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Halophilic Degradation of Low Density Polyethylene using Novel Mineral Medium

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Abstract: Bio-Degradation plays an important role in the polymer fields apart from the discoveries made in this field. This project is a try to degrade virgin low density polyethylene with the help of “HALOPHILE” microbe belonging to the kingdom of “Archea” along with novel synthetic mineral medium of specified composition. Halophiles generally known as Salt Loving Microbes were incorporated into the compost taken from the saltern bed after visually examining the colour of the saltern soil. For a period of 90 days at 52 ^{O}C a comparison study was made between natural and novel synthetic mineral medium which has been used to degrade the low density polyethylene. This study will prove itself beneficial for further study in the field of using Halophiles with polymers.

Introduction

Bio degradation, an eventually wanted field to protect environment for future generation in the case of plastics. Halophiles, a type of microbe belonging to the kingdom of Archea. These halophilic microbes are the organisms requiring at least 0.2M concentration of salt (NaCl) for their growth. These halophilic microbes survive in conditions of salty lake beds, frequently in temperatures over 40^{O}C with subsurface water Temperature of 65^{O}C. The Halophiles can be found anywhere with the concentration of salt five times greater than the salt concentration of the ocean. Such lakes exist in California, Israel (Dead Sea), Kenya, Australia and Mongolia[1-11].

Experimental

Material and Methods

The Halophile Agar medium was purchased from Hi-Media labs (M590) and other mineral was purchased from Merck Products such as Itaconic acid, Ammonium ferric citrate, Zinc acetate dihydrate, Magnesium sulphate and double distill water

Preparation of low density polyethylene

Low density polyethylene in the form of granules was purchased from Reliance Industrial Pvt. ltd. The granules were predried to remove any moisture and then were manufactured in the form of film using Blow film extrusion process. A film of thickness 50 micron was obtained. They were checked visually for any defects and voids to ensure its quality. The film was cut in a form of square of dimension 5cm X 5cm.

Preparation of compost

According to literature [Rodriguez 1979] the microbe is said to prevail in saltern soil with 2 M NaCl and the soil would be distinctively red color. On the basis of this the compost for the project was taken from kelambakam, Tamil Nadu. The compost was collected in glass bottles. The compost was bright red in colour. Then it was brought to the lab conditions (30 ^{O}C ±2). As shown in Figure 1. The halophiles usually can be examined visually in "Red – Pink color". This photograph explains the presence of the microbe "HALOPHILE". Before incorporating the films into the compost, the compost was weighed for around 800g. The compost was pre-dried at a temperature of 52^{O}C in order to maintain 50% of the moisture content. These films were incorporated into the compost.

Figure 1: Visually examined red color compost indicating presence of halophile

(a) **(b)**

Preparation of Mineral Medium

As presented in the (Table- 1) various minerals such as Itaconic acid, magnesium sulphate, zinc acetate dehydrate, Ammonium ferric citrate and Halophilic agar medium were taken in specific composition for the constant growth of the microbe. The final pH (at 25^{O}C) is 7.2±0.2.

Table 1: Composition of Mineral Medium

Minerals	**Composition (g/10ml)**
Halophilic agar	3.25
Itaconic acid	0.1
Ammonium ferric citrate	0.063
Zinc acetate dehydrate	0.0023
Magnesium sulphate	0.2

Compost Conditions

The films were incorporated into the compost and was stirred uniformly using a glass rod in order to prevent any damage to the film. The specified composition of the novel synthetic mineral medium was taken and stirred with 10ml of distilled water and then poured into the compost. This was done daily in order to maintain 50% of the moisture content for the microbe to grow. The temperature of around 52^{O} C was maintained in hot air oven for a period of 90 days with constant stirring at regular intervals to ensure growth of the microbes. A change in smell was identified which indicated the growth of the microbes.

Washing of Films

After 90 days, the films were taken out from compost using glass rod. The films were kept in a porcelain tub immersed in distilled water. The soil particles attached to the surface of the films were washed away. Around 400ml of distilled water was taken in a beaker and was heated at a temperature of 85^{O}C for one hour bringing it to well boiled condition. This was done to ensure that the water was free from any presence of microbes. It was soon covered with an aluminium foil and was kept aside for it to cool to the room temperature.

