



Evaluation of Biodegradation Efficiency of Xylene Pretreated Polyethylene Wastes by Isolated *Lysinibacillus fusiformis*

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ABSTRACT

The ability of the bacterial degradation of low-density polyethylene (LDPE) waste by *Lysinibacillus fusiformis* isolated from hydrocarbon-contaminated soil was investigated in the present study. The potential of the bacterial isolate to utilize LDPE waste bags of two different thicknesses in a month as a sole carbon source in mineral salt media was assessed. Further, the effect of pretreatment by xylene on the bacterial degradation of LDPE waste bags (0.5 percent w/v) in 30 days was investigated. The isolated *Lysinibacillus fusiformis* was able to degrade 9.51 percent of LDPE with 30 μ m thickness but able to degrade only 1.45 percent of LDPE having 50 μ m thickness. The bacterial biomass was 1.77 times higher on LDPE- 30 μ m containing media in comparison to LDPE- 50 μ m. The xylene pretreatment of LDPE wastes enhanced the biodegradation efficiency of isolated *Lysinibacillus fusiformis* to 12.09 and 1.97 percent respectively in 30 μ m and 50 μ m thick LDPE bags. The xylene pre-treatment improved the bacterial growth on media with LDPE of both thicknesses. The adherence of bacterium on the surface of LDPE was found more on 50 μ m thick xylene treated LDPE compared to its untreated LDPE than 30 μ m thick LDPE films. The xylene pre-treatment of polyethylene waste had an additive effect on the biodegradation of waste LDPE films with a significant effect on thickness.

INTRODUCTION

The generation and dumping of different types of plastic wastes after consumption into the environment had increased manifold in the present era of expeditious industrialization. The global annual production of plastic is around 400 million tonnes and nearly 8 to 13 million tonnes of plastic wastes are ended with direct ocean dumping (Danso et al. 2019). Out of the total plastics produced, polyethylene and polypropylene are major polymers that comprise 92% of the total production. The natural aging and weathering of plastics from marine dumping or landfilling increased their mobility and were easily incorporated into the food chain, thus adversely affecting the organisms (Sen & Raut 2015). The disposal of plastic wastes in landfills generates hazardous chemicals and in turn, contaminates the groundwater (North & Halden 2013). The microplastics formed from plastic waste had the potential to act as a carrier for the adsorption of recalcitrant hydrophobic toxic chemicals such as polychlorinated biphenyls (de Souza Machado et al. 2018).

The different methods for the management of plastic waste are landfilling, incineration, and recycling, (Peng et al. 2018, Ali et al. 2021). But these conventional degradation methods have their limitations such as the negative impact on

climate, space constraints, effect on soil fertility, and leakage of toxic components in water and soil (Hopewell et al. 2009). The indigenous microbial population present in contaminated habitats holds the key to solving most of the challenges linked with the bioremediation of environmental pollutants (Verma & Jaiswal 2016). The microbes having potential for degrading polyethylene is limited. Many studies are focused on searching for polyethylene degrading microbial strains from diverse habitats like oil spillage, sludge, municipal landfills, and plastic dump sites (Duddu et al. 2015, Soud 2019). The bacterial genera namely *Pseudomonas*, *Bacillus*, *Stenotrophomonas*, *Ralstonia*, *Acinetobacter*, *Streptococcus*, *Rhodococcus*, *Staphylococcus*, *Klebsiella*, and *Streptomyces* are reported for polyethylene degradation (Park & Kim 2019, Shahreza et al. 2019).

The pretreatment of polyethylene including thermal oxidation, UV irradiation, and chemical and mechanical breakdown before the microbial degradation plays an important role in the effective cleanup of these synthetic polymers (Olayan et al. 1996, Raut et al. 2015). The chemical pretreatment increased the availability of the plastic substrate to microorganisms for biodegradation (Balasubramanian et al. 2014, Kundungal et al. 2021). The thickness of dumped polyethylene plastics was in the range of 15-50 μ m (Tziour-

rou et al. 2021). The present study attempts to investigate the effect of thickness of low-density polyethylene waste bags on the degradation potential of isolated *Lysinibacillus fusiformis* and also the impact of xylene pretreatment on biodegradation.

MATERIALS AND METHODS

Bacterium Used in Biodegradation Study

The bacterium *Lysinibacillus fusiformis* used in the present study was previously isolated from the soil contaminated with hydrocarbons collected from areas of coal-fired thermal power facilities in Bathinda, Punjab (Kalia 2015).

