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Biodegradation of Low Density Polyethylene by Using Mixed Culture of Bacteria and Fungi

Revati Bhave[1], Sachin Mane[2]
ME student (Environmental Engineering) [1], Assistant Professor[2]
Department of Civil Engineering
D.Y.Patil College of Engineering Akurdi, Pune, Maharashtra, India

Abstract:
Mixed culture of three isolates namely Pseudomonas Spp, Aspergillus Spp and Rhizopus arrhizus A. Fisch has been isolated from polyethylene dumped area. All the isolates showed biodegradation activity against Low density Polyethylene. All the isolates were incubated with LDPE showed high amount of release of CO_2 (0.818 gm of CO_2) within 42 days. These isolates used LDPE as sole carbon source. The clear changes in the form of splitting of polymer compared with control were observed within 42 days of biodegradation with SEM analysis. The efficiency of LDPE degradation was observed through Percentage elongation as it resulted in decreased trend compared to that of controlled LDPE film of 20 micron.

Keywords: Biodegradation, Low density polyethylene (LDPE), microorganisms.

I. INTRODUCTION

Low density Polyethylene is used widely because of its recalcitrant nature and effectiveness. Most of the application of 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 tons/ day out of which 6000 tones remain uncollected and littered as per Central Pollution Control Board. Plastic pollution in most of the countries caused because of poor and improper recycling and waste management system (Jayasiri et al, 2013) [1]. 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.

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

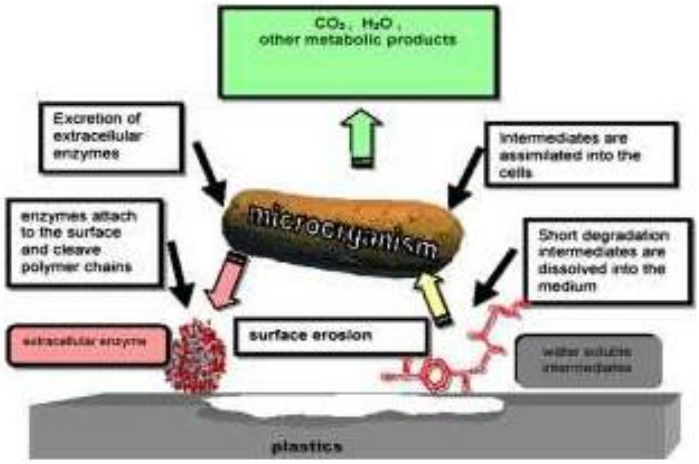

Figure.1. General Mechanism of plastic biodegradation under aerobic

conditions (Mueller, 2003) Microorganisms first attach to the surface. Once the microorganisms attach to the surface, it starts growing by using polymer as carbon source. In primary degradation first the main chain cleaves, then it gets converted into monomers. This degradation is due to the extracellular enzymes secreted by the microorganisms. These low molecular weight compounds are further utilized by the microbes as carbon and energy sources. 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 derivative pathway involved. It has been reported that many soil microorganisms were able to degrade plastic at its natural habitat. The organisms such as Pseudomonas,

Bacillus and the fungal species like Aspergillus were able to degrade the plastic *in vitro*.

II. MATERIALS AND METHODS

Methodology

A) Sample collection

The Gravel Sand and clay Samples were collected from plastic dumping area in Pune region. To check the biodegradable activity, the polythene bags of 40 micron has been taken. Clay and Crushed Gravel Sand collected was then washed with water and dried. Sieve analysis was carried out for both the sample. The Crushes Gravel sand was passed through 2000 mm and retained at 1000 mm whereas Clay sample was passed through 355 mm and retained at 250 mm. These sieved samples were used for further experimentation

B) Isolation of plastic degrading soil bacteria and fungi

The soil collected from the plastic dumping area was serially diluted with saline (0.85%). In short, 1g of soil sample was taken and mixed in 100 ml of saline in a conical flask. After vigorous shaking the sample was serially diluted. The soil bacteria and fungi were isolated by spread plate technique.

0.1 ml aliquot of various dilutions (10^{-2} to 10^{-9}) was spread on Minimal media (NH_4No_3, 1 gm/lit; $MgSO_4.7H_2O$, o.2 gm/lit; K_2HPO_4, 1 gm/lit; $CaCL_2$. $2H_20$ 0.1 gm/lit, KCL 0.15 gm/lit) using plastic as only source of carbon by using spreader (Atefeh Esmaeili, *et.al*, 2013) [2]. The incubation condition was at 37ºC for 24 to 48 hrs for bacteria and 3 to 5 days at room temperature for fungi.

