

**Biodegradation of Low Density-Polyethylene (LDPE)
by biosurfactant producing bacteria isolated
from plastic polluted dumpsites**

**SUMMARY
of
THESIS**

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Petroleum based polymers are used widely in number of applications due to their excellent thermal and physico-chemical properties. Among all the polymers, low-density polyethylene (LDPE), which is a thermoplastic, is used commonly. Polyethylene is used in large quantity for the manufacture of carry bags, bottles, milk packets, garbage containers etc. Low cost of LDPE and its bio-inertness nature are among the prime reasons for its usage as a packaging material. However, the accumulation of LDPE waste in large amount is imposing a great threat to the terrestrial as well as aquatic organisms. LDPE also causes environmental pollution because it is resistant to biodegradation.

Several other chemicals are also used with the manufacturing of such plastic to provide it certain characteristic such as additives, phthalates and flame-retardants. They all have been identified as harmful chemicals, which affects the health of human, and animal by interrupting with their endocrine system. So, the problem of white pollution or plastic pollution has been a major concern for the mankind. Almost, all the parts of the world are suffering with negative consequences of the plastic pollution.

In the current era of globalization some efforts should be made to develop the alternative ways for dealing with such plastic waste, which have eco-friendly approach. So, in relation to the aforesaid context, for dealing with the degradation of LDPE, the objectives designed for the study entitled “**Biodegradation of Low Density-Polyethylene (LDPE) by biosurfactant producing bacteria isolated from plastic polluted dumpsites**” were:

1. Isolation and screening and characterization of biosurfactant producing bacteria isolated from plastic polluted dumpsite.
2. Optimization, characterization and production of biosurfactant.

3. Evaluation of LDPE degradation by biosurfactant and its producing bacteria.
4. Evaluation of LDPE degradation under controlled composting.
5. Analysis of the chemical alterations induced in the LDPE exposed to chemical surfactants.
6. Analysis of the photo-oxidation of LDPE.

Initial objective was to isolate, screen and characterize the biosurfactant-producing bacteria. In this study plastic samples were collected from plastic polluted dumpsites. They were then used for isolating bacteria. In total, fifteen bacteria were isolated among which two bacteria were found to be the active producer of biosurfactant. These two bacteria named as ENV 1 and ENV 4 were then subjected to molecular identification. The analysis identified ENV1 as novel strain of *Bacillus* sp. and it was named as *Bacillus* sp strain 530F_seq260_Env1 whereas, ENV 4 was identified as the novel strain of *Lysinibacillus* sp and it was names as *Lysinibacillus* sp strain DESBBAU2. These two bacteria were found to be positive for the emulsification index test, oil spreading test and phenol-sulfuric acid test, which are the major test for detecting the production of biosurfactant. The morphological characteristic for both the bacteria showed that they were gram positive and rod shaped. For the biochemical test performed, *Bacillus* sp. was found to be positive for protease, amylase, catalase and glucose whereas *Lysinibacillus* sp was found to be positive for oxidase and laccase.

Second objective was to produce, optimize and characterize the biosurfactant. In optimization study, the optimized parameter for *Bacillus* sp were- pH: 6, temperature: 30 °C, Peptone concentration: 10 mg/l, Beef extract concentration: 10 mg/l. For *Lysinibacillus* sp, optimized parameter were- pH: 8, temperature: 30 °C, Peptone concentration: 5 mg/l, Beef extract concentration: 5 mg/l. The characterization of the both biosurfactant extracted from the bacteria showed the total lipid content of 53 mg

(71.62%) in the biosurfactant produced by the *Bacillus* sp strain 530F_seq260_Env1 and 59 mg (75.64%) in the biosurfactant produced from *Lysinibacillus* sp strain DESBBAU2. The total protein content of biosurfactant obtained by *Bacillus* sp strain 530F_seq260_Env1 was 5.981 µg/ml and that by the *Lysinibacillus* sp strain DESBBAU2 was 6.94 µg/ml. The FTIR analysis showed major functional groups such as O-H stretching, stretching of unsaturated C=C bonds, stretching due to carbonyl (C=O) groups, ester functional group, bending of the hydroxyl group (OH) and Sugar C-O stretch, which are the characteristic functional group depicting the biosurfactant. The EDX analysis showed the carbon content of 60.22% and 70.01% in *Bacillus* sp. biosurfactant and *Lysinibacillus* sp biosurfactant respectively, whereas, the oxygen content was 38.9% and 17.1% for *Bacillus* sp biosurfactant and *Lysinibacillus* sp biosurfactant respectively. TGA results revealed that for biosurfactant that was extracted from *Lysinibacillus* sp, the degradation temperature was 255°C and final decomposition temperature was 450°C. The DSC analysis showed that the melting temperature of *Lysinibacillus* sp extracted biosurfactant is greater than the *Bacillus* sp extracted biosurfactant. The DSC data reveals that the biosurfactant of *Lysinibacillus* sp is more thermostable which is important for a biosurfactant to be used in commercial application.

For the production of biosurfactant, the yield of biosurfactant obtained from *Bacillus* sp and *Lysinibacillus* sp was 1.36 gm/l and 1.48 gm/l respectively.

