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Bacterial (*Alcaligenes Faecalis*) Degradation of PET (Poly(Ethylene Terephthalate) Obtained from Old Bottles Wastes

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Abstract

PET bottles present to modern society momentous execution qualities wanted by a wide range of shoppers yet the destiny of poly(ethylene terephthalate) in the earth has turned into a huge management problem. For this study, bacteria were isolated from soil-contaminated PET plastic surfaces. The bacterial biofilm formation, bacterial division stages, and single individual colonization of *Alcaligenes faecalis* (KY026604) bacteria observed by scanning electron microscopy on UV-exposed PET flakes surfaces were compared to UV-untreated PET flakes: UV can provide a starting point for biofilm formation due to their ease of biodegradation. Further, PET degradation was confirmed by FTIR studies, which indicated the formation of a new functional group (C=O bond stretching, C-C bond stretching, C-H bond stretching, C-H bond stretching, C-H bond stretching, C-C-O bond stretching) as evinced in day UV exposed PET.

Keywords: PET, UV light, Biodegradation, FTIR.

Резюме

Бутилките от поли-(етилентерефталат) (PET) предоставят на съвременното общество изключителни качества, които се търсят от широк кръг купувачи, но изхвърлянето им се е превърнало в огромен проблем. За настоящото изследване са изолирани бактерии от почва, замърсена с пластмасови остатъци от бутилки PET. Образуването на бактериални биофилми, етапите на деление на бактериите и колонизирането с единични клетки на *Alcaligenes faecalis* (KY026604) върху UV-експонирани повърхности от PET люспи, в сравнение с UV необработените са наблюдавани чрез сканираща електроннна микроскопия. UV-лъчите могат да дадат началото на образуване на биофилм поради лесното им биоразграждане. Разграждането на PET е потвърдено от проучвания с FTIR, които показват формирането на нова функционална група (C = O, C-C, CH, CCO връзка), както е доказано в деня на облъчване на PET с УВ лъчи.

Introduction

Polyester (poly(ethylene terephthalate) is universally used as packaging films, synthetic fibers, bottles for beverage and food, and engineering plastic components, owing to excellent thermal and mechanical properties, high chemical resistance, and low gas permeability (Goodman *et al*., 1969). In recent years, there has been a growing public concern over environmental deterioration associated with the disposal of conventional plastics. These issues have given plastic waste a significant focus within the management of solid waste as the plentiful assortment of PET waste is of ecological worry because of its non-biodegradability that could be a noteworthy deterrent of PET transformation by customary techniques, such as land filling and combustion (Kumar *et al*., 2007).

Weather is responsible for the deterioration of most exposed materials. Abiotic contributors to these conditions are moisture in its variety of forms, non-ionizing radiation, and atmospheric temperature. The ultraviolet (UV) component of the solar spectrum contributes ionizing radiation, which plays a significant role in initiating weathering effects in plastics. The hydrophobic nature of PET poses a significant barrier to microbial colonization

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of the polymer surface thus attenuating effective adsorption and access by hydrolytic enzymes to accomplish polymer degradation (Atefehoff *et al*., 2007). The biotic contributors can strongly assist colonization by providing the necessary nutrients for microbial growth. Hydrophilic surfaces may provide more suitable sites for colonization. Readily available exoenzymes from the colonized area can initiate the degradation process.

Many studies have incontestable partial biodegradation of synthetic resin has been found (Albertsson *et al*., 1987). It appears that the biodegradation of synthetic resin is enhanced by oxidation pretreatment that increases surface hydrophilicity by the formation of carbonyl groups which will be used by microorganisms (Cornell *et al*., 1984; Albertsson *et al*., 1987). Microbial biodegradation of plastics is a widely accepted option and its effectiveness is still being improved (Lee *et al*., 2012)**.** There is a growing interest in non-degradable synthetic polymer biodegradation using effective microorganisms (Boonchan *et al.,* 2000). Polymers that undergo controlled biological degradation by microorganisms have become of remarkable interest during the last years (Shakina *et al*., 2012). In this study bacteria were isolated from soil-contaminated PET bottle surfaces. UV- exposed PET inoculated with bacteria and biofilm formation confirmed by SEM images and further FTIR analysis of chemical changes.

Materials and Methods

Isolation of bacteria from PET waste

Poly(ethylene terephthalate) waste bottles were collected from garbage dump site Tiruchirappalli, Tamil Nadu, India. The soil particles on the surface of the PET waste were removed and washed with sterile distilled water and inoculated into nutrient broth. After 24 hours of incubation, 100 µl of broth culture was inoculated into nutrient agar plates. After 24 hours of incubation, the bacterial isolates were identified by the methods described in Bergey's Manual of Determinative Bacteriology (Sneath *et al*., 1994). Dominant bacteria were selected for further study and 16srRNA sequences were analyzed the Institute of Microbial Technology (CSIR-IMTECH), Chandigar. Bacterial gene sequences and phylogenetic analysis and the accession number KY026604 are given in previous article.

