

Biodeterioration Studies of Thermoplastics in Nature using Indigenous Bacterial Consortium

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ABSTRACT

Thermoplastics, poly vinyl chloride and low-density polyethylene were treated in the presence of indigenously developed bacterial consortium in laboratory and natural conditions. The consortium was developed using four bacteria, selected on the basis of utilization of PVC as primary carbon source, namely P. otitidis, B. aerius, B. cereus and A. pedis isolated from the plastic waste disposal sites in Northern India. The comparative in-vitro treatment studies as revealed by the spectral and thermal data, illustrated the relatively better biodegradation potential of developed consortium for PVC than the LDPE. Further, the progressive treatments of both the thermoplastics were conducted for three months under natural conditions. For this purpose, bioformulation of consortium was prepared and characterized for the viability up to 70 days of storage at 25±1°C. The consortium treated polymer samples were monitored through SEM and FT-IR spectroscopy. Analytical data revealed the biodeterioration potential of the developed consortium for PVC and LDPE, which could help in disposing the plastic waste.

Key words: Biodeterioration, Bioformulation, Thermoplastic, FT-IR, SEM, TG-DTG-DTA

INTRODUCTION

Nowadays, wider applications of thermoplastics with particular emphasis on poly vinyl chloride (PVC) and low-density polyethylene (LDPE) in domestic, scientific and technological applications have raised serious issue of waste disposal, leading to pollution (Kawai 2010). Over the past few years, stabilization and degradation of such recalcitrant thermoplastic-based materials and issues related to their applications, health, safety and environmental impact have been of immense academic and technological interest. In this context, among various strategies of degrading

recalcitrant polymers, biodeterioration and biodegradation offer a mild means for their recycling and disposal (Wypych 2008; Arutchelvi et al. 2008; Yoon et al. 2012; Sharon and Sharon 2012). Although, biodegradation is necessary for both water-soluble and water-immiscible polymers because they eventually enter the streams, which can neither be recycled nor incinerated (Barnes et al. 2009). Several microorganisms such as *Pseudomonas aeruginosa*, *Aureobasidium pullulans*, *Rhodotorula aurantiaca* and *Kluyveromyces* sp. have been reported to cause *in situ* colonization and substratum deterioration of plasticized PVC (Booth and Robb 2007). Further,

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the colonization of *A. pullulans* displays a significant substratum damage of the plasticized PVC, followed by *R. aurantiaca* and *Kluyveromyces* sp., suggesting that microbial succession occurs during the colonization process and that *A. pullulans* has been critical to the establishment of a microbial community on the polymers (Webb et al. 2000). The plasticizer content of PVC has been reported to degrade in the presence of *P. fluorescens* (Zeng et al. 2002), *Coryneform bacterium* and *Mycobacterium* sp. (Nakamiya et al. 2005). The blend of PVC/polycaprolactone has also been reported to degrade in the presence of *Curvularia* sp., *Trogia buccinalis* and *Phanerochaete chrysosporium* (Sandra et al. 2010).

Similarly, polyethylene's has been reported to degrade in the presence of *Pseudomonas*, *Streptococcus*, *Staphylococcus*, *Micrococcus* (Kathiresan 2003, Yoon et al. 2012), *Xanthomonas*, *Flavobacterium*, *Agrobacterium* and *Bacillus* sps (Gupta et al. 2010). However, degradation of thermoplastic in natural condition is a global challenge of this time (Nowak et al. 2011). Hence, there is an urgent need to develop efficient consortia and their products to solve the burning issue, i.e., plastic waste management in nature. Thus, the present study focused on the *in vitro* and *in situ* treatment of two thermoplastics in the presence of potential bacterial strains and their bioformulation, respectively.

MATERIALS AND METHODS

Starting Materials

PVC and LDPE beads were procured from Sigma-Aldrich Inc., St. Louis, MO, U.S.A. and converted into powdered form by boiling with xylene, followed by solvent evaporation at room

temperature (28°C). The powdered form was successively washed with 70% ethanol and dried at 50±1°C for 1 h and used as a primary carbon source for *in vitro* studies. The above processing was done in order to increase the surface area of polymer for better interaction with the bacteria in the liquid medium. Further, commercially available PVC and LDPE films were also used for the *in situ* studies. The talc, which was used as a carrier for bioformulation preparation was purchased from HiMedia Lab Pvt. Ltd, Mumbai, India. It was composed of talcum steatite, talc fine powder and hydrous magnesium silicate. Nutrient broth (HiMedia, India) was taken as medium for the bacterial growth and Minimal broth Davis without (w/o) dextrose (HiMedia, India) as the medium for the *in vitro* studies.

