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Effect of Used Engine Oil and UV-Thermal Pretreatments on Biodegradation of Low-Density Polyethylene by *Lysinibacillus fusiformis* TPB

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The present study focused on the impact of Used Engine Oil (UEO) and abiotic pretreatments by ultraviolet (UV) radiation and thermal treatment at 70°C for 144 h on the potential of *Lysinibacillus fusiformis* TPB isolated from hydrocarbon contaminated soil for the biodegradation of low-density polyethylene (LDPE) in mineral salt medium at 30°C and 150 rpm for 30 days. The isolated *L. fusiformis* TPB degraded 9.51% of LPDE films without any treatment and used as the sole carbon source for biomass production. The supplementation of used engine oil (0.5% v/v) enhanced biodegradation of untreated LDPE films to 11.96% comparable to a non-ionic surfactant Tween 80. The abiotic pretreatments had also facilitated metabolism of LDPE by *L. fusiformis* TPB. The biodegradation of UV treated LDPE by *L. fusiformis* TPB was 13.78% and was significantly higher than thermally treated LDPE with 12.89% biodegradation. The Fourier Transform Infrared spectrum revealed structural and morphological changes in the LDPE films by abiotic pretreatments and were associated with addition of carbonyl groups and change in double bond index. The Scanning Electron Microscopy analysis of LDPE films from UEO and UV-thermal pretreated LDPE supplemented mineral salt media confirmed the improved bacterial colonization and biofilm formation. The isolated *L. fusiformis* TPB had LDPE degradation potential and biodegradation had improved by UEO supplementation and UV-thermal pretreatments.

Keywords: Biofilm, Carbonyl indices, Colonizing agent, Oxidation, Weight loss

Introduction

Low density polyethylene (LDPE) is very commonly used polymer in domestic and industrial sectors due to its versatility and low cost. The proper disposal of polyethylene in the environment is of great concern due to its ubiquitous and persistent nature, low bioavailability, and high resistance to biodegradation. It is estimated that world plastic production surpassed to 360 million tonnes in 2018 and thermoplastics demand in India exceeded 18910 kilotonnes in 2019-2020.(1) The LDPE affect terrestrial as well as aquatic ecosystems and life forms from dumping in open areas and landfills adding up the pollution level. Every year approximately 1.5 to 4% of global plastic production ends up in the ocean.[2]

The products of photooxidation of plastics can enter easily into the food chain, causing fatal damages to organisms. Different techniques used for environmental cleanup of LDPE are chemical disintegration, photo-oxidation, gasification, catalyzed cracking and pyrolysis.[3] Studies have reported microbial degradation and metabolism of plastics under controlled conditions.[4] The direct biodegradation of LDPE by the microorganisms in environmental cleanup is challenging due to its hydrophobicity and high molecular weight.[5,6] A number of microbial species belonging to genera *Streptomyces, Bacillus, Aspergillus, Pseudomonas, Chelatococcus, Rhizopus, Acinetobacter, and Phanerochaete* are potential polyethylene degraders.[7–9]

The oxidative pretreatment of polyethylene increase photodegradation and thermo-oxidative surface weathering and thereby increases surface hydrophilicity by the formation of carbonyl groups.[10–12] The abiotic pretreatment prior to biotic degradation stimulated microbial colonization, biofilm formation and generation of other hydrophilic functional groups that facilitate the microbial attack. The improved polymer degradation and bacterial attachment by thermal treatment and photooxidation of LDPE were reported by different researchers.[13,14] The addition of bacterial colonizing agents such as surfactants played an important role in effective biodegradation of the polyethylene without pretreatment.[15,16] The present investigation focused on the effect of UEO in comparison to the role of prior oxidative UV and thermal pretreatments on biodegradation of LDPE by

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the bacteria isolated from hydrocarbon contaminated soil and further compared with commercial surfactant Tween 80.

