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Research Paper

FTIR Spectral Analysis of *Bacillus sp.,* Mediated Chemical Changes in UV Exposed Polystyrene Foam

Meenashi Subramani*[1] and Umamaheswari Sepperumal[2]

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A B S T R A C T

The existence of synthetic polymeric wastes in the environment could have adverse impact on the biota. Although polystyrene has wide applicability in the industries, their mode of disposal should be in an ecofriendly manner. With this view, the present study was designed to elucidate the potential of bacteria persisting in Polystyrene (PS) waste dumped site to degrade polystyrene. FTIR spectral studies reveal that *Bacillus sp.* has induced chemical changes in polystyrene. Further, UV treatment has also caused chemical changes in PS. The formation of CH, C=C and OH group in the FTIR spectra of PS inoculated with *Bacillus sp.* for a period of one week and disappearance of absorption peak at 1373 cm^{-1} (CH) on prolonged incubation of PS for four weeks was evident. Furthermore, incubation of UV treated PS with *Bacillus* sp. has resulted in the formation of C-O (1288 cm^{-1}) and disappearance of CH group (1492 cm^{-1}) when compared to the UV untreated PS. Moreover, prolonged incubation for four weeks in MSM caused the evolution of new absorption peak at 1369 cm^{-1} (CH) and disappearance of absorption band at 1288 cm^{-1} (C-O). These observations indicate that *Bacillus sp.* could degrade polystyrene.

***Key words*:** Polystyrene, Biodegradation, *Bacillus,* UV, FTIR

Plastics are considered as markers of anthropocene [1]. The persistence of synthetic polymeric wastes like polyethylene terephthalate, polystyrene, polypropylene, polyurethane etc., in the environment for a longer period is a threat to the delicate ecological balance of the earth [2]. Although strategies like incineration, recycling, landfilling are adopted to dispose of these wastes, viable remediation methods using microorganisms harboring the synthetic polymeric waste dumped sites, to degrade these polymers into their precursors could be an ecofriendly option. Several studies have focused using microorganisms like *Pseudomonas sp.*, *Bacillus sp.*, *Enterobacter sp*., to degrade these synthetic polymers [3-5].

The potential of mcrobes like *Brevibacillus borstelensis, B. weihenstephanensis, Comamonas sp., Delftia sp., Stenotrophomonas sp., Achromobacter xylosoxidans, Bacillus YP1; Bacillus amyloliquefaciens, Bacillus pumilus M27, Kocuria palustris M16; Lysinibacillus xylanilyticus , Bacillus mycoides, Bacillus subtilis, Pseudomonas aeruginosa* PA01 (ATCC 15729), *Pseudomonas aeruginosa* (ATCC 15692), *Pseudomonas putida KT2440* (ATCC 47054), *Pseudomonas syringae* DC 3000 (ATCC 0862), *Rhodococcus ruber*, *Aspergillus sp.* to degrade the polymers like LDPE (Low Density Polyethylene), HDPE (High Density Polyethylene) have been demonstrated by several researchers [6-16).

A growing body of research has focused on the ability of the microbes like bacteria, fungi and Actinomycetes to utilize synthetic polymers like polyethylene terephthalate, polystyrene, poly propylene as a sole source of carbon for their growth (*Micrococcus*, *Streptomyces*, *Rhodococcus*, *Arthrobacter*, *Corynebacterium*, *Pseudomonas*). Physical and chemical agents alter the structure of the polymers in the form of cracks, roughness and cause molecular changes [17]. Further, microorganisms mineralize these plastics to carbon dioxide and water. With this perspective, the present research was designed to evaluate the ability of bacteria prevalent in polystyrene dumped site to degrade polystyrene through FTIR studies.

MATERIALS AND METHODS

Isolation of bacteria from PS waste dumped soil

Soil samples were collected from polystyrene waste dumped sites. 1 g of soil was dissolved in 99 ml sterile distilled water and serially diluted. The diluted samples were inoculated on nutrient agar plates and the bacterial isolates were identified using Bergeys manual of Systematic Bacteriology [18].

