structure were observed.

In SEM study, control film was appeared with smooth surface without any pits, cracks or any particles attached on its surface while in treated, the attachment of fungi, Aspergillus Spp. and Fusarium Spp. on LDPE surface and formation of various holes and irregularities were observed.

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Soil sample were collected from Municipal solid Waste (Das and Kumar 2014) [11].

3. Fourier Transform Intra-Red (Ftir) Analysis:

Chemical changes in polymeric chains can be observed by this method. In study of (Jose Maria Rodrigues da Luz, et al. 2015) [10], chemical alteration shows evidence of oxidation of Green polyethylene by P. Ostreatus. The micro-destruction of the small samples is widely analyzed by an important tool such as Fourier Transform Infrared spectroscopy (FT-IR), and due to the recent up-gradation of this instrument the map of the identified compounds on the surface of the sample can be documented via collection of large number of FT-IR spectra (Manisha k Sangale, et al.2012) [9]. Structural changes induced by degradation were analysed by FI-IR analysis using characteristic bond ranging from 1700 to 1760 cm-1. Changes in C=O bond were magnified (Sahebnazar Zahra, et al, 2009) [12]. LDPE devoid of fungi compared to fungal treated irradiated film revealed a number of peak shifts that indicate the changes in the macromolecular segments of the LDPE and induced bioactive hydrolysis in polymer film (Sana Sheik, et al. 2015)[13].

4. Gravimetric analysis:

Test and control bottles containing SM supplemented with LDPE powder will be prepared. Fungal strain will be added in the test bottle. Then sterile air will be allowed to flow through 1M KOH solution containing bottles (air pre-treatment bottles). The CO2 free air will be passed to the test bottles or conical flask can be taken instead. The CO2 free air will be utilized by the inoculum for releasing of CO2 (after metabolizing LDPE) in the absorption bottle. The test will be performed at room temperature for 1week. After 1week the amount of CO2 produced in absorption bottle will be calculated by adding 0.1 M Bacl2 which forms a precipitate of barium carbonate. CO2 released will be gravimetrically calculated by measuring the weight of the precipitate formed (Barium carbonate). Difference in the values obtained in the control and test will be noted. The dissolved carbon dioxide present in the medium was also measured volumetrically using titration method. Briefly, sample (medium- 25ml) was taken in a conical flask and 0.05ml of 0.1N Thiosulphate solution was added. After the addition of 2 drops of methyl orange indicator, this solution was titrated against 0.02M Sodium Hydroxide solution. End point was the change in colour from orange red to yellow. Following this, two drops of phenolphthalein indicator was added and titration continued till a pink color developed. Method of (R. Pramila and k. Vijaya, 2011) [6]. Volumes of the titrant used were noted and the amount of CO2 evolved was calculated using the formula:

$$\frac{A \times B \times 50 \times 1000}{V}$$

Where,

A= mL of NaOH titrant
B= normality of NaOH
V= mL of the sample

5. Tensile Strength Measurement:

Tensile strength measurement is done with tensile tester. It give mechanical properties. Tensile strength was calculated as:

$$\text{Tensile strength N/cm}^2 = \frac{\text{Breaking load in N}}{\text{Cross sectional area in cm2.}}$$

6. The elongation at break by a mechanical tester, elongation percentage and elasticity by dynamic mechanical thermal analysis can be carried out to find degradation of plastic or polymer.

7. The Polymer biodegradation describe the thermal evolution by using the differential scanning calorimeter that gives the glass transition temperature (Tg), cold crystallisation temperature (Tcc) and/or melting temperature (Tm), (Weiland et al., 1995; Ratto et al., 1999; Hakkarainen et al., 2000; Ki and Park, 2001; Abd El-Rehim et al., 2004; Rizzarelli et al., 2004; Marten et al., 2005; Zhao et al., 2005; Bikiaris et al., 2006; Kim et al., 2006b; Morancho et al., 2006; Tserki et al., 2006; Tsuji et al., 2006) [14].

8. The estimation of hydrolysis is determined by "CLEAR ZONE TECHNIQUE". It is used to screen the microbial ability to hydrolyse a specific polymer. Firstly appropriate agar medium is prepared by adding selective media to it for growth of microorganisms. Agar medium is used for solidification. Then flask is allowed to autoclave at 121oC for 15min. After cooling it is poured on to in incubator at room temperature for 3-4 days in order to see the growth of microorganisms. Once the growth is observed isolate the specific microbe and add LDPE film to it and keep it for degradation. Continuous monitoring and measurement has to be done during this period. The formation of a clear halo around the microbial colony indicates the biosynthesis and the excretion of depolymerisation.