Incubation of PET flakes in MSM inoculated with bacteria

Purchased PET bottles were cut into small flakes (1×1 cm) and exposed to UV radiation for different time durations (10, 20p and 30 days) using UV light (500W) with an optical filter (250–380 nm) at room temperature. The distance between the PET flakes and the lamp was 3 feet. Then the PET flakes were washed with 70% ethanol and again washed with distilled water and finally the samples were kept at 45^{o}C to dry. After that, UV-exposed and UV-unexposed PET flakes samples were directly inoculated into minimal salt medium containing *Alcaligenes faecalis*. They were kept in an orbital shaker for a period of one month at 37^{o}C temperature at 120 rpm (Control PET flakes: untreated with bacteria).

Scanning electron microscopy

Scanning electron microscope (SEM) (VEGA3 TESCAN) was used to determine the changes on the surface of PET flakes and bacterial colonisation. Control and bacterial treated samples are generally sputter-coated with gold or some metal ions before SEM examination. Analysis was carried out using low vacuum 0.68 Torr mode, 10 to 30 kv at different magnifications 6.13 kx to 500 kx and LFD (large field detector) (Kumar *et al*., 2007).

FTIR spectroscopic analysis of bacterial degradation of PET flakes

Fourier-transform infrared (FT-IR) measurements were carried out with a Perkin Elmer Spectrum two (Version 10.03.09) in the range of 4000-400 cm^{-1}. FT-IR spectra were recorded at a resolution of 2 cm^{-1} and at an accumulation of 32 scans. FTIR analysis was done to detect the chemical changes of PET inoculated in MSM containing *A. faecalis.*

Results

The differences in the chemical composition of PET samples can be revealed by comparing the IR spectra. Compilation of FTIR spectral peaks band assignment of PET and 10, 20 and 30 days-UV exposed PET flakes revealed the appearance of new peaks and disappearance of a few peaks (Fig. 1, Table 1).

The signature peaks of PET flakes included 3781 cm^{-1} (O-H bond stretching), 1716 cm^{-1} (C=O bond stretching), 1407 cm^{-1} (C-H bond stretching), 1339 cm^{-1} (C-H bond stretching), 1242 cm^{-1} (C-C-O) bond stretching), 1093 cm^{-1} (O-C-C bond stretching), 1017 cm^{-1} (C-H bond stretching), 871 cm^{-1} (C-H bond stretching) and 723 cm^{-1} (C-H bond stretching). On exposure of PET flakes to UV for 10 days, new peaks appeared (1237 cm^{-1}: C-O bond stretching; 845 cm^{-1}: C-H bond stretching; 790 cm^{-1}: C-H bond stretching) was evinced.

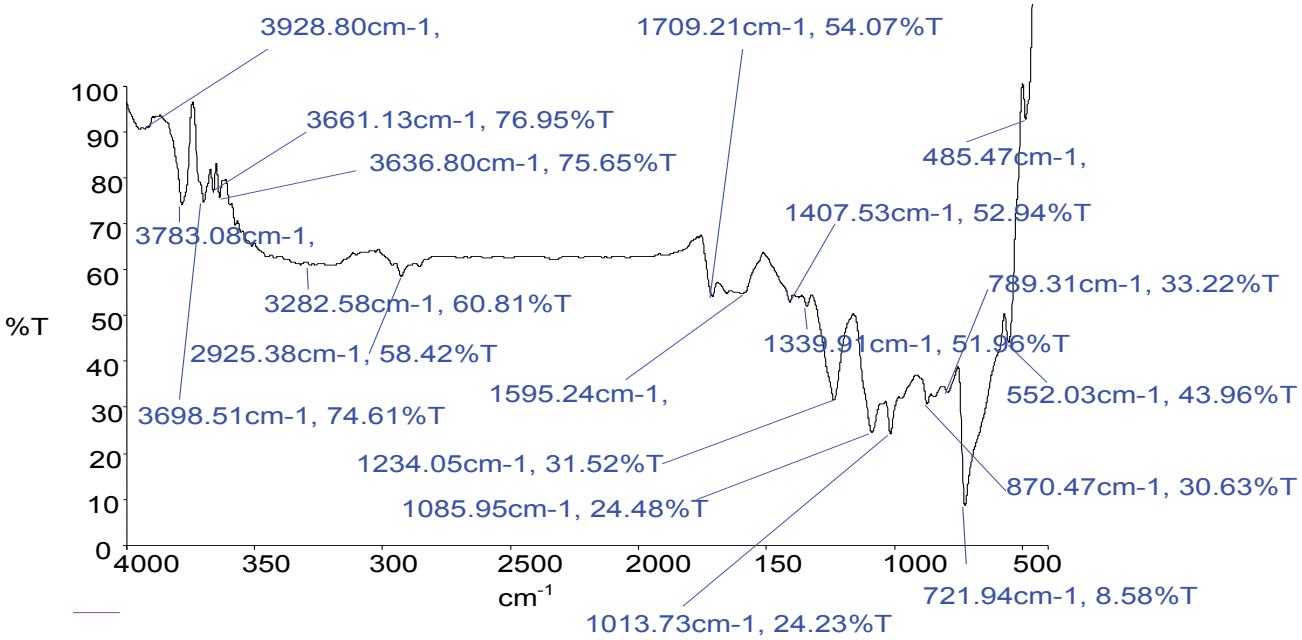

Fig. 1. FTIR spectra of PET flakes inoculated with *A. faecalis* in MSM

Table 1. Band assignment of FTIR spectra of PET flakes inoculated with *A. faecalis* in MSM