Bacterial Consortium

A total of four bacterial cultures were retrieved from the departmental culture collection of Microbiology, CBSH, G. B. Pant University of Agriculture and Technology, Pantnagar, India (Table 1). These cultures were selected on the basis of their pre-identified potential to act upon PVC (Anwar 2011) and LDPE (Kapri et al. 2010). The cultures were revived on nutrient broth and maintained on nutrient agar at optimum initial pH (7.0±0.2) and temperature (37°C). A single colony of each bacterial culture was inoculated into 50 mL flask containing 10 mL of nutrient broth and active cultures were prepared by incubating the flask for 16 h with continuous shaking at 120 rpm. The calculated amount (cfu mL⁻¹) of each strain was mixed for the development of consortium. The compatibility of the used strains for the preparation of the consortium was tested and reported earlier (Satlewal et al. 2008; Negi et al. 2009; Soni et al. 2009; Goel et al. 2010; Kapri et al. 2010; Anwar 2011; Goel et al. 2011).

Table 1 - Bacterial strains used in the study.

Bacterial strains	Accession no.
<i>Pseudomonas otitidis</i> strain SPT1	GU598256
<i>Bacillus aerius</i> strain SPT2	GU598257
<i>Acanthopleuribacter pedis</i> strain SPT3	GU598258
<i>Bacillus cereus</i> strain SPK1	GU598261

Laboratory Scale Treatment of Thermoplastics

For the *in vitro* studies, 100 mL Minimal broth Davis w/o dextrose (pH 7.0 ± 0.2) was taken in

five 250 mL Erlenmeyer flasks. One flask containing 300 µl of active consortium in the absence of any polymer was taken as positive

control. Two flasks containing PVC and LDPE powder (added separately) at a concentration of 5.0 mg/mL without the consortium were taken as negative control. The remaining two inoculated with above said amount of consortium and both the polymers separately were taken as the experimental flasks. All the flasks were incubated at 37°C with continuous shaking (120 rpm). The shift in λ -max in the treatments and negative controls was determined after the regular intervals of 24 h during the incubation period, till the consortium attained the stationary phase. The treated samples were recovered from the broth through the filtration of flask content and then the filtrate as well as the residue was collected separately. The filtrate was centrifuged at 5000 rpm for 15 min to remove the bacterial biomass and the supernatant was kept in an oven at 50°C for overnight to evaporate the water and the residual samples was recovered and analyzed.

Treatment in Natural Condition

Preparation and viability determination of talc-based bioformulation

Prepared active consortium (200 mL) was spun at 5000 rpm for 15 min to remove the bacterial cells. Then, 10 g talc was weighed and added to the pellets under sterile conditions and mixed properly. The mixture was then kept at room temperature (28±1°C) aseptically for drying. The viability of bacterial isolates in the bioformulation was ascertained by serial dilution plating method. For this purpose, 1.0 g of talc-based formulation was mixed in 1.0 mL of sterile distilled water. The suspension was further mixed with 9.0 mL of sterilized distilled water. Likewise, the dilution plating was done in nutrient agar medium. The plates were incubated at 37±1°C and the cfu mL⁻¹ counts were checked initially after 2 and 4 days. Thereafter, the viability was determined after regular interval of 7 days for subsequent three weeks, followed by 15 days interval. The above pattern was followed keeping in view the rapidity of changes in viable counts during the storage. The plate counts were carried out in triplicates and the average of the three respective readings were taken.

In-situ treatment

Top-soil was dug from a barren land at Pantnagar, India, autoclaved at 121°C for 25 minutes and then filled into 45×34 cm² sinks. The PVC and LDPE film coupons of dimensions 2.5 x 2.5 cm² (1.0

inch²) were surface sterilized with ethanol (70%) for 10 min and subsequently mounted vertically inside the soil at varying depths. The soil of the respective treatments was inoculated with the bioformulation and incubated in natural condition. The moisture and aeration in the experimental sinks were taken care by adding autoclaved distilled water and shovelling the soil at regular intervals of two weeks. The PVC and LDPE film coupons incubated in the soil devoid of bacterial consortium were taken as control. Both the film coupons were kept in soil for three months, thereafter recovered and then sterilized with ethanol (70%) for 10 min and dried in a desiccator for 24 h under vacuum.