Preparation of PS samples

The Polystyrene foam were cut into beads to equal sizes and used for degradation studies.

***Meenashi Subramani**
meenashi73@gmail.com

[1]P.G. and Research Department of Zoology, Government Arts College (Autonomous), Karur (Affiliated to Bharathidasan University) Tamil Nadu, India

[2]P.G. and Research Department of Zoology, Periyar EVR College (Autonomous), Tiruchirappalli, (Affiliated to Bharathidasan University) Tamil Nadu – 620 023, India

UV irradiation

Polystyrene beads of the same size were placed under the UV light (TUV25W) for a period of one month.

Degradation of PS by bacteria in MSM

The dominant bacterial isolate *Bacillus sp.*, was used for PS degradation studies. 10 µl of the broth culture of *Bacillus sp.* was inoculated in 100 ml sterile MSM (Minimal Salt media) containing PS (Polystyrene) and kept in the shaker at 37°C with 120 rpm for a period of 1 month. The PS samples were subjected to FTIR studies.

FTIR spectroscopy analysis

Fourier Transform Infrared Spectroscopy analysis was used for detecting the formation of new functional groups or changes in the amount of existing functional groups [19]. Fourier transform infrared (FTIR) measurement were carried out with a BIO-RAD spectrometer (model FTS 40A) in the range of 4000-650 cm^{-1}). The FTIR spectra were recorded at a resolution of 2 cm^{-1} and an accumulation of 32 scans.

RESULTS AND DISCUSSION

The signature peak of polystyrene is depicted in the FTIR spectra in (Fig 1) and their band assignments in (Table 1). Absorption peaks at 3025 cm^{-1}, 2918 cm^{-1}, 2853 cm^{-1}, 1942 cm^{-1}, 1366 cm^{-1}, 907 cm^{-1}, 756 cm^{-1}, 694 cm^{-1} and 545 cm^{-1} corresponds to C-H bond stretching.

Table 1 Band assignment of FTIR spectra of polystyrene flakes

Wave Number (cm^{-1})	Functional Group	Relative Intensity	References
3025.69	C-H	W	Sarker and Rashid (2013); Anuradha and Anu Kaliani, (2009), Adan-Yovani Leon-Bermudez and Ramiro Salazar (2008); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
2918.80	C-H	M	Sarker and Rashid (2013), Simion Jitian (2011); Anuradha and Anu Kaliani (2009); Shovitri *et al.* (2017); Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
2853.08	C-H	W	Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
1942.74	C-H	W	Umamaheswari and Murali (2013)
1596.76	C=C	M	Umamaheswari and Murali (2013); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
1448.19	CH_2+C=C	M	Simion Jitian (2011); Anuradha and Anu Kaliani (2009); Adan-Yovani Leon-Bermudez and Ramiro Salazar (2008)
1366.04	C-H	W	Shiv Govind Prasad *et al.* (2011); Hadi Al Kadhmey *et al.* (2016)
1173.54	C-O	W	Silverstein *et al.* (1990); Nikolic *et al*. (2013); Shovitri *et al*. (2017)
1025.03	C-O	M	Umamaheswari and Murali (2013)
907.50	C-H	M	Vijayakumar and Rajkumar (2012); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
756.50	C-H	Vs	Vijayakumar and Rajkumar (2012); Kaniappan and Latha (2011); Anuradha and Anu Kaliani (2009); Shovitri *et al.* (2017); Sekhar *et al.* (2016); Jaleh *et al.* (2011); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
694.65	C-H	Vs	Umamaheswari and Murali (2013); Anuradha and Anu Kaliani (2009); Meenakshi and Umamaheswari (2016); Jaleh *et al*. (2011); Sekhar *et al.* (2016); Hadi Al Kadhmey *et al.*, (2016); Addullah Al Ashraf (2015)
545.36	C-H	W	Coates *et al.* (2000)

