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Role of Microorganisms Like Bacteria and Fungi in Polythene Degradation

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Abstract: *Polythene degradation is a challenge all over the world. It is a serious problem throughout the globe to reduce polythene garbage. Every year 500 billion to 1 trillion polythene carry bags are being consumed around the globe. It is a huge amount to be used across the globe. Its degradation process is very slow as compare to other wastes and takes many years to decompose. It also have adverse effect on the environment and living organisms. In this review paper we are trying to attract attention on polythene pollution, it's possible methods to decrease the polythene level, its degradation process and future needs on polythene degradation.*

Keywords: *Polythene, Degradation, Pollution, Waste.*

I. INTRODUCTION

Polythene is one of the widely used products in today's world. It is widely used in packaging of food, gift, vegetables, snacks, and in different type of material packaging. India is highly populated country. In India polythene is used at large scale by different industries. The use of polythene is increased with the increase in the life style and modernization of the countries. In developing countries its safe disposal is the biggest problem. Now a day's large amount of garbage due to the polythene is accumulated in these countries. Scientist's works on it for its safe disposal. Polythene and plastics takes millions of years to decompose completely. Its excess use causes the environmental pollution. So, we should need to take serious steps for stopping the use of polythene and plastics. We should use other alternate sources in place of polythene and plastics. Polythene finds a wide range of applications in human's daily use because of its easy processing for various products used for carrying food articles, for packaging textiles, for manufacturing laboratory instruments and automotive components (Arutchelvi et al., 2008) [2]. Polythene constitutes 64% of the total synthetic plastic as it is being used in huge quantity for the manufacture of bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes (Lee et al., 1991). Polyethene bags are made of polyethylene. The synthetic polymers are high hydrophobic level and high molecular weight. Annually 500 billion to 1 trillion polythene bags are being used routinely all over the world. Bacteria and fungi have been implicated in this process albeit slow rates. According to Bhatia M, et al (2014) [3], Pseudomonas species are most highly implicated in the biodegradation of LDPEs. They isolated Pseudomonas citronellolis EMBS027 which had 17.8% weight reduction on polyethylene sheets. Hadad D et al (2005) [4], isolated Brevibaccillus borstelensis strain 707 which upon 30 days incubation at 50°C reduced the gravimetric and molecular weights of polyethylene sheets by 11 and 30% respectively. Fungal isolates: Fusarium sp. AF4, Aspergillus terreus AF5 and Penicillium sp. AF6 were found attached to Polyethylene sheets mixed with sewage sludge for ten months Shah AA et al (2008)[1]. Ability of Bacillus subtilis to degrade polyethylene was also demonstrated by Vimala PP, et al (2016) [5] in the presence and absence of bio-surfactants. Vinay BR et al (2016) [6] isolated several fungal genera that were able to degrade polyethylene sheets with Aspergillus niger showing the highest weight reduction of 4.32%. Degradation of polythene is a great challenge as the materials are increasingly used. An estimated one million birds and ten thousand marine animals die each year as a result of ingestion of or trapping by plastics in the oceans. Recently, the biodegradation of plastic waste and the use of microorganisms to degrade the polymers have gained notable importance because of the inefficiency of the chemical and physical disposal methods used for these pollutants, and the environmental problems they cause (Kawai, 1995) [7]. The present study focused to check the capability of fungi and bacteria in plastic biodegradation from plastic contaminated soil.

II. MATERIALS AND METHODS

A. Rich Source Of Polythene Degrading Microbes

The rich sources of polythene degrading microbes have been found in nature are dumping site of garbage, polythene buried in soil, rhizosphere soil of mangroves, marine water etc.

B. Isolation of Soil Bacteria

The soil bacteria were isolated by spread plate technique (Kathiresan, 2003). 1g of plastic contaminated soil sample was taken and mixed in 100 ml of distilled water in a conical flask and serially diluted. 0.1 ml aliquot of various dilutions (10⁻³ to 10⁻¹⁰) was spread on nutrient agar medium (Himedia, Mumbai) by using L-rod and incubated at 37°C for 24 hrs (M. Ariba Begum et al. 2015) [10].