Analysis of Treated Polymer Samples

The changes in the polymer samples treated were preliminary determined by measuring the λ -max shift in comparison to negative control through Perkin Elmer UV-Vis Spectrophotometer. In order to eliminate the discrepancy between the consortium-treated polymer samples and their untreated controls, the thermal properties were studied through simultaneous thermogravimetric (TG)-differential thermogravimetric (DTG)-differential thermal analysis (DTA) over Perkin Elmer (Pyris Diamond) thermal analyzer under nitrogen atmosphere (200 mL min⁻¹) from 50 to 1000°C with heating rate of 5°C min⁻¹ on a platinum pan. The weight loss (%) of the samples was evaluated as a function of temperature through TGA. The temperature with the maximum weight loss was illustrated as DTG peak temperature and the heat of degradation (ΔH) involved in the thermal behaviour of the samples representing the exotherm and endotherm was estimated through DTA.

The chemical changes in the residuals and the recovered polymer films obtained after the treatment were determined through Perkin Elmer Fourier transform infrared (FT-IR) Spectrophotometer and the spectra were recorded on KBr disc, taking pure PVC and LDPE as the references. The change in the morphology of the recovered polymer films were ascertained through the microscopy studies. For this purpose, the samples were metallized with gold particles (three discharges of 40 mA/50s in argon atmosphere) in a high vacuum metalizer (Bal-Tec SCD 005) and analyzed by scanning electron microscopy (SEM) (Leo, 435VF, U.K.) at 15.00 kV EHT under three successive magnifications (1.5, 3.0 and 5.0 KX).

RESULTS AND DISCUSSION

Comparative Growth Profiling of Consortium

The growth profile of the used consortium, compared in the absence and presence of the polymers (PVC and LDPE) revealed that both the polymers didn't affect the growth pattern of the consortium as stationary phase was achieved within two days, as in case of control (Table 2). However, in the presence of polymers, the consortium showed reduction of lag phase by 24 h, earlier onset of log phase and more biomass (revealed by the OD₆₀₀), which indicated the sustained growth of consortium as compared to the control (Figures 1 A, B). Further, a prominent shift in λ max of PVC (from 206.3 nm to 218.84 nm) was observed within 24 h of incubation and

subsequently attained a value of 218.84, 218.15 and 217.13 nm after a successive period of incubation, i.e., 3rd, 4th and 5th day, respectively (Fig. 1A). However, shift in λ max of LDPE (from 202.1 nm to 209.5 nm) was observed after 2nd day and subsequently up to 4th days of incubation (Figure 1B). These findings suggested that the microbial action on PVC could be fast in comparison to the LDPE. Further, the results indicated that both the polymers were used by the bacterial consortium for their survival as the growth medium was not containing any other carbon source. Also, higher bacterial biomass and changes in the polymer structure were achieved during the incubation period as reported by Kapri et al. (2010) and Sah et al. (2010).

Table 2. Growth profile of bacterial consortium grown in the presence of PVC and LDPE as sole carbon source in liquid medium during incubation.

Treatment	Days	OD ₆₀₀ values in triplicate			λ max
Consortium	0	0.001	0.001	0.001	-
Consortium	1	0.016	0.015	0.014	-
Consortium	2	0.13	0.12	0.14	-
Consortium	3	0.11	0.11	0.12	-
Consortium	4	0.08	0.09	0.08	-
Consortium	5	0.114	0.115	0.116	-
Consortium + PVC	0	0.001	0.001	0.001	203
Consortium + PVC	1	0.196	0.195	0.192	204
Consortium + PVC	2	0.17	0.18	0.18	206
Consortium + PVC	3	0.17	0.16	0.17	207
Consortium + PVC	4	0.162	0.161	0.16	208
Consortium + PVC	5	0.163	0.162	0.161	209
Consortium + LDPE	0	0.001	0.001	0.001	207
Consortium + LDPE	1	0.134	0.135	0.133	216
Consortium + LDPE	2	0.156	0.155	0.156	218
Consortium + LDPE	3	0.153	0.152	0.154	219
Consortium + LDPE	4	0.135	0.136	0.135	217
Consortium + LDPE	5	0.182	0.183	0.182	216