Vs-Very strong; W-weak; M-medium

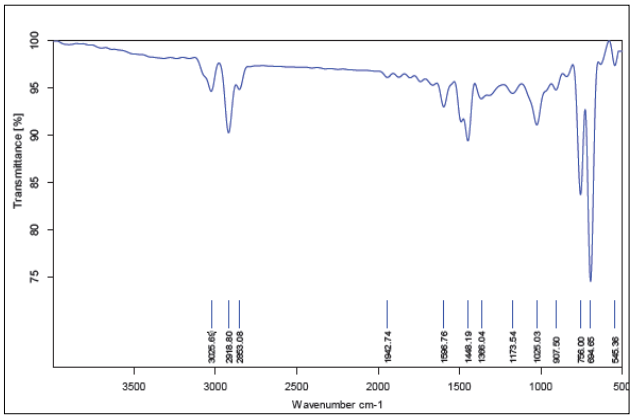

Fig 1 FTIR spectra of polystyrene flakes control

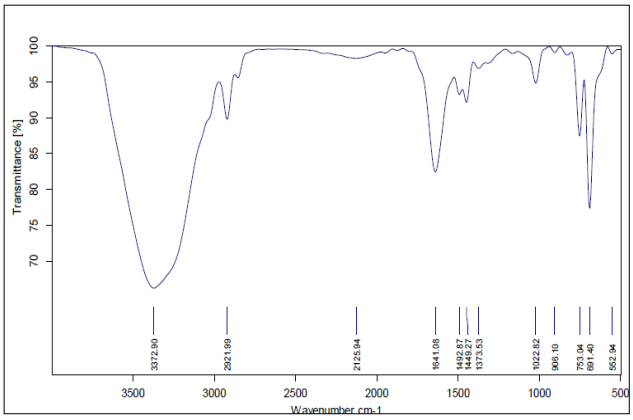

Fig 2. FTIR spectra of polystyrene flakes inoculated with *Bacillus* sp., in MSM for a period of one week

The spectral bands at 1173 cm^{-1} and 1025 cm^{-1} has been attributed to C-O bond stretching. The absorption peak at 1596 cm^{-1} has been linked to C=C bond stretching. The absorption peak at 1448 cm^{-1} indicates CH_2+C=C bond stretching. FTIR spectra of polystyrene flakes inoculated with *Bacillus sp.* in MSM for one week is illustrated in (Fig 2, Table 2).

Table 2 Band assignment of FTIR spectra of polystyrene flakes inoculated with *Bacillus sp.*, in MSM for a period of one week

Wave Number (cm^{-1})	Functional Group	Relative Intensity	References
3372.90	O-H	Vs	Coates (2000); Jaleh *et al*. (2011); Addullah Al Ashraf (2015)
2921.99	C-H	M	Sarker and Rashid (2013); Simion Jitian (2011); Shovitri *et al*. (2017); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009); Adan-Yovani Leon-Bermudez and Ramiro Salazar (2008); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
2125.94	C-H	W	Nikolic *et al*. (2013)
1641.08	C=C	S	Umamaheswari and Murali (2013); Meenakshi and Umamaheswari (2016)
1492.87	C-H	W	Nikolic *et al*. (2013), Chen *et al*. (2012); Meenakshi and Umamaheswari (2016); Jaleh *et al*. (2011)
1449.27	CH_2+C=C	W	Simion Jitian (2011), Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009)
1373.53	C-H	W	Lee *et al*. (2012); Hadi Al Kadhmey *et al.* (2016)
1022.82	C-O	W	Umamaheswari and Murali (2013)
906.10	C-H	W	Shiv Govind Prasad *et al*. (2011); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
753.04	C-H	S	Vijayakumar and Rajkumar (2012), Kaniappan and Latha (2011); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009); Jaleh *et al*. (2011); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
691.40	C-H	Vs	Umamaheswari and Murali (2013); Jaleh *et al*. (2011); Meenakshi and Umamaheswari (2016), Anuradha and Anu Kaliani (2009); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
552.94	C-H	W	Chen *et al*. (2012)

