Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
e-ISSN: 2395-3454

Vol. 17 | No. 2 | pp. 649-655 | 2018

Original Research Paper | Open Access

Comparative Biodegradation Studies of LDPE and HDPE Using *Bacillus weihenstephanensis* Isolated from Garbage Soil

R. R. Ingavale and P. D. Raut†
Department of Environmental Science, Shivaji University, Kolhapur-416004, Maharashtra, India
†Corresponding author: P. D. Raut

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 1-03-2018
Accepted: 14-03-2018

Key Words:
Polyethylene
Biodegradation
FT-IR spectrophotometry
Bacillus weihenstephanensis
Garbage soil

ABSTRACT

Polyethylene has achieved an inseparable place in our life due to its recalcitrant nature, durability and cost, especially as a packaging material. Attributable to its inactive nature and degradation resistant property, its collection in nature has turned out to be tremendous. In the present study, polyethylene degrading bacterium was isolated from the garbage soil from Kolhapur and screened under *in vitro* condition. Through the 16S ribosomal RNA gene partial sequence, the isolated bacterium was identified as *Bacillus weihenstephanensis*. Polyethylene sheets, only source of carbon, along with synthetic media were incubated on a rotary shaker at 30°C and 110 rpm for 6 months. The biodegraded samples of LDPE and HDPE exhibited weight loss (7.02% and 7.08%, respectively). The biodegradation of LDPE and HDPE sheets was further investigated through FTIR spectroscopy which has confirmed the weakening and breaking of existing bonds and also the formation of new functional carbonyl group (C-O) at 1262 cm^{-1}, 1745 cm^{-1} and 799 cm^{-1} which is a result of microbial activity.

INTRODUCTION

Polyethylene is a thermoplastic polymer which consists of elongated hydrocarbon chains and forms a base of polymer (Kumari et al. 2009). Polyethylene has been the most essential element of today's era due to its wide application including food cling wraps, grocery bags, detergent bottles, automobile fuel tanks, few to list, thus making it more popular worldwide. This is due to enhanced physical and chemical characteristics of polyethylene such as strength, light weight, resistance to water, low-price, light weight, robust, durable, non-rust material and high thermal and electrical insulation characteristics. Plastic waste thus generated induces its disposal problem leading to ecological disturbance. The durability, lightness and processability of polyethylene causes it to remain persistent in nature for hundreds of years and finally sent to dumping grounds and/or natural aquatic bodies (Jang et al. 2002). Hydrophobic property, high molecular weight and three-dimensional structure make the polyethylene recalcitrant in nature and thus they are not easily available to microorganisms (Hadad et al. 2005, Shah et al. 2009). Wide studies have been conducted for biodegradation of polyethylene blended with starch. However, if the biofilm is formed on the surface of polymer and utilized it as a nutrient or carbon source, the degradation would be more efficient (Albertsson et al. 1993, Shah et al. 2009).

The rate of degradation of polyethylene is quite slow if it is subjected to natural conditions and this has caused an immense threat to environment (Premraj & Dobley 2005). If the right microbial strain is isolated, polyethylene, considered to be inert, can be biodegraded (Pramila & Vijaya 2011). It is found that some of the microbes are potential to degrade polyethylene. They are *Acinetobacter baumannii, Brevibacillus parabrevis, Pseudomonas citronellolis, Mucor circenollides* (Pramila & Vijaya 2011), *Streptomyces* sp., *Bacillus* sp. and *Aspergillus* sp. (Usha et al. 2011). It was also found that polyethylene blended with starch was degraded fast by some fungi like *Streptomyces* sp. and *Phanerochaete chrysosporium* (Premraj & Dobley 2005).

In the present study low density polyethylene (LDPE) and high density polyethylene (HDPE) were used as they have wide applications in our daily life.

MATERIALS AND METHODS

Polyethylene: LDPE and HDPE were provided by Laxman Udyog Samuh, Plastics and Allied Industries, Gokul Shirgaon MIDC, Kolhapur in the form of pellets. They were later converted into thin sheets by melting and pressing in the laboratory. The prepared sheets were sterilized by dipping in alcohol for few minutes, and air dried. Proper precautions were taken so as to avoid contamination. During these procedures no physical changes were observed.