Wave number (cm^{-1})	Functional group	Relative intensity
3928	O-H	VW
3783	O-H	VW
3698	O-H	VW
3661	O-H	VW
3636	O-H	VW
3282	O-H	VW
2925	C-H	VW
1709	C=O	W
1595	C-C	W
1407	C-H	W
1339	C-H	W
1234	C-O	W
1085	C-O-C	W
1013	C-H	W
870	C-H	W
789	C-H	W
721	C-H	S

On prolonged exposure of PET flakes to UV for 20 days, the appearance of new peaks at 3429 cm^{-1} (O-H bond stretching), 2877 cm^{-1} (C-H bond stretching), 1576 cm^{-1} (C-C bond stretching), 1238 cm^{-1}(C = O bond stretching) were evinced, when compared to control PET flakes. On prolonged (30 days) exposure of PET flakes to UV, several new peaks appeared (3910 cm^{-1}: O-H bond stretching; 3422 cm^{-1}: O-H bond stretching; 2971 cm^{-1}: C-H bond stretching; 2872 cm^{-1}: C-H bond stretching; 1578 cm^{-1}:C-C bond stretching; 1482 cm^{-1}, 1432 cm^{-1}, 1372 cm^{-1}: C-H bond stretching; 1237 cm^{-1}: C-O bond stretching; 790 cm^{-1}: C-H bond stretching). This phenomenon demonstrates that the degradation mechanism of PET flakes exposed to UV for different periods (10, 20 and 30 days) is different. Thus, it was established that absorption peaks at 1407 cm^{-1} and 1242 cm^{-1} in PET flakes spectra disappeared on UV exposure irrespective of the time of exposure. The virtually unaltered position of the main C=O bond indicates that the main portion of C=O group remains bonded in the same configuration. UV treatment resulted in reduction in the intensity of peaks in the FTIR spectra of PET. On the other hand, on inoculation of PET flakes with *A. faecalis,* many new peaks in the region 4000 to 3000 cm^{-1} (3928 cm^{-1}, 3698 cm^{-1}, 3661 cm^{-1}, 3636 cm^{-1}, 3282 cm^{-1}, (O-H bond stretching), 2925 (C-H bond stretching), 1595 cm^{-1} (C-C bond stretching), 1234 cm^{-1} (C-O bond stretching), 1085 cm^{-1} (C-O-C bond stretching) and 789 cm^{-1} (C-H bond stretching) were noticed. Further, few peaks disappeared at 1242 cm^{-1}, and 1093 cm^{-1}. In addition, a shift in absorption peaks was evinced from 3781 to 3783 cm^{-1}; 1716 to 1709 cm^{-1}, 1017 to 1013 cm^{-1}, 871 to 870 cm^{-1} and 723 to 721 cm^{-1} was observed. UV and bacterial treatment of PET resulted in the disappearance of the absorption peak at 1242 cm^{-1} which was a common phenomenon observed. In general, an increase in the intensity of characteristic peaks of PET was evinced on exposure to *A. faecalis*.

The FTIR spectral analysis of 10 days UV-exposed and 10 days UV + *A. faecalis* inoculated PET allows explanation of the structural differences of these materials. Absorption peaks that appeared in the FTIR spectra of 10-day UV-treated PET disappeared on inoculation with *A. faecalis* (1237 cm^{-1},