The films were then immersed in the distilled water and stirred for 30 minutes using a magnetic stirrer. The films were taken out and kept in a petridish. After that, 2 to 3 grams of surfactants was added to boiled and cooled distilled water. The films were immersed into the distilled water containing the surfactants and were allowed to stir in a magnetic stirrer for a period of 30 minutes. The films were taken out and kept in a petridish.

The films were subjected to sodium hydroxide treatment. The films were washed with 10% of sodium hydroxide using magnetic stirrer for a period of 30 minutes. The films were taken out and kept in a petridish. Then they were subjected to 1N HCl treatment. The HCl was used to wash the film. The films were stirred using magnetic stirrer for a period of 30 minutes. The films were taken out and placed in petridish each separately and dried in a hot air oven for a period of one hour. The films were then taken out and checked visually to ensure there was no moisture content. After ensuring films were weighed using Metler Toledo Analytical balance. A loss in weight was observed.

Fourier Transform Infrared Spectroscopy (FTIR)

Fourier transform infra red spectroscopy (FTIR) is a technique which is used to obtain an infra red spectrum of absorption. This FTIR was used to analyze the structural characteristics of the polyethylene film subjected to microbial action. The wave number ranges from 400 cm^{-1} to 4000 $cm^{-1.}$. The films were analyzed using NICOLET 6700 USA. A change in the structural characteristics was noticed that is discussed in

Scanning Electron Microscope (SEM)

Scanning electron microscope (SEM) is a type of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. SEM was used to analyze visually the changes on the surface of the polyethylene film subjected to microbial action. Polyethylene being non-conducting in nature was subjected to gold sputtering before subjecting it to the action of electron beams. The magnification of 5000,10000,15000,20000 and 25000 was obtained. Significant changes were observed on the surface of the polyethylene film which proved microbial degradation.

Density Measurement

The density of films were measured as per ASTM D 792 using Mettler Toledo analytical balance equipped with density measurement accessory. Isopropyl alcohol with a purity of 99.5% (density 0.786 g/cm^3) was used as the liquid medium.

Results and Discussion

Weight Analysis

After several washing of the polyethylene films in different medium the films was dried at $85^{O}C$ in hot air oven for a period of 1 hour. The films were weighed before and after weight of the polyethylene film due to the microbial action was analyzed. A change in the weight was observed as displayed in (Table- 2).

Table 2: Comparison of % of weight loss in different medium

Medium	% of weight loss
Natural medium (sea water)	No change
Novel synthetic mineral medium	1.2

Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier transform infrared spectroscopy was done to both the films subjected to natural and synthetic medium. Various changes were observed but significant change was observed in that of polyethylene film subjected to novel synthetic mineral medium. The presence of carbonyl group in the range 1721cm^{-1} with the excess of hydroxyl group peaks was observed in that of the polyethylene film subjected to novel synthetic mineral medium, while the hydroxyl group peaks were only observed in that of polyethylene film subjected to natural medium which is discussed in (Table- 3 &4). (Figure- 2) represents the FTIR result for using natural sea water for the degradation of polyethylene film. (Figure- 3) represents the FTIR results for using novel synthetic mineral medium for degradation of the polyethylene film.

Table 3: Characteristic peak value in the FTIR spectra for LDPE with natural sea water

Frequency (cm^{-1})	Band and Functional group
3610	O-H stretching vibration
2918, 2664	C-H asymmetric and symmetric vibration
2372,2345	O-H stretching (Carboxylic acid)
1599	C=O stretching vibration
1463	CH_3 (alkane) scissoring vibration
1302	CH_2 (alkane) rocking vibration
1035	C-O, alcohol, ester, ether
729,720	C-H Bending vibration

Table 4: Characteristic peak value in the FTIR spectra for LDPE with novel synthetic mineral medium

Frequency (cm^{-1})	Band and Functional group
3904-3650	O-H stretching vibration
2922, 2662	C-H asymmetric and symmetric vibration
2428,2255,2172,2131	O-H stretching (Carboxylic acid)
1721	C=O stretching vibration
1507	C=C stretching vibration
1462	CH_3 (alkane) scissoring vibration
1376,1368,1339,1303	CH_2 (alkane) rocking vibration
1082	C-O, alcohol, ester, ether
951 ,941	R CH=CH-R, C-H Bending of Alkenes
888,729,720	C-H Bending vibration