Low-Density Polyethylene Waste Bag Collection

The low-density polyethylene waste bags used in the present study were collected from local dumping sites in Bathinda, Punjab, India. The polyethylene waste bags were sorted based on thickness into two categories namely LDPE- 30 μm and LDPE- 50 μm . The LDPE waste bags having thicknesses of 30 μm and 50 μm were cut into uniform pieces of 1x1 cm^2 dimensions.

Xylene Treatment of Polyethylene Waste Bags

The LDPE waste bags of 30 μm and 50 μm thickness cut into uniform pieces of 1x1 cm^2 were subjected to xylene treatment by boiling for 15 min. The LDPE waste bags after xylene treatment was subjected to ethanol washing followed by drying in a hot air oven at a temperature of 60°C. The xylene pretreated samples were stored at room temperature and used for evaluating the effect of xylene on biodegradation study by the bacterium (Das & Kumar 2015).

The untreated (control) and xylene-treated waste LDPE films of 30 μm and 50 μm thickness were analyzed by FT-IR spectroscopy (FTIR Bruker, Model: Tensor 27) to detect the changes in surface functional groups on polyethylene in the spectral range of 4000-600 cm^{-1} (Albertsson et al. 1987, Sudhakar et al. 2008).

Biodegradation of Waste Polyethylene by *Lysinibacillus fusiformis*

The mineral salt media used in the present biodegradation study contained $(\text{NH}_4)_2\text{SO}_4$, K_2HPO_4 and NaCl in 1 g.L^{-1} , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 0.5 g.L^{-1} , $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 0.002 g.L^{-1} , KH_2PO_4 in 0.2 g.L^{-1} , $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 0.001 g.L^{-1} with pH maintained at 7 (Das & Kumar 2015).

The degrading efficiency of bacterial isolate *Lysinibacillus fusiformis* was tested by supplementing waste LDPE bags of two thicknesses, 30 μm and 50 μm at 0.5 percent (w/v) in the mineral salt medium as the sole carbon source (Gilan et

al. 2004, Balasubramanian et al. 2014). The biodegradation study of untreated and xylene-treated waste LDPE bags was performed with isolated *Lysinibacillus fusiformis* in a 250 mL Erlenmeyer flask incubated in a rotatory incubator shaker at 30°C and 180 rpm for 30 days. The weight loss of polyethylene bags of two thicknesses from initial weight was used to measure the biodegradation efficiency of *Lysinibacillus fusiformis*.

The waste LDPE samples collected from the culture media after the degradation study was washed with aqueous sodium dodecyl sulfate solution (2% v/v) followed by distilled water washing (Gilan et al. 2004). The weight loss of the films from initial to final weight indicated the degradation as per the formula given below.

$$\text{Biodegradation (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Growth of Isolated *Lysinibacillus Fusiformis* on Low-Density Polyethylene Waste Bags

The bacterial population on the surface of untreated and xylene treated LDPE was estimated from the amount of extractable protein present on the samples. The LDPE samples were collected from the growth medium at 5 days intervals. The samples were boiled for 30 min in 0.5 N NaOH, centrifuged and the supernatant was collected. The protein concentration was determined spectrophotometrically by the Lowry method (Lowry et al. 1951).

Statistical Analysis

The biodegradation study was conducted in triplicates. The statistical analysis for testing of significance by ANOVA single factor and correlation studies were carried out.

RESULTS AND DISCUSSION

Waste Polyethylene Biodegradation Potential of *Lysinibacillus fusiformis*

The *Lysinibacillus fusiformis*, previously isolated from hydrocarbon-contaminated soil was able to grow in the liquid, mineral salt medium enriched with waste LDPE- 30 μm and LDPE- 50 μm films as sole carbon sources without any treatment. The biomass growth was measured in terms of protein content confirming the growth on the surface of both LDPE waste bags (Fig. 2). The 30 μm thick LDPE waste bags had more *Lysinibacillus fusiformis* growth than the 50 μm thick bags. The increase in growth of *Lysinibacillus fusiformis* from incubation was 2.01 and 2.19 times respectively for 30 μm and 50 μm thick LDPE wastes in 5 days. The growth on the 15th day on the LDPE 30 μm and LDPE- 50 μm films

were 62 percent and 42 percent higher than on the 10th day of incubation. The bacterial proliferation got reduced further and only an 8.16 percent increase in protein concentration was reported from 25 to 30 days period of bacterial growth using untreated LDPE- 30 μm films in vitro. The protein concentration on the 20th day was 32 percent higher than on the 15th day of incubation. Further, a decrease in the rate of increase in protein concentration and was only a 12 percent increase was noticed in the next five days in 30 days of incubation using waste LDPE- 50 μm films. The thickness of LDPE had a significant effect on bacterial proliferation and the more the thickness, the lower the growth. The LDPE films are hydrophobic in nature and restrict the easy attachment of microbes. A few bacterial strains were able to synthesize biosurfactants with an emulsifying activity that reduced the surface and inter-surface tension to increase the polymer bioavailability (Hassanshahian et al. 2014).

