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Biodegradation of Low Density Polyethylene(LDPE) by *Nocardiopsis alba* from municipal landfill in Chennai

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Abstract

Low density polyethylene, a synthetic polymer is widely used in packaging material in our day to day life. It constitutes majority of the total municipal waste generated in the environment. This LDPE waste disposal remains as a major environmental concerns faced globally. Only a small fraction of this plastic waste is recycled and the rest of them were dumped into the landfills as a disposal procedure. The use of plastic is increasing every day and hence its removal demands a great challenge. Recently biodegradation has gained its importance for the safe disposal of plastic. The chemical and physical methods are inefficient in their mechanism moreover they were additionally polluting the environment. In the present study, *Actinomycetes* were isolated from municipal landfill and they were used for the biodegradation study. LDPE sheet was subjected to biodegradation for a period of 1,3 and 6 months of time interval by *Nocardiopsis alba* isolated from landfill. The biodegraded LDPE sheets were evaluated by weight loss, scanning electron microscopy and Fourier transform infrared spectroscopy analysis after the specified time period. The present investigation concludes that our isolated strain has the efficiency in degrading the polyethylene sheet and can be involved in solving the current environmental issue. The *Actinomycete* was identified and characterized as *Nocardiopsis alba* by 16s r RNA sequencing.

Key words: LDPE, *Actinomycetes*, *Nocardiopsis alba*, Weight loss, SEM, FTIR.

1. Introduction:

Plastics with unique properties compared to other materials started dominating the world in the past three decades. Because of diversified and versatile nature of these plastics, they are used to make a vast array of products in almost all the fields (Andrady,2011). Polyethylene is a recalcitrant synthetic polymer which is commonly used in our day to day life in almost all the facets of the environment. They are highly advantageous because they are strong, durable, lightweight and easily moldable. One cannot imagine the world without plastics, it become a part and parcel of our environment. Polyethylene either LDPE (low density polyethylene) or HDPE (high density polyethylene) is a thermoplastic made by monomers of ethylene, used primarily as thin films and packaging sheets (Albertsson *et al.*,1987). Among these plastics, low density polyethylene (LDPE) is widely used as a packaging material in agriculture and other industrial applications. Because of their excessive usage of plastics, increased pressure has been laid on the means for its safe disposal. Only a fraction of this plastic waste is recycled whereas, majority of the wastes started draining into the landfills (Lederberg, 2000 and Moore, 2008). Thus entering the food chain and become hazardous. This plastic pollution interferes with almost all living beings both marine and terrestrial life and make them more miserable to survive. These pollutants created a great negative impact in the ecosystems and pose a serious threat to human life and hence this LDPE

waste disposal remains one of the major environmental concerns faced by the world today.

In current scenario, major focus has been given to enhance the biodegradation of LDPE by microbial species. In the present study, degradation of low density polyethylene was performed by *Actinomycetes* from soil source. The high progress of their growth in soil and the growth development and penetration into other sites through hyphal filaments makes them more favorable for degrading polyethylene **(**Kim *et al.*,2003). LDPE film degradation by *Actinomycetes* is the aim of this study because of their compatibility with a landfill and composting environment which presents a natural condition for efficient degradation.

2. Materials and Methods:

2.1 Collection of soil sample:

The soil samples were collected from the municipal solid waste landfill area, Pallikaranai, Chennai, India at a depth width of 2-3 cm in a sterile container and air dried at room temperature.

2.2 Preparation of LDPE powder:

Pure grade Low density polyethylene (LDPE) sheets were commercially procured. LDPE films were cut into small pieces, immersed into xylene and boiled for 15 min and they were crushed to make fine powder. The obtained powder of LDPE was later washed with ethanol and dried in hot air oven at 70^{o}C overnight to get dry powder. It was stored at room temperature for future use.