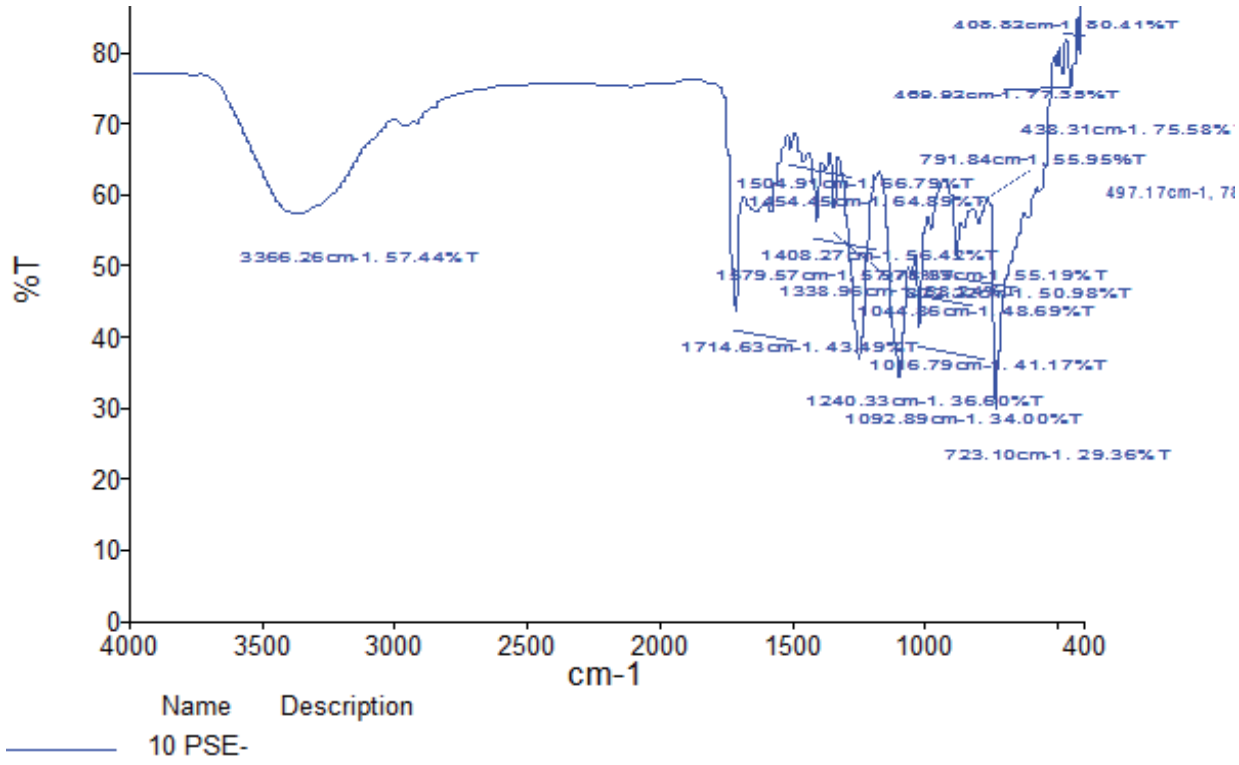

Fig. 2. FTIR spectra of 10 day UV treated PET flakes inoculated with *A. faecalis* in MSM

845 cm^{-1}). As indicated in Fig. 1, there is a doublet of C-C vibration mode around the 1500 cm^{-1} region. On exposure to *A. faecalis,* PET appears to undergo some molecular conformational changes as indicated by the appearance of new peaks at 1579 cm^{-1} (C-C bond stretching), 1504 cm^{-1} (C-C bond stretching), 1454 cm^{-1} (C-H bond stretching), 1408 cm^{-1} (C-H bond stretching), 1338 cm^{-1} (C-H bond stretching), 1240 cm^{-1} (C-C-O bond stretching), 1044 cm^{-1} (C-O bond stretching), 971 cm^{-1}, and 872 cm^{-1}. Shift in absorption at 1711 to 1714 cm^{-1}, 1091 to 1092 cm^{-1}, 1017 to 1016 cm^{-1}, 790 to 791, 725 to 724 cm^{-1} was observed. A broad band was recorded at 3366 cm^{-1}, and a strong sharp band was noticed at 1714 cm^{-1}, 1338 cm^{-1}, 1240 cm^{-1}, 1092 cm^{-1}, 1016 cm^{-1}, 971 cm^{-1}, 872 cm^{-1}, 791 cm^{-1}, and 723 cm^{-1}. A doublet band was exhibited at 1454 cm^{-1} and 1408 cm^{-1}. Inoculation of 20-days UV-exposed PET with *A. faecalis* resulted in the formation of new absorbance in the region 3000 to 4000 cm^{-1} peaking around 3288 cm^{-1} (O-H bond stretching) and bands at 1504 cm^{-1} (C-C bond stretching), 1453 cm^{-1} (C-H bond stretching), 1408 cm^{-1} (C-H bond stretching), 1092 cm^{-1} (O-C-C bond stretching), 970 cm^{-1} (C-H bond stretching), 846 cm^{-1} (C-H bond stretching), and 792 cm^{-1} (C-H bond stretching). On the other hand, a shift in absorption peaks was evinced at 1716 to 1713 cm^{-1}, 1340 to 1339 cm^{-1}, 1015 to 1016 cm^{-1}, 871 to 872 cm^{-1}, and 724 to 723 cm^{-1}. Furthermore, disappearance of absorption bands at 3429 cm^{-1}, 2877 cm^{-1} and 1576 cm^{-1} was noticed (Fig. 2, Table 2).

Table 2. Band assignment of FTIR spectra of 10-day UV treated PET flakes inoculated with *A. faecalis* in MSM

Wave number (cm^{-1})	Functional group	Relative intensity
3366	O-H	VW
1714	C=O	S
1579	C-C	W
1504	C-C	W
1454	C-H	W
1408	C-H	W
1338	C-H	W
1240	C-C-O	S
1044	C-O	W
1092	O-C-C	S
1016	C-H	W
971	C-H	W
872	C-H	W
791	C-H	W
723	C-H	S

The intensity of absorption peak at 1238 cm^{-1} increased in *A. faecalis* inoculated 20-days UV-exposed PET when compared to the un-inoculated ones. An intensive sharp band was noticed at 1713 cm^{-1}. Doublet peaks were evident at 1408 and 1339 cm^{-1}. Strong absorption peaks were evinced at 1238 cm^{-1}, 1092 cm^{-1}, 1016 cm^{-1}, 872 cm^{-1}, and 723 cm^{-1} (Fig. 3, Table 3).