Subsequently, after initial bacterial attachment on the polymer surface, the *Lysinibacillus fusiformis* was able to utilize 1.17 percent of supplied LDPE- 30 μm films in 10 days. But the degradation of LDPE- 50 μm films in 10 days was only 0.55 percent of the incubated concentration. The degradation percentage of *Lysinibacillus fusiformis* was increased by 3.96 times in the next 10 days of incubation in growth media enriched with waste LDPE- 30 μm films in comparison to an increase of 0.62 percent in LDPE- 50 μm . Further increases in degradation percent in 30 days for LDPE- 30 μm and LDPE- 50 μm were 4.38 percent and 0.28 percent respectively.

The bacterial isolate was able to utilize 9.51 percent and 1.44 percent of the untreated waste LDPE- 30 μm and LDPE- 50 μm films in vitro respectively in 30 days (Fig. 3). The bacterial isolate *Lysinibacillus fusiformis* was able to accumulate 1.78 times more protein concentration and 6.58 times more degradation from untreated LDPE- 30 μm films than LDPE- 50 μm films as the sole carbon source. The bacteria with polyethylene degrading capabilities isolated from contaminated environments were also reported by Duddu et al. (2015). The adherence of the bacterial population on the surface of LDPE had a significant effect on the degradation pattern (Montazer et al. 2018). The bacterial strains from hydrocarbon-contaminated sites marked the presence of catabolic genes that encode alkane hydroxylases needed for polyethylene degradation (Gilan et al. 2004, Tanase et al. 2013, Lima et al. 2019).

The comparatively lesser growth and low degradation of LDPE- 50 μm films were due to the higher thickness. The increase in polymer thickness decreased the contact between the surface exposed to the hydrolytic enzyme and microbial attachment, thereby reducing the degradation rate (Yang

et al. 2005). The higher thickness also reduced the oxygen diffusion in the core of the polymer and thereby reduced the degradation rate (Lin & Anseth 2013).

Effect of Xylene Treatment on Biodegradation of Polyethylene Waste Bags

The waste LDPE- 30 μm and LDPE- 50 μm films were further subjected to xylene treatment. The xylene treatment fragmented the polymer and converted it to powder (Fig. 1). The structural changes in the high-density polyethylene by pretreatment of *p*-xylene were reported by Blackadder & Keniry (1972). The dissolution of LDPE by xylene as a safe pretreatment method was reported by Wong et al. (2014). The solubility of high-density polyethylene and polypropylene in xylene was also studied by Richards (1946) and Arkan et al. (2017).

The structural changes in the polyethylene were analyzed by the FTIR analysis of the powdered polymer after xylene treatment. The FTIR spectra of untreated LDPE films had peaks at 723 cm^{-1} (C-H), 1086 cm^{-1} (C-O stretch), 1459 cm^{-1} (CH_2 bending) and 2,660 cm^{-1} (CHO stretch). This was in accordance with the peaks obtained for the IR spectrum of LDPE reported by Das & Kumar (2015). The spectra of LDPE films treated with xylene were shown some different peaks. The FTIR spectra of xylene-treated LDPE- 30 μm (X_LDPE-30 μm) film showed peaks at 1484, 2872, and 3426 cm^{-1} . The powdered LDPE- 50 μm films after xylene treatment (X_LDPE-50 μm) had marked peaks at 1375, 1459,