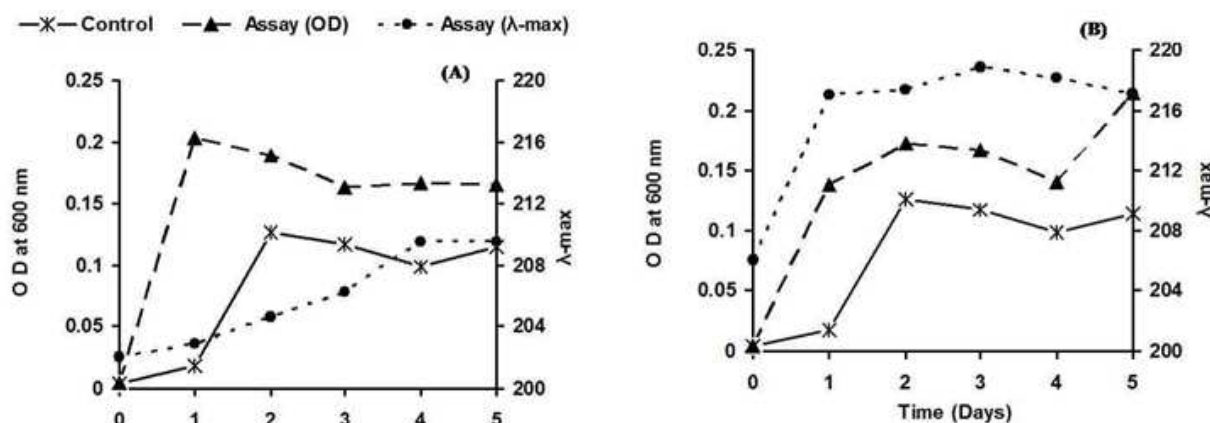


Figure 1 - Growth profile of bacterial consortium grown in the presence of PVC (A) and LDPE (B) as sole carbon source in liquid medium.

Shelf Life of Bioformulation

The viability of the consortium was maintained in the talc-based bioformulation for a period of 70 days at ambient temperature (Table 3).

These observations suggested that the consortium was viable in the developed carrier-based formulation. The consortium in the form of powdered bioformulation was further used for the pilot-scale experiments. The viability test for

PGPR bioinoculants was also conducted by Arora et al. (2008), wherein sawdust was used as the carrier. The clay-based formulation of Pseudomonads for degrading the petrol has been reported by Ting et al. (2010). The talc as carrier has also been reported for PGPR strains for management of various plant pathogens (Shanmugam et al. 2011).

Table 3 - Shelf life of bioformulation under ambient storage temperature.

Cfu/ml* ($\times 10^6$) at subsequent time intervals (days)							
2 nd	4 th	11 th	18 th	25 th	40 th	55 th	70 th
223 \pm 2	216 \pm 2	210 \pm 2	176 \pm 2	171 \pm 2	90 \pm 2	76 \pm 2	36 \pm 2

*Mean of three replicates.

Analysis of Treated Polymers Simultaneous TG-DTG-DTA

The thermal data of PVC and LDPE treated by the consortium with reference to control, are shown in Table 4.

The thermogram of control polymers showed single step decomposition. The thermal degradation of control PVC started at 301°C with weight loss (WL) of 3.1% and was terminated at 500°C with complete decomposition. The TG data was further supported by a DTG profile. A triplet was observed in DTG profile at temperature ranging 400-469°C with highest rate of decomposition (0.111 mg/min). It also showed a

pair of DTA exotherm at 403°C (115 μ V) and 476°C (104 μ V) with heat of decomposition Δ H (mJ/mg), 138 (Fig. 2A). In case of control LDPE, the thermal degradation started at 400°C with 4.5% WL and it was terminated at 466°C with weight residue of 0.6% as reported by Tajeddin et al. (2009). The single step thermal decomposition was observed with a DTG profile at 451°C and the rate of decomposition was 1.79 mg/min. A pair of DTA endotherm has been reported at 107°C (-3 μ V) and 457°C (-31.4 μ V) with Δ H, 129.0 and 53.0 mJ/mg, respectively. Along with this temperature, a weak exotherm was also observed at 476°C (104 μ V) with Δ H -14.6 mJ/mg (Fig. 2B).

Table 4 - Thermal analysis of consortium treated PVC and LDPE powder with reference to respective control.