Materials and Methods

LDPE Sample Collection

The LDPE bags were collected from municipal waste dumping site in Bathinda, India (30°10'54" N, 74°57'10" E). Vernier calipers was used to measure thickness of LDPE bags used for the study. The basic characteristics and thermal stability of waste LDPE sample was measured by Thermogravimetric analysis (Thermo Analyser TG-DTA; Shimadzu DTG 60H) under nitrogen atmosphere within a temperature range of 25 to 600°C at a heating rate of 10°C/min.[17] Chemicals and solvents used in the present study were of analytical grade and obtained from Sigma, Himedia and Loba Chemie.

Bacterial Strain and Culture Media

The bacterial strain (*Lysinibacillus fusiformis* TPB) used in the present study was previously isolated from hydrocarbon contaminated soil collected from nearby areas of Guru Nanak Dev thermal power plant, Bathinda (30°13' 59.57" N, 74°55' 48.92" E) by serial dilution and enrichment method followed by 16S r-DNA sequencing.[18] The polyethylene degradation potential of *L. fusiformis* TPB was investigated by culturing in LDPE enriched synthetic medium containing $(NH4)_2SO_4$, 1.0; $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 1.0; NaCl, 1.0; $CaCl_2.2H_2O$, 0.002; KH_2PO_4, 0.2; $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, and $CuSO_4.5H_2O$, 0.001 (in $g{\cdot}L^{-1}$) and pH of the media was maintained at 7.[19]

Preparation and Pre-treatment of LDPE Films

LDPE waste bags were cut into small strips of 10 × 10 mm^2 and washed with distilled water followed by ethanol (70% v/v) washing. The sterilized polyethylene strips were air dried, weighed and stored aseptically for further use. These LDPE films were subjected to pretreatment by UV-radiation and thermal treatment for 144 h. The UV treatment of LDPE samples with UV lamps of 16 W (Philips TUV, made in Poland) placed on racks at 5 cm from the lamp. Low density polyethylene strips were pretreated thermally in a preheated hot air oven at 70°C for a period of 144 h.

Characterization of LDPE Film

The untreated, UV irradiated and thermal pretreated LDPE films were subjected to Fourier Transform Infrared Spectroscopy analysis (FTIR Bruker, Model: Tensor 27) to detect the changes in functional groups on the polymer surface in the spectral range of 4000–600 cm^{-1}. The indices such as ester carbonyl index (1740 cm^{-1}/1465 cm^{-1}), keto carbonyl index (1715 cm^{-1}/1465 cm^{-1}) and double bond index (1650 cm^{-1}/1465 cm^{-1}) were estimated to analyze the changes.[4]

UEO Collection

The UEO used in this study was obtained from local motor workshop in Bathinda (India) (30°12' 38.23" N, 74°56' 45.47" E) and stored at 4°C till further use.

Biodegradation of LDPE by *L. fusiformis* TPB

The effect of UEO, UV and thermal treatments on biodegradation of LDPE strips were studied. The degradation study was performed in mineral salt media in Erlenmeyer flasks and incubated at 30 ± 2°C and 150 rpm in a rotary shaker for an incubation period of 30 days. The biodegradation of LDPE by *L. fusiformis* TPB was studied in LDPE enriched synthetic medium with untreated, UV irradiated and thermally treated LDPE films in separate flasks. The effect of UEO (0.5% v/v) was compared with untreated, pretreated and Tween 80 (0.5% v/v) on the LDPE biodegradation by *L. fusiformis* TPB. Each flask was inoculated with 5% of 18 h old inoculum of *L. fusiformis* TPB grown in Luria-Bertani broth. The LDPE samples were collected from the media at 5 days intervals. The residual LDPE samples were washed with 2% (v/v) aqueous sodium dodecyl sulfate (SDS) solution followed by washing with distilled water to remove the bacterial biofilm attachment from the LDPE surface.[20] The extent of LDPE biodegradation was then estimated from the weight loss of the LDPE films from initial to final weight in percent.[21]

Estimation of Bacterial Biomass on LDPE Film

The bacterial biomass was measured indirectly by determining the concentration of total extractable protein from the bacterial colonies adhered to the polymer surface.[22] The biomass on LDPE samples were recovered by boiling with 0.5 $mol{\cdot}L^{-1}$ NaOH for 30 min followed by centrifugation. The protein extracted in the supernatant was determined spectrophotometrically by the Lowry method.[23] SEM analysis was also performed for the samples with different treatments.[24]

Statistical Analysis

All the experiments were performed in triplicates, and results are represented as mean ± standard deviation. Analysis of Variance (one way ANOVA) was performed to statistically analyze the results, and significantly different values were expressed at p value ≤ 0.05.

