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POLYPROPYLENE DEGRADATION POTENTIAL OF MICROBES ISOLATED FROM SOLID WASTE DUMPING SITE

PRAMILA OLIYA[1], SANJEEV SINGH[1], NAVODIT GOEL[2], UDAI PRATAP SINGH[2] AND ABHINAV KR. SRIVASTAVA[2]*

[1]Department of Bioengineering and Biosciences, Lovely Professional University, Phagwara 144 411, Punjab, India
[2]Amity Institute of Biotechnology, Amity University Uttar Pradesh, Sector 125, Noida 20 1303, Uttar Pradesh, India

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ABSTRACT

Modernisation has increased the demand and uncontrolled use of plastics (polypropylene) in day to day activities. Lengthy natural degradation time of plastic causes soil, water and air pollution and serious environmental and health hazards. The physical and chemical methods for plastic disposal have proved to be ineffective. Hence, alternative methods of plastic degradation which are faster and eco-friendly are being considered to serve the purpose. In recent studies, several fungi and bacteria have been observed to perform such plastic degradation. Keeping this in view, the present study aimed at analysing the potential of bacteria and fungus in their efficacy to decompose plastic and convert it into inorganic harmless compounds. The dumping site soil around the Jalandhar city of Punjab region was the source of potential plastic degrading bacteria and fungus. The isolated bacteria and fungus which showed plastic decomposing properties were identified as *Staphylococcus* sp. and *Aspergillus fumigatus* respectively. The ability of polypropylene degradation by these microbes was tested under *In vitro* broth conditions over an incubation period of 2, 4 and 6 months. The biodegradation was calculated in terms of weight loss percentage of the polymer. Both the microbes demonstrated promising capacity to degrade plastic under laboratory conditions. Upto 18.08% degradation of plastic material was achieved by the isolated microbes. The scanning electron microscopic analysis of the residual polymer after microbial action on it revealed the presence of cracks and porosity on plastic strip surface and its FTIR analysis confirmed the stretching of functional group, thus validating the decomposition of polypropylene.

KEY WORDS : Polypropylene, Plastic degradation, Biodegradation, Waste management

INTRODUCTION

Polypropylene (commonly termed as plastic) is a polyolefin which has found wide range applications in agriculture, telecommunication, construction, packaging, medical, domestic, industrial and day to day utilities. Since it has the capacity to withstand degradation and decomposition under natural conditions for hundreds of years, polypropylene waste keeps on accumulating in the environment, consequently resulting in to pollution and long-lasting detrimental effects on it (Arutchelvi *et al*., 2008). Several methods for plastic disposal have been adopted over the decades such as land-filling, incineration and recycling etc. Although these methods have shown promising results, yet they could not contain environmental pollution effectively. With ever-increasing global usage of plastic year by year, its unplanned utilisation, high level of immunity towards biodegradation by most of the microbes and lack of disposal and decomposition adaptations have raised a serious concern which, if not met, might lead to damages to the environment persisting for several centuries or even millennia (Shah *et al*., 2008).

Under these circumstances, it becomes

imperative to explore surrogate methods of plastic disposal which should be fast, cost effective, reproducible and above all eco-friendly. Biodegradation of polypropylene by microorganisms can be the solution to all the problems presently being faced for plastic disposal (Kale *et al*., 2015). The focus on biodegradation of polypropylene and other such polymers has led to several studies in the last two decades or so. A few microbes including bacteria and fungi have been discovered to perform plastic biodegradation in a very small duration under natural conditions, thus converting the otherwise persistent plastic into non-toxic inorganic chemicals which are actually beneficial to soil fertility (Longo *et al*., 2011; Skariyachan *et al*., 2018). Broad range application of several microbes and their ability to efficiently decompose a variety of non-degradable polymers has been studied by Shimao (2001) and Sivan (2011). The evaluation of biodegradability of microbes is attributed to changes incurred both in the chemical structure of the polymer as well as on its physical properties such as weight loss, melting point, glass transition temperature, crystallinity, storage modulus, *etc*. (Tokiwa *et al*. 2009).

Quite a few microbes have been discovered to have plastic degrading properties in recent years. *Pseudomonas* spp. and *Aspergillus* spp. (Arutchelvi *et al*., 2008) have been observed to utilize plastic films as a carbon source, thus flourishing on them and eventually degrading them completely (Raziyafathima *et al*., 2016). Sepperumal and Markandan (2014) reported crystallisation and flake degradation of polypropylene plastic waste (confirmed by FTIR studies) and cracking, granule formation and increased roughness of its surface (confirmed by SEM imaging) upon exposure to *Pseudomonas* spp. More than 50% of the plastic degradation has been noted by *Microbacterium paraoxydans* and *Pseudomonas aeruginosa* (Rajandas *et al*., 2012). Increased porosity and fragility as observed by SEM imaging, and up to 12% loss in weight of plastic has been proven as a result of degradation by *Aspergillus* spp. under laboratory conditions (Raaman *et al*., 2012). The decrease in intrinsic viscosity and average molecular weight of gamma irradiated polypropylene strips inoculated with *Aspergillus* sp. has been studied earlier (Sheik *et al*., 2015). Bhardwaj *et al*. (2013) have also discussed the biodegradation of plastic materials by microbial agents including and *Aspergillus* spp.

The present investigation, thus aimed at elucidating the microbes (both bacteria and fungi) for their polypropylene degrading properties. The soil samples for isolation of bacteria and fungi were collected from waste disposal sites near Jalandhar (Punjab, India). The biodegradation of the polymer by isolated microbes was studied both on liquid broth under laboratory conditions. The parameters of analysis were reduction in weight of the polymer, physical properties such as morphology of the degraded residual plastic (by SEM) and chemical structure changes (by FTIR).