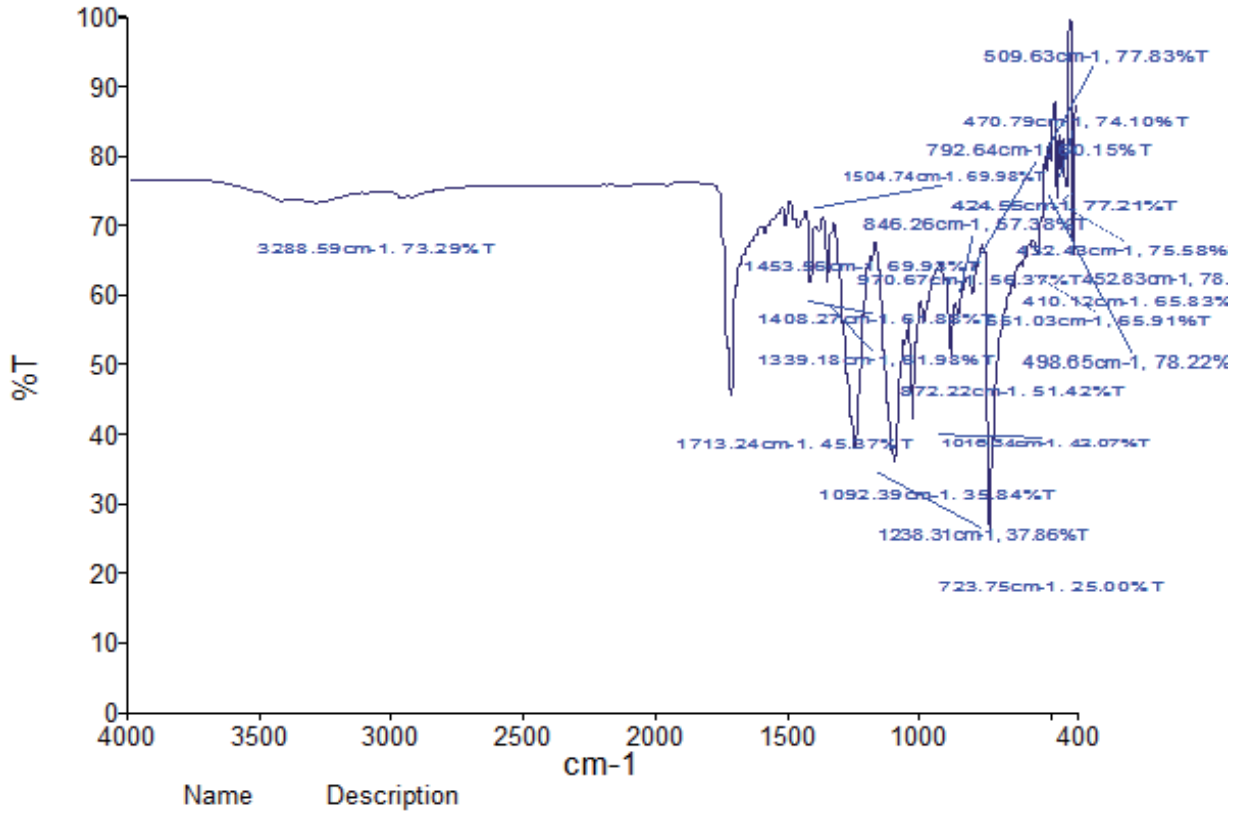

Fig. 3. FTIR spectra of 20-day UV treated PET flakes inoculated with *A. faecalis* in MSM

Table. 3. Band assignment of FTIR spectra of 20 day UV treated PET flakes inoculated with *A. faecalis*

Wave number (cm^{-1})	Functional group	Relative intensity
3288	O-H	W
1713	C=O	S
1504	C-C	W
1453	C-H	W
1408	C-H	W
1339	C-H	W
1238	C=O	S
1092	O-C-C	S
1016	C-H	M
970	C-H	W
872	C-H	W
846	C-H	W
792	C-H	W
723	C-H	S

A. faecalis resulted in chemical changes in 30-days UV-treated PET, which is represented by the appearance of new peaks in the FTIR spectra (1954 cm^{-1}: C=O bond stretching, 1505 cm^{-1}: C-C bond stretching, 1454 cm^{-1}: C-H bond stretching, 1408 cm^{-1}: C-H bond stretching, 1339 cm^{-1}: C-H bond stretching, 1240 (C-C-O bond stretching), 1175 cm^{-1}: C=O bond stretching, 1043 cm^{-1} (C-O bond stretching), 970 cm^{-1}, 872 cm^{-1}, and 847 cm^{-1}: C-H bond stretching), and a shift in absorption peaks from 3422 to 3423 cm^{-1}, 2971 to 2965 cm^{-1}, 1709 to1714 cm^{-1},1372 to 1371 cm^{-1}, 1015 to 1016 cm^{-1},790 to 792 cm^{-1}, 725 to 723 cm^{-1}. In addition, several peaks disappeared (3910 cm^{-1}, 3784 cm^{-1}, 2872 cm^{-1}, 1482 cm^{-1}, 1432 cm^{-1}, 1237 cm^{-1}). Moreover, the intensity of absorption peaks at 1714 cm^{-1}, 1578 cm^{-1}, 1408 cm^{-1}, 1339 cm^{-1}, 1240 cm^{-1}, 1093 cm^{-1}, 1016 cm^{-1}, 970 cm^{-1} 847 cm^{-1}, 792 cm^{-1} and 723 cm^{-1} increased on inoculation of the 30 days UV-treated PET flakes with *A. faecalis* when compared to the un-inoculated PET flakes (Fig. 4, Table 4). On the other hand, a doublet was noticed at 1408 and 1339 cm^{-1}. Irrespective of the period of UV exposure of PET flakes, certain new peaks appeared in all the *A. faecalis* inoculated PET (10 days UV + *A. faecalis*: 1504 cm^{-1}, 1454 cm^{-1}, 971 cm^{-1}; 20 days UV+ *A. faecalis*: 1504 cm^{-1}, 1453 cm^{-1}, 970 cm^{-1}; 30 days UV + *A. faecalis*: 1505 cm^{-1}, 1454 cm^{-1}, 970 cm^{-1}). Vibrational peaks were registered in the FTIR spectra of UV unexposed, 10, and 20-day UV treated PET flakes on inoculation with *A. faecalis.* Strong and sharp absorption peaks were recorded in the FTIR spectra of 30 days UV-treated PET on inoculation with *A. faecalis* (1714 cm^{-1}, 1240 cm^{-1}, 1093 cm^{-1}, 1016 cm^{-1}, 970 cm^{-1}, 847 cm^{-1}, 792 cm^{-1}, 723 cm^{-1}).