Vs-Very strong; W-weak; M-medium

Table 3 Band assignment of FTIR spectra of polystyrene flakes inoculated with *Bacillus sp.*, in MSM for a period of four weeks

Wave Number (cm^{-1})	Functional Group	Relative Intensity	References
3362.25	O-H	Vs	Coates (2000); Jaleh *et al.* (2011); Addullah Al Ashraf (2015)
2922.87	C-H	M	Moinuddin Sarker *et al*. (2013), Simion Jitian (2011); Shovitri *et al*. (2017); Meenakshi and Umamaheswari 2016; Anuradha and Anu Kaliani (2009); Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
2143.79	C-H	W	Prasad SG *et al.* (2011); Nikolic *et al*. (2013); Meenakshi and Umamaheswari (2016)
1640.10	C=C	Vs	Umamaheswari and Murali (2013); Meenakshi and Umamaheswari (2016)
1493.90	C-H	W	Nikolic *et al*. (2013), Chen *et al*. (2012); Meenakshi and Umamaheswari (2016); Jaleh *et al*. (2011)
1449.20	CH_2+C=C	W	Simion Jitian (2011); Meenakshi and Umamaheswari (2016); Adan-Yovani Leon-Bermudez and Ramiro Salazar (2008)
1026.03	C-O	W	Umamaheswari and Murali (2013)
751.47	C-H	S	Vijayakumar and Rajkumar (2012); Kaniappan and Latha (2011); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009); Jaleh *et al*. (2011); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
691.30	C-H	Vs	Umamaheswari and Murali 2013; Anuradha and Anu Kaliani (2009); Meenakshi and Umamaheswari (2016); Jaleh *et al*. (2011); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
544.87	C-H	M	Yusilawati *et al*. (2010)

Vs-Very strong; W-weak; M-medium

Compared to the untreated polystyrene, the FTIR spectra of *Bacillus sp.*, inoculated polystyrene in MSM for a period of one week caused the appearance of new absorption peaks at 3372 cm^{-1}, 2125, cm^{-1} 1492 cm^{-1} and 1641 cm^{-1} which are characteristic absorptions of hydroxyl (O-H), methyl (C-H) and C=C groups, respectively. On the other hand, several peaks have disappeared (3025 cm^{-1}, 2853 cm^{-1}, 1942 cm^{-1}: CH; 1596 cm^{-1}: C-C cm^{-1}; 1173 cm^{-1}: C-O) in the FTIR spectra of PS inoculated with *Bacillus sp.*, when compared to the FTIR spectra of untreated PS. Moreover, the intensity of these peaks were found to decrease when compared to the corresponding absorption peaks in the FTIR spectra of control polystyrene. In addition, shift in absorption peaks were observed from 2918 cm^{-1} to 2921 cm^{-1}, 1448 cm^{-1} to 1449 cm^{-1}, 1366 cm^{-1} to 1373 cm^{-1}, 1025 cm^{-1} to 1022 cm^{-1}, 907 cm^{-1} to 906 cm^{-1}, 756 cm^{-1} to 753 cm^{-1}, 694 cm^{-1} to 691 cm^{-1} and 545 cm^{-1} to 552 cm^{-1}. During prolonged exposure of PS to *Bacillus sp.*, for a period of four weeks resulted in the disappearance of absorption peak at 1373 cm^{-1} (Fig 3, Table 3). On the other hand, if polystyrene is treated with UV and then inoculated in MSM for a period of one week, appearance of new absorption peak at 1288 cm^{-1} was evident, which has been assigned to C-O bond stretching, while absorption peak at 1492 cm^{-1} disappeared (Fig 4, Table 4). The FTIR spectra of UV treated PS inoculated for a period of four weeks as compared with one week revealed the disappearance of absorption peak at 1373 cm^{-1} (CH) (Fig 5, Table 5). The evolution of CH group was evinced at 1369 cm^{-1} and