1) Isolation of Actinomycetes:

Soil extract media was used as a natural media without any added ingredients except LDPE as a sole source of carbon. 5gm of soil was diluted in 30 ml of sterile distilled water and was kept in the shaker under proper aeration and agitation at 30^{o}C for two to three days. The broth was taken intermittently and was examined for *Actinomycetes* by wet mount and LPCB staining. Further, the inoculum was taken from the broth and was spread plated onto soil extract agar medium. The plates were incubated at 30^{o}C for one week.

2) **Identification and characterization of *Actinomycetes:***

The characterization of *Actinomycetes* culture was done using aerial mass colour, reverse side pigmentation, melanoid pigment, spore chain morphology and spore morphology. *Actinomycetes* were further identified based on standard biochemical methods (Shirling and Gottlieb, 1966 ; Pridham and Gottlieb, 1948).

3**)Molecular characterization of *Actinomycetes:***

Identification by 16S rRNA sequencing:

The Actinomycete isolate was phenotypically characterized and subjected to molecular characterization by 16SrRNA Sequencing and blasted as per the standard protocol (Edgar,2004; Talavera and Castresana,2007 and Dereeper, *et al*.,2008).

4) Determination of weight loss of the biodegraded low density polyethylene by the potential *Actinomycete* isolates:

The plastic films after exposure to all four bacterial suspensions were taken and washed thoroughly with 2% SDS for 4 hr (Orr *et al.*,2004). The strips were then dried at 60^{o}c overnight and the percentage weight loss was determined by calculating the percentage of weight loss of plastics. The percentage of weight loss was calculating by the following formula (Kyaw *et al*., 2012).

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

5) Characterization of the degraded LDPE sheets:

a) Fourier transforms infrared spectroscopy (FTIR) analysis

It is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects the high spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. FTIR analysis was done to detect the degradation of LDPE sheets after culturing in liquid media on the basis of changes in the soil extract.

b) Scanning electron microscopy (SEM) analysis.

It is a type of electron microscope that produces images of a sample by scanning it with a focussed beam of electrons. The electrons interact with atoms in the sample, producing various signals that can be detected and that contain information about the sample surface topography and composition. The morphology of the surface of LDPE sheets were analysed

through scanning electron microscope to check for the structural changes.

3. Results:

Compost soil samples were collected and were processed by standard procedures for isolation, identification and its degradation activity on low density polyethylene by various strategies were determined.

3.1 Isolation and Identification of *Actinomycetes:*

Phenotypic characterization was performed and the actinomycetes were identified and characterized by its distinct morphological characters (plate 1 and plate 2)

- **i)** Aerial mass colour- It forms brown tan, peaked, circular, erose, rough, and opaque with aerial mycelia that develop over time.
- **ii)** Reverse side pigmentation – Not distinct
- **iii)** Melanoid and soluble pigmentation- Distinct
- **iv)** Spore chain morphology- Bi verticillus spira.
- **v)** Microscopic observation- Cells are gram positive, acid fast positive, branched, and filament like.

Plate 1. Shows Aerial mass colour

Plate 2. Showing Spore chain morphology

3.2 Molecular identification of *Actinomycetes* by 16S rRNA sequencing:

The genotypic characterization was performed and the *Actinomycetes* strains was subjected to 16S rRNA sequencing and blasted for its confirmation. The strain was identified as *Nocardiopsis alba.*

3.3 Determination of weight loss of the biodegraded low density polyethylene by the potential *Actinomycete* isolates:

The *Nocardiopsis* strain was subjected to biodegradation of LDPE sheets for incubation period of one month. The inoculated strips were evaluated for its degradation by weight loss measurements, and it was found that the LDPE strips had reduced to some extent in their weight compare to the initial weight (un-inoculated). The percentage weight loss was determined by standard formula. The result suggests that this *Nocardiopsis* strain had utilised the polyethylene film as a sole source of carbon resulting in partial degradation by forming biofilm on LDPE sheets. (Table 1)

Table 1: Weight loss of LDPE

Sample	Initial Weight of LDPE	Final Weight of LDPE (After 1 month)	Weight loss %
Control	0.031	0.031	0
Nocardiopsis alba	0.031	0.021	32.25

