Bio-Degradation of LDPE Materials by Using *Acrophialophora Fusispora*

SHALINI, R
PG and Research Department of Biotechnology, Jamal Mohamed College (Autonomous), Tiruchirappalli District, Tamil Nadu, India.

Abstract— This article reveals the bio-degradation of low-density polyethylene by Acrophialophora fusispora. LDPE sheets were immersed in MSM contains A. fusispora. After the period of incubation, the LDP materials were collected from the MSM (mineral salt media) and washed with ethanol and air drayed. The collected sample was undergone to analyze the rate of bio-degradation and morphological changes through Weight loss method and FTIR, SEM analysis.

Index Terms- Acrophialophora fusispora, Zone, SEM, FTIR, LDPE.

I. INTRODUCTION

Trillions of plastic bags are consumed each year and were thrown in the street or road side after their use. These waste materials were collected by the sweeper and dumped in the garbage area. It takes more than a thousand years for complete degradation. To make pollution free metro pollutant city we apply various methods to degrade them, but it doesn't show sufficient result, so by using microorganisms or biological additives, known as biodegradation is employed to degrade LDPE. In this process of degradation, plastic reacts with oxygen from the air and then the microorganisms, facilitate this degradation process by secreting polyethylene degrading enzymes to oxidize or break down the products for its energy into smaller byproducts such as carbon dioxide and water (Nupur Ojha *et al.,* 2017). Compare to another method, it is natural oriented one and degradation rate also much higher compare to other. Efforts should be intense on emergent eco-friendly methods of degrading LDPE materials by utilizing the potential of fungi (Sudhakara, *et al.,* 2008).

The present study concentrates on isolating the potential fungal isolates responsible for the degradation of LDPE materials was isolated from a soil sample of the Tiruchirappalli garbage area. The study also focuses to catalysis the media for improving the degradation rate. To analysis the percentage of degradation, several analytical methods have been used in the degradation of the LDPE materials.

II. MATERIALS AND METHODS

- Isolation of LDPE degrading fungal species:

After the successful growth of the microorganisms, each single colony was identified (based on colony morphology and color) and re-streaked as primary inoculants on the surface of the PDA for fungi. The plates were then incubated at 27 ± 2°C for 6 days and the isolated strains were maintained on PDA (fungi) slants at low temperature (4 ± 1^0C) for further use.

- Identification of fungal isolated from the garbage area of the LDPE contaminated site of Tiruchirappalli D.t:

Identification of every chosen fungus was done by observing colony morphology and fungal staining (Carmichael and Bryce Kendrick, 1980; Domsch and Games, 1993 and Barnatt and Hunter, 1998).

- Examination of colony morphological characters:

The colony morphological character *i.e.,* colour was studied on the selected fungal strains and the results were recorded and obtainable.

- Rapper and fennel method for identification of fungi:

1 or 2 drops of lacto phenol cotton blue strain are poured in the clear slide, to that 6 days old fungal mycelium was added into the same slide and smear it until the mycelium spread over the strain, excess solution was removed with blotted paper, and covered it with the cover slip with the help of vaseline and kept

for few minutes and observed under the oil immersion microscope.

- Screening of Low-density polyethylene (LDPE) degrading microbes' fungi through clear- zone formation:

The agar plate is emulsified with LDPE, a pure fungal culture was spreader over it and incubate the plate at 30ºC for 2 to five days, a clear zone is observed around the LDPE sample were determined modified method of (Augusta *et al.*, 1993), microbes were screened based on the diameter of zone formation for further degradation studies.

- Inoculation of Polythene Strips into Mineral salt medium:

LDPE bags (purchased local market) were cut into small strips (each 3cm size) and inoculated into the mineral salt medium and kept it for 3,6-,9- and 12-month of incubation period.