disappearance of CO group (1288 cm^{-1}) on prolonged inoculation of UV irradiated polystyrene for a period of four weeks in the FTIR spectra. The absorption peak at 907 cm^{-1} attributed to CH bond stretching disappears in the FTIR spectra of PS inoculated with *Bacillus sp.*, for four weeks and UV irradiated PS inoculated with *Bacillus sp.*, for a period of 1 and 4 weeks. These observations coincides with the findings of [20] who have reported appearance and disappearance of absorption peaks in the FTIR spectra of Coumarin doped PS before and after irradiation with alpha rays.

Table 4 Band assignment of FTIR spectra of UV treated polystyrene flakes inoculated with *Bacillus sp.*, in MSM for a period of one week

Wave Number (cm^{-1})	Functional Group	Relative Intensity	References
3366.17	O-H	Vs	Coates (2000); Jaleh *et al*. (2011); Addullah Al Ashraf (2015)
2922.62	C-H	M	Sarker and Rashid (2013), Simion Jitian (2011); Maya Shovitri *et al*. (2017); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009); Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
2139.14	C-H	W	Prasad *et al.* (2011), Nikolic *et al*. (2013); Meenakshi and Umamaheswari (2016)
1640.37	C=C	S	Umamaheswari and Murali (2013); Meenakshi and Umamaheswari (2016)
1449.24	CH_2+C=C	W	Simion Jitian (2011); Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009)
1288.20	C-O	W	Umamaheswari and Murali (2013); Meenakshi and Umamaheswari (2016); Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008)
1024.80	C-O	W	Vijayakumar *et al*. (2012); Umamaheswari *et al*. (2013)
752.62	C-H	M	Vijayakumar and Rajkumar (2012), Kaniappan and Latha (2011); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009); Jaleh *et al.* (2011); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
689.85	C-H	Vs	Umamaheswari and Murali 2013; Anuradha and Anu Kaliani (2009); Meenakshi and Umamaheswari 2016); Jaleh *et al*. (2011); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)

Vs-Very strong; W-weak; M-medium

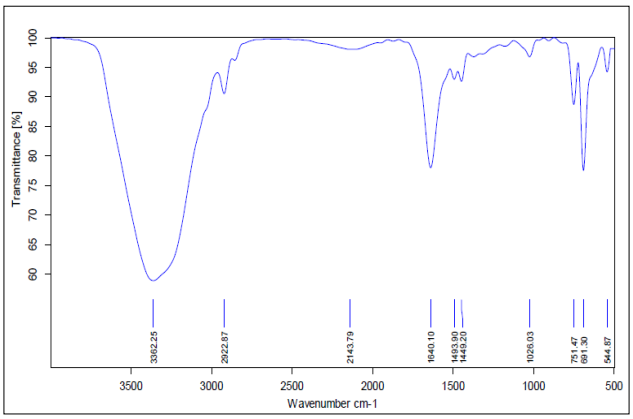

Fig 3 FTIR spectra of polystyrene flake inoculated with *Bacillus* sp., in MSM for a period of four weeks

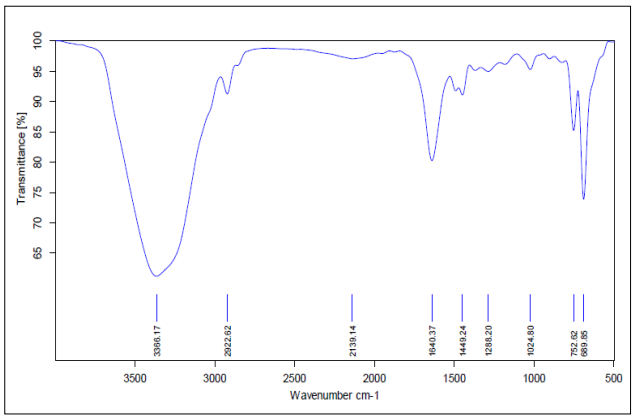

Fig 4 FTIR spectra of UV treated polystyrene flake inoculated with *Bacillus* sp., in MSM for a period of one week