C. Identification of Soil Bacteria

The selected bacterial isolates were identified by morphological and biochemical characterization. In morphological characterization, macroscopic characteristics like shape, size, structure, texture, appearance, elevation and colors were studied.

Phenotypic characteristics such as microscopic characterization of gram reaction, motility and biochemical test including catalase, oxidase, indole, methyl red, Voges-Proskauer, triple sugar iron, citrate utilization, urease, nitrate reduction and carbohydrate fermentation test were performed.

The isolates were characterized by various morphological and biochemical test, according to Bergey's manual of determinative bacteriology (Holt et al., 1994).

D. Mechanism of Polythene Biodegradation

The degradation of polythene begins with the attachment of microbes to its surface. Various bacteria like *Streptomyces viridosporus* T7A, *Streptomyces badius* 252, and *Streptomyces setonii* 75Vi2 (Pometto 3rd AL et al. 1992) [25] [26] and wood degrading fungi produced some extracellular enzymes which leads to degradation of polythene (Iiyoshi Y et al. 1998) [27]. The degradation of polythene by bacteria was studied by following the method of Kathiresan (2003) [13].

E. Surface Sterilization of Polythene Bag

The collected plastic bags were cut into small pieces and cleaned with tap water and surface sterilized with ethanol. Then washed with distilled water, 0.1% mercuric chloride and again washed with distilled water.

F. Degradation of Polythene Bag

Nutrient broth was prepared and autoclaved at 121°C for 15 minutes. 200 ml of cooled, nutrient broth was poured into eight 250 ml sterile conical flasks.

The sterile pre weighed polythene bag pieces were aseptically transferred into nutrient broth. A loopful of bacterial cultures such as *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* was inoculated into nutrient broth. One 250 ml of flask containing the polythene bag pieces without bacterial cultures was maintained as control.

These flasks were incubated at 37°C for 10, 20 and 30 days. The polythene bag pieces were carefully removed from the culture (by using forceps) after different days of incubation. The collected pieces were washed thoroughly with tap water, ethanol and then distilled water.

The pieces were shade dried and weighed for final weight. The data's were recorded. The same procedure was also repeated for all the treated samples.

G. Determination of Degradation of Polythene Bag

The percentage of degradation of polythene bag pieces by *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* was determined by calculating the percentage of weight loss of plastics. The percentage of weight loss was calculating by the following formula (Usha, et al. 2011).

$$\text{Percentage of weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

III. STUDIES ON POLYTHENE DEGRADATION

Various studies were made on polythene degradation, all these studies shows polythene degradation is very slow and its accumulation in our environment causes pollution of soil, water and air. Polythene degradation become one of the major problem not only in India but also in all over the world. Some of the researches on polythene degradation are shown in Table.1.

| Sr.No. | Title of Paper | Source of Microbes | Major findings | Level of Identification | References |
|--------|--|---|---|--|--|
| 1. | BIODEGRADATION OF POLYTHENES BY BACTERIA ISOLATED FROM SOIL | Soil microbes collected from different location of dehradun | During 10 day's time interval maximum degradation for 10 and 40 micron polythene was shown by PIC (<i>Bacillus</i> sp.) with the degradation rate of 10% and 25% respectively and no degradation was shown by isolates P1A (<i>Staphylococcus</i> sp.), P1B (<i>Pseudomonas</i> sp.) and P1D (Consortium). | Morphological keys and Biochemical tests | Gauri Singh*, Ashok Kumar Singh and Kalpana Bhatt [17] |
| 2. | Potential biodegradation of low density polythene(LDPE) by <i>Acinetobacter baumannii</i> . | A bacterial culture was isolated from a municipal land fill area and identified as <i>Acinetobacter baumannii</i> . | Increase in the carbonyl index (CI) of LDPE treated with <i>A.baumannii</i> after 30 days of incubation indicates the formation of carbonyl group. | Not specified | R.Pramila and K. Vijaya Ramesh [22] |
| 3. | Plastic Degrading ability of <i>Aspergillus oryzae</i> isolated from the garbage dumping sites of Thanjavur, India | The soil samples were collected in sterile zip lock bags from plastic contaminated places in Thanjavur, Tamil Nadu. | The results among all the fungal species that are identified to degrade the plastics <i>A. Oryzae</i> also involves in effective in Exsitu degradation of plastics. | Morphological keys and Biochemical tests | A. Indumathi 1* and T. Gayathri2 [21] |