Results and Discussion

Characteristics of LDPE Bags

The LDPE bags used in the present study were translucent, semi rigid and of 30 ± 2 μm thickness. The results of TGA analysis revealed 95.08% weight loss in LDPE samples at the temperature range of 25 to 530°C. The TGA curve for the LDPE samples had only an initial 26.01% mass loss at the temperature range of 25 to 400°C stating the thermal stability. The maximum weight loss percentage of 63.5 was recorded at 400 to 475°C due to loss of volatile fractions. Further increase in temperature to 530°C, the weight loss percentage was reduced and declined to 5.57%.

Structural Changes from FTIR Spectra and SEM Images of Thermally Treated and UV Irradiated LDPE Bags

The thermal treatment at 70°C and UV irradiation of LDPE bags for 144 h had induced polymer surface modulations and structural deformations. The variations in band intensity and generation of marked peaks in pretreated LDPE films of bags were observed at different regions by FTIR analysis (Fig. 1) and compared with untreated LDPE films (control). The presence of carbonyl groups in FTIR spectra were confirmed in thermally pretreated and UV irradiated LDPE samples by the peaks at 1713 cm^{-1} and 1714 cm^{-1} and between 1710–1750 cm^{-1} respectively. The thermal treatment at 70°C had a new absorption band at 1019 cm^{-1}. The new absorption bands at 1368 cm^{-1} was observed in both LDPE samples with thermal treatment at 70°C and with UV irradiation confirmed the oxidized fraction containing OH group. Previous studies have also reported the introduction of polar groups such as –OH, C = O, COOH and COO- on the polymer matrix by the photo and thermo-oxidative treatments.[24,25] The shifting of the peak values of functional groups in pretreated films indicated the conformational changes in the LDPE.[19] The thermal treatment of LDPE films for 144 h increased the ester carbonyl residue up to 23.88%. UV irradiation of LDPE films for 144 h enhanced the ester carbonyl residue to 33.96% (Table 1). The thermal and UV pretreatments also increased the keto carbonyl and double bond index (DBI) from the unoxidized control LDPE films. The carbonyl index and double bond index of LDPE films irradiated by UV for 144 h were higher than thermally oxidized LDPE films by 19.50% and 13.98% respectively.

The carbonyl groups are major photooxidation products and indicator of polymer chain scission.[26,27] LDPE had high affinity to oxidation due to branched structure and oxidation of stabilizers and plasticizers increasing the carbonyl residue. The generation of free radicals makes them easily available to microbial degradation.[11] Earlier studies have also confirmed the expressive role of photodegradation and thermo-oxidative reaction in structural deformation such as cross linking of carbon backbone and chain scission.[28,29] Martínez-Romo and coworkers testified the similar pattern showing increase in carbonyl index in LDPE after exposed to UV-B radiation with 3.5×10^{-4} W/cm^2 UV-B lamps for five days.[25] The prior

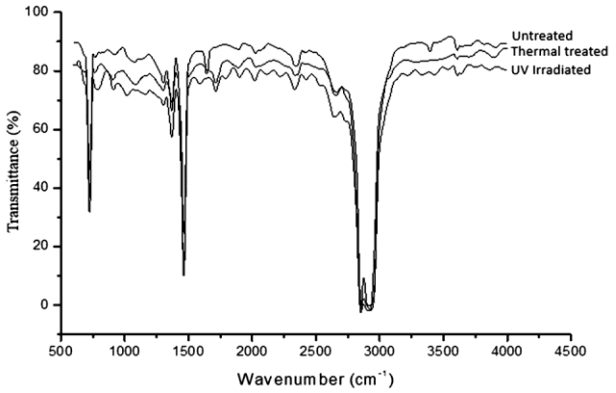

Fig. 1 — FTIR spectra of untreated LDPE films, thermally treated LDPE films at 70°C for 144 h and photo-oxidized (UV irradiated) LDPE films for 144 h