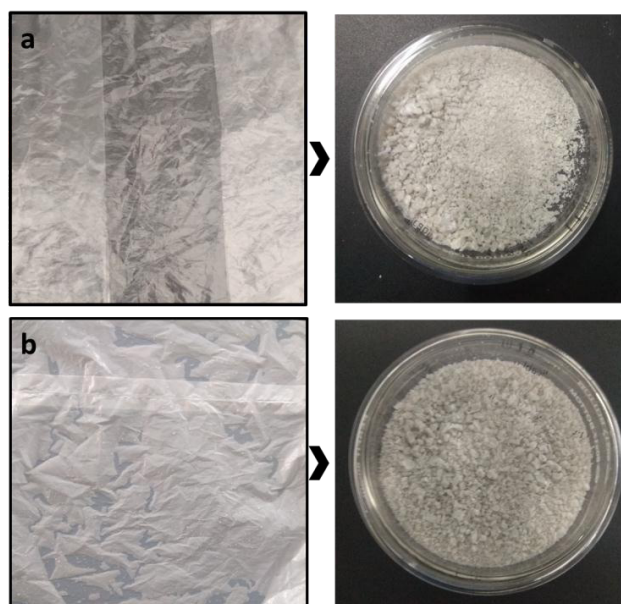


Fig. 1: Waste polyethylene films a) LDPE- 30 μm and b) LDPE- 50 μm before and after treatment with xylene.

2832, 2876, 2959, and 3426 cm^{-1} . The changes in the peaks value for every functional group and generation of new peaks in xylene-treated LDPE films confirmed the changes in the structure by the treatment of xylene.

The bacterial isolate was able to adhere to the xylene-treated LDPE films supplied in the culture broth. The treatment with xylene improved the growth in both 30 μm and 50 μm thick LDPE wastes. The biomass increase in *Lysinibacillus fusiformis* after xylene treatment was 2.54 and 2.39 times for 30 μm and 50 μm thick LDPE wastes respectively in 5 days. The xylene treatment of waste LDPE- 30 μm and LDPE- 50 μm films increased the biomass growth as evident from extractable protein concentration was 21 percent and 27 percent respectively than the untreated films of LDPE-30 μm and LDPE- 50 μm by *Lysinibacillus fusiformis* in 10 days of incubation (Fig. 2). The percent increase in protein concentration on xylene treated LDPE- 30 μm was 21 percent in 10 days which decreased to 10.78 percent in 30 days in comparison to untreated LDPE- 50 μm . Interestingly the percent increase in protein concentration on xylene treated LDPE- 50 μm was 28 percent in 10 days which increased to 41.5 percent in 30 days in comparison to untreated LDPE- 50 μm .

The biodegradation of xylene treated LDPE-30 μm and LDPE- 50 μm films by *Lysinibacillus fusiformis* were 12.09 percent and 1.97 percent respectively after 30 days of incubation (Fig. 3). The xylene treatment of the waste polyethylene films had an additive effect on biodegradation

as it increased the degradation percentage of LDPE- 30 μm and LDPE- 50 μm films to 27.15 and 36.21 percent than the untreated LDPE films respectively. The xylene treatment of waste LDPE films improved the bacterial biomass (protein concentration) by 10.78 and 41.50 percent than the untreated waste LDPE- 30 μm and LDPE- 50 μm films respectively. The biodegradation of xylene pretreatment of LDPE wastes was highly significant with thickness. The biomass growth was highly correlated with biodegradation of untreated and xylene treated LDPE of 30 μm and 50 μm thickness.

The biofilm formation significantly increases the biodegradation potential of the bacterial isolate (Balasubramanian et al. 2010). The improvements in protein concentration and a weight loss percentage of LDPE- 30 μm and LDPE- 50 μm films were attributed to the structural changes induced by the xylene treatment before incubation with *Lysinibacillus fusiformis*. The dissolution of polyethylene films using xylene fragmented the polymer and resulted in changes in molecular weight distribution and morphology. The pattern of biodegradation of LDPE was similar to the reports of Albertsson (1980) and Das & Kumar (2015).

The pre-treatment helped to increase the availability of LDPE to microorganisms (Cornell et al. 1984, Koutny et al. 2006). The chemical treatments of polyethylene increased the polymer susceptibility for microorganism by inducing functional groups on the polyethylene surface that favors the microbial attachments (Chaudhary & Vijayakumar 2020).

