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Research Article

EXAMINING AND IDENTIFYING BACTERIA-MEDIATED POLYETHYLENE TEREPHTHALATE BOTTLE WASTE DEGRADATION BYPROPS

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ABSTRACT

Plastic waste accumulation raises concerns about global environmental risk due to persistence of Polyethylene terephthalate (PET) which degrades slowly and release harmful compounds. Hence, it becomes increasingly imperative to remove plastic waste from the environment. In light of this, the present study examined the PET degradation capacity of naturally existing bacteria obtained from sites where plastic garbage was dumped. *Bacillus subtilis* was isolated from the old PET plastic waste bottles. Pre-treated PET (Ultraviolet light, Sunlight and untreated PET) plastics were cultured with *Bacillus subtilis for six months at 37º C to examine their biodegradability in Minimal Salt Medium.* The functional groups of PET wastes and deteriorated by-products in MSM were analyzed for change using Gas chromatography–mass spectrometry (GC-MS) and Fourier–transform infrared spectroscopy (FTIR). It revealed that the bacterial biodegradation led to appearance of new peaks such as alkyl aryl ether and alkene groups in ultraviolet-pretreated PET microplastics when compared to sunlight and control PET microplastics. After six months of incubation of PET microplastics, Bis(2-ethylhexyl) phthalate, fatty acids, amides, and ketones were detected in the supernatants of Ultraviolet-treated and sunlight-treated PET microplastics in minimal salt medium. The soil bacteria showed the potential to degrade PET and hence could be employed for eliminating PET from plastic contaminated sites.

Keywords: Biodegraded compounds, FTIR, GCMS, PET.

INTRODUCTION

In today's world, plastics are extensively utilized; an estimated 320 million metric tonnes are produced worldwide each year (Ragusa *et al*., 2021). On the other hand, the durability and persistence of plastic materials have resulted in significant environmental problems, including the accumulation of plastic waste in landfills, waterways, and oceans. Approximately 8 million tonnes of plastic waste enter the ocean annually (Mendoza and Balcer, 2018). After being introduced to the water environment, plastic can undergo degradation caused by factors like microbiological activity, radiation, and mechanical stress, leading to the disintegration and fragmentation of larger plastic items into smaller particles called microplastics (Silva *et al*., 2018; Wang *et al*., 2021). Due to their potential effects on both human health and the environment, microplastics are an especially dangerous type of plastic pollution. In this form, plastics easily pollute marine environments, which has led to their getting into the food chain for both humans and animals. Clodagh M. Carr *et al*., have connected this to a number of harmful health impacts, including cancer, immunological problems, and congenital defects (Clodagh M. Carr *et al*. (2020). Recent studies have identified MPs in the air, water, soil, fresh water, drinking water, lakes oceans, aquatic and terrestrial environments, food products, human placenta, and stools (Felismino *et al*., 2021; Li *et al*., 2021; Malla Pradhan *et al*., 2022; Ragusa *et al*., 2021)**.** Therefore, it is upmost necessity to study about the various treatment techniques that are useful in Plastics waste management and the effectiveness of these techniques in the removal of microplastics in environment (Krishnan *et al*., 2023). In the case of polymers, such as PET, a continuous chain of

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repetitive ethylene units makes it resistant to degradation. The hydrophobic nature of polymer hinders the attachment of microorganisms to its surface. Physical treatments, which include UV, thermal, and chemical, lead to oxidation of the polymer surface and also decrease the hydrophobicity of the surface (Sudhakar *et al*., 2008), which ultimately helps in the formation of microbial biofilm on its surface (Gilan *et al*., 2004). Thus, treatment leading to oxidation of the polymer can be effectively used as a pretreatment strategy before subjecting it to biodegradation. An increase in the biodegradation of polyethylene was observed with an increase in the time of exposure to UV (Hadad *et al*., 2005). In earlier investigation, various microorganisms, including bacteria, have been reported to be able to consume PET plastics, Bacillus cereus SEHDO3 and *Agromyces mediolanus* PNP3 (Patricia Torena *et al*., 2020), *Vibrop alginolyticus*, *Pseudoalteromonas caenipelagi, Microbulbifer pacificus, Pseudomonas marincola* and *Bacillus subtilis*. The ability of these bacteria to biodegrade plastics represents an opportunity to effectively remove persistent pollutants from the environment (Aqil Azizi *et al*.,2024). The current study investigated the biodegradation of pre-treated PET microplastics by bacteria. PET plastics and examined the biodegradation of PETMPs using GC-MS and FTIR.