MATERIALS AND METHODS

Polypropylene cup: Polypropylene cups were purchased from local merchant (Tajinder Singh merchant, Hoshiarpur Road, Rama Mandi, Jalandhar) of Jalandhar market.

Soil: The Wariana dumping site of Jalandhar in Punjab region of India was selected for collection of soil due to high probability of finding potential plastic degrading microbial strains in the areas it being one of the oldest dumping sites. The soil was collected from a depth of 28-30cm using the sterile equipments and preserved in sterile containers until isolation of microbes from it.

Isolation and identification of microbes from soil: Pure isolates of bacteria and fungi from sampled soil were obtained by dissolving 1 mg of soil in 1 L of autoclaved water followed by serial dilution up to 10^{-5} times. One mL of the dilution was inoculated on nutrient agar plate. The inoculated plate was incubated at 37 °C for 24 hrs. Sub-culturing was performed to obtain pure culture and single cell colonies of the bacteria. The dilution was also inoculated on Potato Dextrose Agar and incubated at room temperature for 7 days for obtaining fungal cultures. Further sub-cultures were performed for obtaining pure fungal isolates. The isolates were subjected to standard identification procedures i.e morphological characteristics and biochemical tests for bacteria and 18S rRNA sequencing for the fungus (Zhang *et al*., 2009; Rohilla and Salar, 2012).

Sterilization of polypropylene cup: The collected polypropylene cups were cut into small pieces of dimensions 2x2cm^2. The pieces were rinsed with running tap water (to remove dust particles), surface sterilized using 70% ethyl alcohol and exposed to UV light in a laminar air flow for 20 minutes and then weighed.

Culture media preparation and inoculation:

Nutrient broth (NB) and Potato dextrose broth (PDB) were prepared as per standard procedure for bacterial and fungal inoculation respectively. The pH of NB was maintained at 7.4 and that of PDB at 3.5. Both the media were sterilised by autoclaving them at 121 °C for 15 min. 200 mL of cooled broths was poured into 4 flasks each for bacterial and fungal inoculation respectively. The sterile pre-weighed polypropylene pieces were aseptically placed into all 8 flasks. Out of the 4 flasks for fungal inoculation, 3 were inoculated with 1 mL liquid culture of the fungus isolated from soil sample and 1 was kept as control. These flasks were incubated at 25 °C. Similarly, out of the remaining 4 flasks, 3 were inoculated with 1 mL liquid culture of the bacteria isolated from soil sample and 1 was kept as control. These flasks were incubated at 37 °C. The experiment was set up in triplicates. The analysis of the decomposed polypropylene strip was carried out at 2, 4 and 6 months post-inoculation.

Biodegradation studies

Dry weight determination of residual plastic: The plastic strips used for the experiments were recovered from respective culture media, washed with 2% (v/v) sodium dodecyl sulphate (SDS) and rinsed with autoclaved distilled water. The strips were then dried overnight at 60 °C before weighing for determining the loss in weight of the plastic strip using the following formula (Kyaw *et al.*, 2012).

$$Weight\ Loss\ (\%) = \frac{Initial\ weight - final\ weight}{Initial\ weight} \times 100$$

Spectroscopic analysis by FTIR (Fourier transform infrared): The changes in chemical structure of the polypropylene structure i.e alterations in their chemical bonds and other changes in different functional groups was analysed using FTIR spectroscopy. The polypropylene strips subjected to degradation by the microbes were analyzed at the different sampling intervals (Gajendiran *et al.*, 2016).

Scanning electron microscopy (SEM) analysis: The change in the morphology of the plastic cup strip surface before and after biodegradation at 2, 4 and 6 months of sampling intervals was analysed by scanning electron microscope. The treated samples were subjected to SEM analysis after washing with 2% (v/v) SDS and distilled water for few minutes and flushing with 70% ethanol to remove the inoculated microbial cells. The sample was pasted onto the SEM analysis stub using acarbon tube, coated with gold for 40s and analyzed under high-resolution scanning electron microscope (EVO LS15; Carl Zeiss, Germany) (Gajendiran *et al.*, 2016).

RESULTS

Identification of plastic degrading microbes: The morphological characteristics and biochemical tests confirmed the bacteria isolated from dumping site to be *Staphylococcus* sp. and 18S rRNA sequencing confirmed that the isolated fungus was *Aspergillus fumigatus*.

Weight loss % studies: The analysis of residual plastic weight from the liquid broth demonstrated that the isolated microbes *Staphylococcus* sp. and *A. fumigatus* were able to degrade upto 9.5% and 18.08% of the plastic respectively over a period of 6 months (Figure 1).