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|----|---|---|--|--|--|
| 4. | Biodegradation of PEG and Polythene Bag using PGPR Isolated from the Rhizosphere of <i>Celosia cristata</i> L | Soil sample was collected from the root region (rhizosphere) of <i>Celosia cristata</i> L. and put into polythene bags, labeled and stored at 4°C until analysis. | It has been found that the <i>Pseudomonas aeuriginosa</i> ; strain accession number MCC-3198 is effective against the PEG and it is also affective against polythene but at a lesser rate than PEG. | For identification purpose the isolates were sent to MCC for identification 16S rDNA sequencing and general strain deposition. | Sunanda Dutta, Avishek Sarkar, Sikha Dutta* [8] |
| 5. | Biodegradability of polyethylene by bacteria and fungi from Dandora dumpsite Nairobi Kenya | Soil samples were collected randomly from the five selected sampling blocks of the dumpsite. | Fungi of the genus <i>Aspergillus</i> and bacteria of the genus <i>Bacillus</i> had the highest capacity of degradation compared to the other genera in this study. | FTIR spectra | Christabel Ndahebwa Muhonja1*, Huxley Makonde2, Gabriel Magoma1, Mabel Imbuga3 [9] |
| 6. | Biodegradation of Polythene Bag using Bacteria Isolated from Soil | The plastic contaminated soils were collected from plastic contaminated place in Thanjavur and polythene bags were collected from a stationary shop in Thozhkopier square, Thanjavur (Dt.), Tamil Nadu, India | The biodegradation efficacy of two isolates such as <i>Desulfotomaculum nigrificans</i> and <i>Pseudomonas alcaligenes</i> were investigated by using polythene bag | Morphological and biochemical characterization of isolated bacteria | M. Ariba Begum1*, B.Varalakshmi 1 and K.Umamagheswari2 [10] |
| 7. | Biodegradability of polythene and plastic by the help of microorganism: a way for brighter future | Five sources: Medicinal Garden soil, (B) Sewage Water Soil, (C) Energy Park soil, (D) Sludge Area soil, (E) Agricultural Soil | After one month of incubation in both bacterial and fungal isolates the maximum degradation by fungi (<i>Aspergillus niger</i>) and bacteria (<i>Streptococcus lactis</i>) was found as 12.25% and 12.5 % respectively | Morphological keys and biochemical tests | Priyanka N, Archana T (2011) [11] |
| 8. | Biodegradation of photo-degraded mulching films based on polyethylenes and steirates of calcium and iron as pro-oxidant additives | Polythene films were scattered in agricultural vegetable field and after 30 days were used for the isolation of microbes | Polythene films 75-85% (containing Fe stearate) and 31-67% (containing Ca stearate) at 45 C leads to reduction in carbonyl index | Molecular level (16S rRNA gene sequencing) | Abrusci C, Pablos JL, Corrales T, Lopez-Marín J, Marín I, et al. (2011) [12] |