- Biodegradation of LDPE materials were characterized through various analytical techniques:

Weight loss method:

Determination of dry weight of residual LDPE for the accurate measurement of dry weight of residual LDPE, the LDPE films were recovered from the degradation medium and they were washed with 2 % (v/v) sodium dodecyl sulfate (SDS) solution and further rinsed with distilled water (Gilan *et al.* 2004). The washed LDPE film was air dried overnight at 60^0 c before weighing and the percentage of weight loss was determined using the formula (Kyaw *et al.* 2012). Based on this the following weight loss percentage was determinate using the following formula:

$$\text{Weight loss (\%)} = \frac{\text{Initial weight} - \text{Final weight} * 100}{\text{Initial weight}}$$

- Fourier Transform Infrared (FTIR) Spectroscopic 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.

- Scanning Electron Microscopy analysis:

The treated samples over a period of incubation were washed with 2 % (v/v) aqueous SDS and distilled water for a few minutes and flushed with 70 % ethanol to remove the cells. After that the sample was pasted onto the SEM analysis stub using a carbon tube and the sample was coated with the gold for 40 s and analyzed under high-resolution scanning electron microscope (EVO LS15; Carl Zeiss, German) for analysis.

III. RESULTS AND DISCUSSION

Isolation and Identification of *Acrophialophora fusispora* from the garbage area of Tiruchirappalli D.T:

Acrophialophora fusispora is isolated from garbage area contaminated with LDPE materials in Tiruchirappalli D.T. They were identified based on Raper and fennel key. It's pure culture and its microscopic view were given in picture format in figure 1.

Figure 1. *Acrophialophora fusispora* and its microscopic view

Screening of Low-density polyethylene (LDPE) degrading microbes fungi through clear- zone formation:

Acrophialophora fusispora was inoculated into the medium containing LDPE powder and kept for 2 to 3 days for incubation. A halo zone was formed around the fungus indicate the chosen fungus have the ability to degrade the LDPE materials. The clear zone formation was measured with a ruler and its shows 1.5 cm. Zone formation and its diameter of zone were predicted in table 1 and figure 2.

Table 1. Diameter of clear zone of *Acrophialophora fusispora*

S.No	Name of the Organisms	Diameter of Zone
1	*Acrophialophora fusispora*	1.5cm

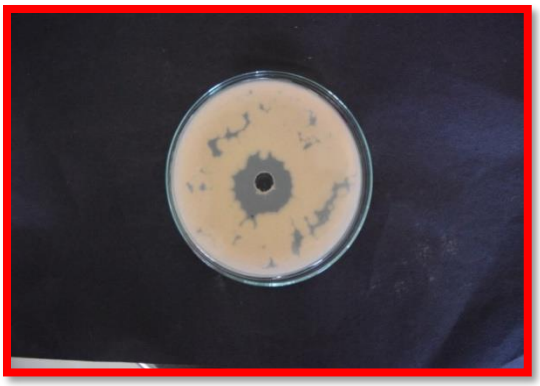


Figure 2. Zone formation of *Acrophialophora fusispora*

The gradual increase in zone formation around the colony was measured in centimeters with the ruler every day. It implies the initiation of biodegradation (Vatselductt, and S.Anbuselvi. 2014) that can be interacted and made changes in mechanical properties of tensile strength, optical changes of cracking, erosion and decolorization. It is clear that the organisms are at least able to de polymerize the polymer. Because it secrete the extracellular enzymes which degrade the polymeric substances into water soluble materials, resulting into the formation of a clear zone around the microbial culture, indicating utilization of polyethylene powder as the sole carbon source., which is the first step of biodegradation. Augusta *et al.*, 1993 have reported that the zone of clearance around the colony is due to extracellular hydrolyzing enzymes secreted by the target organisms into suspended polyester agar medium. This method is usually applied to screen organisms that can degrade a certain polymer (Nishida and Tokiwa, 1993; Abou-Zeid, 2001), but it can also use to obtain semi-quantitative results by analyzing the growth of clear zones.