MATRIALS AND METHODS

Bacterial isolation and identification

The old PET waste bottle was collected from the waste dumping site (St. Joseph University, Dimapur Nagaland, India). The waste bottle was cut into small pieces and washed properly with distilled water and let it to dry. It was then directly inoculated in Nutrient Broth and incubated at 37˚C for 24 hours allowing for the bacterial growth. After 24 hours of incubation the bacteria were isolated and cultured separately using slant culture method. One bacterium was isolated and one has been selected for further study and 16srRNA sequences were analyzed. Bacterial Gene sequences were submitted in NCBI to analyze pair wise similarity and receive accession number (MK128437).

Polyethylene terephthalate microplastic (PET MP) sample preparation

PET plastic bottles were purchased and cut into small flakes. It was further crushed to make microplastics, thereafter it was exposed to UV light (Laminar Airflow chamber UV light) to yield UV-PETMPs(UV ray exposed PET Microplastics)for 15 days and also exposed sunlight for 15 days SL-PETMP (PETMP Placed open place for sunlight exposed).Pre-treated (UV-PETMP and SL-PETMP) and control PET powder (Un pre-treated (UT-PETMP) sample inoculate with *Bacillus subtilis* in the Minimal salt media (MSM: Minimal Salts media was prepared by dissolving 1.73g K_2HPO_4, 0.1g $MgSO_4.2H_2O$, 4g NaCl, 0.03 $FeSO_4.7H_2O$, 1g KNO_3, 0.02g $CaCl2.H_2O$ in 1000ml of distilled water). The whole culture media was incubated for a period of six month at a temperature of 37˚C. End the experiment the supernatant of MSM were subjected by GCMS and PET microplastics were subjected by FTIR.

FTIR analysis

Chemical changes occurring on the surface of the PET were analysed using FTIR spectrophotometer Fourier transform infrared (FTIR). Measurements were carried out with the Perkin Elmer Spectrum two (version 10.03.09) in the range of 4000-400 cm^{-1}. FTIR spectra were recorded at a resolution of 2cm^{-1} and at an accumulation of 32 scans.

GC-MC analysis

After six-month incubation, the supernatant of MSM medium subject to analysis GC-MS. GC-MS analysis was carried out using GC-MS (QP2010 PLUS Shimadzu, Japan). The column oven temperature was 60.0˚C and injection temperature was 260˚C. A pressure of 73.3 kPa was maintained with a total flow of 16.3 mL/min and a column flow of 1.21 mL/min. The linear velocity was 40.1 cm/sec, purge flow of 3.0 mL /min and a split ratio of 10.0. The GC program ion source temperature was 230.00˚C, interface temperature 270.00˚C with a solvent cut time of 3.50 min. The MS program start time was 4.00 min and ended at 44.00 min. the event time was 0.20 sec at a scan speed of 1666µl/sec. Mass spectra were recorded and the range was m/z 30-500 amu. The total running time was 40 minutes. Identification of components**:** The National Institute of Standard and Technology's (NIST) database and WILEY 8 were used to interpret the mass spectrum of the GC-MS. The name, structure and molecular weight of the components present were ascertained. The percentage of each compound present was calculated by comparing the individual peak area to the total area.