IV. MICROBES INVOLVED IN BIODEGRADATION OF POLYETHYLENE

Most of the microorganisms from the research include isolation from agriculture soil, compost soil, landfill soil, from sea water, garbage were plastic was degraded, oil contaminated soil etc. Bacteria and fungi played pivotal role in degradation of Low density Polyethylene. Most of the Microbes has shown good degradability results with 120 days of exposure.

LDPE strips inoculated with Aspergillus sp., Paecilomyces lilacinus from H. brunonis and Lasiodiplodia theobromae from Psychotria flavida indicate fungal efficiency in plastic degradation. (Sana Sheik, et al. 2015) [13]. The degradation with 150 days of exposure with microbes has shown good results as 1.53 %, 1.67 %, 1.50 %, 5.06 %, 40.65 % and 54.33 % for pure LDPE, 10 %, 20 % 30 %, 40 % and 50 % starch blended plastics was found by inoculums of bacterial consortium (K.S. Veethahavya, et al. 2015) [18]. As per the research the positive isolates were confirmed as Kocuria palustris M16, Bacillus pumilus M27 and Bacillus subtilis H1584 based on the 16S rRNA gene sequence homology. The weight loss of polyethylene was 1%, 1.5% and 1.75% after 30 days of incubation with the M16, M27 and H1584 isolates, respectively. The 32% of cell surface hydrophobicity was observed in M16, followed by the H1584 and M27 isolates (Kumar Harshavardhan, et al. 2013) [15]. The fungal sps. were identified by plating and staining techniques. Aspergillus sps. Fusarium sp, Mucor sp. and Penicillium sp. Were able to degrade polymer (Jyoti singh and K.C.Gupta, 2014) [16]. Fungi identified as Mucor circinilloides and Aspergillus flavus for biodegradation of LDPE shown cracks, pits formation, sporangia and spores on LDPE by Scanning Electron Microscope Analysis. Results of CO_2 evolution were as assessed both gravimetrically and volumetrically for the isolate of Mucor circinelloides and Aspergillus Flavus. . CO_2 evolved was 5.9g/L for Mucor circinelloides and Aspergillus Flavus evolved around 4.4g/L (R. Pramila and Vijaya Ramesh, 2011) [6]. Isolated fungal strains were identified as Aspergillus Niger, A. japonicus, A. terreus A. flavus and Mucor sp. Predominant fungal strains Aspergillus Niger and A. japonicus were selected for polythene degradation under laboratory conditions. Their effectiveness on the degradation of commercial polythene carry bags of low density polyethylene (LDPE) was studied over a period of 4 weeks in shaker culture under laboratory conditions. Biodegradation was measured in terms of mean weight loss, which was nearly 8 to 12% after a period of 4 weeks. Further SEM (Scanning electron microscopy) analysis confirmed the degradation by revealing the presence of porosity and fragility of the fungal degraded polythene surface. Aspergillus japonicus showed 12% degradation potential when compared to A. Niger of 8% degradation in one month period (N. Raaman, et al. 2012)[17]. Penicillium oxalicum NS4 and Penicillium chrysogenum NS10 were able to degrade high and low density polyethene for the exposure of 90 days. (Nupur Ojha, 2016) [19].

V. CONCULSION

It is true that plastic has now-a-days become our need for day to day life, but after its use, effects of plastic are not addressed properly and most of the ecological imbalance is increasing. Plastic accumulation, particularly in the world's oceans, lakes, landfill, in the area of city garbage dumps is of increasing environmental concern. Polyethylene become the major source of pollution especially low density polyethylene bags and micro plastics have become major threat to aquatic life and also entirely on environment. Bioremediation and biodegradation of polyethylene is an attractive for environmentally friendly and efficient disposal of plastic waste but still potential of screening specific biodegradable microbes is yet a challenge. Laboratory scale research has been done till now but still it lacks in practical application polyethylene degradation. Study reveals that fungi as well as bacteria have good potential to degrade polyethylene. More techniques could be developed for practical application in order to solve the problem of plastic pollution in environment.

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