Ecofriendly bio-degradation of low density polyethylene materials by using a screened *Rhodococcus rubber* from Tiruchirappalli District

SHALINI, R[1*] AND SASIKUMAR, C[1]
[1]Department of Biotechnology, Nehru Memorial College (Autonomous), Puthanampatti-621007, Tamil Nadu, India.

ABSTRACT: ***These articles expose the bio-degradation of low density polyethylene materials by Rhodococcus rubber and proven its degradation efficiency through insilico method. LDPE sheets were immersed in MSM along with Rhodococcus species and kept for different period of incubation, after that LDPE sheets were collected from the MSM (mineral salt media) and washed with ethanol and air drayed. The collected sample were undergone to analyzes the rate of bio-degradation and morphological changes through Weight loss method and FTIR,SEM analysis and its degradation efficiency is proven via insilico method.***

KEYWORDS: Rhodococcus rubber, SEM, FTIR, LDPE and insilico

Introduction:

Being a versatile, light weight, strong and potentially transparent material, they are ideally suited for a variety of applications. Their low cost, excellent oxygen/moisture barrier properties, bio-inertness and light weight make them excellent packaging materials (Andrady, 2011). Andrady and Neal, 2009. Estimated about 500 billion to 1 trillion polyethylene bags are consumed worldwide, which is about over one million a minute and most of them end up in the dustbin in a few minutes. Ohtake *et al*., 1998, states that it takes about 300 years to degrade LDPE films. It evokes big ecological issues. Because the efficient discarding of these polyethylene materials is a herculean task as they are not easily degradable and pose detrimental effects on the environment(Njeru, 2006). To overcome this issues Da Luz *et al*., 2014., Some physical and chemical methods are being employed to degrade LDPE, but it did not showed as satisfactory results. Thus, attention has been focused on alternative means of degrading the polyethylene material to bring out by the action of microbes (Sadocco *et al.,* 1997). So far that; biological methods brag to be eco-friendly however they are not expenditure.

The current study has also made an attempt to degrade LDPE by bacteria isolated from Tiruchirappalli garbage area. To analysis the percentage of degradation, several analytical methods have been used in the degradation of the LDPE materials. Our study revealed an Ecofriendly degradation of LDPE materials from Tiruchirappalli District to make a pollution free state by using bacteria species catalyst by enriched techniques.

MATERIALS AND METHODS

Study location and collection of sample:

The sampling spot was located in Tiruchirappalli District. It was highly contaminated with polyethylene waste due to huge population and their need in need. Soil sample is collected from the spot (garbage) area and stored in zip lock cover and stored in a refrigerator at 4c for further studies.

Identification of bacterial isolates for the biodegradation of LDPE material:

Each selected bacteria were identified based on three colony morphological characters, three microscopic characters and by twelve various biochemical studies as suggested by Cappucino and Sherman (1999).

Isolation and maintenance of pure culture

After successful growth of the microorganisms, each single colony was identified (based on colony morphology and colour) and re-streaked as primary inoculants on the surface of the NA media (bacteria). The plates were then incubated at 27 ± 2°C for 1 to 2 days and stored at low temperature (4 ± 1^{0}C) for further use.

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

The agar plate is emulsified with LDPE, a pure bacteria culture were spreaded over it and then, incubate the plate at 30^o c for 2 to 5 days, a clear zone is observed around it {modified method of (Augusta *et al.,* 1993)}, the zone of clearance is measured with scale. Based on diameter of zone the microbes were screened for further degradation studies.

Low density polyethylene

Fresh low density polythene carry bags were used for this study and they were obtained from the paper market of Thiruchirappalli, Tamil Nadu. LDPE 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.

Inoculation of Polythene Strips into Mineral salt medium:

LDPE bags were cut into small strips (each 3cm size) and inoculated in to the mineral salt medium and kept it for 3,6,9 and 12 month of incubation periods.

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:

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

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. 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.

Scanning Electron Microscopy analysis:

The treated samples after period of incubation was washed with 2 % (v/v) aqueous SDS and distilled water for 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 analyzed under high-resolution scanning electron microscope.