3.4 Characterization of the degraded LDPE sheets:

The LDPE sheets were inoculated with *Nocardiopsis* strain in soil extract broth and were allowed to undergo degradation for a time interval (1, 3 and 6 months) of incubation. After the specified period of incubation, the degraded sheets were subjected to physico-chemical parameters viz.,

a) FTIR analysis.
b) SEM.

a) Fourier transforms infrared spectroscopy (FTIR) analysis:

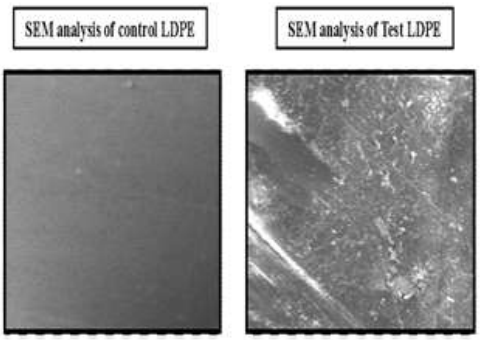
The changes in the polymer bonds due to biodegradation were determined using FTIR spectrophotometer. The LDPE sheets of different incubation periods (1, 3 and 6 months) which were exposed to the test strain of *Actinomycetes*, that is *Nocardiopsis alba* were analyzed. In control LDPE sheets, the spectrum lack many peaks indicating the absence of degraded products whereas, in the degraded, sheets, many

new peaks emerged indicating the extent of degradation activity (Ratanakamnuan and Aht-Ong, 2006). New peaks were formed, indicating the formation of many double bonds after a specified period of incubation. These bonds showed the formation of carboxylic acids, aldehydes, alcohols, esters, ethers, aromatics, and phenol groups indicating the degraded products by *Nocardiopsis alba.*

b) Scanning electron microscopy(SEM):

The changes in surface morphology of the LDPE films, before and after biotic exposure were investigated using SEM. The degraded LDPE film samples were prepared for SEM along with a control LDPE film. The plastic strips were taken out after 0, 30, 90 and 150 days of incubation. The surface morphology of the LDPE films degraded by Nocardiopsis was analyzed. On observation, it was found that, there was attachment of the spores and biofilm formation with the respective films were observed after 30 days of incubation. Few structural changes like grooves, cracks, damaged layer, pits and roughening of the surface were observed after 90 and 150 days of degradation. No apparent structural changes were found on the control film which was incubated under the same conditions that remain un-inoculated. (Figure 1)

Figure 1: SEM analysis of LDPE



4. Discussion:

LDPE is used widely because of its recalcitrant nature and effectiveness. Hence they possess a vast array of applications in day to day life as plastic carry bags, wrappers, food packaging materials, plastic bottles, lab equipments, pipes etc. Despite its broad application, it has major disadvantages being piled up into the landfill, garbage or into water bodies. These hazardous activities of this plastic litter causes tremendous pollution in the environment and remain there for many years damaging the ecosystem, environment and human health, thereby, it demands a safe disposal. However, if proper remedial action is not taken, this pollutant will create a big issue and a great nuisance to the environment globally.

Hence many methods have been tried for degrading these plastic contaminants like physical and chemical methods. Even though these methods were quite efficient in their actions, they were costly and they left toxic residues behind their activity which added up the pollution and made them more harmful. Hence in the present scenario, the biodegradation of low density polyethylene by employing microbes becomes simple, eco friendly and viable treatment option to reduce pollution.

McCarthy, 1987 reported that, *Actinomycetes a*re widely distributed in natural environments, such as soils and composts where they make an important contribution to nutrient recycling and humification. They are therefore a potentially useful source of plant biomass-degrading enzymes, and activity against the major components (lignin, hemicellulose and cellulose) has been identified in many strains. Various species of *Bacteria and Streptomyces* were similarly observed to biodegrade plastics, including polyethylene, according to El-Shafei, *et al.*,1998, of which Streptomyces was capable of degrading polyethylene containing 6% starch.