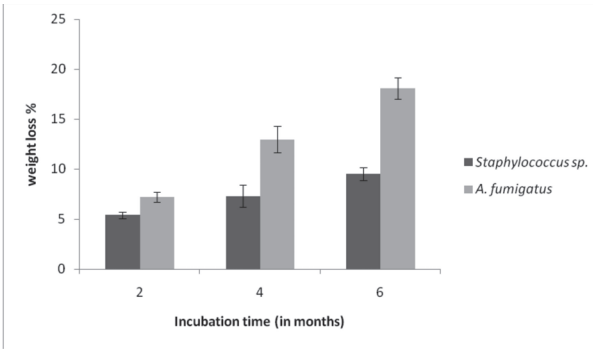
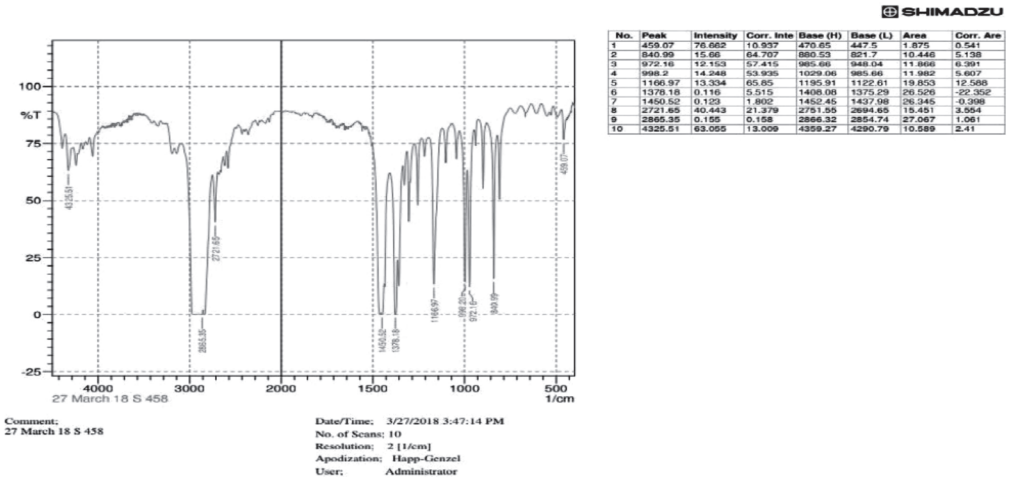


Fig. 1. Weight loss % in the residual polypropylene plastic after incubation with isolated *Staphylococcus* sp. and *Aspergillus fumigatus*. The bars represent the average weight loss % ± SE (n=3)

FTIR analysis: The FTIR analysis of the degraded plastic revealed the stretching of several functional groups after incubation with the isolated bacteria or fungus. This was confirmed by the differences in the peaks of the FTIR spectrum amongst the control and test samples (Figures 2 to 9). Tables 1 and 2 summarize the type of functional group involved in stretching by microbial action, the wave number and the position of infra-red band on the polymer. The action of *Staphylococcus* sp. lead to stretching in C-O and C=O of aldehyde group at 1378.18 and 2865.35 cm^{-1} respectively within 2 months of polypropylene incubation with it. After 4 months of incubation, a cyclo alkane was observed to undergo stretching by bacterial action at 2865.55 cm^{-1}. Also, a bound OH group showed peak for stretching at 3165.29 cm^{-1}. After 6 months of incubation of the plastic material with the bacteria, stretching of as many as 3 functional groups (C-H of cellulose and lignin and

=-CH_3 group of the phenol ring) was observed in the residual plastic FTIR spectra at 1378.18cm^{-1}. *Aspergillus fumigatus* also had severe impact on the bonds of the functional group in the polypropylene polymer. The FTIR spectra of the residual plastic after fungal action demonstrated that the microbe had significant stretching effects on the functional groups. Within 2 months of fungal action on the polymer, a new peak in the residual plastic was observed in the spectra at 459 and 2863.42 cm^{-1} corresponding to the stretching of a C-I and C-H and C=O respectively. A shift in the peak was observed in the residual plastic after 4 months of fungal action at 2845.10 cm^{-1}, proving the stretching of C-H and C=O of aldehyde group. Cleavage of the O-H group was confirmed by the shift of a peak from 2580.84 cm^{-1} in control to 2501.76 cm^{-1} in the test sample. Stretching of CH_3 group at 1450.52 cm^{-1} and C=O, C=C, C=N groups at 1701.92 cm^{-1} was observed in the residual plastic after 6 months of fungal incubation.

SEM analysis of residual plastic: No visible changes were noted in the surface morphology of control samples. The SEM images of residual polypropylene strips after being degraded by *Staphylococcus* and *A. fumigatus* for 6 months demostrated the appearance of significant cracks and pores on the surface. Granule formation of



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	459.07	76.662	10.937	470.65	447.5	1.875	0.541
2	840.99	15.66	64.707	880.53	821.7	10.446	5.138
3	972.16	12.153	57.415	985.66	948.04	11.866	6.391
4	998.2	14.248	53.935	1029.06	985.66	11.982	5.607
5	1166.97	13.334	65.85	1195.91	1122.61	19.853	12.588
6	1378.18	0.116	5.515	1408.08	1375.29	26.526	-22.352
7	1450.52	0.123	1.802	1452.45	1437.98	26.345	-0.398
8	2721.65	40.443	21.379	2751.55	2694.65	15.451	3.554
9	2865.35	0.155	0.158	2866.32	2854.74	27.067	1.061
10	4325.51	63.055	13.009	4359.27	4290.79	10.589	2.41

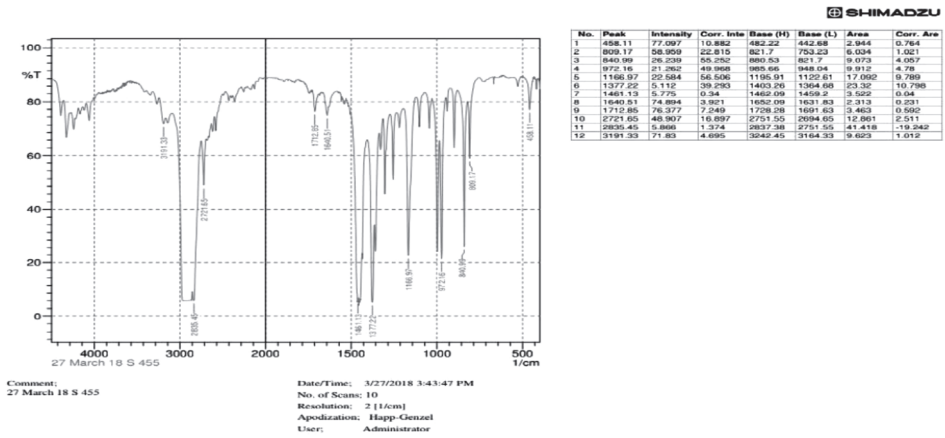
Fig. 2. FTIR spectra of bacterial control polypropylene sample.