Before subjecting to degradation, initial parameters of LDPE and HDPE sheets were recorded. Initial thickness of LDPE and HDPE sheets was measured by using a microm-

eter screw gauge while the initial diameter was measured by a Vernier calliper. Also, initial weight of sheets was taken on a weighing balance.

Soil samples: The site was selected where garbage containing plastic waste has been dumped since years. Soil sample was collected from Kasba Bawda site of Kolhapur city where large amount of municipal solid waste is dumped with large amount of plastic waste. Land was dug at about 5 cm and soil was collected from the trapped plastic material and stored in the air-lock bags and later air dried at room temperature for further studies.

Isolation of microorganisms from soil: Enrichment technique was followed for the isolation of microorganisms from the garbage soil. The rationale behind the enrichment method was to make strong selective conditions utilizing powdered PE as the main source of carbon. The synthetic medium (Hadad et al. 2005) served as a growth medium for isolation of strains capable of growing on polyethylene as the sole carbon source. Composition of synthetic medium (SM) contains distilled water (dw): 1000 mL, NH_4NO_3: 1.0 g, $MgSO_4.7H_2O$: 0.2 g, K_2HPO_4: 1.0 g, $CaCl_2.2H_2O$: 0.1 g, KCl: 0.15 g, Yeast extract (Difco): 0.1 g, $FeSO_4.6H_2O$: 1.0 mg, $ZnSO_4.7H_2O$: 1.0 mg, $MnSO_4$: 1.0 mg. This medium was autoclaved at 121°C for 20 minutes. One gram of soil sample was added into sterilized 100 mL of synthetic medium into 500 mL flask. The enrichment medium was prepared by adding 0.1 g PE powder (LDPE and HDPE powder, separately) to 100 mL of growth medium. The flasks were kept for incubation at RT on shaker at 120 rpm for 6 weeks.

The initial isolation of microorganisms was performed in solid media (synthetic mineral medium-agar) containing PE powder as the sole source of carbon, FECs were subjected to serial dilutions (1:2, 1:5, 1:10) and plating out on culture media in which sole source of carbon was PE powder. Culture medium was autoclaved at 121°C for 15 minutes. After sterilization, medium was allowed to cool and poured in sterilized Petri plates. On solidification of plates, 0.2 mL of suspension from each dilution was streaked out on culture medium. These plates were incubated at 37°C for 1 week. After a week, the isolated colonies, colony number and colony characteristics were observed (Burd 2008).

Depending on the difference in morphology and colony characteristics, the isolates were purified by streaking single colony on solid medium. Thus, 3 bacterial and 1 fungal strains were obtained on purification and screening of available isolates. Among all, Isolate-2 was used for further analysis. It was sent to Agharkar Research Institute, Pune for molecular identification.

Submerged cultivation procedure: LDPE and HDPE sheets were exposed to submerged cultivation process for investigating the biodegradation of PE. Microbial degradation was performed in Erlenmeyer flasks in shaking condition. Each flask contained sheets of LDPE and HDPE and 100 mL of the synthetic medium.

Aliquots (100 mL) of synthetic medium were transferred into 500 mL Erlenmeyer flask and sterilized in an autoclave for 20 min at 121°C. On cooling, the medium was inoculated with a wire loop of Isolate-2. The fermentation broth was incubated at room temperature on a rotary shaker at 110 rpm. Set was incubated for 6 months for assessment of biodegradation of PE. PE sheets, which were the sole source of carbon in the medium. The control was maintained without inoculating any microbial strain. The set was maintained in triplicate.

Each month, LDPE and HDPE sheets were taken out from the flasks, washed with absolute alcohol followed by distilled water and air dried. On labelling, LDPE and HDPE sheets were subjected to measuring for final parameters like weight, thickness and diameter. After 6 months of incubation, sheets were sent for analysis of additional parameter FTIR (Fourier Transform Infrared Spectroscopy) at Common facility Centre (CFC), Shivaji University, Kolhapur. Finally, all the analysed parameters of LDPE and HDPE sheets were compared with the control.