C) Identification of bacteria and fungi

The bacteria were selected randomly on the basis of differentiation in colony morphology. The selected bacteria was identified on the basis of Gram staining and motility. Further the organisms were classically identified by using carbohydrate fermentation pattern. Fungi were identified by using staining technique by cotton blue and selected on the basis of microscopic observation of mycelium and spore structure.

D) Selection of organisms for further studies

The selected organisms were spared on minimal media containing plastic discs. After incubation of plastic with organisms for 30 days at Room temperature the plastic films were collected and checked for SEM analysis. Maximum changes on plastic film were observed when the plastic film was incubated with the organisms Pseudomonas spp, Aspergillus spp and Rhizopus. Pseudomonas Spp. confirmed with Gram staining, motility and classical identification method.

Aspergillus spp identified on the basis of spore staining while the maximum changes in plastic film in the form of degradation was observed with Rhizopus arrhizus A. Fisch. which was further confirmed based on morphological characters in in-vitro culture by ARI Pune. These three organisms were further used of actual experimental studies.

E) Experimental set up

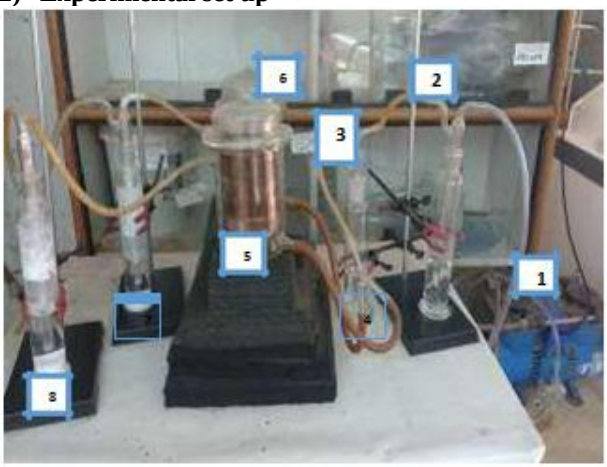

Figure.2. Pilot Scale set up for LDPE degradation

1. Aeration Motor (1/4 HP)
2. Gas Washing Bottle containing water
3. Gas Washing Bottle containing saturated NaOH
4. 'T' Shaped air distribution device
5. Container1 for Experiment (Clay+Sand+ LDPE+ microorganisms)
6. Container2 for Control (Clay + Sand + LDPE)
7. Gas Washing bottle containing saturated Ba $(OH)_2$ for detection of CO2 evolution for Container2.
8. Gas Washing bottle containing saturated Ba $(OH)_2$ for detection of CO2 evolution for container1

F) Confirmation of LDPE Degradation

Low density polyethylene degradation will be confirmed by CO2 evolution, Tensile testing, thickness by Digimatic micrometer, Field Emission Scanning electron microscope (FESEM).

a. Tensile testing and percentage elongation

For tensile strength measurement, test strips were retrieved after 42 days of biodegradation of LDPE films, washed with distilled water and films were kept for drying before tensile testing. Changes in mechanical properties, i.e. tensile strength, elongation at break and modulus, were studied according to ASTM standard, in a Universal Testing Machine. Two samples of each were strained at a rate of 5mm/min and average values of tensile strength, elastic modulus and elongation at break were determined. The length between the jaws at the start of each test was fixed as 20mm. The tests were undertaken in an air conditioned environment at 22ºC and relative humidity 50%. (Ambika Devi k *et.al*, 2015)[3]

b. CO_2 evolution test

The preliminary degradation ability of Aspergillus Spp, Pseudomonas Spp, and *Rhizopus arrhizus A. Fisch* was studied by measuring CO_2 evolution. The mixed culture of fungal strains and bacterial suspension (24hrs old broth containing 10^9 CFU/ml) were used. The air is allowed to flow through bottle containing 150ml of 10% Sodium hydroxide solution. The sterile CO2 `free air was passed in control as well as in experimental Container. The Sterile air was provided for respiration of microorganisms. CO2 free air was utilized by microorganisms and released carbon dioxide during respiration which is trapped with the help of barium hydroxide in Gas washing bottle. The

amount of CO2 trapped in gas washing bottle was calculated by using titration method. To check the evolved CO_2, 5 ml aliquot of barium hydroxide in both bottles were taken in a conical flask and 2 drops of phenolphthalein was added as indicator. This solution was titrated against 0.1 N HCL Solution. The colour changed from pink to colourless as endpoint. All experiments were repeated for thrice.