Figure 2: Polyethylene film subjected to Natural medium

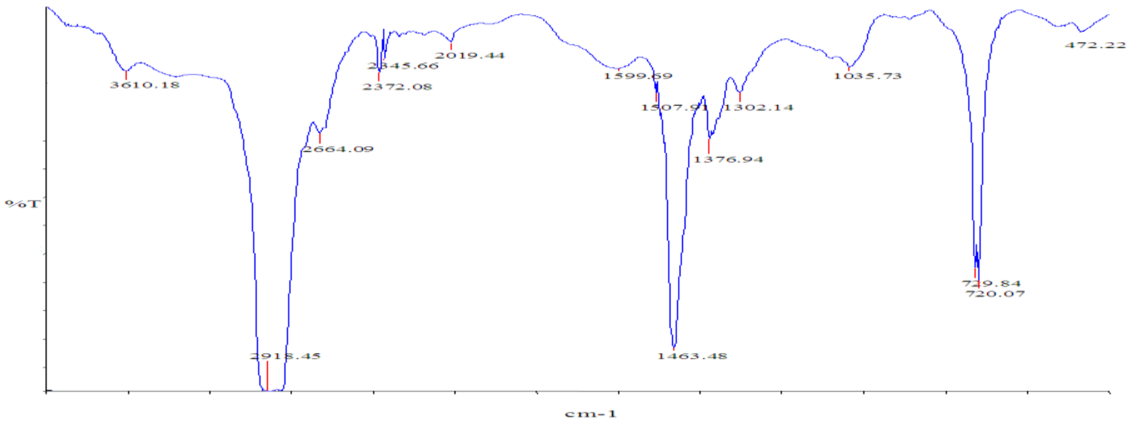
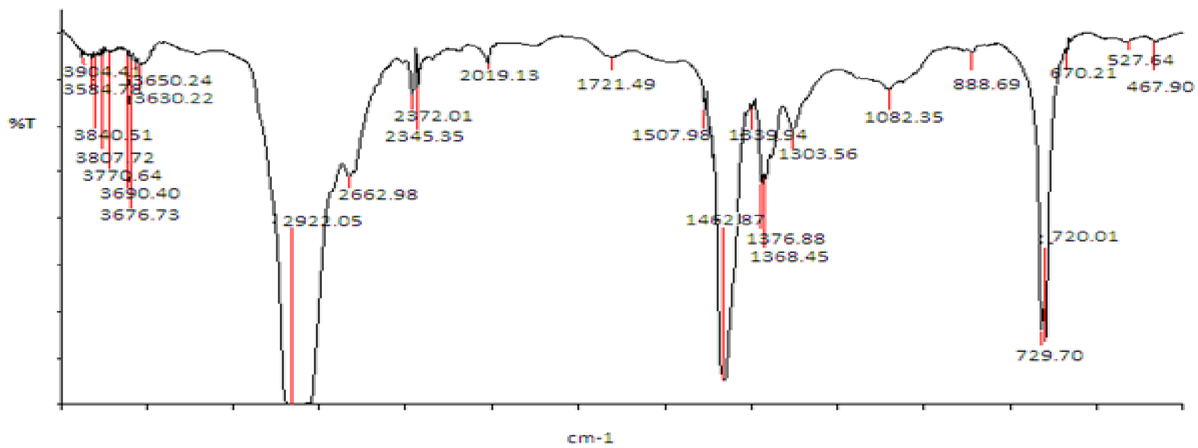


Figure 3: Polyethylene film subjected to novel synthetic mineral medium



Scanning electron microscope

The SEM analysis was performed to visually notice the changes on the surface of the polyethylene film subjected to microbial action. The SEM result of virgin polyethylene film was obtained as shown in (Figure- 4). The SEM results for the polyethylene film subjected to microbial degradation was obtained as shown in (Figure- 5(a, b, c, d))