RESULTS AND DISCUSSION

Identification of isolated microorganisms: Isolate-2 was identified by 16S rRNA gene sequencing technique at Agharkar Research Institute of MACS, Pune. Identification report of Isolate-2 by 16SrRNA gene sequencing approach shows closest phylogenetic affiliation to *Bacillus weihenstephanensis* (T) 16S ribosomal RNA gene partial sequence with 100% maximum identification in the phylogenetic tree of *Bacillus* sp. The bacterial strain was deposited with Gene Bank accession number CP000903. Satlewal et al. (2008) isolated bacterial strains from waste disposal sites and identified them as *Bacillus cereus, Bacillus pumilus* and *Arthrobacter* species by 16S rRNA sequencing technology. Soni et al. (2009) also identified the isolates as Bacterium Te68R, *Bacillus cereus, Proteobacterium* sp. and *Arthrobacter luteolus* on the basis of 16S rDNA sequences. Pramila et al. (2012) isolated bacterial strains from the soil which was collected from the municipal solid waste landfill area and by using 16S rRNA gene sequencing technique. They were identified as *Brevibacillus parabrevis, Acinetobacter baumannii* and *Pseudomonas citronellolis*. Mahalaxmi (2013) identified the bacterial and fungal isolates having potential to degrade PE. Confirmation of bacterial isolates was carried out based on 16s rDNA analysis and were identified as *Bacillus megaterium* and *Pseudomonas mediterranea* while the fun-

gal strains were identified as *Rhizopus arrhizus, Penicillium* species and the *Aspergillus* species.

Weight loss (%) of LDPE and HDPE sheets incubated with *Bacillus weihenstephanensis:* PE sheets treated with *Bacillus weihenstephanensis* showed reduction in weight. The maximum weight reduction was observed in 6th month of incubation i.e., 7.02% and 7.08% for LDPE and HDPE respectively (Fig. 1). Vijaya & Reddy (2008) noticed the weight loss of LDPE and HDPE as 11.01% and 3.68%, respectively after 12 months of incubation. Chonde et al. (2012b) have observed similar pattern of weight loss of nylon 6 sheets on incubating it with fungus *Phanerochaete chrysosporium* NCIM 1073 and recorded the weight loss of nylon 6 sheets from 0.013 g to 0.006 g after 75 days. Mukherjee & Chatterjee (2014) revealed 32.61% and 35.64% weight loss on treatment with *Bacillus weihenstephanensis* for thick and thin plastic, respectively after 6 months.

Seneviratane et al. (2006) have reported that *Bacillus* sp. is able to produce extracellular enzyme viz. oxidoreductase to adapt itself in the nutrient deficient environment. The presence of extracellular enzymes such as oxidoreductase could be the key variable for the biodegradation of LDPE and HDPE along with H_2O and CO_2, shorter hydrocarbons, alcohols, organic acids, ketones, aldehydes, etc. are also formed (Albertsson & Karlsson 1990). But reduction in the weight can be attributed to the usage of PE as a only way of obtaining carbon and energy. Hence, it may be expected that when the basal supplements from the medium were totally used by the microbes, polyethylene acted as the prompt source of carbon and energy (Nanda & Sahu 2010). Hadad et al. (2005) have also stated that carbon deficiency stimulates use of PE as a only carbon source leading to weight loss of PE strips.

Thickness reduction (%) of LDPE and HDPE sheets incubated with *Bacillus weihenstephanensis*: PE sheets treated with *Bacillus weihenstephanensis* also showed reduction in thickness. Both the LDPE and HDPE showed similar thickness loss. The percent thickness reduction for LDPE was 5.83% and 5.93% for HDPE in 6th month and is shown in Fig. 2. Chonde et al. (2013) have recorded reduction in thickness for nylon 6 and nylon 6, 6 as 46% and 49% re-