Deepika and Jaya (2015), in their study on screening the ability of different microorganisms in degrading polyethylene, observed that *Streptomyces sp*. were more efficient than bacteria and fungi. They produce extracellular enzymes that degrade a wide range of complex organic compounds in addition; the frequently occurring filamentous growth favours the colonization of soil particles (Thampayak *et al.*, 2006).

Another interesting feature within this group of microorganisms, especially in the biodegradation of hydrophobic substance is their surfactant producing activity (Ensign, 1978). Many *Actinomycetes*, mainly *Nocardioform actinomycetes* are known hydrocarbon degraders (Kästner *et al.*,1994; Iwahori *et al.*,1995 and Whyte *et al.*, 1999**)** and moreover, they represent the dominant group among degraders (Johnsen *et al.*, 2002). Our results goes along with the other reports stating that among the soil microflora, *Actinomycetes* dominates them and they were found to be more efficient degraders without any

added additives which enhance the degradation activity and being native to the environment.

A simple and quick way to measure the biodegradation of polymers is by determining the weight loss. Microbes that grow utilizing the polymer lead to an increase in weight due to the adherence of microbes, whereas a loss of polymer integrity leads to weight loss. Weight loss of LDPE is proportional to the surface area, since biodegradation usually is initiated at the surface of the polymer. After the degradation period, the LDPE films were treated with SDS as surfactant which denatures the cells and completely wash off from the surface. The reduction in weight was observed after the biodegradation of LDPE. In our study, 35 % weight loss of LDPE films was observed after 90 days of incubation with *Nocardiopsis alba*, whereas in control there was no weight loss of LDPE films

The evaluation of visible changes in polythene can be performed in almost all the methods. Changes observed in degradation includes roughening of the surface, formation of holes or cracks, defragmentation, changes in color, or formation of biofilm 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 SEM or atomic force microscope (AFM) (Ikada, 1999). In our present study, the biodegradation of LDPE was assessed by physicochemical methods like SEM and FTIR. These methods showed surface changes on LDPE sheets which show roughening, formation of holes, cracks and biofilm formation. Our results go in consistent with the previous results. 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 magnetic resonance spectroscopy (NMR), x-ray photoelectron spectroscopy (XPS), X-ray diffraction (XRD) (Shah *et al.*, 2008). This technique is generally analyzed for visualizing the surface changes in LDPE. Erosion of the film surface was observed in the vicinity of the microorganisms and decay of oxidation products in the surface of the polymer film was measured by FTIR measurement and was found to be associated with the formation of protein and polysaccharides attributable to the growth of microorganisms.

FTIR was used to confirm biodegradation by determining the formation of new functional groups or disappearance of groups in the polymer (Milstein *et al.*, 1994). Changes in the polyethylene structure following natural weathering and subsequent incubation with the fungal isolates were analyzed by FTIR spectroscopy. The carbonyl index was measured from the FTIR spectrum in the transmittance mode, by comparing the relative intensities of the carbonyl band at approximately 1712 cm-1 to that of the methylene band at approximately 1465 cm-1.

Sowmya *et al.*, (2014) reported that degradation was monitored by observing weight loss and changes in physical structure by SEM and FTIR spectroscopy. Our findings infers that these methods prove as an efficient tool in this degradation studies and therefore in future, these methods can be employed as a preliminary assessment criteria for the biodegradation activities.

5. Conclusion:

Microorganisms are capable of degrading inorganic and organic pollutants present in the soil, which evokes a great interest in isolating and identifying the microbes which are responsible for degradation of polymers especially LDPE. Biofilm formation improves the degradation efficiency followed by the mineralization of polymers which is the initial step in degradation. The results of this study showed Actinomycetes exhibited great potential for LDPE biodegradation under natural conditions, such as those found in soil. In the near future, these microorganisms can be suggested as an alternative in the reduction of plastic pollutants which has been accumulating in the environment for quite a long time.

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