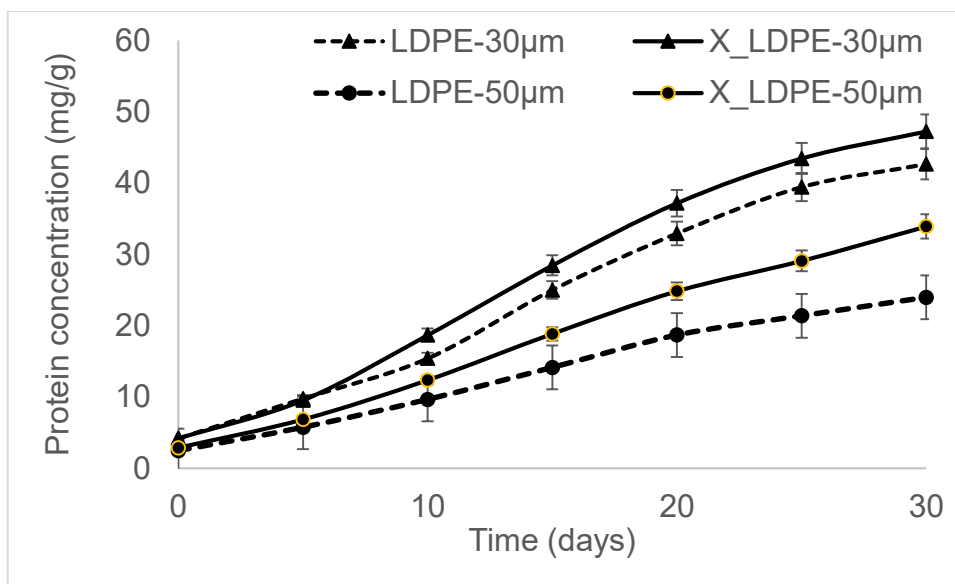


Fig. 2: Effect of xylene treatment on protein concentration in media inoculated with *Lysinibacillus fusiformis* using untreated and xylene pretreated waste LDPE- 30 μm and LDPE- 50 μm films.

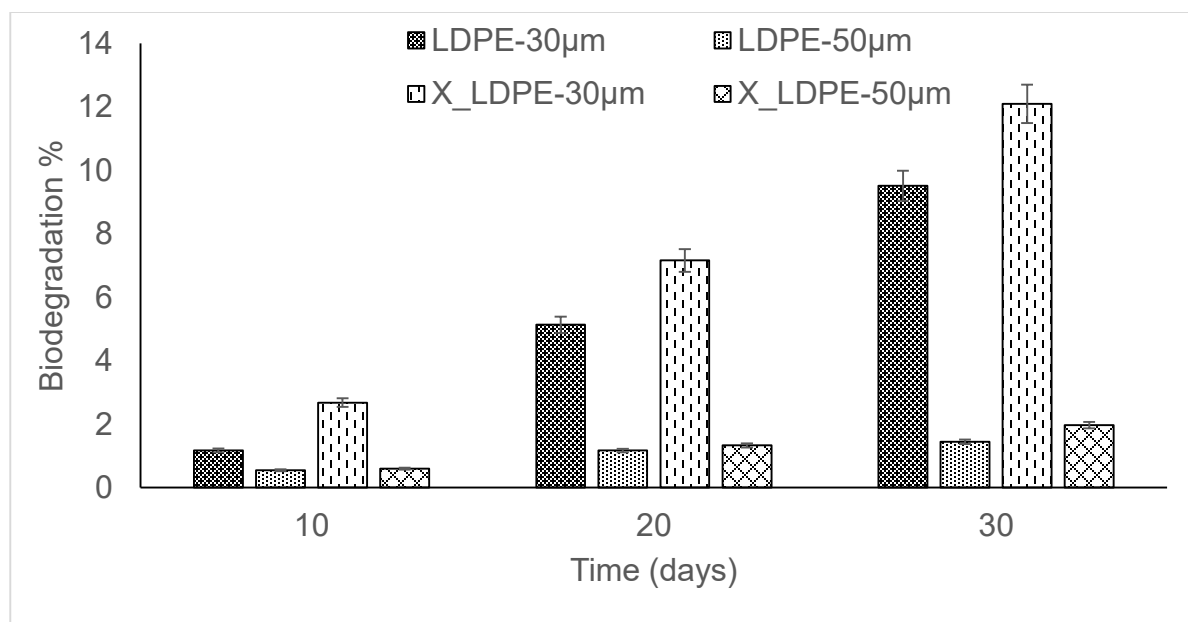


Fig. 3: Comparison of biodegradation efficiency of untreated and xylene treated LDPE- 30 µm and LDPE- 50 µm waste bags after 30 days of incubation with *Lysinibacillus fusiformis*.

CONCLUSION

The isolated *Lysinibacillus fusiformis* had the potential to degrade the low-density polyethylene. The efficiency of biodegradation varied with the thickness of LDPE. The xylene treatment was found to enhance the biomass growth on the LDPE surface with a faster degradation. The naturally occurring microbial strain with abiotic pretreatment help in the biodegradation of more recalcitrant organic pollutants more efficiently and effectively.

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