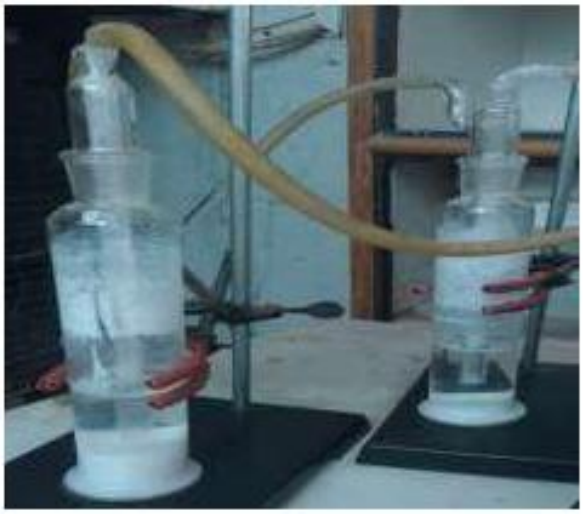

Figure.3. Ba (OH) 2 precipitate of Contain 1 and Container 2

The amount of CO2 evolved was calculated by

$$\text{Gm of } CO_2 = \frac{(C.R - E.R) \text{ X } 0.0044 \text{ X Total ml of } Ba(OH)_2}{\text{ml of } Ba(OH)_2 \text{ taken}}$$

C.R= Control Reading
E.R = Experimental Reading

c. Field emission scanning electron microscope SEM operated at 20 to 30 KV. The SEM microphotographs were recorded at a magnification of 1000X Magnification to 3000X Magnification. SEM analysis was carried out to investigate micro cracks, pits, erosion of surface, cracking and polymer adhesion. Samples were dehydrated before being coated with palladium.

III. RESULTS AND DISCUSSIONS

Low density polyethylene degrading bacterial and fungal strains were isolated from polyethylene dumped area. A total 20 organisms were isolated. These microorganisms were capable to grow on Low density polyethylene. The highest LDPE degradation has been observed with Pseudomonas spp which is confirmed with identification by Gram Staining, motility and certain biochemical identification. Fungal strains identified as Aspergillus Spp and Rhizopus Arrhizus.

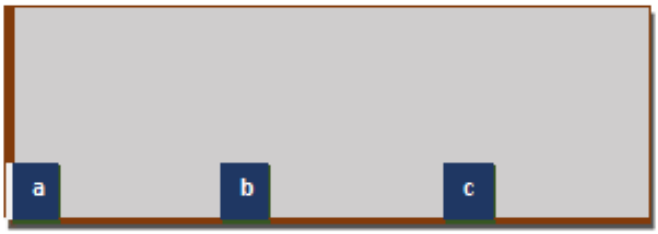

Figure.4. a) Growth of pseudomonas after 48 hrs of incubation at 37^0c b) Growth of Aspergillus spp.

after 3 days at room temperature c) Growth of Rhizopus Arrhizus after 4 days at room temperature These mixed cultures of stains were used for LDPE degradation for 42 days. Aeration has been given with every intermediate day. After 42 days of degradation, thickness of Low density polyethylene was report in Table 1.

Table.1. Thickness measurement of LDPE sample by using

Sample	**Initial Thickness (mm)**	**Final Thickness (after Biodegradation)**		
		42 days	**50 days**	**70 days**
LDPE	0.040	0.040	0.038	0.038

a) FE-SEM analysis:

During FE-SEM analysis it was observed that LDPE films with Control film had appearance of smooth Surface with no defects + as shown in fig 3. SEM analysis of Low density Polyethylene treated with mixed culture of Pseudomonas Spp, Aspergillus Spp and Rhizopus arrhizus A. Fisch showed structural changes such as Splitting of polymers, cracks, and holes through LDPE film as shown in fig 4.