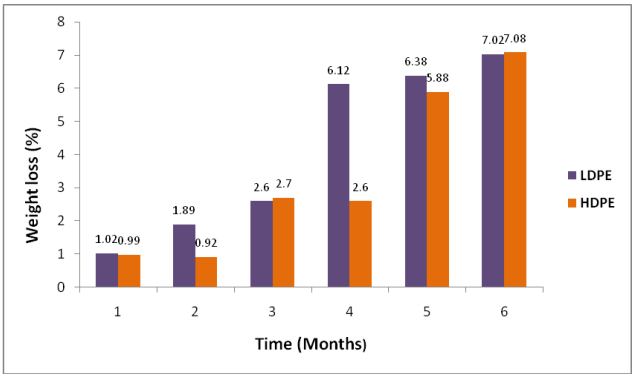

Fig. 1: Weight loss (%) on treatment with *Bacillus weihenstephanensis.*

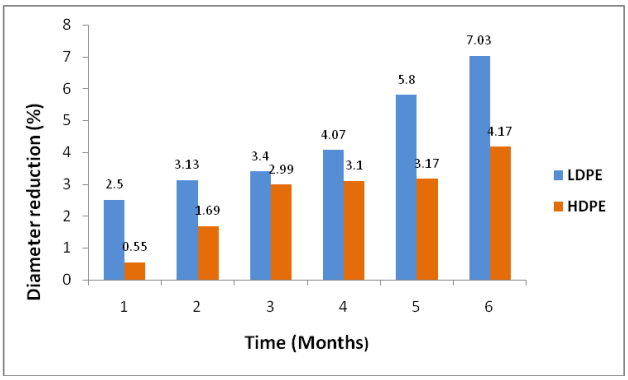

Fig. 3: Diameter reduction (%) on treatment with *Bacillus weihenstephanensis.*

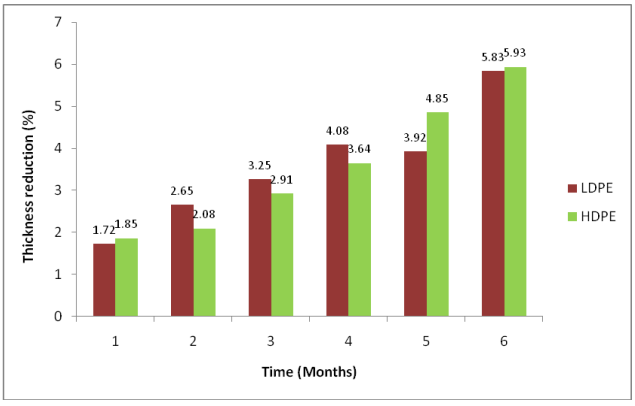

Fig. 2: Thickness reduction (%) on treatment with *Bacillus weihenstephanensis.*

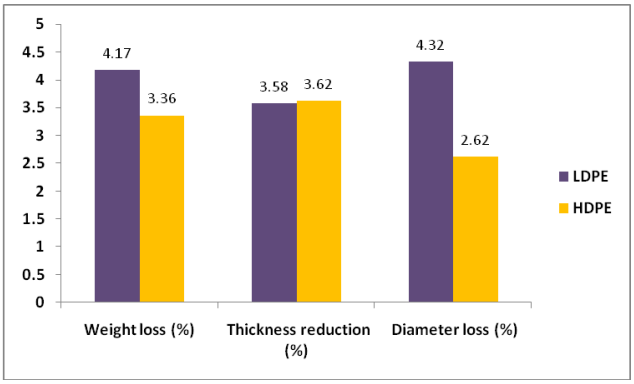

Fig 4: Average degradation of LDPE and HDPE on incubation with *Bacillus weihenstephanensis* for 6 months.

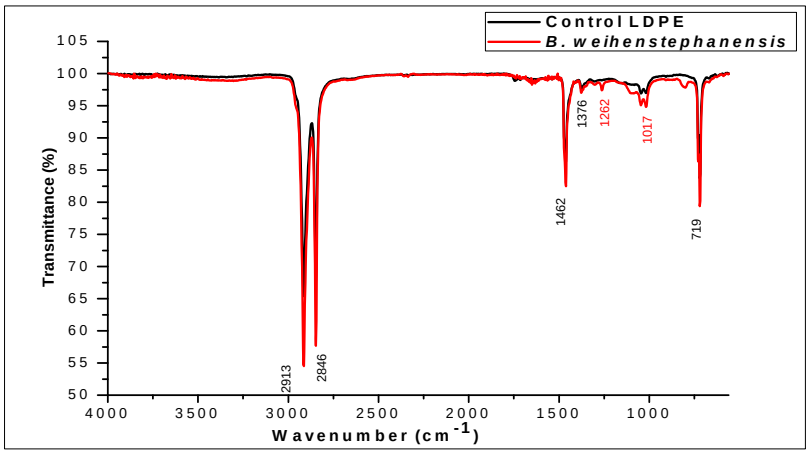

Fig. 5: FTIR spectrum of LDPE control and treated with *Bacillus weihenstephanensis* for the range 4000-560 cm^{-1}.