Table 1. Comparison of IR band position in the residual polypropylene cup strips after incubation with isolated *Staphylococcus* sp. and control.

S.No	Incubation period (sampling interval)	IR band positioncontrol-test sample (cm^{-1})	Functional Group involved
1	2month	1378.18-1377.22	C-O
		2865.35-2833.45	C-H and C=O
4	4 month	2865.55-2876.92	Cyclo alkane
		3165.29-3142.15	Bonded OH group
3	6 month	1378.18-1373.36	CH cellulose, lignin and =-CH_3

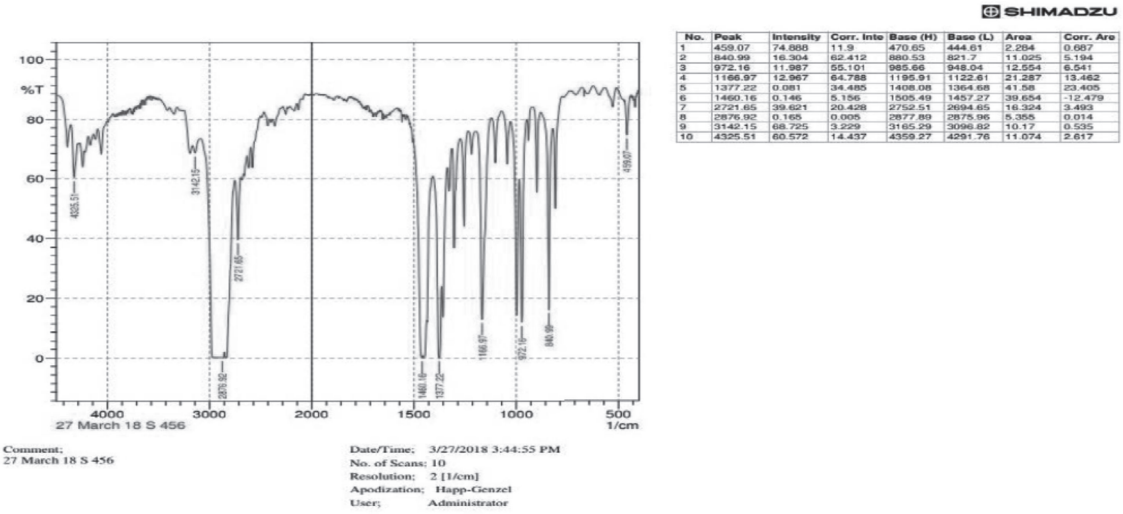
Table 2. Comparison of IR band position in the residual polypropylene cup strips after incubation with isolated *A. Fumigates* and control.

S.No.	Incubation period (sampling interval)	IR band positioncontrol-test sample (cm^{-1})	Functional Group involved
1	2 month	459-458.1	C-I
		2863.42-2837.38	C-H and C=O
2	4 month	2580.84-2501.76	O-H
		2863.42-2845.10	C=O and C-H
3	6 month	1450.52-1449.55	CH_3
		1701.92-1702.24	C=O, C=C, C=N



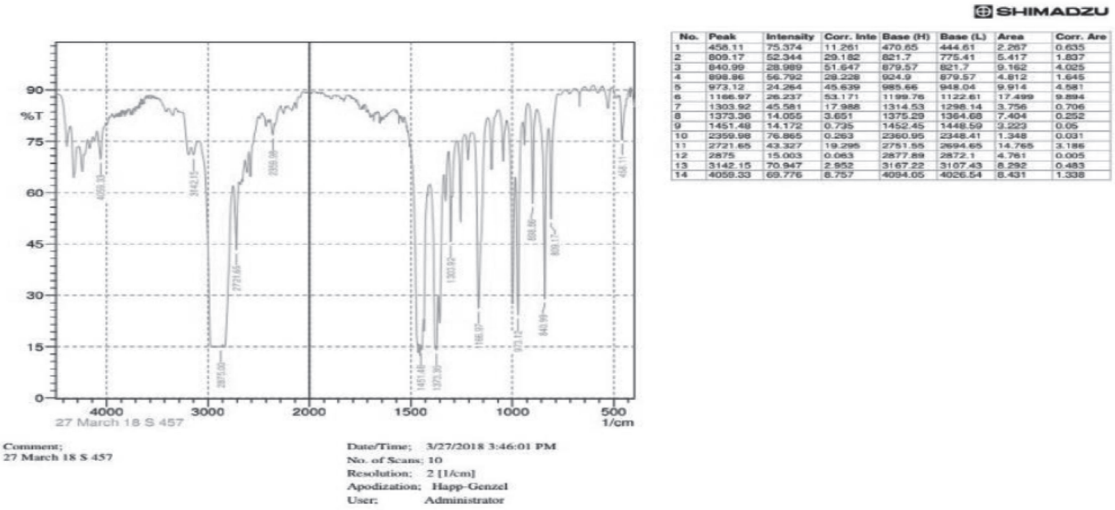
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	458.11	77.097	10.882	482.22	442.68	2.944	0.764
2	809.17	58.959	22.815	821.7	753.23	6.034	1.021
3	840.99	26.239	55.252	880.53	821.7	9.073	4.057
4	972.16	21.262	49.968	985.66	948.04	9.912	4.78
5	1166.97	22.584	56.506	1195.91	1122.61	17.092	9.789
6	1377.22	5.112	39.293	1403.26	1364.68	23.32	10.798
7	1461.13	5.775	0.34	1462.09	1459.2	3.522	0.04
8	1640.51	74.894	3.921	1652.09	1631.83	2.313	0.231
9	1712.85	76.377	7.249	1728.28	1691.63	3.463	0.592
10	2721.65	48.907	16.897	2751.55	2694.65	12.861	2.511
11	2835.45	5.866	1.374	2837.38	2751.55	41.418	-19.242
12	3191.33	71.83	4.695	3242.45	3164.33	9.623	1.012

Fig. 3. FTIR spectra of polypropylene sample incubated with *Staphylococcus* sp. for 2 months.