RESULTS AND DISCUSSION

FTIR analysis showed the change in spectra between the control and treated sample at different wavelengths. The Peak value and Functional groups as showed in the table (Table-1,2). Some new peak was detected in the bacterial treated UV PET-MP. C=O Stretching of the alkyl aryl ether group were observed as peak at 2582 cm^{-1}, 2388 cm^{-1}, 2285 cm^{-1}, 2117 cm^{-1}, 1721 cm^{-1}. C=C bending of the alkene group were all shown by the peaks at 1579 cm^{-1} and 1506 cm^{-1}. C-H stretching in the alkene groups was responsible for the peaks at 1408 cm^{-1}, 1342 cm^{-1} and 1020 cm^{-1}. Moreover, new speaks was detected in the sun light treated and Bacterial inoculated PETMPs. C=O stretching of alkyl was observed as peaks at 2520 cm^{-1}, 1959 cm^{-1},1715 cm^{-1} and 1507 cm^{-1} (C-C) in Sun light-PETMPs. (Figure1, Figure 2, Figure 3, Figure 4 and Table-1)

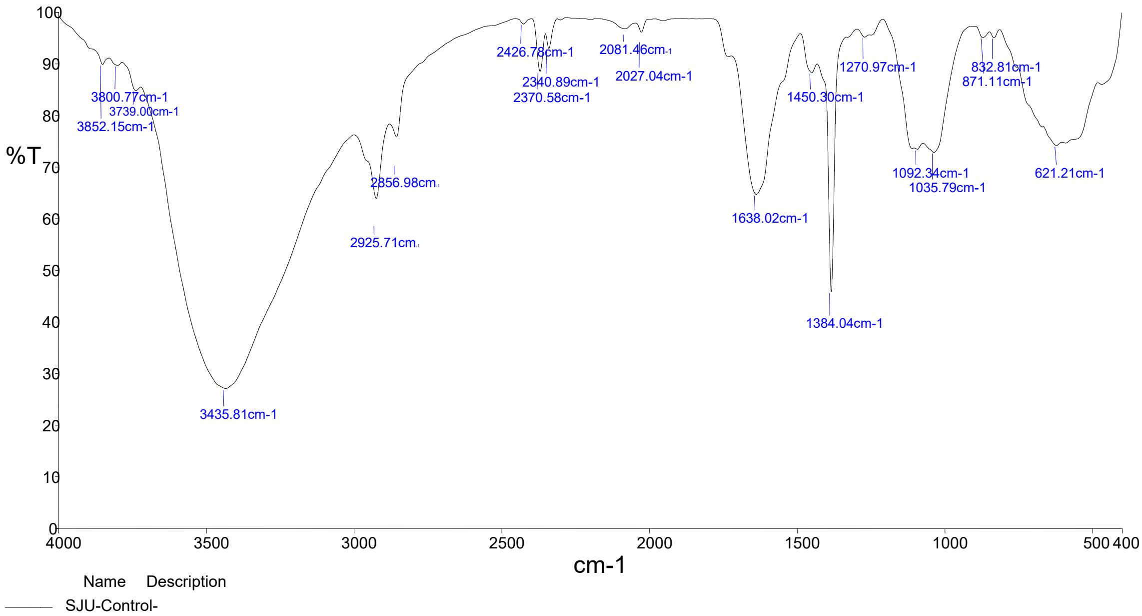

Figure 1. FTIR spectroscopy of Control PET MP.