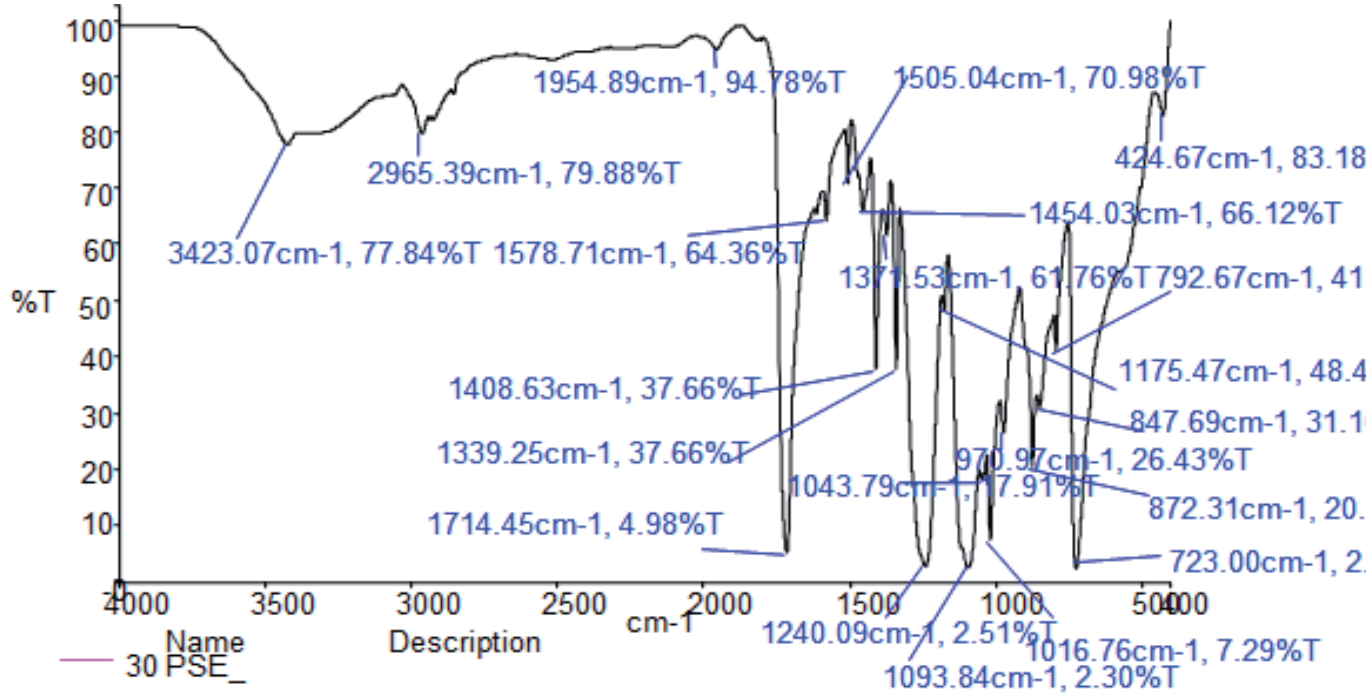

Fig. 4. FTIR spectra 30 day UV treated PET flakes inoculated with *A. faecalis* in MSM

Table. 4. Band assignment of FTIR spectra 30 day UV treated PET flakes inoculated with *A. faecalis* in MSM

Wave number (cm^{-1})	Functional group	Relative intensity
3423	O-H	W
2965	C-H	W
1954	C=O	W
1714	C=O	VS
1578	C-C	W
1505	C-C	W
1454	C-H	W
1408	C-H	S
1371	C-H	W
1339	C-H	S
1240	C-C-O	S
1175	C=C	W
1093	O-C-C	S
1043	C-O	W
1016	C-H	S
970	CH	W
872	C-H	M
847	C-H	W
792	C-H	W
723	C-H	S

After one month of incubation with *A. faecalis,* massive bacterial colonies were found on the surface of 10, 20 and 30-day UV-exposed PET flake surfaces when compared to UV-unexposed PET flakes, which showed adherence of bacterial colonization, bacterial cell divided stages and individual bacterial can be seen on the surfaces of UV-exposed PET flakes (Plate 1).

