

# Efficacy of Microbial Consortium on Degradation of Low Density Polythene Material Through FTIR Spectroscopy

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## Abstract

Microorganisms were isolated from the garbage soil with the help of suitable media, and they were constructed to form a microbial consortium. Then the consortium was mixed with garden soil along with LDP materials and it was enriched with suitable media for regular interval. After incubation, the LDP materials were collected from the soil and analyzed its surface changes through FTIR spectroscopy.

**Keywords:** FTIR, LDP, Microorganisms, Surface change, Consortium

## I. INTRODUCTION

Low density polyethylene (LDP) is one of the most used polymers in the society (Merina Paul Das and Santosh kumar, 2013). It is a hydrophobic synthetic polymer of high molecular weight and it is characterized by good toughness, resistance to chemicals, flexibility and clarity. These properties make LDP an important plastic grade widely used for manufacturing various laboratory containers, dispensing bottles, tubing, plastic bags, food containers and corrosion-resistant work surfaces etc (Negi, et al., 2011; Sowmya et al., 2014). For the last 60 years, the use of synthetic polymers has grown progressively because of their low cost reproducibility and resistance to physical aging and biological attack (Hiroko Kitamoto et al., 2011).

If polyethylene found in the soil, it reduces water penetration into the soil, reduces soil fertility and prevents the growth of plant life and thus, poses environmental problems (Vijaya and Mallikarjuna, 2007). Some possible measure employed for the purpose is biodegradation and biocycling (Vishnu Singh et al., 2002). Microbial degradation of polythene is caused by enzymatic activities leading to a chain cleavage of the polymer into oligomeres and monomers after which they are further metabolized by the microbial cells (Sonil et al., 2010). To this environmentalists have given an alarm about the ill consequences of polythene and plastic wastes on earth. Hence, scientists are now in the way to prepare for degradation in a safer way by using effective microbes. The present study was aimed to isolate microorganisms from garbage soil, then it was mixed with garden soil to prepare microbial consortium along with LDP materials further it was enriched with suitable media for regular interval. After incubation, the LDP materials were collected from the soil and analyzed its surface changes through FTIR spectroscopy.

## II. MATERIALS AND METHODS

### A. Sample Collection

Soil sample was collected from the garbage of Thottiyam of Thiruchirappalli District, Tamil Nadu and stored in zip lock covers and then kept at 4°C for further studies.

### B. LDP

Fresh low density polythene carry bags were used for this study and they were obtained from the paper market of Thiruchirappalli, Tamil Nadu. LDP bags were cut into (3x3 cm) pieces and then washed with 70% ethanol for 30 min, then followed by distilled water, and air dried for 15 minutes in the laminar air flow chamber and then used for future studies.

### C. Serial Dilution Method

One gram of collected soil sample was weighed using electronic balance and then it was mixed with 99ml of distilled water and shaken well, then 9 sterile test tubes were taken, each of which contains 9ml of sterile distilled water, 1ml of sample was transferred serially to all the tubes to make about of  $10^{-1}$  to  $10^{-8}$  dilution.

#### **D. Media Preparation**

About 200 ml of potato dextrose broth for fungi and nutrient broth for bacteria were prepared and sterilized in an autoclave at 15 lbs pressure for 15 minutes. Then it was allowed to cool at room temperature.

#### **E. Inoculation of Microorganisms**

From the serial dilution,  $10^{-5}$  dilution was taken and added into 0.1ml of the same dilution into 100 ml nutrient broth and potato dextrose broth separately and kept in rotary shaker for 7 days.

#### **F. Garden Soil**

About 2 kg of garden soil was collected from our College garden and filled in the tray. Water was sprinkled over the tray to maintain the humidity.

#### **G. Microbial Consortium**

The well grown bacteria and fungi from the medium were taken and mixed together to form a microbial consortium.

#### **H. Introduction of Microbial Consortium into Garden Soil**

The microbial consortium was introduced into the garden soil and mixed well further it was kept it for 2 hours incubation.

#### **I. Inoculation of LDP Materials into the Tray Containing Microorganisms**

LDP materials were inoculated into the tray which containing microorganisms and then allowed for 5 month incubation.

#### **J. Enriched With Suitable Media**

Every month, during the incubation period the sample was enriched with potato dextrose broth and nutrient broth. After that, the sample was mixed thoroughly for proper aeration.

#### **K. FTIR Studies**

Fourier transform infrared spectroscopy analysis was performed for detecting the formation of new functional groups or changes in the amount of existing functional group. After the incubation period, the sample was collected and washed with water and followed by ethanol to remove debris and again washed with distilled water to remove excess precipitation and then allowed it to dry. The surface changes made on LDP pieces were analyzed through FTIR studies.

### **III. RESULT AND DISCUSSION**

The growth of microorganisms present in the suitable media was visible by naked eye. After the incubation, the surface changes like cracks, pits were analyzed through FTIR studies.