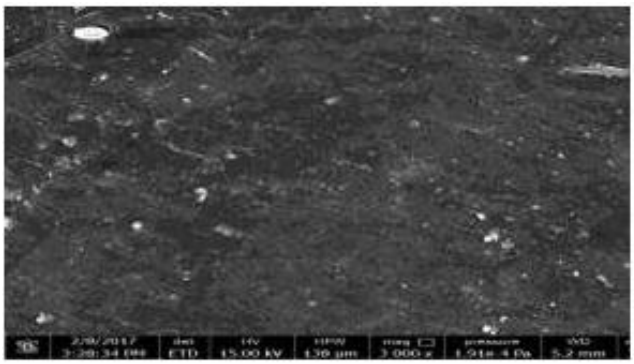

Figure.5. Scanning Electron microscopy before the LDPE film exposed with mixed bacterial culture

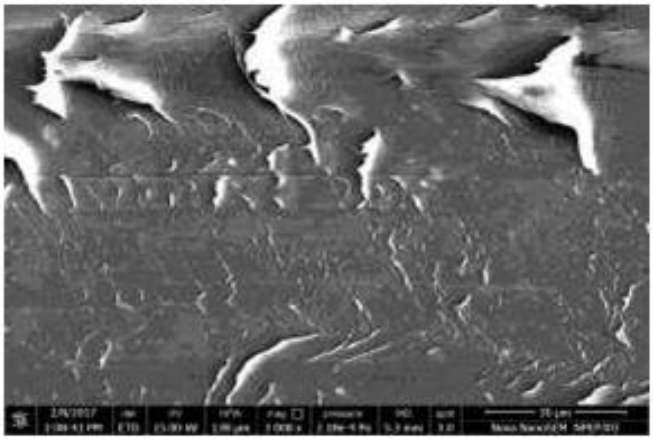

Figure.6.Scanning Electron microscopy after 42 days of LDPE film degradation with mixed bacterial culture.

b) CO_2 Evolution Test:

The total amount of CO2 evolved for isolates of mixed culture of Pseudomonas Spp, Aspergillus Sp and Rhizopus arrhizus A. Fisch was increased within 7 days of internal. The details are given in below mentioned graph. (Fig No.2). The continuous increase in CO_2 evolution has been observed with 42 days. The similar results were observed in research conducted by Pramila R *et.al.* 2011 [4]. However the fungal stain used in the studies was *Aspergillus Flavus.*

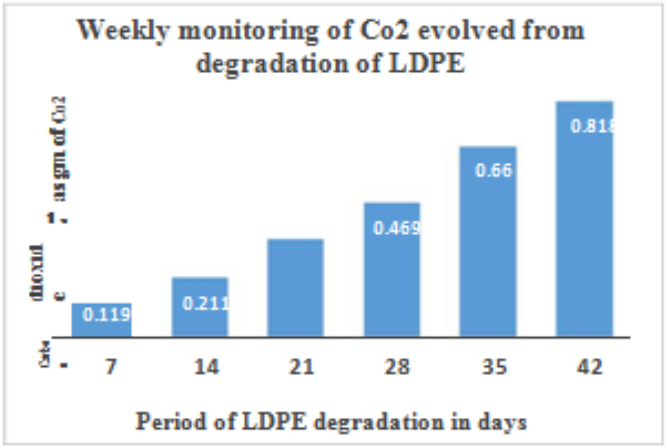

Figure.7. CO_2 evolution after LDPE degradation for 42 days

c) Tensile Testing and percentage elongation:

The tensile strength, percentage elongation at break and modulus of elasticity measures the stress at the fracture LDPE film and the extension of LDPE under load respectively. 42 days incubated LDPE films with mixed culture of Pseudomonas Spp, Aspergillus Niger and Rhizopus arrhizus A. Fisch had shown following results of mechanical properties as noted in Table No.2

- 142.70% elongation is seen in controlled LDPE film of 20 micron
- 69% elongation is seen in uncontrolled LDPE film of 20 micron

Table.2. Mechanical properties of LDPE of control and treated after 42 days of incubation.

LDPE Sample 20 micron	**Tensile Strength N/mm^2**	**% elongation**
Controlled LDPE	0.635	142.7
Treated LDPE	0.855	69

Reduction in percentage elongation of LDPE film is observed after biodegradation Process. Similar reduction in percentage elongation of polyethylene films after biodegradation was reported by Nowak *et.al;* 2011

IV. CONCLUSION

In this paper, Biodegradation of low density polyethylene was studied using mixed culture of Pseudomonas Spp, Aspergillus Spp and Rhizopus arrhizus A Fisch which were isolated from polyethylene dumped area. CO_2 evolved after degradation of Low Density Polyethylene shown good results with 42 days. LDPE was the only carbon source provided for microorganisms as a food for their survival it was seen that degradation has started in very short period of time.

V. ACKNOWLEDGEMENT

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