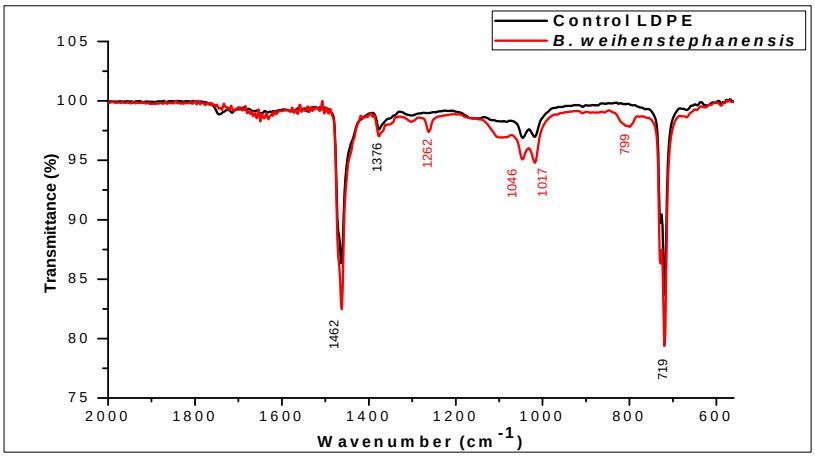

Fig. 6: FTIR spectrum of LDPE control and treated with *Bacillus weihenstephanensis* for the range 2000-560 cm^{-1}.

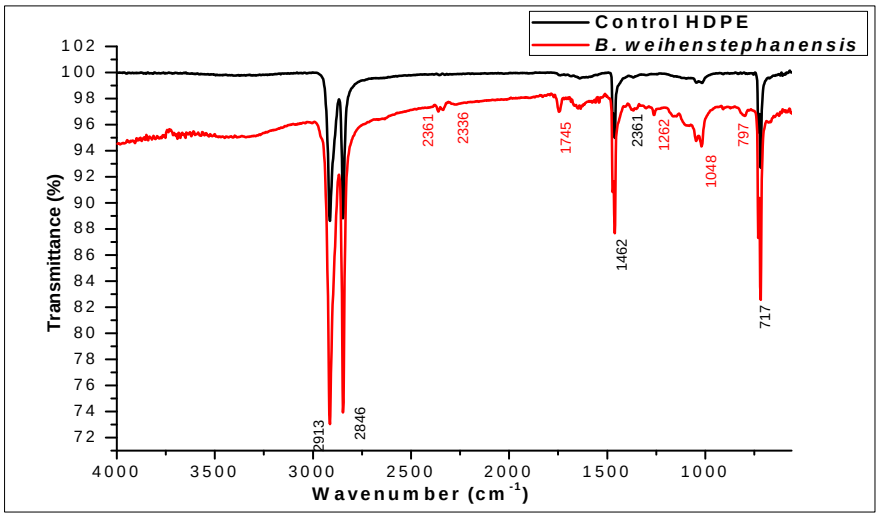

Fig. 7: FTIR spectrum of HDPE control and treated with *Bacillus weihenstephanensis* for the range 4000-560 cm^{-1}.

spectively on treating it with *Pseudomonas aeruginosa* NCIM 2242 for 6 months. Chonde et al. (2012a) have observed similar thickness loss for nylon 6 on treating it with fungus *Trametes versicolor* NCIM 1086 after 75 days.

Diameter reduction (%) of LDPE and HDPE sheets incubated with *Bacillus weihenstephanensis*: PE sheets treated with *Bacillus weihenstephanensis* showed reduction in diameter. The maximum diameter reduction for LDPE was observed in 6th month i.e., 7.03% and 4.17% for HDPE. The results are shown in Fig. 3.

Thus, on an average the weight loss for LDPE was 4.17%, and for HDPE 3.36% after 6 months of incubation. The thickness reduction recorded for LDPE was 3.58%, and for HDPE 3.62% while the diameter reduction for LDPE was observed as 4.32%, and for HDPE 2.62% when incubated with *Bacillus weihenstephanensis* for 6 months. The comparative degradation is depicted in Fig. 4.