Discussion

The bacteria *A. faecalis* isolated and identified in this study was found capable of utilizing UV-exposed PET as a sole carbon source. During the one-month incubation with *A. faecalis*, with UV- exposed and UV-unexposed PET, biodegradation was measured by FITR, the peaks in the 4000 to 3000 cm^{-1} region became broader after degradation, which was due to the formation of hydroxyl and carboxyl groups. This was also confirmed by the shift in the absorption peak of C=O stretching vibration after degradation. This observation is well supported by the findings of Weng *et al.*, (2013) who have also demonstrated that biodegradation of p (3 HB, 4 HB) films in soil was mainly caused by microorganisms and many low molecular weight polymers were produced (Weng *et al.*, 2013). Broadening of absorption peaks in the 4000 to 3000 cm^{-1} region was observed in the FTIR of PET flakes exposed to UV for 30 days. This observation is in line with the findings of Arkatkar *et al.*, (2010) who have opined that PP–SUV (Short UV treated Polypropylene) were more hydrophilic in the presence of microorganisms. Roughness of UV-exposed PET evinced in this study coincides with that of Don Rosu *et al.* (2009) who have confirmed through FTIR study that UV-irradiated polyurethane (PU) results in a decrease in the band corresponding to the stretching vibration of N-H group (3328 cm^{-1}) and have attributed it to the loss of urethane structure as a result of UV irradiation. Further, the appearance of new peaks in the region between 3910 to 2872 cm^{-1} is in agreement with

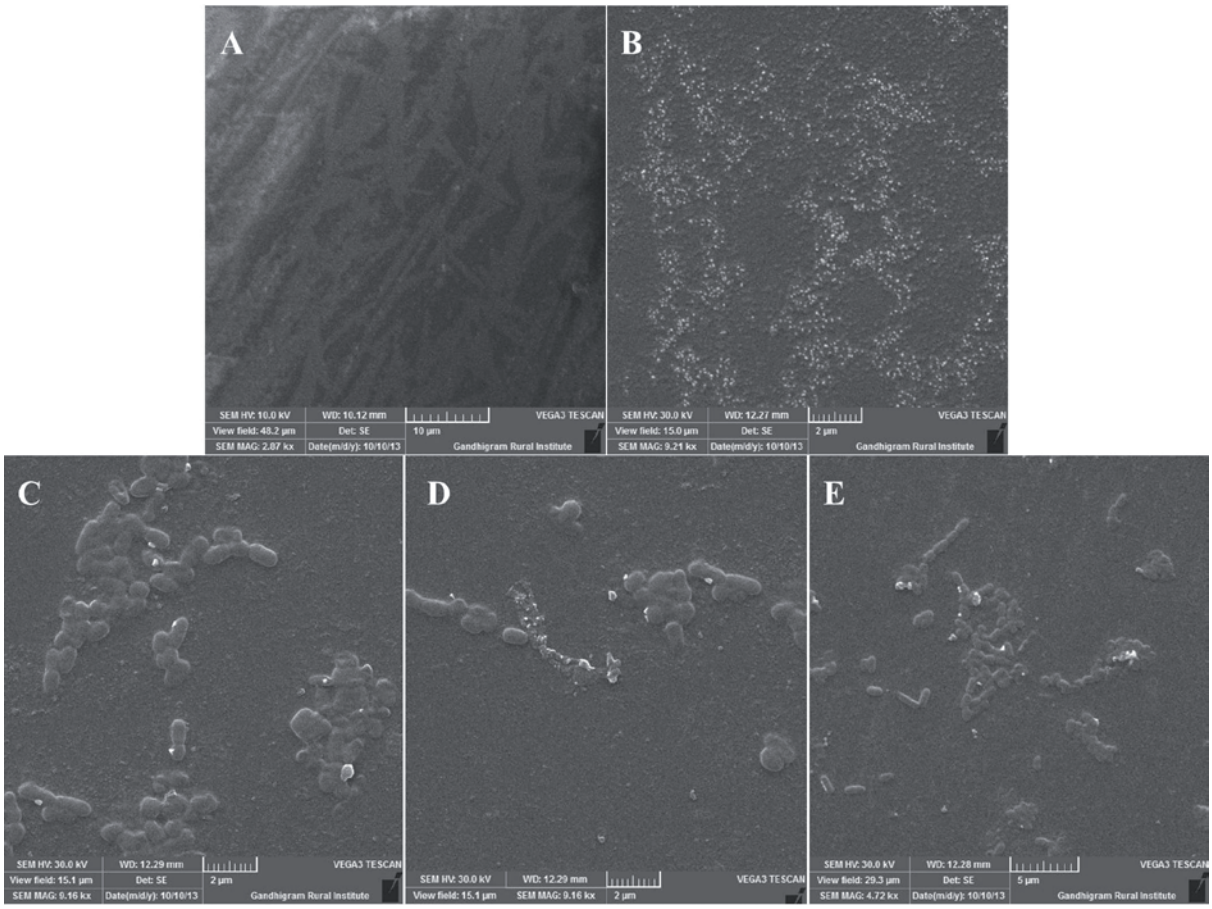

Plate 1. SEM micrographs of PET flakes inoculated with *A. faecalis (AF)* in MSM: A) control; B) UV unexposed; C) 10 days UV exposed; D) 20 days UV exposed; E, F, G) 30 days UV exposed.