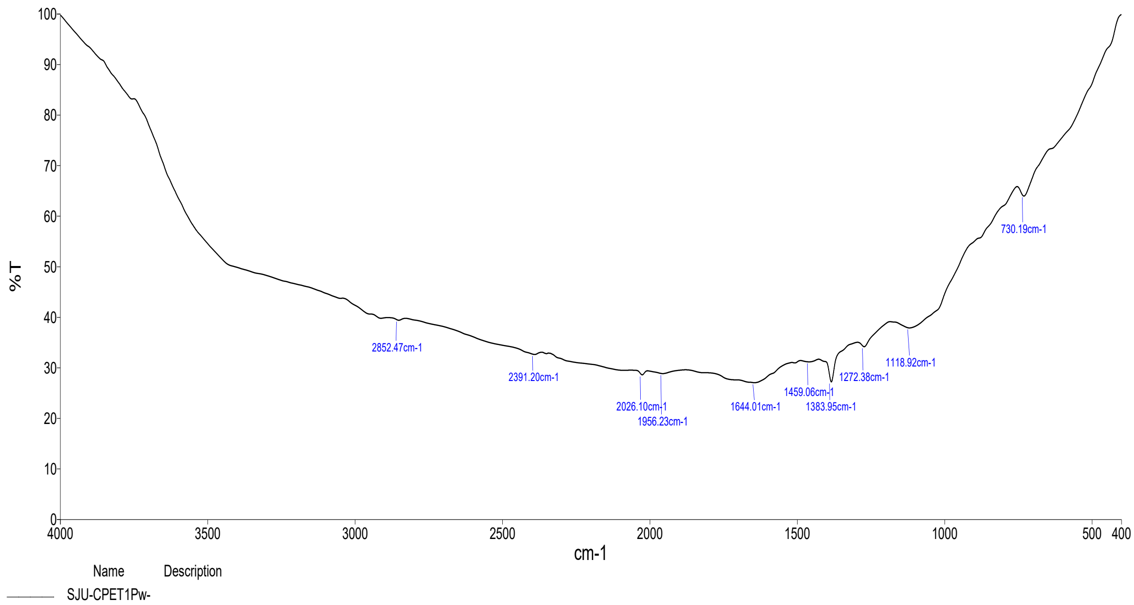

Figure 2. FTIR control with bacterial treated.

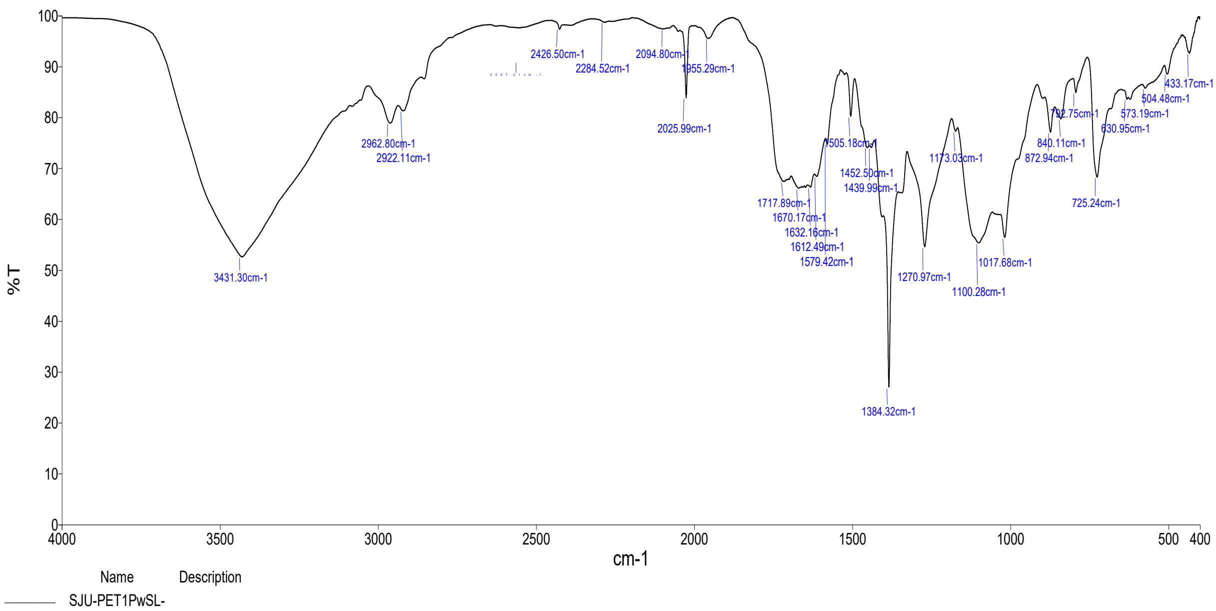

Figure 3. FTIR Spectroscopy of *B.Subtillis* treated SL-PETMPs.