Bioinformatics approach- Pach Docking

The effectively degrading microbes were collected from Protein data bank and save as in Pdb format. After that Low density polyethylene structure were drawn with the help of Chemdraw (version 13 Cambridge soft corporation (US) and saved as in MDL MOL file format. The microbes act as a receptor and Low density polyethylene materials act as a ligand, Both the receptor and ligand structures were docked with the help of pachdock (docking tool). The scoring function employed was based on the level of docked poses were calculated.

Results and discussion

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

After inoculation with microorganisms, the formation of a clear halo zone around the colony indicates that the organisms are at least able to de polymerize the polymer, which is the first step of biodegradation. This method is usually applied to screen organisms that can degrade a certain polymer (Nishida and Tokiwa, 1993; Abou-Zeid, 2001), Growth was started within the 5-7 days at respective incubation temperatures. Initially an opaque zone was observed around the colony, however its growth rate is slow at 12 days at 30c. The gradual increase in zone formation around the colony was measured in centimeters with the ruler every day. Augusta *et al* 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. The gradual increase in zone formation around the colony was measured in centimeters with the ruler every day. In our result the identified *Rhodococcus rubber* formed 1.9 cm diameter of zone around the colonies predict in table 1 and figure 1a. These zones indicate that they have the ability to utilize the LDPE as a sole carbon source in the medium

Figure 1a. Clear zone of *Rhodococcus rubber*, Figure 1b. Monoculture of *Rhodococcus rubber* in 12 month of incubation periods for biodegradation of LDPE under mineral salt media

Table 1. Diameter of clear zone of *Rhodococcus rubber.*

S.No	Name of the Organisms	Diameter of Zone
1	***Rhodococcus rubber.***	**1.9cm**

Monoculture of *Rhodococcus rubber* in various incubation periods for biodegradation of LDPE under mineral salt media:

After that LDPE strips were inoculated into the mineral salt medium and kept it for 3, 6, 9 and 12 months of incubation periods (Figure 1b). After the incubation period the strips were collected and cleaned with ethanol and air dried.

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.

Weight loss method:

After the degradation period, the LDPE films were treated with ethanol as surfactant which denature the cells and completely washed off from the surface. The reduction in weight was observed after the biodegradation (Merina Paul Das and Santhosh kumar 2015). Mona goundar *et al*., 2012 defined as degraded of polyethylene is a surface erosion process. Based on this the following weight loss percentage was determinate using the following formula followed by Wang *et al*., 2006. :

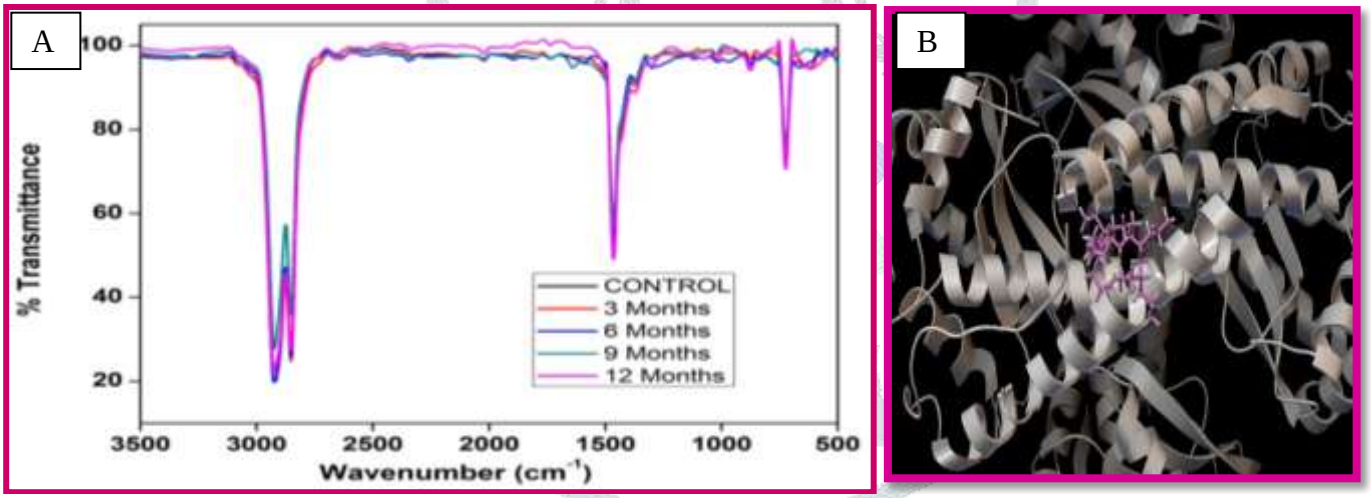
$$\text{Weight loss (\%)} = \frac{\text{Initialweight} - \text{Finalweight}}{\text{Initial weight}} \times 100$$