Monoculture of *Acrophialophora fusispora* in various incubation periods for biodegradation of LDPE under mineral salt media:

Acrophialophora fusispora was inoculated into the mineral salt media (MSM) along with LDPE materials (3*3cm) and kept for 3, 6, and 9 month of incubation period. After the incubation period, the LDPE strips were taken and washed with ethanol and air dried and undergo for analytical studies for calculating the percentage of degradation rate. *Acrophialophora fusispora* in various incubation periods were given in figure 3.

Figure 3. Monoculture of *Acrophialophora fusispora* in various incubation periods for biodegradation of LDPE under mineral salt media:

A

B

C

A: 3 month of incubation period of *Acrophialophora fusispora + LDPE* material in MSM
B: 6 and 9 month of incubation period of *Acrophialophora fusispora + LDPE* material in MSM
C: 12 month of incubation period of *Acrophialophora fusispora + LDPE* material in MSM

Biodegradation of LDPE materials were characterized through various analytical techniques:
After 3, 6, 9 and 12 month of incubation period, the LDPE strips were taken and washed with ethanol and air dried. After that they were undergone for analytical studies.

IV. WEIGHT LOSS METHOD

LDPE strips were kept in weight balance to determine the weight loss percentage method. The reduction in weight was observed after the biodegradation said by (Merina Paul Das and Santhosh kumar 2015) Mona goundar *et al*., 2012, states that it, usually enzymatic degradation of polyethylene is a surface erosion process. The weight loss can be used to measure the enzymatic cleavage of the polymer. The microbial enzyme catalyzed the depolymerization and thus there was weight reduction (Merina Paul Das *et al*., 2014). The reading was predicted followed by the formula and results were predicated in table 2.

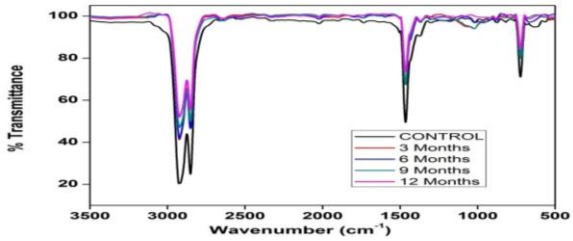
Table 2. Percentage of degradation LDPE

S.No	Period on incubation	Name of the Organisms with LDPE materials	Percentage of degradation
1	3 month	*Acrophialophora fusispora* + LDPE	04%
2	6 month		09%
3	9 month		11%
4	12 month		16%

V. FOURIER TRANSFORMS INFRARED (FTIR) SPECTROSCOPIC STUDIES

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 LDPE pieces were analyzed through FTIR studies. Based on carbonyl group, the percentage of LDPE degraded by the microorganisms was calculated.

Figure 4. FTIR analysis for monoculture of *Acrophialophora fusispora* in various incubation periods for biodegradation of LDPE under mineral salt media



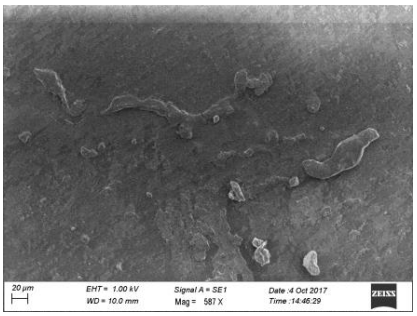
Different month's incubation period of *Acrophialophora fusispora* + *LDPE* material in MSM

A band around1461.11 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 cm^{-1} revealed a bending deformation but it is however been shown rocking deformation due to microbial growth (Ibine *et al*., 2013).The changes in the polymer bonds due to biodegradation were determined using FTIR spectrophotometer. The LDPE film exposed to the isolates was analyzed after 3, 6, 9 and 12 month of incubation period. The result was predicted in figure 4. It is used as an analytical technique in many biodegradation studies reported by (Kiatkamjornwong *et al.*, 2007; Dirmal *et a.,l* 2007, Kirbas *et al.*, 1999; Arboleda *et al.*, 2004 and Shalini *et al.*, 2014). Since it is known that the degradation of polymer can proceed 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., that the mechanisms of polymer degradation can be determined by measuring the levels of ketone carbonyl, ester carbonyl and internal double bond absorbance peaks said by (Sudhakar *et al*., 2008).