Table 1 — Effect of UV radiation and thermal treatment on FTIR spectra (ester carbonyl, keto carbonyl index and double bond index) of LDPE films

LDPE	Ester carbonyl index	Keto carbonyl index	Double bond index
Untreated	0.716 ± 0.07	0.715 ± 0.05	0.719 ± 0.04
UV irradiated	0.952 ± 0.15	0.815 ± 0.09	0.805 ± 0.11
Thermally oxidized (70°C)	0.887 ± 0.12	0.730 ± 0.10	0.731 ± 0.06

abiotic treatment increased the amount of carbonyl residue that increased polymer availability by introducing hydrophilic functional groups.[30,31] The surficial changes caused by the irregular distribution of short branches and degradable product introduced after abiotic pretreatments were also reported in thermal aged (70°C) and UV irradiated LDPE .[32]

Biodegradation of LDPE by *L. fusiformis* TPB

The untreated, thermally and UV treated LDPE films were studied for biotic degradation potential of *L. fusiformis* TPB isolated from hydrocarbon contaminated soil for 30 days. The growth of bacterial biomass on LDPE films were observed by computing the total protein concentration during incubation period.

L. fusiformis TPB had grown in synthetic media with untreated LDPE films and used as sole carbon source, colonized on the polyethylene surface and formed a sparse biofilm. The total protein content extracted from the media confirmed the gradual increase in biomass production of *L. fusiformis* TPB. Bacterial isolate was able to degrade LDPE films and 9.51% weight loss was reported after 30 days of incubation. The bio-film formation on the surface of polyethylene aids polymer metabolism by conversion of carbonyl groups into unsaturated hydrocarbon.[33] The previous reports have also confirmed the polyethylene degradation potential of different species of *Lysinibacillus.*[8,34]

Effect of Thermal Pretreatment (70°C) and UV Irradiation on Biodegradation of LDPE by *L. fusiformis* TPB

The enhanced biodegradation by isolated *L. fusiformis* TPB was reported using thermally pretreated LDPE at 70°C for 144 h. The isolated bacterial strain was able to degrade 12.89% of thermally treated LDPE films in 30 days with improving biodegradation percentage by 35.54 than untreated LDPE films. The UV irradiation of LDPE for 144 h also increased biodegradation by *L. fusiformis* TPB to 13.78% in 30 days of incubation. The photooxidation of LDPE films increased the weight loss percentage by 44.90 and 6.09 respectively in comparison to untreated LDPE and thermally pretreated LDPE films at 70°C. The biomass production of *L. fusiformis* TPB on thermally pretreated and UV irradiated LDPE films was increased with 1.07 and 3 times than the protein concentration of untreated LDPE in 30 days. The protein concentration recorded for the LDPE films after oxidative treatments were significantly higher than LDPE films without treatment (*p* value at 0.05 level).

Effect of UEO on Biodegradation of LDPE Films by *L. Fusiformis* TPB

The bacterial biomass on untreated LDPE film supplemented with UEO was also measured from total protein concentration. The protein concentration of 52.59 µg/cm^2 was recorded in UEO supplemented media enriched with untreated LDPE films. The biomass production of *L. fusiformis* TPB was 23.84% higher than untreated LDPE films. The result data depicted in Fig. 2a shows a biphasic pattern of bacterial co-metabolism using LDPE films and UEO. The first phase was characterized by a gradual increase in protein content in 5 days of incubation followed by a phase of steep upsurge in protein concentration. The addition of UEO in media enhanced the protein concentration by 1.16 times and 1.10 times respectively than thermally pretreated and UV irradiated LDPE films (Fig. 2a).