Don Rosu *et al.* (2009) who have also observed new peaks in the region between 3080 cm^{-1} and 2880 cm^{-1} in the FTIR peak of UV- irradiated PU. They have also reported that the aromatic structures from PU are not stable to light and are susceptible to rapid degradation during UV exposure. They have concluded that UV light modifies the chemical structure of PU. The absorption of UV induces the degradation of PU and photo-oxidation of the CH_2 groups. Chonde Sonal *et al.* (2012) have confirmed through FTIR spectroscopy that *Trametes versicolor* NCIM 1086 mediated degradation of the synthetic polymer nylon 6 by the appearance of new groups like CH_3, $CONH_2$, CHO and CHOOH and have reasoned that it could be due to hydrolysis or oxidation. They have further attributed it to the cleavage of the C-C bond in CH_2-CH_2 adjacent to the nitrogen atom and weakening C=N stretching. In addition, they have also noticed a decrease in the strength of characteristic band of C (O) NH occurring around 3300 cm^{-1}, 1640 cm^{-1}, 1550 and 1018 cm^{-1} after 90 days. The absorption peak observed in the FTIR spectra of PET is in parallel to the FTIR spectra of PET films reported by Lee *et al.* (2012). They have observed that the intensity of the band at 1716 cm^{-1} decreased with increasing the UV irradiation time (0, 30, 60, and 90 days). In the present study the intensity of the bands of PET decreased on exposure to UV. In addition, a positive cross-correlation was observed peak at (1716 cm^{-1}, 1340 cm^{-1}), (1716 cm^{-1}, 1247 cm^{-1}) in the power spectrum extracted along the diagonal line in the synchronous 2D correlation spectrum (1340 cm^{-1}, 1247 cm^{-1}) and have attributed it to the intensity of the ester linkage in the terephthalate moiety and the ethylene group decrease together with increasing the UV irradiation time. They have ascribed the changes in the intensity at 1716 and 1247 cm^{-1} in power spectrum to the influence of the ester linkage by photodegradation of PET and have suggested that the ester moieties in the terephthalate moiety as well as CH_2 groups are strongly involved in the photo-degradation of PET. This explanation could be extended to the present result. Moreover, through synchronous 2D correlations FTIR spectra, they have suggested that photo-degradation of PET films induces spectral changes of the CH_2 group adjacent to the ester linkage, ester (C (=O)-O and the phenyl ring sequentially and have concluded that photo-degradation of PET films induces faster spectral changes of

the methylene groups than that of the terephthalate moieties. The emergence of a keto group noticed in this study due to UV treatment in the FTIR spectra of PET exposed to UV for 20 days period gains support from the observations of Arkatkar *et al*. (2010) who have evinced the occurrence of a 1700 to 1800 cm^{-1} region in FTIR spectra indicating the presence of oxidized groups. In addition, they also observed peaks at 1715 cm^{-1} or 1711 cm^{-1} and 1748 cm^{-1} corresponding to the formation of keto carbonyl and ester carbonyl groups after pretreatments of polypropylene and have attributed it to oxidation of the polymer. These peaks either became stronger and sharper or disappeared at the end of the twelfth month. They have concluded that the post-pretreatment FTIR spectrum of PP-ART, PP-FRT and PPSUV showed formation of a keto carbonyl peak whereas PP-FRT and PP-TT showed formation of an ester carbonyl peak indicating oxidation, which probably enhanced the attack of microbes. This is in contradiction to the present finding that UV induced chemical changes in PET.

In the SEM result, bacteria colonized and bacterial division stages were evinced on UV-exposed PET surface and formed a massive biofilm on it, a process that seemed to be a prerequisite for biodegradation. The adherence of selected bacteria *A. faecalis* (10-, 20-, and 30 days UV-exposed PET flakes surfaces) partially agrees with that of Atefeh Esmaeili *et al.* (2013) who have also evinced colonisation of mixed culture of fungi (*Lysinibacillus xylanilyticus* and *Aspergillus niger*) on PET flake surfaces when incubated in soil inoculated with *L. xylanilyticus* and *A. niger* for a period of 126 days in the SEM image.

Conclusions

The observations in the present study reveal that PET waste dumped in soil is exposed to a variety of organisms, especially bacteria. *A. faecalis* isolated from PET waste were reinoculated with UV-treated PET under laboratory conditions. The growth of *A. faecalis* on PET was evinced in the SEM images. Thus, these findings permit us to conclude that *A. faecalis* can colonies and form biofilms on a PET surface, which indicates that bacteria are able to utilize PET as a carbon source for their growth. However, the involvement of bacteria in degrading PET has to be confirmed by further studies.

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