Thermoplastic	DTG Peak Temperature		DTA Exotherm		DTA Endotherm	
	°C	Decomposition Rate (μ g/min)	°C	Δ H (mJ/mg)	°C	Δ H (mJ/mg)
PVC Control	400	101	403,476	6.28	132	138
	436	111				
	469	930				
LDPE Control	451	1790	467	14.6	107	129
Treated PVC	57	161.7	-	-	62	92.5
	117	76.4				
	154	158.0				
	222	101.3				
Treated LDPE	148	710	-	-	149	155
	218	150				
				576	83.4	

The consortium treated PVC was undergoes three-step decomposition with an early onset of thermal decomposition temperature. The first step of decomposition occurred at 65°C, second step at

107°C and final step at 230°C with 4.6, 5.4 and 11.9% WL, respectively. The three steps decomposition was verified through three DTG profile with the highest rate of decomposition

(0.161 mg/min) at 57°C. The TG data was further supported by DTA endotherm at temperature ranging 62°C (-11.0 μV) to 225°C (-15.86 μV) with ΔH , 92 to 147 mJ/mg, respectively (Fig. 2C). However, for LDPE, two steps thermal decomposition was observed by the treated with consortium. The TG data revealed the first step decomposition at 120°C with 3.6% WL and second step decomposition at 202°C with 16.8% WL. TG data was further verified through two DTG profile with the highest rate of decomposition (0.71 mg/min) at 148°C. These data were further supported by a pair of DTA endotherm at temperature ranging 149°C (-17.7 μV) and 224°C

(-13.6 μV) with ΔH , 155 and 92.2 mJ/mg, respectively (Fig. 2D). However, the similar significant changes in the thermal profiles of biodegraded samples of polycarbonate (Goel et al. 2008) and LDPE (Sah et al. 2010) have been reported showing the biodegradation potential of the used consortium for PVC and LDPE. Thus, the consortium was responsible for multiple step decomposition, changes in the thermal profile and increased weight loss of the treated polymer. Further, the comparative analysis of the change in the thermal profiling suggested that the consortium could better use PVC as sole carbon source in comparison to the LDPE.