Based on weight loss percentage, after three months it shows that 0.2% of degradation rate in *Rhodococcus rubber* and 18% of degradation was observed in 12 months of incubation periods. They were predicting 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	*Rhodococcus rubber*. + LDPE	02%
2	6 month		07%
3	9 month		12%
4	12 month		18%

Figure 2a. FTIR analysis for monoculture of *Rhodococcus rubber* in various incubation periods for biodegradation of LDPE under mineral salt media, Figure 2b. *Rhodococcus rubber* docked with LDPE materials via Patchdock



FOURIER TRANSFORMS INFRARED (FTIR) SPECTROSCOPIC STUDIES:

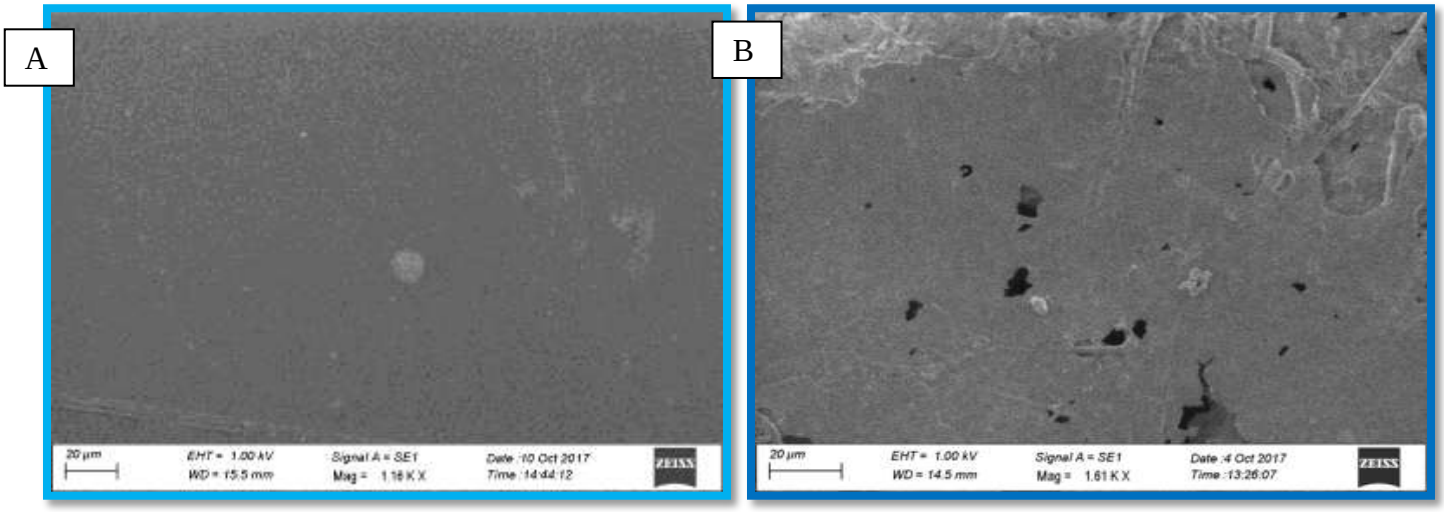
Fourier Transform Infrared spectroscopy analysis was used for detecting the formation of new functional groups or changes in the amount of existing functional groups or change in the amount of existing functional groups (Milstein *et al.*, 1994). It is used as an analytical techniques in many biodegradation studies (Kiatkamjornwong *et al.*, 2007; Dirmal *et a.,l* 2007, Kirbas *et al.*, 1999; Arboleda *et al.*, 2004 and Shalini *et al.*, 2014).