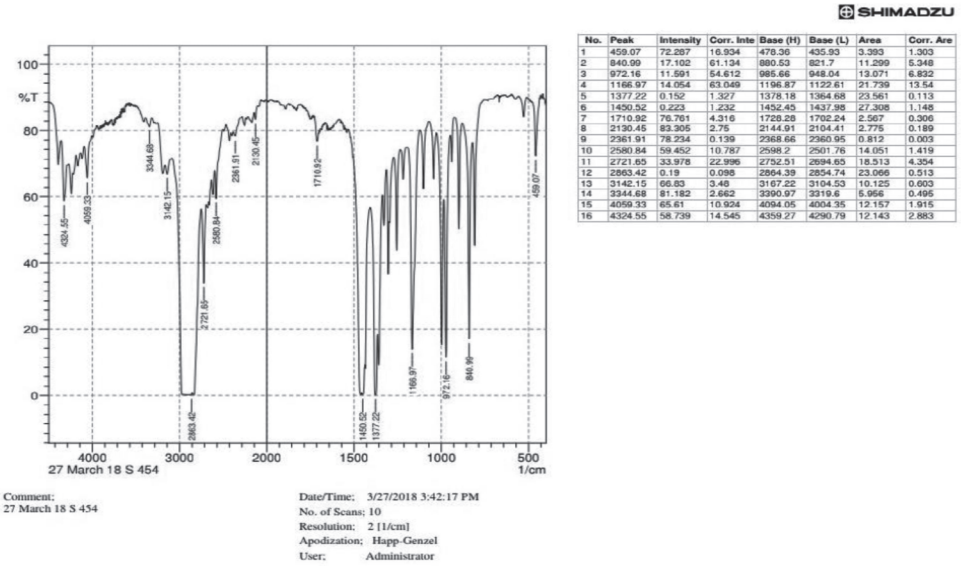
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	459.07	74.888	11.9	470.65	444.61	2.284	0.687
2	840.99	16.304	62.412	880.53	821.7	11.025	5.194
3	972.16	11.987	55.101	985.66	948.04	12.554	6.541
4	1166.97	12.967	64.788	1195.91	1122.61	21.287	13.462
5	1377.22	0.081	34.485	1408.08	1364.68	41.58	23.405
6	1460.16	0.146	5.156	1505.49	1457.27	39.654	-12.479
7	2721.65	39.621	20.428	2752.51	2694.65	16.324	3.493
8	2876.92	0.165	0.005	2877.89	2875.96	5.355	0.014
9	3142.15	68.725	3.229	3165.29	3096.82	10.17	0.535
10	4325.51	60.572	14.437	4359.27	4291.76	11.074	2.617

Fig. 4. FTIR spectra of polypropylene sample incubated with *Staphylococcus* sp. for 4 months.



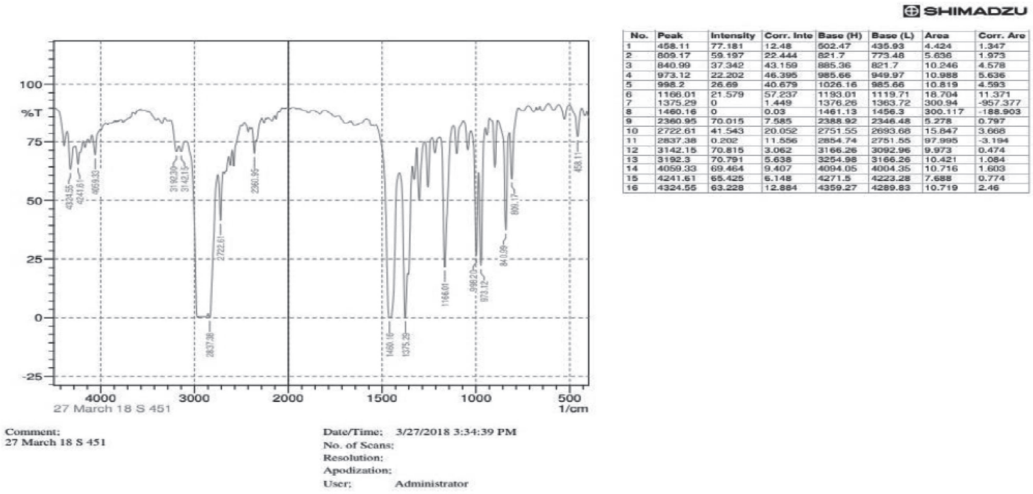
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	458.11	75.374	11.261	470.65	444.61	2.267	0.635
2	809.17	52.344	29.182	821.7	775.41	5.417	1.837
3	840.99	28.989	51.647	879.57	821.7	9.162	4.025
4	898.96	56.792	28.228	924.9	879.57	4.812	1.645
5	973.12	24.264	45.639	985.66	948.04	9.914	4.581
6	1166.97	26.237	53.171	1199.76	1122.61	17.499	9.894
7	1303.92	45.581	17.982	1314.53	1298.14	3.756	0.706
8	1373.36	14.055	3.651	1375.29	1364.68	7.404	0.252
9	1451.48	14.172	0.735	1452.45	1448.59	3.223	0.05
10	2359.98	76.865	0.263	2360.95	2348.41	1.348	0.031
11	2721.65	43.327	19.295	2751.55	2694.65	14.765	3.186
12	2875	15.003	0.063	2877.89	2872.1	4.761	0.005
13	3142.15	70.947	2.952	3167.22	3107.43	8.292	0.483
14	4059.33	69.776	8.757	4094.05	4026.54	8.431	1.338

Fig. 5. FTIR spectra of polypropylene sample incubated with *Staphylococcus* sp. for 6 months.