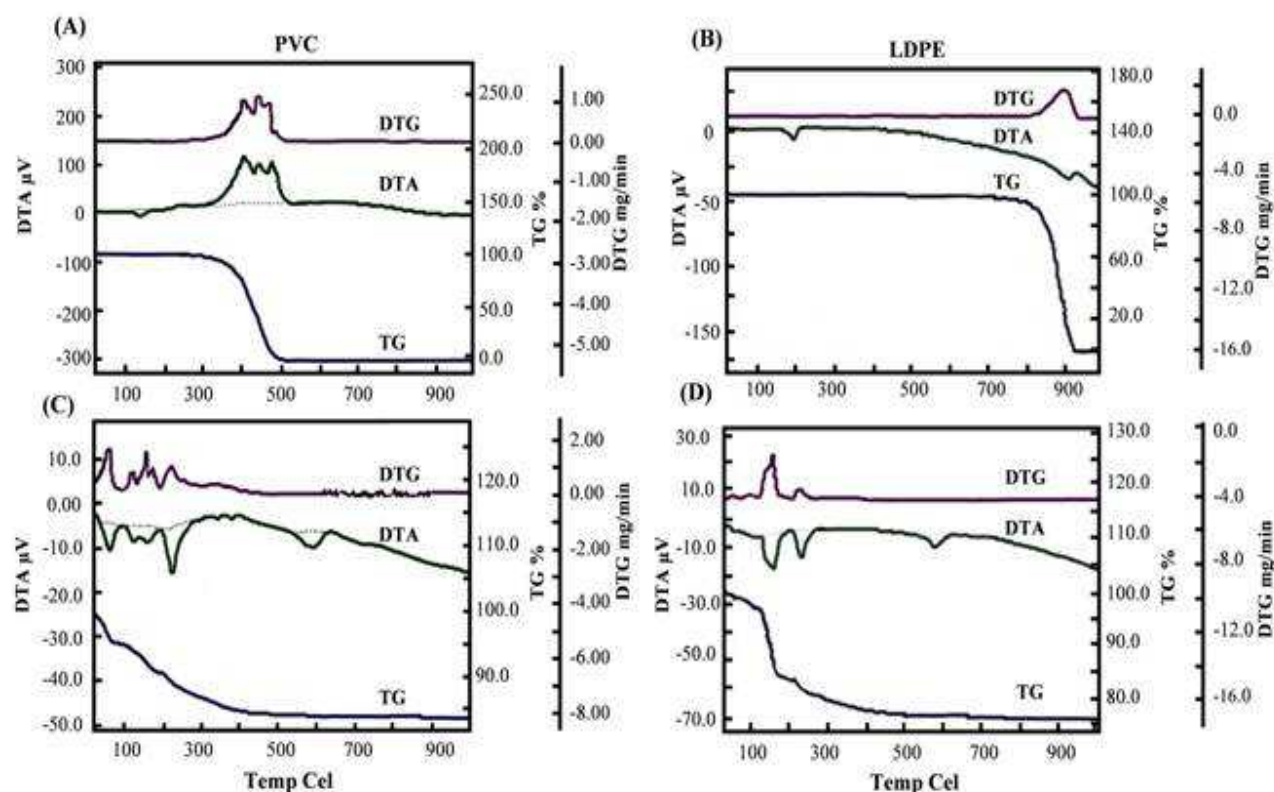


Figure 2 - Comparative TG -DTG-DTA thermogram of treated PVC (C) and LDPE (D) with reference to their control (A) and (B), respectively.

FT-IR spectra

The potential of consortium towards the biodegradation of PVC and LDPE powder was also reflected through the shift in the wave numbers (cm^{-1}) in comparison to the untreated one (Fig. 3). The changes in the polymers incubated in the presence of consortium were monitored in the range of 4000 to 450 cm^{-1} . The control PVC powder (Fig. 3A) and film (Fig. 3B) showed

characteristic wave numbers (cm^{-1}) at 2962.8-2363.0 (ν CH_2), 1469.6-1463.0 (δ CH_2), 1376.3-1371.8 (δ CH), 1084.7-1073.2 (ν C-O-C), and 745.9-721.5 (ν C-Cl) as reported by Mekki and Belbechir (2007) for vinyl chloride-vinyl ether copolymers. Additional characteristic peaks reported at (cm^{-1}) 3455.9 (ν OH), 1631.2 (δ OH), 535.6 (δ C-C-C) (Fig. 3A) and at 1726.3 (ν C=O), 1280.8 (γ CH), 1127.6 (δ C-C), 652.5 (ν C-C-C)

(Fig. 3B) represented to PVC powder and film, respectively. Similar characteristic peaks were also reported by Ramesh and Yi (2008) in the plasticized PVC.

The PVC powder treated in the presence of consortium in the laboratory-scale showed characteristic wave number (cm^{-1}) corresponding to 3417.0 (ν OH), 1663.0 (δ OH), 1476.3 (δ CH_2), 1094.9 (ν C-O-C), 530.8 (δ C-C-C), respectively. However, the biodeterioration of PVC was ascertained through the introduction of the additional wave numbers at 985.4 and 863.17 cm^{-1} corresponding to ρ CH_2 and ω CH_2 , respectively. The consortium imparted simultaneous absence of wave number of ν_{as} CH_2 group and increase in the transmittance of the C-O-C group over control PVC. The removal of ν C-Cl at 721.5 cm^{-1} depicted the removal of some chlorine atom from the treated sample (Fig.3A).

The PVC film treated in natural conditions using the bioformulation showed wave number (cm^{-1}) corresponding to 3450.7 (ν OH), 2924.5 to 2854.5 (ν CH_2), 1463.8 (δ CH_2), 1216.4 (γ CH), 1080.7 (ν C-O-C), 763.5 (ν C-Cl) and 673.0 (ν C-C-C), respectively (Fig. 3B). The consortium introduced the absorption corresponding to the formation of hydroxyl group on the macromolecular chain of the PVC as well as the absence of wave numbers corresponding to δ C-C. The simultaneous shift in the wave numbers of various groups indicated the microbial action on the polymeric structure.

The control LDPE powder (Fig. 3C) and the film (Fig. 3D) showed characteristic wave numbers (cm^{-1}) absorptions corresponding to 3435.6-3426.0 (ν OH), 3030.8 (ν_{as} CH_3), 2930.5-2919.9 (ν_{as} CH_2), 2850.6-2848.2 (ν_{s} CH_2), 2368.0-2342.2 (ρ CH_2), 2151.8 (δ CH_2), 1595.1-1594.2 (δ OH), 1465.5-1461.5 (δ_{as} CH_2), 1352.6-1351.6 (δ_{s} CH_3) and 768.4-720.2 (ρ CH_2), respectively (Kapri et al. 2010). The FT-IR spectra of LDPE powder treated by the consortium in the laboratory rendered spectral absorptions corresponding to 3494.9 (ν OH), 2923.4 (ν_{as} CH_2), 2365.2 (ρ CH_2), 1570.5 (δ OH), 1465.8 (δ_{as} CH_2), and 720.2 (ρ CH_2), respectively. However, this sample showed the absence of various absorptions corresponding to methyl and methylene groups as observed in the control LDPE at 3030.8, 2850.6, 2151.8 and