The FTIR spectra of pre- treated polythene film in Mineral salt medium shows the following peaks. The characteristic absorption bands were at 719 cm^{-1} (C-H bend-mono), 1,472 cm^{-1} (C=C stretch), 2,660 cm^{-1} (CHO stretch) and 2,919, 2,850 cm-1(both due to C-H stretch).After the incubation periods, some bond get stretched and some new bond get appeared. A reduction in CI was observed from 6 month of incubation period of LDPE materials. (Figure 2a)Carbonyl peak was found with respect to the internal bond absorbance.FTIR results showed the formation of ketone, aldehyde, carboxylic acids, alcohol, phenol, ester and aromatic compounds at different frequencies indicating their degradation by selected microbes. A band around 1461-1466 cm^{-1} reveled a bending deformation and another band at 720-724 cm-1 indicates a rocking deformation. The carbonyl band corresponds to the ketone and ester carbonyl groups and it is a typical product of oxidative degradation of polythene (Gilan *et al*., 2004, Hadad *et al*., 2005, Ibiene., 2013). The methane C-H exhibits C-H stretching and bending vibration at 2890 and 1340m1 respectively and are weak having no practical utility in structural elucidation, The Methylene group, however exhibits the two bands near 2924 and 2850 cm^{-1} respectively with slight modification made by microorganisms. An isolated hydrogen in m-DI, unsymmetrical tri-symmetric tetra-, un -symmetric tetra and Penta substituted benzene exhibits absorption in the region 890-835 cm^{-1}

SCANNING ELECTRON MICROSCOPY ANALYSIS:

The SEM analysis was performed to monitor the changes in the surface of the films. The adhesion of microorganisms to the polymeric surface is fundamental for biodegradation to take place. The changes in surface morphology of the LDPE films were investigated after 3month, 6month, 9month and 12month of biotic exposure, after removal of LDPE in MS medium and kept it for a few hours to dry. The surface changes on the LDPE materials were analyzed after washing with ethanol. The microbial degradation of polyethylene is caused by certain enzymatic activities that lead to a chain cleavage of the polymer into oligomers and monomers (Figure 3).

Figure 3. SEM analysis for monoculture of *Rhodococcus rubber* in various incubation periods for biodegradation of LDPE under MSM media; A: Control (LDPE), B: 12 month of incubation period of *Rhodococcus rubber* +*LDPE* material in MSM



From the above SEM image showed that cracks (even it visible at lower magnification) and cavity formation while it intake LDPE sheets. At three month period of incubation times, microbes start to penetrate the edges of the LDPE materials in MSM (mineral salt medium).After the period of 6 months, it secretes their enzyme which began to break down the LDPE molecules and cleavage occurred in the polyethylene chains. Whereas 12 months of incubation periods, the microorganisms need some source to alive for long life, so far that they started to take LDPE as carbon source, and produced the end product like CO_2, H_2O and CH_4

Docking studies of LDPE degrading bacteria with authentic LDPE materials

The research further focused on docking studies. It plays an important role in the rational design of drugs as it predicts the intermolecular complex formed between two or more molecules. Sinoshskariya *et al*., 2014. The ligand and enzyme interaction were carried by molecular docking, the three dimensional structure of LDPE was drawn with the help of Chem. draw software. It was docked with the receptor *Rhodococcus rubber*. were retrieved it from PDB (protein data bank). The docking score was obtained as 5850 (Figure 2b).