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|-----|---|--|--|--|---|
| 9. | Polythene and plastics-degrading microbes from the mangrove soil | Mangroves rhizosphere soil | 20.54 ± 0.13 (<i>Pseudomonas</i> sp.) 28.80 ± 2.40 (<i>Aspergillus glaucus</i>) percent of weight loss per month in shaker culture | Morphological keys were used | Kathiresan K (2003) [13] |
| 10. | Polythene degradation potential of <i>Aspergillus niger</i> | Polythene dumping site | 25% of weight was observed after 8 months with regular shaking | Morphological keys | Aswale P, Ade A (2011) [14] |
| 11. | Synergistic effect of chemical and photo treatment on the rate of biodegradation of high density polyethylene by indigenous fungal isolates | High density polyethylene (HDPE) film buried in soil 3 months and then used as a sources of microbes | <i>Aspergillus oryzae</i> leads 72% reduction in percentage of elongation and abiotically treated HDPE film clearly showed generation of carbonyl peak at 1718.32 cm as compare to control | Molecular level (16S rDNA sequencing) | . Konduri MKR, Anupam KS, Vivek JS, Kumar RDB, Narasu ML (2010) [15] |
| 12. | Plastic degradation by thermophilic <i>Bacillus</i> sp. BCBT21 isolated from composting agricultural residual in Vietnam | The bacteria strain BCBT21 was isolated from the thermophilic phase of agricultural waste composting in Vietnam. | A thermophilic bacteria <i>Bacillus</i> sp. BCBT21 isolated from composting agricultural residual was capable of degrading biodegradable and oxo-biodegradable plastics from various resources. | Morphological identification and FTIR spectrum | Thi Cam Ha Dang et al 2018 Adv. Nat. Sci: Nanosci. Nanotechnol. 9 015014 [16] |
| 13. | Diversity of cellulolytic microbes and the biodegradation of municipal solid waste by a potential strain | Municipal solid waste, soil and compost | With the potential strain (<i>Trichoderma viride</i>) out of the 250 isolates (49 cellulolytic) after 60 days, the average weight loss was 20.10% in the plates and 33.35% in the piles | Morphological keys and biochemical tests | Gautam SP, Bundela PS, Pandey AK, Jamaluddin, Awasthi MK, et al. (2012) [20] |
| 14. | Screening of polyethylene degrading microorganisms from garbage soil | Garbage soil samples (waste disposable site dumped with polythene bag and plastic cup | <i>Actinomycetes</i> (<i>Streptomyces</i> KU8) leads to 46.16% weight loss of the polythene whereas bacteria (<i>Pseudomonas</i> sp) and fungi (<i>Aspergillus flavus</i>) degraded only 37.09% and 20.63 % after six months | Morphological keys and biochemical tests | Usha R, Sangeetha T, Palaniswamy M (2011) [18] |

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|-----|---|--|--|--|--|
| 15. | <i>Studies on the biodegradation of natural and synthetic polyethylene by Pseudomonas spp</i> | Three sites: 1. Soil from domestic waste disposal site. 2. Soil from textile effluents drainage site and 3. Soil dumped with sewage sludge | The highest weight loss percentage of natural polythene (46.2%) and synthetic polythene (29.1%) was reported with <i>Pseudomonas</i> sp. collected from sewage sludge dumping site | Morphological keys and biochemical tests | Nanda S, Sahu S, Abraham J (2010) [19] |
|-----|---|--|--|--|--|

IV. FUTURE NEEDS

Polythene degradation is a typical process and takes longer time to degrade. So it is necessary to create awareness in the peoples about the ill effects of uses of polythene and plastics. People encourage for stopping the use of polythene and plastic in their daily life. Govt. of states as well as center should take initiatives towards the less use of polythene and plastics. Biodegradable polythene and jute bags, paper bags should be used in place of plastic/polythene bags. The microbes responsible for the degradation of polythene should be isolated from all the sources, screened to know the efficient isolates. The efficient microbes are needed to characterize at molecular level. Some extracellular enzymes are responsible for the biodegradations of the polythene (Aswale P, et al. 2008) [23]. These enzymes needed to be characterized and the genes responsible for those enzymes should be worked out. Once the genes responsible for the degradation of polythene would be known, the genes would be used to enhance the polythene degrading capacity of the other easily available microbes. After field trials, the most efficient polythene degrading microbes should be multiplied at large scale to decompose the polythene at commercial level (Manisha K Sangale et al. 2012) [24].

V. SUMMARY AND CONCLUSION

As we all know that polythene is very useful and important part of our life. Polthene is used in packaging of food items, gifts, packaging of cloths, medicines, lamination, wrapping of scientific instruments, packaging of electronic items etc. According to the different survey almost one million marine animals and many land animals, birds died due to the consumption of polythene and its toxic products. In the review paper many methods have been studied for polythene degradation but cheapest method is natural degradation by microorganisms. But all these methods are insufficient to degrade such a huge amount of polythene. More research work should be required for polythene degradation because it is produced at large amount commercially but its degradation process is very slow.

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