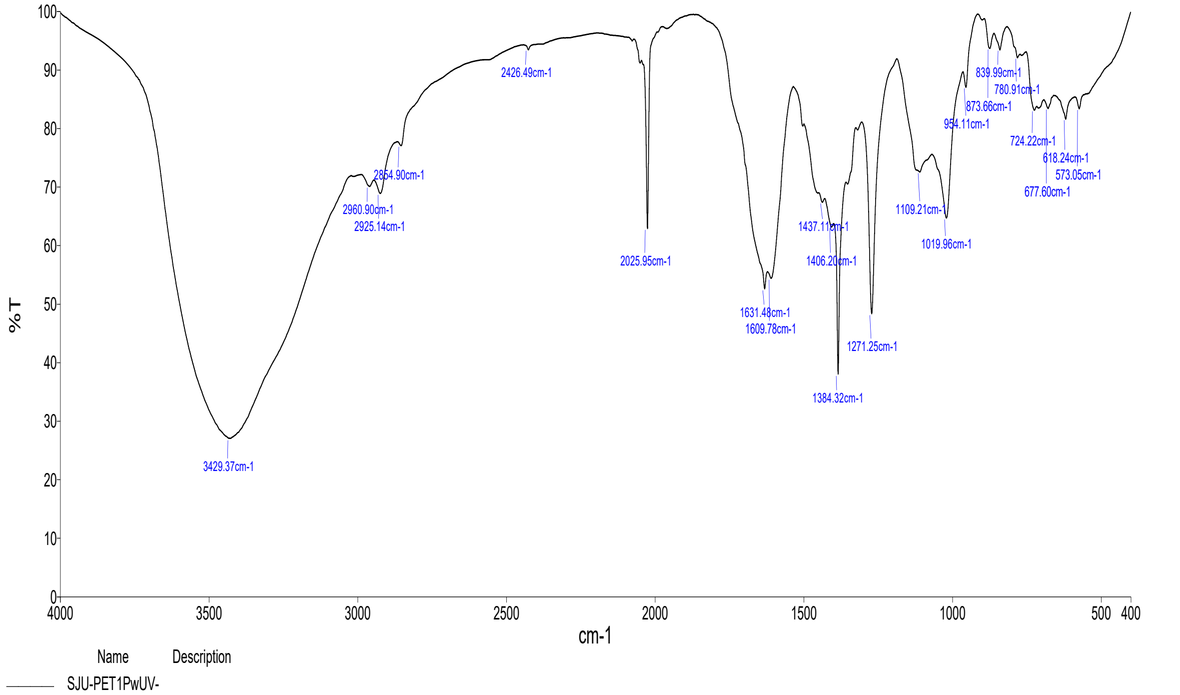

Figure 4. FTIR spectroscopy of *B.Subtillis* treated UV- PET MPS.

Table 1. FTIR Analysis of PET plastics.

Control PET MPs	Functional group	*Bacillus subtilis* + PET MPs	Functional group	*Bacillus subtilis* + UV - PET MPs	Functional group	*Bacillus subtilis* + *SUN-PETMPs*	Functional group
3800.77	O-H	3431.97	O-H	3430.40	O-H	**3854.46**	O-H
3739.00	O-H	3060.53	C-H	3063.11	C-H	3808.72	O-H
3852.15	O-H	2963.57	C-H	2962.17	C-H	3746.10	O-H
3435.81	O-H	2909.03	C-H	2918.34	C-H	3434.59	O-H
2925.71	C-H	2532.59	C=O	2582.72	C=O	2923.85	C-H
2856.98	C-H	2374.35	C=O	2388.96	C=O	2857. 84	C-H
2426.78	C=O	2283.40	C=O	2285.09	C=O	**2520.77**	C=O
2370.58	C=O	2109.15	C=O	2117.55	C=O	2424.02	C=O
2340.89	C=O	1955.97	C=O	1958.20	C=O	2310.68	C=O
2081.46	C=O	1724.21	C=O	1721.99	C=O	2101.60	C=O
2027.04	C=O	1579.07	C-C	1579.18	C-C	**1958.97**	C=O
1638.02	C=C	1505.63	C-C	1506.13	C-C	**1715.92**	C=O
1450.30	C-H	1408.19	C-H	1454.02	C-H	1642.12	C=C
1384.04	C-H	1383.89	C-H	1408.30	C-H	**1507.50**	CC
1270.97	(C=O)-O	1287.47	(C=O)-O	1383.82	C-H	1450.18	C-H
1092.34	C-C-O	1116.06	C=O	1342.12	C-H	1342.91	C-H
1035.79	C-H	1021.18	C-H	1263.06	(C=O)-O	1 246.16	C-C-O
871.11	C-H	875.36	C-H	1099.48	C-C-O	1097.66	C-C-O
832.81	C-H	727.93	C-H	1022.48	C-H	1021.26	C-H
				874.25	C-H	873.92	C-H
				797.77	**C-H**	791.37	C-H
				727.03	**C-H**	725.37	C-H

