SUMMARY AND CONCLUSION

Plastics, the lightweight and long-lived material has potential application in day to day life. Polyethylene, the most common petroleum-based plastic, is the principal type of plastic used today due to its easy processibility, excellent chemical resistance, high durability, flexibility, and freedom from odor and toxicity.

The amount of plastic waste in municipal solid waste has grown progressively in recent years. This enormous plastic solid waste remains a challenging task in many countries. Accumulation of plastic waste has become a serious environmental hazard. Municipal corporation in many developing countries like India, view the carry bags made of polyethylene as the chief pollutant behind the environmental deterioration.

The incineration of plastics has been criticized because it generates large amounts of bottom ash and various toxic air pollutants, like polycyclic aromatic hydrocarbons, as well as dioxins in case of halogen-containing plastics. The recycling of plastic solid waste is a highly desirable way for resource, energy conservation and reduction in waste gas emissions. But the recycling of plastics is not used extensively because of lack of suitable infrastructure for sorting, processing and composting.

To maintain sustainable environment, biodegradation approach is safe and ecofriendly. Microbial strains naturally present in contaminated soils are capable of biodegrading organic pollutants. The isolation and characterization of polyethylene degrading microorganisms from the contaminated source and a better understanding of the enzymatic system involved in polyethylene degradation could be helpful in the development of remediation approaches for plastic wastes, which could eliminate plastic pollution.

The present study aimed to isolate and identify a potent polyethylene degrading bacterial strain from the polluted soil and to study its degrading efficiency in a laboratory scale. The selected strain was further screened for the presence of enzymes such as laccase, manganese peroxidase and lignin peroxidase activity and biosurfactant production during polyethylene degradation. The bioremediation potential of the selected strain in low density polyethylene (LDPE) polluted soil under laboratory conditions was investigated.
The present research work was organized into four phases

Phase I of the study was conducted to isolate polyethylene degrading bacteria from soil samples collected at two different sites, which include petrochemical oil contaminated site and plastic waste disposal site. The bacterial strains were isolated by enrichment technique in a synthetic medium (SM) containing polyethylene as the sole carbon source and utilizing the soil samples as source of inoculum. The bacterial isolates were identified based on the morphological and biochemical characteristics. Cell surface hydrophobicity of the isolated bacterial strains was assessed. Further, they were screened individually for their low density polyethylene (LDPE) degrading efficiency by *in vitro* biodegradation assay. Among the isolated bacterial strains, the more potent strain was selected and its phylogeny was analyzed by 16S rDNA sequencing studies.

The efficiency of the potent strain to colonize on the LDPE surface was demonstrated in Phase II. The biofilm growth and viability of the selected bacterial strain were investigated. Biofilm imaging was performed to study the surface attached selected bacterial strain on LDPE. Further, degraded products of low density polyethylene were analysed by HPLC and GC-MS after the biodegradation study which was conducted for a long incubation period by inoculating the selected bacterial strain in SM with LDPE as the carbon source. After the incubation period, the viability of the selected bacterial strain on the surface of the low density polyethylene was confirmed by fluorescent microscopy and the biodegradation was assessed by FTIR analysis.

Phase III was designed to identify the role of extracellular secretory factors on polyethylene degradation by the selected strain. The extracellular production of ligninolytic enzymes and biosurfactant by the bacterial strain were investigated. Initially the selected bacterial strain was screened for ligninolytic enzymes such as laccase, manganese peroxidase and lignin peroxidase and biosurfactant production. Further, the activity of ligninolytic enzymes were determined during low density polyethylene degradation in SM supplemented with LDPE. The obtained crude biosurfactant was characterized by thin layer chromatography and FTIR.

Phase IV was performed to explore the bioremediation potential of the selected bacterial strain in low density polyethylene polluted soil using bioaugmentation strategy under laboratory conditions. Three soil model systems were developed wherein naturally attenuated, biostimulated, and selected bacterial strain inoculated (bioaugmented) soil samples were examined for their ability to degrade LDPE. LDPE films were added to
each of these microcosms and incubated for 60 days. After the incubation period, soil samples from each treatment were subjected to analysis for physicochemical parameters such as pH, electrical conductivity, available nitrogen, available phosphorus, available potassium and organic carbon. The LDPE films were removed from each treatment soil and subjected to FTIR analysis. During the incubation period the evolved CO₂ was measured.