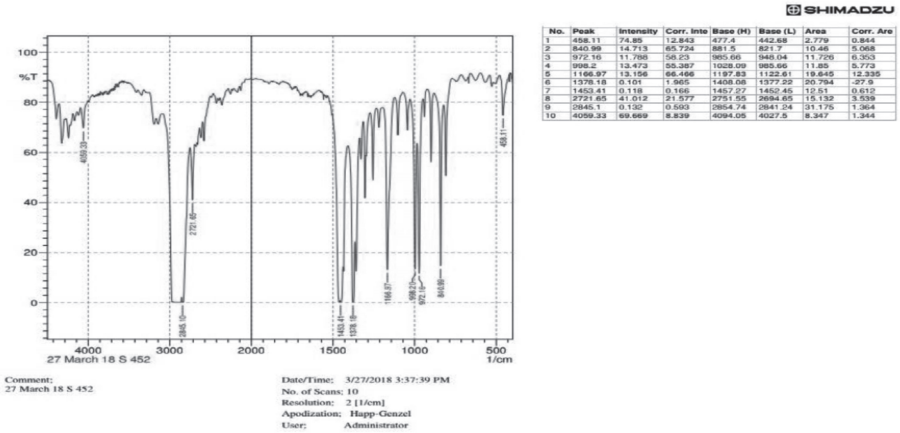
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	459.07	72.267	16.934	478.36	435.93	3.393	1.303
2	840.99	17.102	61.134	880.53	821.7	11.299	5.048
3	972.16	11.591	54.612	985.66	948.04	13.071	6.832
4	1166.97	14.054	63.049	1196.87	1122.61	21.739	13.54
5	1377.22	0.152	1.327	1378.18	1364.68	23.561	0.113
6	1450.52	0.223	1.232	1452.45	1437.98	27.398	1.148
7	1710.92	76.761	4.316	1728.28	1702.24	2.567	0.306
8	2130.45	83.305	2.75	2144.91	2104.41	2.775	0.189
9	2361.91	78.234	0.139	2368.66	2360.95	0.812	0.003
10	2580.84	59.452	10.787	2598.2	2501.76	14.051	1.419
11	2721.65	33.978	22.996	2752.51	2694.65	18.513	4.354
12	2863.42	0.19	0.098	2864.39	2854.74	23.066	0.513
13	3142.15	66.83	3.48	3167.22	3104.53	10.125	0.603
14	3344.68	81.182	2.662	3390.97	3319.6	5.956	0.495
15	4059.33	65.61	10.924	4094.05	4004.35	12.157	1.915
16	4324.55	58.739	14.545	4359.27	4290.79	12.143	2.883

Fig. 6. FTIR spectra of fungal control polypropylene sample.



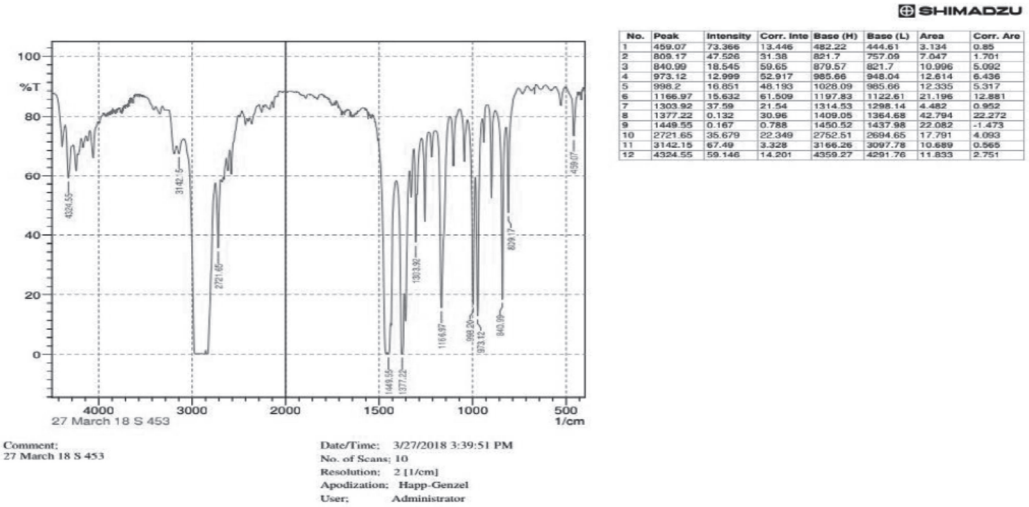
No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	458.11	77.181	12.48	502.47	435.93	4.424	1.347
2	809.17	59.197	22.444	821.7	773.48	5.636	1.973
3	840.99	37.342	43.159	885.36	821.7	10.246	4.578
4	973.12	22.202	46.395	985.66	949.97	10.988	5.636
5	998.2	26.69	40.679	1026.16	985.66	10.819	4.593
6	1166.01	21.579	57.237	1193.01	1119.71	18.704	11.371
7	1375.29	0	1.449	1376.26	1363.72	300.94	-957.377
8	1460.16	0	0.03	1461.13	1456.3	300.117	-188.903
9	2360.95	70.615	7.585	2388.92	2346.48	5.278	0.797
10	2722.61	41.543	20.052	2751.55	2693.66	15.847	3.668
11	2837.38	0.202	11.556	2854.74	2751.55	97.995	-3.194
12	3142.15	70.815	3.062	3166.26	3092.96	9.973	0.474
13	3192.3	76.791	5.638	3254.98	3166.26	10.421	1.084
14	4059.33	69.464	9.407	4094.05	4004.35	10.716	1.603
15	4241.61	65.425	6.148	4271.5	4223.28	7.688	0.774
16	4324.55	63.228	12.884	4359.27	4289.83	10.719	2.46