Fourier transform infrared spectroscopy (FTIR) of polyethylene sheets incubated with isolated bacterial and fungal strains: Fourier transform infrared spectroscopy (FTIR) investigation is an important tool to define the development of new or disappearance of functional groups (Sudhakara et al. 2008). Along with it, the reduction of native bonds observed in the FTIR-ATR spectrum of polyethylene proves that the polymer has been fragmented to shorter chains (Soni et al. 2009).

The biodegradation of polyethylene was initially started by abiotic process. Oxidation of polymer chain took place because of the presence of oxygen in the air which leads to the development of carbonyl groups. Later, the carbonyl groups form the carboxylic groups, undergo β-oxidation and lastly move in the citric acid cycle ultimately forming CO_2 and H_2O (Albertsson et al. 1987).

In the observations made by Gulmine et al. (2002), distinct peaks were observed at wave numbers 2919, 2851, 1473, 1377 and 720 cm^{-1} on FTIR analysis of pure LDPE. These peaks correspond to the native bonds present in the polymer. In the current study, FTIR analysis of undegraded/pure LDPE (control) showed absorbance at 2913 cm^{-1}, 2846 cm^{-1}, 1462 cm^{-1}, 1376 cm-1 and 719 cm^{-1}. Absorbance at 2850 to 3000 cm^{-1} region corresponds to the alkyl groups (CH_3CH_2, CH). Band at 2914 cm^{-1} and 2847 cm-1 corresponds to CH_2 group with strong intensity and possess asymmetric and symmetric stretching respectively. The strong band at about 1462 cm^{-1} in polyethylene is due mainly to bending mode of the CH_2 group (Gulmine et al. 2002). Absorbance at 1376 cm-1 corresponds to CH_3 symmetric deformation with weak intensity (Krimm et al. 1956). 719 cm^{-1} is assigned to rocking deformation mode of CH_2 group with medium intensity (Gulmine et al. 2002, Krimm et al. 1956).

In the spectra of LDPE and HDPE treated with *Bacillus weihenstephanensis* i.e., Isolate-2, a noticeable reduction in the percentage transmittance was observed on incubation for 6 months. Native bands remained intact. Following additional peaks were observed in the spectrum of LDPE treated with *B. weihenstephanensis* and are showed in Fig. 5 as 1262 cm^{-1}, 1046 cm^{-1}, 1017 cm^{-1} and 799 cm^{-1}. To understand the chemical changes in the fingerprint region, the FTIR spectrum for range 2000-560 cm^{-1} is elaborated in Fig. 6.

On the spectrum of HDPE treated with *B. weihenstephanensis* following additional peaks were observed: 2361

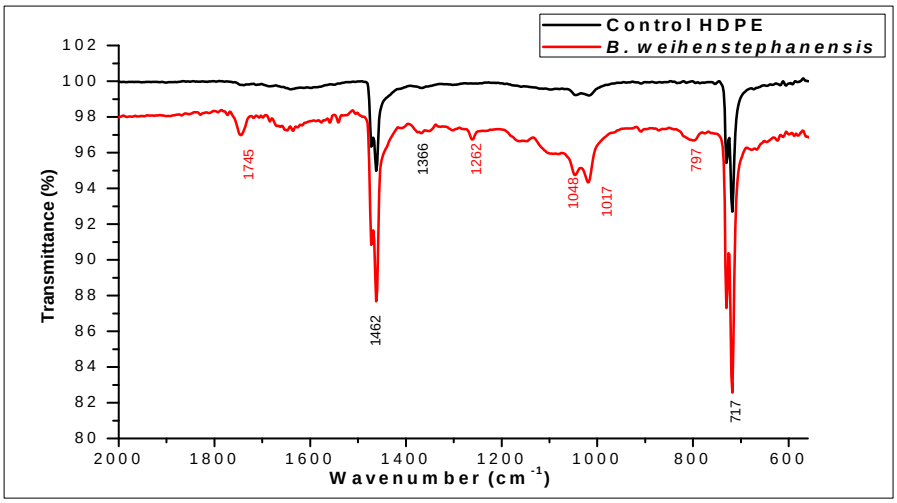

Fig. 8: FTIR spectrum of HDPE control and treated with *Bacillus weihenstephanensis* for the range 2000-560 cm^{-1}.