Summary and conclusion:

The goal of this research is to provide deeper understanding of the microorganism mediated mechanisms of catalysis of LDPE will facilitate the development of new methods to enhance the biodegradation at contaminated sites. From the research findings, we conclude that the microorganisms tested for biodegradation of LDPE, *Rhodococcus rubber* under mono culture conditions were efficiently degrade the LDPE. Furthermore, our results revealed that the biological treatment of soil contaminated with LDPE should be a more efficient, financially affordable and adaptable choice than physicochemical treatment, because it provides potential advantages such as the complete degradation of the pollutants, in naturally at lower cost treatment with greater safety**.** The effectively degrading microbes were docked with LDPE materials However, further research is needed to enhance it efficiency for effective degradation with suitable carrier materials for the large scale production with low cost technology. This will facilitate the LDPE degradation and make pollution free India within a short period.

ACKNOWLEDGEMENT

We thank the Management and the Principal of Nehru Memorial College (Autonomous), Puthanampatti, Thiruchirappalli, Tamil Nadu for providing laboratory facilities to carry out this investigation.

REFERENCES

[1] Andrady, A.L. 2011, 'Microplastics in the marine environment'. *Marine Pollution Bulletin*. 62: 1596-1605.
[2] Njeru, J.2006. The urban political ecology of plastic bag waste problem in Nairobi. Kenya. *Geoforum*. 37:1046–1058.
[3] Da Luz JMR., Paes, S.A., Bazzolli, D.M.S., Totola, M.R., Demuner, A.J. and Kasuya, M.C.M.2011. Abiotic and biotic degradation of oxo-biodegradable plastic bags by *Pleurotus ostreatus*. *PLoS ONE*.
[4] Sadocco, P., Nocerino, S., Dubini-Paglia E., Seves, A. and Elegir, G.1997. Characterization of a poly (3-hydroxybutyrate) depolymerase from *Aureobacterium saperdae*: active site and kinetics of hydrolysis studies. *J. Environ Polym Degrad*. 5:57–65.
[5] 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.

[6] 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.
[7] Ikada, E. *1999.* Electron microscope observation of biodegradation of polymers. *Journal of Environmental Polymer Degradation.*7: 197-201
[8] Tandon, HLS(Ed.). 2005. Methods of analysis soils, plants, fertilizers and organic manures. Fertilizers development and Organization, New Delhi, India. pp.204 + xii.
[9] Cappuccino and Sherman.1999. Microbiology. A laboratory Manual. Dorling Kindersley Pvt. Ltd. India. 507pp.
[10] 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.
[11] Abou-Zeid, D.M., Müller, R.J. and Deckwer, W.D. 2001. *Anaerobic biodegradation of natural and synthetic polyesters, Dissertation*, Technical University Braunschweig, Germany, Internet: http://opus.tu-bs.de/opus/volltexte/2001/246
[12] 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
[13] 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.
[14] Merina P.D., Santosh K. 2015. An approach to low density polyethylene biodegradation by *Bacillus amyloliquefaciens*. *3 Biotech.* 5: 81-86.
[15] 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
[16] Kiatkamjornwong,S., Thakeow, P and Sonsuk, M. 2017. Chemical modification of cassava starch for degradable polyethylene sheets. Polym Degrad Stab. 73(2): 363-375
[17] 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.
[18] Arboleda, C.E., Mejıa, A.I.G., Lo´pez, B.L.O. Poly (vinylalcohol-coethylene) biodegradation on semi solid fermentation by *Phanerochaete chrysosporium*. *Acta Farm Bonaer. 2004*..23:123–128
[19] 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
[20] Sinosh Skariyachan, M., Megha., Meghna Niranjan., Kini Kamath., Manali Mukund., Alya Rizvi., and Kiran Vasist. 2015. Selection and screening of microbial consortia for efficient and ecofriendly degradation of plastic garbage collected from urban and rural areas of Bangalore. *India Environ Monit Assess*. 187: 4174-
[21] 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.
[22] Klrbas, Z., Keskin, N. and Gu¨ner, A. 1999. Biodegradation of polyvinylchloride (PVC) by white rot fungi. *Bull Environ Contam Toxicol*. 63: 335–342.