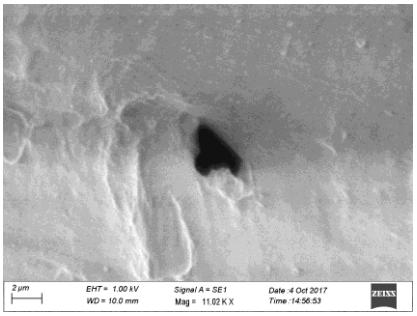
VI. SCANNING ELECTRON MICROSCOPY ANALYSIS

H V Sowmya *et al.*, 2014 states that the analyzed degraded products showed formation of cavities and erosion. In our work, also LDPE treated with selected microbes shows the formation of cavities and erosions. Revealed the presence of cracks and fungal and bacteria growth in the LDPE materials. Increasing the degradation time increased the cracks Mona gounda *et al*., 2012. Before processing, the surface has a smooth surface with no cracks. The surface changes made on LDPE pieces were analyzed through scanning electron microscopy in different magnification and results were predicted in figure 5. However after incubation with the selected microorganisms surface erosion and the formation of pits and cavities on the surface of the samples can be observed (Atefeh Esmaeilli *et al*., 2013). Kawai *et al* 1995 suggested that the strong pressure caused by growth of the roots of fungi and bacteria leads to such crack formation in the case of biodegradation.

Figure 5. SEM analysis for monoculture of *Acrophialophora fusispora* in various incubation periods for biodegradation of LDPE under mineral salt media:



A



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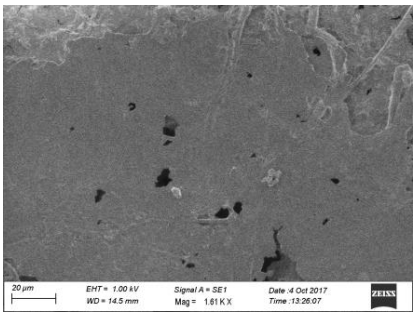

C

A: 3 and 6 month of incubation period of *Acrophialophora fusispora* + *LDPE* material in MSM

B: 9 month of incubation period of *Acrophialophora fusispora* + *LDPE* material in MSM
C: 12 month of incubation period of *Acrophialophora fusispora* + *LDPE* material in MSM

CONCLUSION

From this investigation, we concluded that *Acrophialophora fusispora* is able to grow well MSM with LDPE. Weight loss method clearly proved the percentage of degradation. The FTIR analysis again conformed, where some new bond appears and some bond get deformation also predict the same result above said. Finally SEM analysis clearly exhibited that microbes grown the film makes surface changes like cracks, erosion and formation of pits. Further it proves that microorganisms have the ability to degrade low density polyethylene material when it cultured in MSM and kept for long time incubation period.

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REFERENCES

[1] Abou-Zeid, D.M., Müller, R.J. and Deckwer, W.D. 2001. *Anaerobic biodegradation of natural and synthetic polyesters, Dissertation*, Technical University Braunschweig, Germany.

[2] Arboleda, C.E., Mejıa, A.I.G., Lo´pez, B.L.O. *2004*. Poly (vinylalcohol-coethylene) biodegradation on semi solid fermentation by *Phanerochaete chrysosporium*. *Acta Farm Bonaer*.23:123–128

[3] Atefeh Esmaeili1., Ahmad Ali Pourbabaee., Hossein Ali Alikhani., Farzin Shabani. and Ensieh Esmaeili. 2013. Biodegradation of Low-Density Polyethylene (LDPE) by Mixed Culture of *Lysinibacillus xylanilyticus* and *Aspergillus niger* in Soil. *PLOS one.* 8:1-10.

[4] Augusta, J., Muller, R.J. and Widdecke, H. A. 1993. rapid evaluation plate-test for the biodegradability of plastics. *Appl. Microbiol. Biotechnol*. 39: 673-678.