GC-MS had been used in the present study; this approach initially aimed to identify very volatile compound form MSM medium (UT-PETMP,SL-PETMP, UVPETMP), GCMS analysis for control PET microplastics, Octadecane (RT: 10.78),1-Dodecanol (12.44) , Spiro[cyclopentane-1,2'(1'h)-quinoxaline],(RT : 13.67), Diethyl Phthalate (RT: 15.02), Hexadecanoic acid, methyl ester (RT: 18.59), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (RT:20.22), Methyl stearate (RT:20.51), Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl (RT: 21.63), cis-13-Eicosenoic acid, methyl ester (RT :22.06), Eeicosanoicacid,methylester (RT: 22.28), cis-9-Hexadecenal (RT: 22.94), Oleoyl chloride (RT: 23.12), 9-Octadecenoic acid (Z)-, oxiranylmethyl ester (RT: 23.51), 13-Docosenoic acid, methyl ester (RT: 23.71), 1-Cyclohexyldimethylsilyloxybutane (RT: 24.84),9-Octadecenoic acid (Z)-, oxiranylmethyl ester (25.08), Cis-15-tetracosensaeure, methylester (RT: 25.24), 3-Acetoxy-12-ursanol (RT: 25.79), 13-Docosenamide (RT : 25.84), cis-13-Docosenoyl chloride (RT:26.17), Glycidyl (Z)-9-nonadecenoate (RT :26.54), Verrucarol (RT:27.51), Stigmast-5-en-3-ol, oleat (RT: 28.19), Methyl 9-(acetyloxy)-3,6b,10,10,12a,12b,14a-he (RT : 28.62),(Table-2).

Due to the action of bacterial in the UV pre-treated PET MPs plastic in 250 ml of flask of MSM medium, PET powders were degraded into various by products. GCMS analysis of these byproducts revealed some compounds. Dodecane (RT :6.715), DECANE, 2,5,9-TRIMETHYL (RT:6.89), Heptane, 3,3,5-Trimethyl(RT:7.02), Docosane (RT :7.29), Acetyl valeryl (RT :7.38), Benzene, 1,3-bis(1,1-dimethylethyl)-(RT :7.466), Decane, 3-ethyl-3-methyl (RT :7.60), Dodecane, 4,6-dimethyl-(RT:7.78),Pentadecane (RT :9.12), Tetradecane(RT :9.51), Decanedioic acid, didecyl ester (RT : 9.71), Undecane, 4,4-dimethyl(RT :10.32), Octadecane (RT :10.64), 2,4-Di-tert-butylphenol (RT : 11.02), 1-iodotetradecane (RT:11.21), 1R,2S,4R)-P-MENTH-8-ENE-2-OL (RT :11.57), 1,2-Benzenedicarboxylic acid, Diethyl ester (RT :11.98), Heptadecane (RT :12.01), 1-(4-isopropylphenyl)-2-methylpropyl aceta (RT :12.88), 2-penten-1-ol, 2-methyl-5-(2-methyl-3-methyl (RT :13.05), Sulfurous acid, hexyl octyl ester (RT:13.10),nonane,5-methyl-5-propyl(RT :13.15), Methanone, (1-hydroxycyclohexyl)phenyl (RT :13.31), decanoic acid, 8-methyl-, methyl ester (RT :13.45), 2-penten-1-ol, 5-(2,3-dimethyltricyclo (RT :13.53), Pentadecane (RT :13.94),tetracosane(RT:14.26),Pentadecanal (RT :14.48), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (RT :15.52), Hexadecanoic acid, methyl ester (RT :15.56),methyl ester of 3-(3,5-di-tert-butyl-4-hydr (RT :15.66), 1,2-Benzenedicarboxylic acid, dibutyl EST (RT :15.93), 1-(2- hydroxyethoxy)tridecane (RT :16.71), 1-