Polystyrene structurally consists of the aliphatic chain with an aromatic ring attached to every other carbon atom. Styrene is the monomer of polystyrene and its degradation by bacteria and fungi is well established in the literature (Naima Atiq et al., 2010)

FTIR spectroscopy is most widely used in determining the structural changes in macromolecules. Since it is known that degradation of polymers can via both hydrolysis and oxidation, with this tool it is possible to estimate the extend of modification of the polymer main chain due to the action of abiotic or biotic factors. It is assumed, that the mechanisms of polymer degradation can be determined by measuring the levels of ketone carbonyl, ester carbonyl and internal double bond absorbance peaks (Bozena et al., 2006).

The band at  $719.20\text{ cm}^{-1}$  is aliphatic chloro compound, it was slightly decreased into  $718.42\text{ cm}^{-1}$  in sample respectively, due to microbial growth, the microorganisms grow in surface, but not break the bond completely and however it makes slight changes.

A band around  $1461.11\text{ cm}^{-1}$  revealed a rocking deformation but it was however showed bending deformation due to microbial growth, the deformation is good in sample which is inoculated with microorganisms. The carbonyl band corresponding to the ketone and ester carbonyl groups and it is a typical product of oxidative degradation of polyethylene

A band around  $1019.04\text{ cm}^{-1}$  revealed a bending deformation but it is however showed rocking deformation due to microbial growth (Ibine et al., 2013).

The band at  $1743.82\text{ cm}^{-1}$  and  $1581.32\text{ cm}^{-1}$  carbonyl compounds is completely removed and new bands like  $788.22\text{ cm}^{-1}$  was formed. The peak in  $3500\text{--}3000\text{ cm}^{-1}$  of amino group but gets slight bending formation due to the activity of some microbial growth, then the carbonyl index of the film, showing an increase after exposure and decreased, however, some band revealing a bending deformation, and another band indicates a rocking deformation due to microbial growth.

The present study focused the degradation of low density polyethylene material by using microbial consortium. The microorganisms found on the surface of LDP sample causing some physical changes like some band arose and de arose, is the evidence in this study by FTIR analysis.

## A. FTIR Analysis

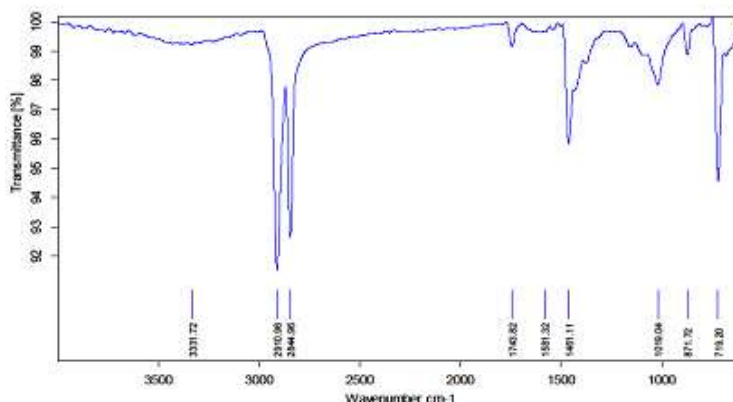


Fig. 1: FTIR spectrum of LDP sample in control soil after incubation (5 months)

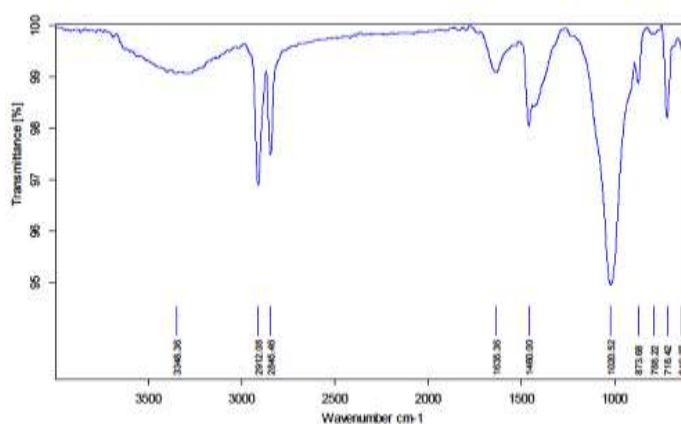


Fig. 2: FTIR spectrum of LDP sample in soil with microbial consortium after incubation (5 months)

Kavitha et al., (2014) observed that the changes in the polyethylene structure with subsequent bacterial inoculation and they were analyzed by FTIR in the frequency range of  $4000 - 800 \text{ cm}^{-1}$ .

FTIR results showed the formation of aldehyde, alcohol, carboxylic acid, aromatic, alkene and ether group formation indicating degradation of polyethylene by isolated bacteria all these results confirmed the partial degradation of LDP material.

## IV. CONCLUSION

Environmentalists have given an alarm about the ill consequences of polythene waste on the earth. This study revealed that the microorganisms present in the garbage soil such as bacteria and fungi were isolated separately and it was mixed together to form a microbial consortium. The microorganisms present in the microbial consortium are able to grow well when it was enriched with suitable media. The microbes grow well when it was kept for long time incubation period and maintenance of proper aeration for their effective growth. After incubation, the microorganisms present in the garbage soil utilize the carbon source very well and degrade the LDP materials on surface level. The FTIR result showed that the microorganisms present in the garbage soil degraded the low density polyethylene material effectively when it was grown with the suitable environment.

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