1352.6 cm^{-1} . The introduction of 1218.2 (ν_{as} C-O-C) and 1071.6 (ν_{s} C-O-C) frequencies was observed in the treated polymer due to the inclusion of O atoms into the hydrocarbon polymer backbone through the microbial action (Fig. 3C). The LDPE film treated in the presence of consortium also showed the absorptions corresponding to 3427.5 (ν OH), 2919.1 (ν_{as} CH_2), 2852.8 (ν_{s} CH_2), 1601.1 (δ OH), 1443.6 (δ_{as} CH_2) and 770.2 (ρ CH_2), respectively. However, the introduction of the additional wave numbers corresponding to 1217.7 (ν_{as} C-O-C), 1066.7 (ν_{s} C-O-C) and 3021.2 (ν_{as} CH_3) and removal of 2342.2 (ρ CH_2), 1351.6 (δ_{s} CH_3) frequencies illustrated the deterioration of LDPE film sample after three months in the soil (Fig. 3D). The variations in the wave numbers in the polymers spectra revealed that both the polymer were used as sole carbon source during the incubation period. The relative potential of the consortium was reflected through the comparative FT-IR study of the treated PVC and LDPE in the whole spectral range cm^{-1} . The shift in the wave numbers of the various functional groups and changes in the backbone of PVC (δ C-C-C) indicated that the consortium was more effective towards the degradation of PVC.

Scanning electron microscopy

The morphological changes on the surface of PVC and LDPE film due to the action of consortium were analyzed after incubation period of three months in natural condition (Fig.4). The control PVC revealed smooth and homogeneous morphology (Fig.4A). But in the case of LDPE film, some wrinkled structures in a diagonal fashion were observed, probably due to the pressure of soil burial (Fig. 4B). The exposure of both the film to consortium imparted heterogeneous morphology with widened fissure, leading to the formation of cavities with wrinkles and white globular areas. The SEM micrograph revealed that the formation of cavities with wrinkles and white globular areas were more widened and prominent in the PVC (Fig.4C) as comparison to LDPE film (Fig. 4D). Thus, the observations suggested the considerable surfacial deterioration of polymer by the virtue of consortium action.