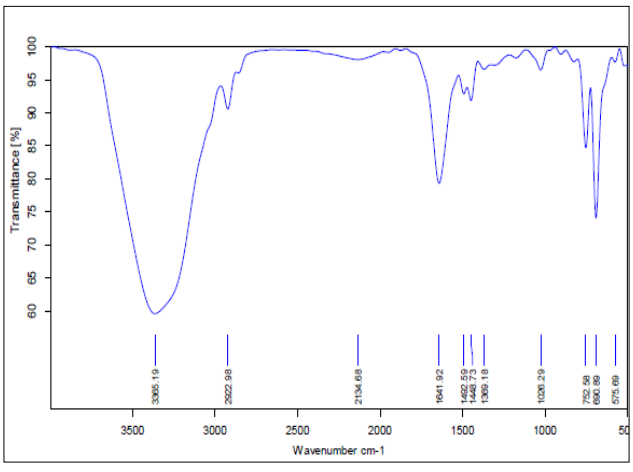

Fig 5. FTIR spectra of UV treated polystyrene flake inoculated with *Bacillus* sp., in MSM for a period of four weeks

The formation of methyl group in PS inoculated with *Bacillus sp.*, in MSM, irrespective of the period of inoculation coincides with the formation of methyl group in the FTIR spectra of PS inoculated with *Micrococcus sp., and Pseudomonas sp.*, in MSM for a period of one-month [21]. According to [22] peaks with OH group signifies monoxygenase activity exhibited by microbes. This explanation could be extended to the present study indicating the evolution of OH group in the FTIR spectra of *Bacillus sp.*, inoculated Polystyrene in MSM when compared to the control polystyrene. Furthermore, [23 has stated that the chemical stability of the polymer is loosened by the addition of OH group, which in turn increased its hydrophilicity and decreased surface tension. Decrease in the intensity of the absorption peaks at 1449 cm^{-1}, 1373 cm^{-1}, 1022 cm^{-1}, 906 cm^{-1}, 753 cm^{-1}, 691 cm^{-1} and 552 cm^{-1} evinced in the FTIR spectra of PS inoculated with *Bacillus sp.*, gains support from the findings of [24] who also have reported a decrease in the

intensity of the absorption peak in the region between 690 – 515 cm^{-1} due to *Enterobacter sp*. The shift in absorption peaks observed in this study is in consistent with the findings of [25] who have also reported shift in the absorption peaks in the FTIR spectra of polystyrene treated with fungi *Cephalosporium species* and *Mucor species.* Similarly, [26] have demonstrated through FTIR studies that biodegradation of polystyrene foam was mainly caused by soil microbes like *Pseudomonas*, *Actinomycetes* and *Pencillium* species, which resulted in the formation of low molecular weight polymers. In contradiction to the present findings, [27] observed in the FTIR spectra of PS incubated in MSM inoculated with *Microbaacterium* sp., *Pseudomonass aeruginosa*., *Bacillus sp*., *Paenibacillus urinalis* for a period of four weeks did not induce any chemical changes in PS. UV-irradiation causes photooxidation in PS, consequently causes changes in the carbonyl and hydroxyl regions of the FTIR spectra due to the formation of aromatic and aliphaticketones of the acetophenone type, and OH/ OOH groups in the main chain [28]. These results are also in partial agreement with [29] have reported the existence of peaks at 1736 cm^{-1} and 3540 cm^{-1}, which has been assigned to carbonyl (C=O and COOH) and hydroxyl group (OH), respectively in the FTIR spectra of UV treated PS beads.