Fig. 7. FTIR spectra of polypropylene sample incubated with *A. fumigatus* for 2 months.



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	458.11	74.85	12.843	477.4	442.68	2.779	0.844
2	840.99	14.713	65.724	881.5	821.7	10.46	5.068
3	972.16	11.786	56.23	985.66	946.04	11.726	6.353
4	998.2	13.473	55.387	1028.09	985.66	11.85	5.773
5	1166.97	13.156	66.466	1197.83	1122.61	19.845	12.335
6	1378.18	0.101	1.965	1408.08	1377.22	20.794	-27.9
7	1453.41	0.118	0.166	1457.27	1452.45	12.51	0.612
8	2721.65	41.012	21.577	2751.55	2694.65	15.132	3.539
9	2845.1	0.132	0.593	2854.74	2841.24	31.175	1.364
10	4059.33	69.669	8.839	4094.05	4027.5	8.347	1.344

Fig. 8. FTIR spectra of polypropylene sample incubated with *A. fumigatus* for 4 months.



No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	459.07	73.366	13.446	482.22	444.61	3.134	0.85
2	809.17	47.526	51.38	821.7	757.09	7.647	1.761
3	840.99	18.545	59.65	879.57	821.7	10.996	5.092
4	973.12	12.999	52.917	985.66	948.04	12.614	6.436
5	998.2	16.851	48.193	1028.09	985.66	12.335	5.317
6	1166.97	15.632	61.509	1197.83	1122.61	21.196	12.881
7	1303.92	37.59	21.54	1314.53	1298.14	4.482	0.952
8	1377.22	0.132	30.96	1409.05	1364.68	42.794	22.272
9	1449.55	0.167	0.788	1450.52	1437.98	22.082	-1.473
10	2721.65	35.679	22.349	2752.51	2694.65	17.791	4.093
11	3142.15	67.49	3.328	3166.26	3097.78	10.689	0.565
12	4324.55	59.146	14.201	4359.27	4291.76	11.833	2.751

Fig. 9. FTIR spectra of polypropylene sample incubated with *A. fumigatus* for 6 months.

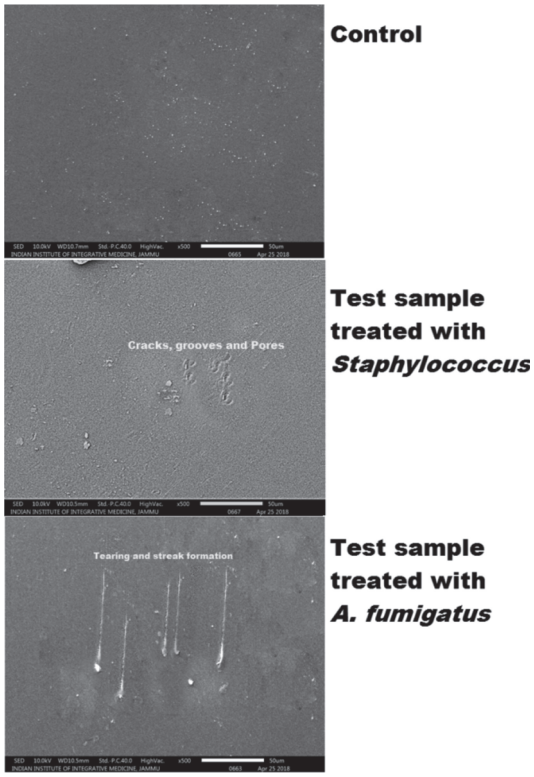


Fig. 10. Comparison of SEM images of the control and test samples after 6 months of incubation.

polypropylene was evident in the polymer degraded by the fungus. The microbial action could perhaps break the polymer into monomeric form. The test samples also showed wrinkles, foldings and physical weakness as compared to the control samples. Tearing of the surface had started after fungal action which was evident from the streaks and rifts developing on the surface due to breakage in it (Figure 10). Biofilm of the inoculated microbes could be observed on the polypropylene surface by SEM analysis which probably indicates that after the exhaustion of the carbon source in the broth medium, the microbes were able to utilize the polymer for growth and reproduction.

DISCUSSION

The study reported here focussed upon investigating upon the plastic degrading capabilities of two microbes viz. *Staphylococcus sp*. and *Aspergillus fumigatus*. Both the microbes were isolated from the local waste dumping sites considering the fact that the possibility of finding such microbes is the highest in such areas due to heavy load of plastic materials being disposed off there since several decades. The results significantly prove that the isolated microbes were able to degrade the polypropylene polymer under *in vitro* conditions in broth culture. The loss in dry weight of the polymer was evident after the weight loss % analysis of the biodegraded residual plastic wherein upto 18.08% of the polymer weight loss was achieved. Moreover, the FTIR studies proved that the microbial action could induce changes in the bonds present in the polymer. These changes were stretching and cleavage of the functional groups, symbolising degradation of the polymer to monomeric forms. Further, the SEM analysis made it evident that the morphology of the residual plastic was very much different from the control one. Several pores, foldings, wrinkling on the surface,

presence of biofilm and formation of granules were observed in the test samples. The fungal isolate was observed to be more efficient in degradation of the polymer as compared to the bacterial isolate.