The bacterial cells of *L. fusiformis* TPB efficiently covered the polymer surface and maximum

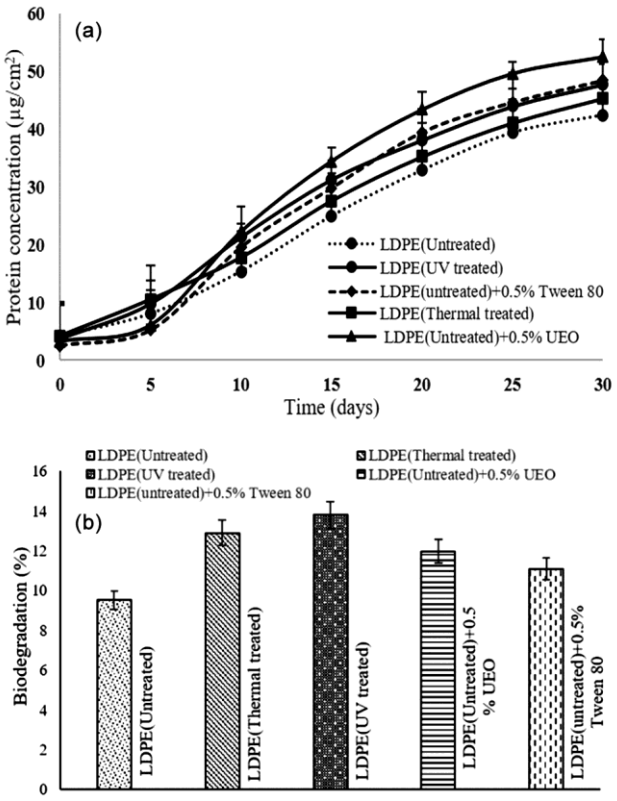

Fig. 2(a)— Protein concentration in media inoculated with *L. fusiformis* TPB using untreated, thermal and UV pretreated LDPE and effect of 0.50% Tween 80 and UEO on biofilm formation (b) Biodegradation of untreated and pretreated LDPE films in mineral salt media by isolated *L. fusiformis* TPB in 30 days

biodegradation of 11.96% of the LDPE film was obtained from LDPE metabolism in media with UEO (Fig. 2b). The supplementation of UEO (0.5% v/v) in media enhanced LDPE biodegradation potential of *L. fusiformis* TPB with an efficiency of 25.76% compared to untreated LDPE films in 30 days. The biodegradation rate of the LDPE films has been altered by supplementation of synthetic and biotic agents that changes hydrophobic interaction between polymers and the microorganisms and helps in stimulation of polymer availability to bacteria.[35] The engine oil is a combination of long-chain (C_{16}–C_{36}) saturated hydrocarbons with base lubricant oil and additive.[36] This also supports the increased bacterial biomass production.

Biodegradation of LDPE Films by *L. fusiformis* TPB in Presence of Tween 80

The LDPE biodegradation in presence of UEO by isolated *L. fusiformis* TPB was further compared with a non-ionic surfactant, Tween 80. The supplementation of Tween 80 improved the protein concentration by 14.23% than untreated LDPE films (Fig. 2a) and weight loss was 11.08% after 30 days of incubation (Fig 2b). The protein concentration in UEO supplemented LDPE enriched media was 8.42% higher than Tween 80 indicating UEO as a comparable colonizing agent. The protein concentration in Tween 80 supplemented LDPE enriched media was also found higher than thermally treated and photo-oxidized LDPE films.

The SEM images confirmed the correlation between bacterial adherence and biodegradation of LDPE films by *L. fusiformis* TPB. The supplementation of Tween 80 in media moderately supported bacterial attachment to the polyethylene surface by reducing hydrophobic interactions than control (Fig. 3 a, b). The surface coverage of bacterial strain in LDPE with UEO supplementation was found comparatively more than Tween 80 supplemented LDPE film (Fig 3 c). The more surface damage was observed for biodegradation of LDPE with abiotic pretreatments. The SEM micrograph of 144 h thermally treated (70°C) and UV irradiated LDPE films incubated with *L. fusiformis* TPB confirmed the improved bacterial colonization on polymer surface (Fig. 3 d & e). The better bacterial adherence and biodegradation were clear with cell-like molded pattern on polymer surface with a number of cracks