Third objective was to evaluate the LDPE degradation by the biosurfactant and its producing bacteria. The two isolated bacteria alongwith their extracted biosurfactant were tested for their potential to degrade LDPE. Different types of analysis like weight reduction test, FTIR, XRD, SEM-EDX, TGA and DSC was performed on the LDPE samples after the treatment to identify the changes occurred in the morphological and

chemical structure of the polymer. The different combinations of bacteria and biosurfactant along with the LDPE as a substrate were maintained in the separate conical flasks for both the bacteria. The different combinations were: (i) Bacteria and its extracted biosurfactant in dried form along with the LDPE in nutrient broth as a medium (ii) Bacteria and LDPE in nutrient broth as a medium (iii) extracted biosurfactant in dried form and LDPE in nutrient broth as a medium (iv) LDPE only in nutrient broth medium. In all the combinations of experiment performed separately for *Bacillus* sp and the *Lysinibacillus* sp the LDPE that was treated with bacteria along with its biosurfactant showed better degradation. Through EDX analysis carbon content was measured which revealed the major reduction of carbon after the degradation. *Bacillus* sp and its biosurfactant treated LDPE showed 73% of carbon and *Lysinibacillus* sp and its biosurfactant treated LDPE showed 58% of carbon content after the degradation, which is lesser than that of untreated LDPE for which the carbon content was 93%. The TGA and DSC analysis showed the lowest decomposition temperature and melting temperature for the LDPE treated with bacteria and biosurfactant together.

Fourth objective was to evaluate the LDPE degradation under controlled composting. In this experiment different concentration of compost with soil was made. The different concentrations of the compost made are: 20%, 40%, 60%, 80% and 100% (w/w). The total amount of compost and soil mixture was 1 kg for the different concentrations of compost. The compost mixture was transferred to the different pots in which LDPE films were buried for the period of 90 days. After treatment period, LDPE films were recovered from the compost mixture and then subjected to various analysis. Significant change of degradation as shown by the analysis post treatment was observed in LDPE treated with 80% compost mixture. Maximum weight reduction of approximately 17% was observed in 80% compost treated LDPE sample followed by 100%, 60%, 40% and

20% compost mixture treated LDPE samples. FTIR analysis performed on the samples revealed the lower crystallinity percent (65%) and higher carbonyl (0.2) and double bond index (1.8) in case of LDPE subjected to 80% compost mixture. SEM analysis showed maximum distortion in case of 80% compost mixture treated LDPE sample. EDX analysis showed lowest carbon weight percent (46%) and highest oxygen weight percent (50%) in case 80% compost mixture treated LDPE. In XRD analysis the comparison of degradation for different LDPE samples was done by comparing the intensity of X-ray at peaks 21° and 23.5° . So, the lowest intensity at the two peaks i.e., 21° and 23.5° was recorded in LDPE subjected to 80% compost mixture. DSC analysis showed highest onset temperature (395°C) and melting temperature (472°C) for the 80% compost treated LDPE. TGA analysis revealed lowest onset (403°C) and final decomposition (486°C) temperature for the LDPE treated with 80% compost.

Fifth objective was to analyse the chemical alterations induced in the LDPE exposed to chemical surfactants. For this experiment, LDPE was subjected to three chemical surfactant which are: Sodium dodecyl sulphate-SDS (anionic), cetyl trimethylammonium bromide-CTAB (cationic), and Tween 80 (non-ionic). Solution of all the three surfactants in various concentrations ranging from 2 to 5% w/v was made separately. The LDPE samples were then transferred to these solutions in the conical flask and kept for chemical treatment at a temperature of 60°C for the period of one month. After the treatment period of one month LDPE films were recovered and subjected to various analysis. From FTIR analysis highest keto carbonyl index (0.48) and double bond index (0.37) was observed in 10% SDS treated LDPE. In case of XRD analysis, maximum number of peaks emerged in LDPE treated with 10% SDS. EDX analysis revealed the carbon and oxygen weight percent of all the LDPE samples. The analysis showed the lowest carbon weight percent (70%) and highest oxygen weight percent (25%) in case of LDPE treated with

10% SDS. So, the maximum change post degradation was observed in LDPE that was treated with 10% SDS.

Sixth objective was to analyse the photo-oxidation of LDPE. The degradation study was done in the presence of sunlight in natural environment. The LDPE sheets cut into a specific dimension was kept over the terrace of a building so that it can be exposed to the maximum intensity of solar radiation for the period of 90 days. The LDPE films were recovered after the period of 90 days and various analyses was performed to check the degradation. SEM analysis signified that solar radiation altered the surface morphology of the polymer. Cracks and grooves were seen. The increased carbonyl index examined by FTIR analysis confirmed the photo oxidation of polymer upon exposure to sunlight. While changes in the elemental composition of the polymer confirms the breakage in the polymer chain. XRD analysis showed the emergence of new peaks in LDPE exposed to the sunlight. TGA and DSC have also shown the change in the thermal behavior of the LDPE after exposure to sunlight. that once the polymer gets brittle their resistance power gets decreased and microbes can make use of the carbon in the polymer chains for their growth and development. The study performed will contribute in the management of the plastic waste in efficient and cost-effective manner. The final melting temperature for the sunlight exposed LDPE was 162°C while that for unexposed one was 138°C. The increase in the temperature can be due to the crystalline region that was left behind after the disruption of amorphous region. The weight reduction obtained over the period of 90 days was 15.67%. So, the above analyses showed that upon exposure to sunlight major changes occur in the molecular structure of LDPE.

In the study basically three forms of LDPE degradation *i.e.*, biodegradation, chemical degradation and physical degradation were compared. The data obtained showed that

biodegradation of the LDPE is the better option for dealing with the LDPE waste in an eco-friendly manner. Under biodegradation two methods were used viz, composting and bacterial degradation using biosurfactant. Between these two methods, LDPE degradation using the two isolated novel strains of *Bacillus* sp strain 530F_seq260_Env1 and *Lysinibacillus* sp strain DESBBAU2 along with their biosurfactant proved better degradation of the LDPE samples compared to composting. Further the isolation of more such strains of bacteria capable of producing biosurfactant could be a promising way towards the easy and better degradation of LDPE.