Table 5 Band assignment of FTIR spectra of UV treated polystyrene flakes inoculated with *Bacillus sp.,* in MSM for a period of four weeks

Wave Number (cm^{-1})	Functional Group	Relative Intensity	References
3365.19	O-H	Vs	Coates (2000); Jaleh *et al.* (2011), Addullah Al Ashraf (2015)
2922.98	C-H	M	Sarker and Rashid (2013), Simion Jitian (2011); Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
2143.68	C-H	W	Shiv Govind Prasad *et al.* (2011), Nikolic *et al.* (2013)
1641.92	C=C	Vs	Umamaheswari and Murali (2013)
1492.59	C-H	M	Nikolic *et al*. (2013), Chen *et al.* (2012)
1448.73	CH_2+C=C	M	Simion Jitian (2011), Adan-Yovani Leon- Bermudez and Ramiro Salazar (2008)
1369.18	C-H	W	Nikolic *et al.* (2013); Hadi Al Kadhmey *et al.* (2016)
1026.29	C-O	W	Umamaheswari and Murali (2013)
752.58	C-H	S	Vijayakumar and Rajkumar (2012), Kaniappan and Latha (2011); Meenakshi and Umamaheswari (2016); Anuradha and Anu Kaliani (2009); Jaleh *et al*. 2011; Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
690.89	C-H	Vs	Umamaheswari and Murali 2013; Anuradha and Anu Kaliani (2009; Meenakshi and Umamaheswari 2016); Jaleh *et al*. (2011); Sekhar *et al*. (2016); Hadi Al Kadhmey *et al.* (2016); Addullah Al Ashraf (2015)
575.69	C-H	W	Donelli *et al.* (2010)

Vs-Very strong; W-weak; M-medium

Table 6 Comparative band assignments of FTIR spectra of polystyrene and UV treated polystyrene inoculated with *Bacillus sp*., incubated in MSM

PS (polystyrene) Control	PS + *Bacillus sp.* (I week)	PS + *Bacillus sp.* (IV week)	UV treated PS + *Bacillus sp.* (I week)	UV Treated PS+*Bacillus sp.* (IV week)
3025.69(C-H)	3372.90(O-H)	3362.25(O-H)	3366.17(O-H)	3365.19(O-H)
2918.80(C-H)	2921.99(C-H)	2922.87(C-H)	2922.62(C-H)	2922.98(C-H)
2853.08(C-H)	2125.94 (C-H)	2143.79(C-H)	2139.14(C-H)	2134.68(C-H)
1942.74(C-H)	1641.08(C=C)	1640.10(C=C)	1640.37(C=C)	1641.92(C=C)
1596.76(C=C)	1492.87(C-H)	1493.90(C-H)	1449.24 CH_2+C=C	1492.59(C-H)
1448.19 CH_2+C=C	1449.27 CH_2+C=C	1449.20 CH_2+C=C	1288.20(C-O)	1448.73 CH_2+C=C
1366.04(C-H)	1373.53(C-H)	1026.03(C-O)	1024.80(C-O)	1369.18(C-H)
1173.54(C-O)	1022.82(C-O)	751.47(C-H)	752.62(C-H)	1026.29(C-O)
1025.03(C-O)	906.10(C-H)	691.30(C-H)	689.85(C-H)	752.58(C-H)
907.50(C-H)	753.04(C-H)	544.87(C-H)		690.89(C-H)
756.50(C-H)	691.40(C-H)			575.69(C-H)
694.65(C-H)	552.94(C-H)			
545.36(C-H)				

CONCLUSIONS

These observations permit us to conclude that chemical changes are mediated by the *Bacillus sp.,* in polystyrene, which is reflected in the FTIR spectra. Thus, microbes existing in polystyrene waste dumped site could deteriorate polystyrene.

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