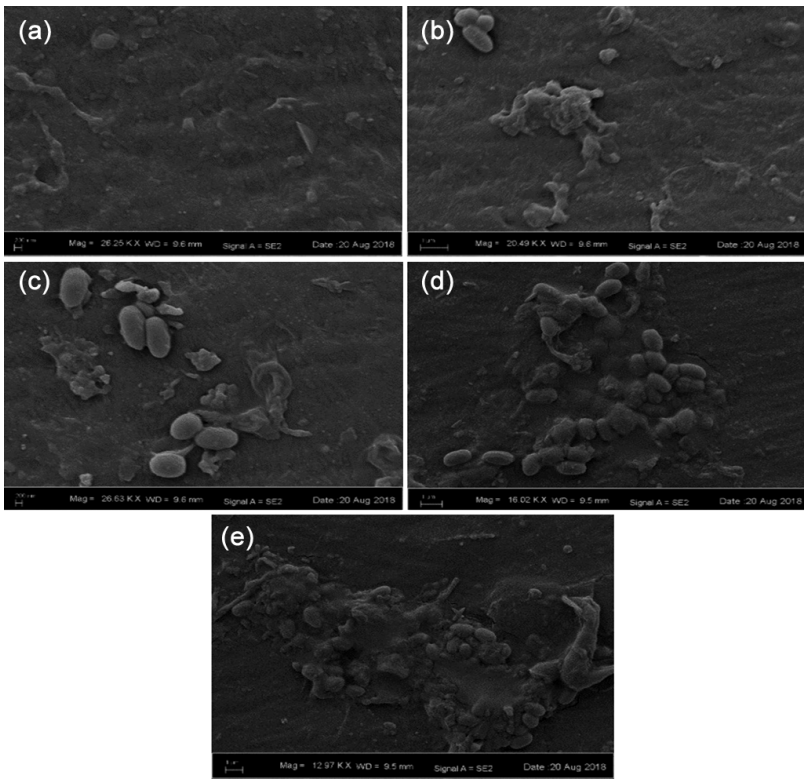

Fig. 3 — Scanning electron microscopic images of biofilm formation by *L. fusiformis* TPB on the LDPE surface (a) untreated LDPE films (b) LDPE with 0.50% Tween 80 (c) LDPE with 0.50% UEO (d) thermally treated LDPE films at 70°C for 144 h (e) UV irradiated LPDE films for 144 h

and grooves. The UV irradiated LDPE films had more bacterial colonization and biofilm formation and hence resulted in more biodegradation of LDPE than untreated films and thermally treated films at 70°C. The present study found UEO supplementation and UV-thermal pretreatments to LDPE had increased bacterial adherence in comparison to 0.5% Tween 80.

The role of indigenous *L. fusiformis* from oil contaminated sites to emulsify petrol, diesel and engine oil by biosurfactant production have been already established in earlier reports.[37,38] The petroleum-based mineral oil proliferates bacterial adherence to polymer surface and metabolism of polyethylene concomitantly increases polymer degradation.[15] The enhanced biodegradation by microbes due to oxidation by UV and thermal treatments was also reported by researchers.[13,14,35] In the present study the biodegradation of LDPE by *L. fusiformis* TPB was found higher than the degradation potential of *Bacillus sp.* reported by Harshvardhan and Jha.[39] Present investigation also reported higher biodegradation potential of *L. fusiformis* TPB isolated from hydrocarbon contaminated sites in comparison to different indigenous strains of bacteria identified by the Kunlere and co-workers.[40]

Conclusions

The indigenous bacterium isolated from hydrocarbon contaminated soil had the potential to use it as sole carbon source and helped in LDPE degradation. The used engine oil supplementation, thermal oxidation and UV irradiation of LDPE films enhanced the colonization of *L. fusiformis* TPB on polymer surface and increased polymer degradation in 30 days. Further study need to be carried out for improvement in LDPE biodegradation by UEO and abiotic pretreatments with potential microbial strains to achieve valuable in-situ plastic waste management.

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