Figure 4: SEM of virgin polyethylene film

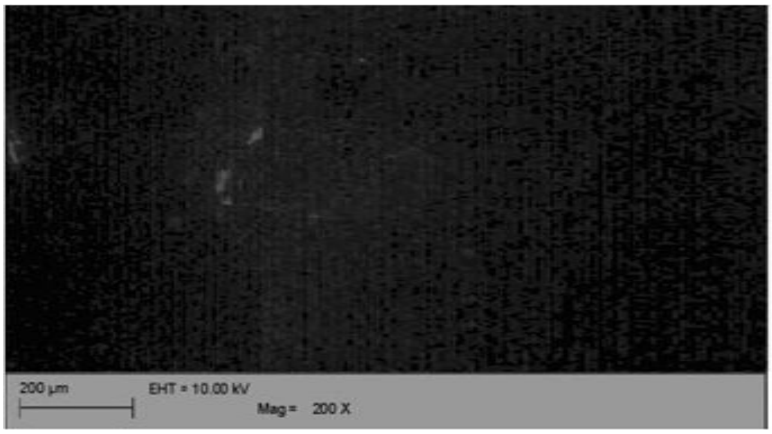
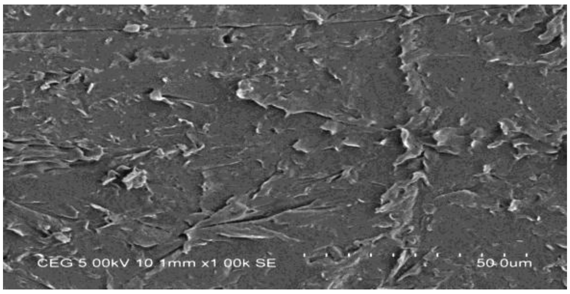
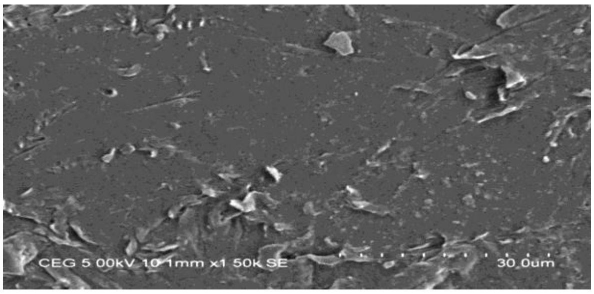


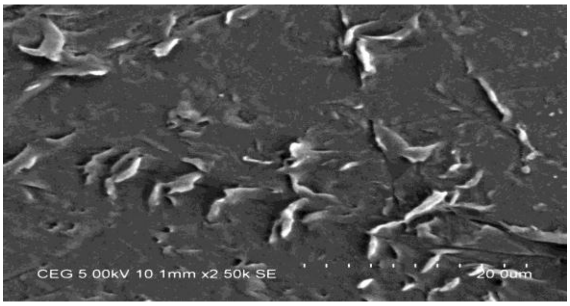
Figure 5(a, b, c, d): SEM result of polyethylene film subjected to microbial action of different magnification. (a) 5000 magnification (b) 10000 magnification (c) 15000 magnification (d) 20000 magnification.



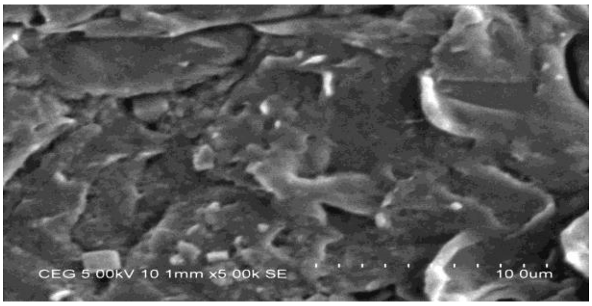
(a)



(b)



(c)



(d)

Density Measurement

The density of LDPE with novel synthetic mineral medium was lower than that of virgin LDPE due to the reduction in molecular weight as presented in (Table- 5).

Table 5: Density Measurement

Sample detail	Density (g/cc)
Virgin LDPE	0.93
Natural	0.92
Synthetic	0.92

Conclusion

This project involves the usage of Halophile microbe with Novel synthetic mineral medium for the degradation of virgin polyethylene film. Better degradation of the polyethylene film has been achieved using this microbe when compared to natural sea water. The results have been proved using weight analysis, Fourier transform infrared spectroscopy and Scanning electron microscope.

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