cm^{-1}, 2336 cm^{-1}, 1745 cm^{-1}, 1262 cm^{-1}, 1048 cm^{-1}, 1017 cm^{-1} and 797 cm^{-1} which are shown in Fig. 7. To understand the chemical changes in the fingerprint region, the FTIR spectrum for range 2000-560 cm^{-1} is elaborated in Fig. 8. The peaks at 2361 cm^{-1}, 2336 cm^{-1}, 1262 cm^{-1} and 1017 cm^{-1} are assigned for C-O stretch. A peak at 1048 cm^{-1} correlates to C-O stretching modes in alkanoate ester and alkoxy ether. FTIR peak at 799 cm^{-1} corresponds to alkenes. These results obtained are similar to the study conducted by Agamuthu et al. (2005) who revealed the formation of carbonyl bond and carbonyl compounds comprising esters, aldehydes and carboxylic acids on FTIR analysis.

CONCLUSION

The present investigation highlights the potentiality of microbes from environment in the degradation of polyethylene. LDPE and HDPE can be biodegraded if the right microorganisms are isolated and inoculated for degradation. The study explores the potential of PE degradation by *Bacillus weihenstephanensis* isolated from waste dumping site which utilizes PE as a source of carbon and energy. Rate of degradation of HDPE is slower than LDPE because the main chains of HDPE have no accessible hydrolysable groups. The oxidation or hydrolysis by microbial enzymes is the primary activity for the biodegradation of high molecular weight polymer to form functional groups which can readily improve its hydrophilicity. Therefore, the principal chains of polymer are degraded forming the polymer of low molecular weight and weak mechanical characteristics, thus, making it more available to facilitate microbial absorption further.

The FTIR technique serves as an indication of PE biodegradation as it quantifies the reduction of transmittance of the native bonds present in LDPE and HDPE. It is evident that the *Bacillus* spp. release extracellular enzymes to degrade the PE, but the detailed characterization is still needed to be carried out.

For commercial and eco-friendly degradation of PE, many more optimized laboratory studies with PE degrading microbes are needed to explore. Further, attention should be provided for biodegradation of PE without any pretreatment or addition of chemicals so as to cope up with the problem of commercially available plastic.

ACKNOWLEDGEMENT

Authors are thankful to the authorities of Common Facility Centre, Shivaji University, Kolhapur and Department of Environmental Science, Shivaji University, Kolhapur for providing facilities to carry out work. Authors are also thankful to Laxman Udyog Samuh, Kolhapur for providing polyethylene.

REFERENCES

Agamuthu, P. and Faizura, N.P. 2005. Biodegradability of degradable plastics waste. Waste Management and Research, 23: 95-100.

Albertsson, A.C. and Karlsson, S. 1990. Polyethylene degradation and degradation products. In: Glass J. E., Swift G. (Eds.), Agricultural and Synthetic Polymers, Biodegradability and Utilization. ACS Symposium Series, Washington DC, 433: 60-64.

Albertsson, A.C., Sares, C. and Karlsson, S. 1993. Increased degradation of LDPE with nonionic surfactants. Acta Polymerica, 44: 243-246.

Albertsson, A.C., Andersson, S.O. and Karlsson, S. 1987. The mechanism of biodegradation of polyethylene. Polymer Degradation Stability, 18: 73-87.

Burd, D. 2008. Plastic not fantastic. Project Reports of the Canada Wide Science Fair, pp. 1-5.

Chonde, S. G., Chonde, S.G., Bhosale, P.R., Nakade, D.B. and Raut, P.D. 2012a. Studies on degradation of synthetic polymer Nylon 6 by fungus *Trametes versicolor* NCIM 1086. International Journal of Environmental Sciences, 2(3): 2435- 2442.

Chonde, S.G., Chonde, S.G., Bhosale, P.R. and Raut, P.D. 2012b. Studies on degradation of synthetic polymer Nylon 6 by lignolytic fungus *Phanerochaete chrysosporium* NCIM 1073. Journal of Environmental Research and Development, 6(3A): 709-714.