Earlier studies have also deliberated upon the abilties of a few bacteria and fungi in biodegradation of the otherwise nondegrading plastic materials. These microbes might have been present in the environment since long and were perhaps not exploited for such capabilities, or it is very much possible that they might have evolved and adapted to utilize plastic polymers as the chief/alternative source of energy. Their action on plastic can remarkably enhance the plastic degradation which remain undegraded for hundreds of years in the environment and cause severe ecological and health hazards. Within 14 days of incubation with *Amycolatopsis*, 60% of the polylactide was degarded under broth culture conditions (Pranamuda *et al*., 1997). Different species of *Pseudomonas, Aspergillus, Staphylococcus* and *Streptomyces* have shown promising plastic degrading results and were able to degrade upto 37.09% of plastic polymers over a period of 6 months (Usha *et al*., 2011). Shah *et al*. (2008) have very comprehensively reviewed the ability of a number of microbes who had shown significant biodegradation of different types of plastic materials such as polyethylene, polyurethane, polyvinyl chloride etc. Physical changes such as alterations in tensile strength, appearance of pores and increased roughness on residual polymer's surface have been highlighted which prove that the microbes were able to flourish over its surface. Formation of biofilms on the plastic dumped into the water bodies was observed by Lobelle and Culiffe (2011), which could be instrumental in plastic decomposition by them. Biodegradation of many poly-ester based polymers by *Paenibacillus amylolyticus* has been observed and attributed to the presence of the protease and esterase enzymes in the broth culture of the incubated polymer (Teeraphatpornchai *et al*., 2003). The production of extracellular enzyme by microbes for bond breakage in the plastic polymer consequently leading to its degradation has been mentioned by Maeda *et al*. (2005) and Caruso (2015).

Fourier transform infrared spectroscopy studies during our study demonstrated alterations in the bonds present on the different functional groups, most of which were either streched or broken by the microbial action. Direct impact on such functional groups can reduce the strength of the polymeric form of the plastic and facilitate its degradation into oligomeric and monomeric forms. Significant chages in the alkyl and carbonyl groups due to their oxidation by *Pseudomonas aeroginosa* led to 2.2% loss in weight of a plastic over a test period of 28 days (Agamuthu and Faizura, 2005). Decrease in carbonyl index, upto 25% dry weight loss and 8.5% loss in tensile strength of a high density polyethylene was observed by Sudhakar *et al*. (2007). Formation of aromatic ether and involvement of hydroxyl groups and ester groups in stretching, bending and cleavage eventually leading to polymer degradation have been reported by Sepperumal and Markandan (2014). The FTIR studies on the residual low density pluethylene demonstrated biodegradation as high as 61% and 50.5% within 2 months of incubation with *Microbacterium paraoxydans* and *Pseudomonas aeruginosa* (Rajandas *et al*., 2012). Polyethylene biodegrading ability of *Zalerion maritimum* was ascertained by FTIR studies of the degraded polymer residue which showed that vibrations were induced in carboxyl, hydroxyl, phenol groups, amides, CH_2 and CH_3 functional groups from lipids or proteins and strectching and cleavage of C=O, C-N and N-H bonds in several proteins (Paco *et al*., 2017). *Bacillus amyloliquefaciens* was observed to tear apart the plastic films and make chemical changes on its surface leading to remarkable degradation within 30 days of incubation with plastic in a carbon-free medium (Zhang *et al*. 2018).

The scanning electron microscopy studies of the plastic residues obtained after their biodegradation by the isolated bacteria (*Staphylococcus* sp.) and fungus *Aspergillus fumigatus*) demonstrated observable morphological differences from the control. Amongst the isolates tested for biodegradation, *A. fumigatus* performed significantly higher degradation of the polymer than *Staphylococcus* sp. The residual polymer suface had significantly eroded along with the appearance of cracks, pores and increased fragility. Erosion on the surface of the polymer due to microbial activity is the primary cause loss in its weight. The observable biofilm of the microbes on the degraded polymer surface strengthen the fact that after the exhaustion of the carbon source in the broth, the microbes survived by actively utilising the plastic material for growth and reproduction. Appearance of crystalline spherolites on the surface due to preferential degradation of the amorphous polymer fraction, etching the slower-degrading crystalline parts out of the material after microbial action has been reported

by Shah *et al*. (2008). Appreciable surface corrosion, folding, cracks and decrease in viscosity due to the extracellular metabolites and enzymes secreted by *A. niger* and *Aspergillus japonicus* have been observed in the polyethylene carry bags by Raaman *et al*. (2012) and Sheik *et al*. (2015). Two fungal strains viz. *Penicillium oxalicum* NS4 (KU559906) and *Penicillium chrysogenum* NS10 (KU559907) reportedly secreted polyhydroxybutyratede (PHB) polymerase responsible for hydrolysing the ester bonds found in PHB and breaking it into oligomers and monomers (3 hydroxybutyrate) in 90 days (Ojha *et al*., 2017).

The results reported in this study seem to be encouraging and promising. The survival of microbes on plastic polymer and degradation of the later by them can prove to be crucial for further studies, which can focus upon the characterisation on the microbial metabolites responsible for bringing about the physical and chemical changes in the polymer. This would enhance our understanding about the mechanism of action the bacteria and fungus causing plastic degradation. The utilization of these microbes for industrial scale biodegradation of polypropylene polymeric plastic can also be exploited.

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