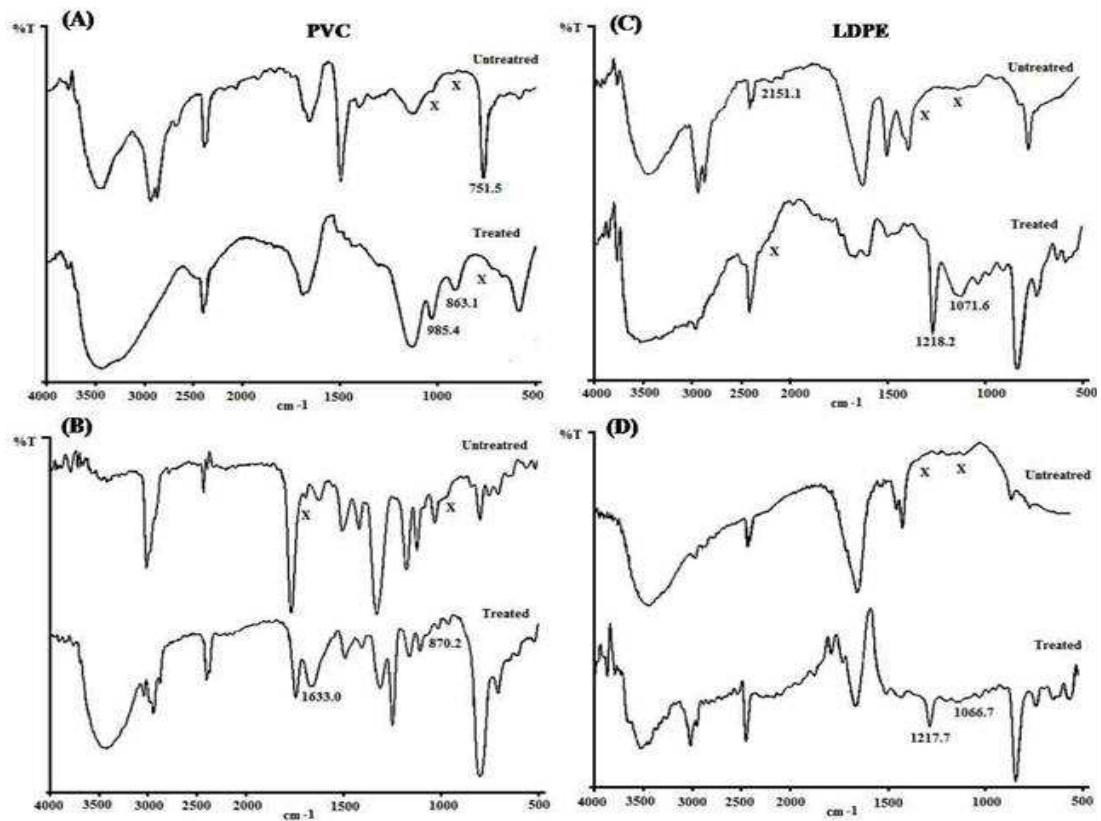


Figure 3 - Comparative FT-IR spectra of consortium treated PVC (A) and LDPE (C) powder *in vitro* and consortium treated PVC (B) and LDPE (D) film in natural conditions with reference to their controls.

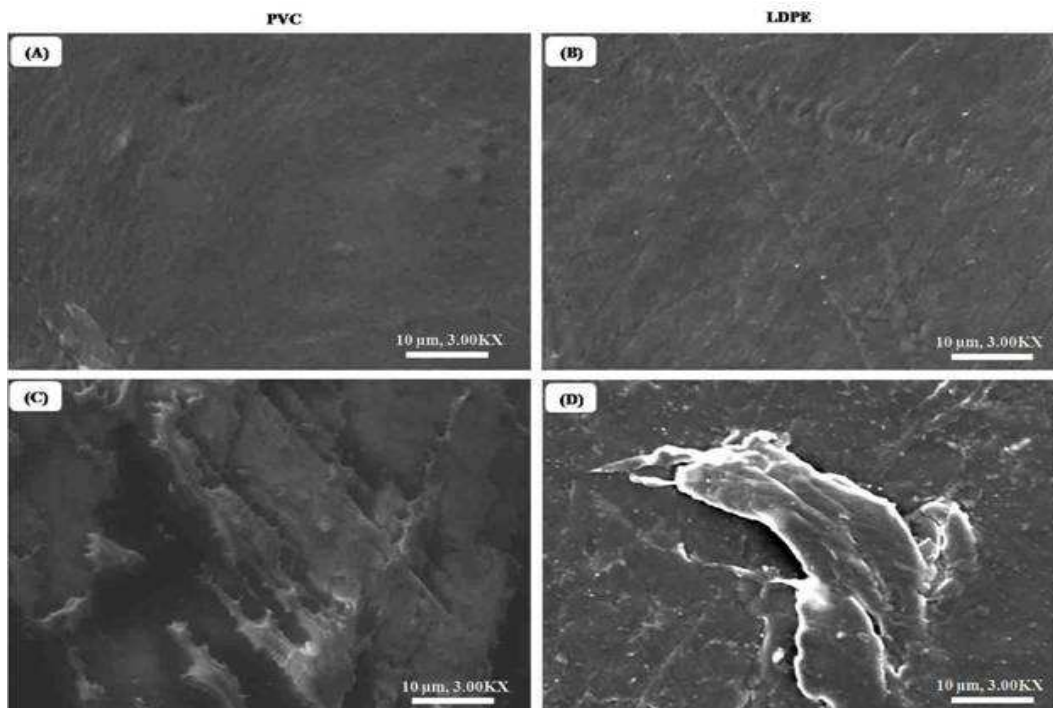


Figure 4 - SEM micrograph of consortium treated PVC (C) and LDPE (D) film in natural condition with references to their controls (A) and (B), respectively.

CONCLUSIONS

This study explored the potential of indigenously developed bacterial consortium to degrade two thermoplastics, i.e., PVC and LDPE powder in broth and their film in the soil. Results revealed that the consortium was capable to act upon the PVC more efficiently than the LDPE. This could have been due to the higher bacterial colonization on the plasticizer content of the PVC. The bioformulation showed prolonged shelf-life and the biodeterioration efficacy of the polymers. Thus, the consortium could be considered for plastic-waste management strategies. This study also established a scale-up procedure with good reproducibility of plastic deterioration and degradation by using the bioformulations at the waste disposal site. This might enhance the capabilities of potential bacterial isolates to act upon, degrade and mineralize the thermoplastic waste on site.

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