Chonde, S.G., Chonde S.G. and Raut, P.D. 2013. Studies on degradation of synthetic polymer Nylon 6 and Nylon 6, 6 by *Pseudomonas aeruginosa* NCIM 2242. International Journal of Emerging Technologies in Computational and Applied Sciences, 4(4): 362-369.

Gulmine, J.V., Janissek, P.R., Heise, H.M. and Akcelrud, L. 2002. Polyethylene characterization by FTIR. Polymer Testing, 21: 557-563.

Hadad, D., Geresh, S. and Sivan, A. 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. Journal of Applied Microbiology, 98: 1093-1100.

Jang, J.C., Shin, P.K., Lee, I.M., Yoon, J.S., Lee, H.S. and Kim, M.N. 2002. Glucose effects on the biodegradation of plastics by compost from food garbage. Polymer Degradation and Stability, 76: 155-159.

Krimm, S., Liang, C.Y. and Sutherland, G.B.B.M. 1956. Infrared spectra of high polymers II polyethylene. The Journal of Chemical Physics, 25(3): 549-562.

Kumari, K., Aanad, R.C. and Narula, N. 2009. Microbial degradation of polyethylene (PE). The South Pacific Journal of Natural Science, 27: 66-70.

Mahalakshmi, V. 2013. Physicochemical, genetic and molecular analysis of polyethylene degrading potential of microorganisms. Ph.D. Thesis, Bharathiar University, Coimbatore, Tamilnadu, India.

Mukherjee, S. and Chatterjee, S. 2014. A comparative study of commercially available plastic carry bag biodegradation by microorganisms isolated from hydrocarbon effluent enriched soil. International Journal of Current Microbiology and Applied Sciences, 3(5): 318-325.

Nanda, S. and Sahu, S.S. 2010. Biodegradability of polyethylene by *Brevibacillus*, *Pseudomonas*, and *Rhodococcus* spp. New York Science Journal, 3(7): 9598.

Pramila, R., Padmavathy, K., Vijaya Ramesh, K. and Mahalakshmi, K. 2012. *Brevibacillus parabrevis*, *Acinetobacter baumannii* and *Pseudomonas citronellolis* - potential candidates for biodegradation of low density polyethylene (LDPE). Journal of Bacteriology Research, 4(1): 9-14.

Pramila, R. and Vijaya Ramesh, K. 2011. Biodegradation of low density polyethylene (LDPE) by fungi isolated from marine water - a SEM analysis. African J. of Microbio. Res., 5(28): 5013-5018.

Premraj, R. and Dobley, M. 2005. Biodegradation of polymers. Indian Journal of Biotechnology, 4: 186-193.

Satlewal, A., Soni, R., Zaidi, M.G.H., Shouche, Y. and Goel, R. 2008. Comparative biodegradation of HDPE and LDPE using an indigenously developed microbial consortium. Journal of Microbiology and Biotechnology, 18(3): 477-482.

Seneviratane, G., Tennakoon, N.S., Weerasekara, M.L.M.A.V. and Nandasena, K.A. 2006. Polyethylene biodegradation by a developed *Pencillium-Bacilllus* biofilm. Current Science, 90: 10.

Shah, A.A, Hasan, F., Hameed, A. and Akhter, J.I. 2009. Isolation of *Fusarium* sp. AF4 from sewage sludge, with the ability to adhere the surface of polyethylene. African Journal of Microbiological Research, 3(10): 658-663.

Soni, R., Kapri, A., Zaidi, M.G.H. and Goel, R. 2009. Comparative biodegradation studies of non-poronized and poronized LDPE using indigenous microbial consortium. Journal of Polymers and the Environment, 17: 233-239.

Sudhakara, M., Doble, M., Sriyutha Murthy, P. and Venkatesan, R. 2008. Marine microbe-mediated biodegradation of low-and high-density polyethylenes. International Biodeterioration & Biodegradation, 61: 203-213.

Usha, R., Sangeetha, T. and Palaniswami, M. 2011. Screening of polyethylene degrading microorganisms from garbage soil. Libyan Agri. Res. Center J. Inter., 2(4): 200-204.

Vijaya, C. and Reddy, R.M. 2008. Impact of soil composting using municipal solid waste on biodegradation of plastics. Indian Journal of Biotechnology, 7: 235-239.