Salient findings

Phase I

Five different bacterial strains (OS1, OS2, PE1, PE2 and PE3) were isolated from the polluted soil. The bacterial isolates OS1 and PE1 were identified as Micrococcus Sp, OS2 was identified as Pseudomonas Sp. and the isolates PE2 and PE3 were Bacillus Sp. The results of the invitro biodegradation assay showed that the bacterial isolates were able to grow in the SM containing LDPE. This suggested that these strains had the potency to use LDPE as the sole carbon source. The FTIR analysis of LDPE treated with the bacterial strains indicated the changes in the chemical properties of LDPE. Scanning electron microscopic results demonstrated that the surface of the treated film appeared to be rough when compared to control film after 30 days of incubation. Among the bacterial strains, the bacterial strain PE3 showed maximum growth, more cell surface hydrophobicity and weight loss and hence it was selected for further studies. The results of the phylogenetic analysis revealed that the 16S rDNA sequence of PE3 shared only 93% sequence similarity with Bacillus cytotoxicus. Hence the strain was designated as Bacillus sp.PE3.

Phase II

Biofilm growth quantification results and scanning electron microscopic images indicated that the strain Bacillus Sp.PE3 was able to colonize and form the biofilm on the surface of LDPE. Flourescence microscopic image of the biofilm on low density polyethylene surface after the long incubation period confirmed the viable biofilm of the Bacillus Sp. PE3 on LDPE. FTIR analysis revealed that the incubation of LDPE films with Bacillus Sp.PE3 led to the formation of new absorbance bands in the hydroxyl region and appearance of new C-O bands at carbonyl region which are assigned to aldehydes, ketones, and acid and ester groups. The degraded products were identified as carboxylic acids, alkanes and alkenes.
Phase III

The strain *Bacillus* Sp.PE3 gave positive results for laccase, manganese peroxidase, lignin peroxidase and biosurfactant production in the screening tests. During the degradation of polyethylene, the activities of these enzymes were observed in the media with *Bacillus* Sp.PE3 and LDPE. The clear band obtained for laccase and lignin peroxidase on native PAGE further confirmed the activity of laccase and lignin peroxidase during polyethylene degradation. Characterisation of the crude biosurfactant by thin layer chromatography and FTIR indicated the lipopeptide nature of the biosurfactant.

Phase IV

Significant changes were noticed in the physicochemical properties that were analysed in the soil samples from different treatments namely T1, T2 and T3. Respirometric analysis revealed that the carbon dioxide evolution was maximum in bioaugmented soil. It is evident from the FTIR analysis, that the values of KCBI, ECBI, VBI and DBI were increased in LDPE film samples taken from biostimulated and bioaugmented soil treatments when compared to the LDPE film taken from naturally attenuated soil. These results revealed the ability of *Bacillus* Sp.PE3 to degrade LDPE in polyethylene contaminated soil. From the results of the Phase IV, it is inferred that the exogenous addition of *Bacillus* Sp.PE3 to LDPE contaminated soil causes an enhanced degradation.

Conclusion

Bacteria capable of utilizing polyethylene as the carbon source were isolated from polluted soil and they were identified as species of *Micrococcus*, *Pseudomonas* and *Bacillus*. The bacterial isolate PE3 was found to possess more degrading efficiency than other bacterial isolates and it was identified as *Bacillus* species and designated as *Bacillus* sp. PE3. The physicochemical properties of the treated LDPE film, biosurfactant production and extracellular ligninolytic enzyme activities during degradation process suggested the degradation of LDPE by the isolated strain. The isolated *Bacillus* sp. PE3 holds considerable potential for degrading LDPE in polyethylene polluted soil. The data thus generated in the present study suggested that there is a possibility of identifying potent microbes from the environment which can degrade synthetic plastics efficiently.
Scope for further study

- In-depth mechanism of attachment of the bacteria with polyethylene surface can be studied.

- Gene regulatory mechanism and the mechanism of action of extracellular enzymes responsible for polyethylene degradation can be studied.

- The microbes responsible for polyethylene degradation can be isolated from other environmental sources and the efficient isolates can be characterized at molecular level. These strains can be applied for field trials. After field trials, the efficient strains must be multiplied at large scale in order to exploit them for commercial applications.

Limitations

- Although the present study has proved the biodegradation of polyethylene, the complete biodegradation of polyethylene could be achieved after a prolonged exposure to the organisms.

- The complete metabolic pathways involved in the biodegradation process and the role of all other enzymes associated in this process could not be studied.