[5] Carmichael, V. J. W. and Bryce, K.W. 1980. Hyphomycetes. *The University of Alberta Press*. 212-224.

[6] Drımal, P., Hoffmann, J and Druz bı k, M. 2007. Evaluating the aerobic biodegradability of plastics in soil environments through GC and IR analysis of gaseous phase. *Polym Test*. 26:729–741

[7] Domsch, K. H. and Games, W. Compedium of soil fungi. IHW-Verlag. 1993.63-67.

[8] Gilan, I., Hadar, Y. and Sivan, A. Colonization, biofilm formation and biodegradation of polyethylene by a strain of Rhodococcus ruber.2004. *Appl Microbiol Biotechnol* . 65: 97–104.

[9] Ibiene, A.A., Stanley, H.O and Immanuel, O.M. 2013. Biodegradation of polyethylene by Bacillus Sp. Indigenous to the Niger delta mangrove swamp. Nigerian Journal of Biotechnology. 26: 68-79.

[10] Ikada, E. *1999.* Electron microscope observation of biodegradation of polymers. *Journal of Environmental Polymer Degradation.*7: 197-201

[11] Kiatkamjornwong, S., Sonsuk, M., Wittayapichet, S., Prasassarakich, P. and Vejjanukroh, P.C. 1999. Degradation of styrene-g-cassava starch filled polystyrene plastics. *Polym Degrad Stab.* 66: 323–335.

[12] Klrbas, Z., Keskin, N. and Gu¨ner, A. 1999. Biodegradation of polyvinylchloride (PVC) by white rot fungi. *Bull Environ Contam Toxicol.* 63: 335–342.

[13] Kiatkamjornwong,S., Thakeow, P and Sonsuk, M. 2017. Chemical modification of cassava starch for degradable polyethylene sheets. Polym Degrad Stab. 73(2): 363-375

[14] Kawai, F. 1995. Breakdown of plastics and polymers by microorganisms. *Adv Biochem Eng Biotechnol*. 52:151–94

[15] Kyaw, B. M., Chmpakalakshmi, R., Sakharkar, M. K., Lim, C. S. and Sakharkar, K. R. *2012.* Biodegradation of low density polythene (LDPE) by *Pseudomonas* species. *Indian Journal of Microbiology*.52 : 411–419.

[16] Nupur O., Neha P., Surjit S., Anil B., Anamika S., Pradip K., Vivek R., and Sutapa B. 2017.

Evaluation of HDPE and LDPE degradation by fungus,implemented by statistical optimization. *Scientific Reports*.. 1-13

[17] Nishida, H. and Tokiwa, Y. 1993. Distribution of poly (β-hydroxybutyrate) and poly (ε-caprolactone) aerobic degrading microorganisms in different environments. *J Environ Polym Degrad*. 1: 227–33.

[18] Merina P.D., Santosh K. 2013. Kinetics study for heat tolerance of LDPE degrading strains. *International Journal of Pharma and Bio Science.* 4:386-391.

[19] Merina P.D., Santosh K. 2015. An approach to low density polyethylene biodegradation by *Bacillus amyloliquefaciens*. *3 Biotech.* 5: 81-86.

[20] Mona, K., Gouda., Azza, E., Swellam., and Sanaa, H. Omar. 2012. Biodegradation of Synthetic Polyesters (BTA and PCL) with Natural Flora in Soil Burial and Pure Cultures under Ambient Temperature. *Research Journal of Environmental and Earth Sciences.* 325-333.

[21] Sudhakara, M., Mukesh Doblea, P., Sriyutha Murthyb, R. and Venkatesan. 2008. Marine microbe-mediated biodegradation of low- and high-density polyethylenes. *International Biodeterioration & Biodegradation.* 61: 203–213.

[22] Vatseldutt, S. and Anbuselvi. 2014. Isolation and characterization of polythene degrading bacteria from polythene dumped garbage. *Int. J. Pharm. Sci*.25: 205 206