octadecene (RT :17.17), methyl stearate(RT :17.49), Oxalic acid, dineopentyl ester (RT :18.13), Nonane, 5-methyl-5-propyl (RT :18.61), Myristic acid glycidyl ester (RT :19.01), Methyl-2,2-dibutyl butyrate (RT :19.25), 1H,5H-cyclopropa[g][1,2,4]triazolo[1,2-a]cinno (RT :19.29), 1H-indole-3-ethanamine (RT :20.45), Pregnane, silane deriv (RT :20.60), Glycidyl palmitate(RT:20.68), Cyclohexaneacetic acid(RT:21.99), Nonadecanoic acid, benzyldimethylsilyl ester (RT :22.14), Cyclohexan, 1,2-bis(hydroxymethyl) (RT : 22.92). (Table-3: Plate-1). Due to the action of bacterial of the Sun light treated PETMPs in 250 ml of flask of MSM medium, Sun light treated PET powders was degraded into various by products. 3-Dodecen-1-Al, Diethyl Phthalate, 1-(4-Isopropylphenyl)-2-Methylpropylacetate, 1, 6-methanonaphthalen-1(2h)-ol, octahydro, Nonanamide, 2-Acetonaphthone,5,6,7,8-Tetrahydro-1,3,5, Hexadecanoic acid, methyl ester, Dibutyl phthalate,7-Tetradecenal,9-Octadecen-1-ol,1-Nonadecene, 9,12-Octadecadienoic acid (Z,Z),Meethyl ester, Methyl stearate, Hexadecanamide, 9-Octadecenal, 2-Isopropyl-10-methylphenanthrene, cis-1,2- Cyclododecanediol,cis-13-eicosenoic acid,methyl ester,9-Octadecenamide, E,E,Z-1,3,12-Nonadecatriene-5,14-diol, 9-Octadecenoic acid (Z)-, oxiranylmethyl ester, 13-Docosenoic acid (Z)-, oxiranylmethyl ester, 13- Docosenoic acid,methyl ester,Bis(2-ethylhexyl)phthalate, 1-cis-vaccenoylglycerol,1-Cyclohexyldimethylsilyloxybutane, 1,2,3- Trisilacyclohexane,15- Tetracosenoic acid,methyl ester, (Z)-9-octadecen-4-olide, 13- Docosenamide, cis-13-Docosenoyl chloride, ethyl 7-oh-me-octanoate,Glycidyl(Z)-9nonadecenoate, Campesterol, Dihydromyrcenol,trimethylsilyl esther, Stigmast-5-en-3- ol, (3 BETA). Stigmasta-3,5-diene.

Table 2. GC-MS noticed a compound from untreated PETMP and *Bacillus subtilis* inoculated media.

S.NO	R. TIME	AREA	AREA%	NAME
1	10.783	37354	0.50	OCTADECANE
2	12.440	23167	0.31	1-DODECANOL
3	13.676	28775	0.39	SPIRO[CYCLOPENTANE-1,2'(1'H)-QUINOXALINE],3'-(
4	15.024	162598	2.19	DiethylPhthalate
5	18.595	194237	2.61	Hexadecanoicacid,methyl ester
6	20.227	207407	2.79	9,12-Octadecadienoicacid(Z,Z)-,methyl ester
7	20.285	408855	5.50	9-OCTADECENOICACID(Z)-, METHYLESTER
8	20.518	128048	1.72	Methylstearate
9	21.639	45506	0.61	Hexadecanoicacid,1-[[[(2-aminoethoxy)hydroxyphosphinyl
10	22.066	356312	4.79	cis-13-Eicosenoicacid,methylester
11	22.282	57755	0.78	EICOSANOICACID,METHYLESTER
12	22.942	88955	1.20	cis-9-Hexadecenal
13	23.124	99072	1.33	Oleoylchloride
14	23.514	197444	2.65	9-Octadecenoicacid(Z)-,oxiranylmethylester
15	23.716	2137380	28.74	13-Docosenoicacid,methylester, (Z)-
16	23.905	77728	1.05	DOCOSANOICACID,METHYLESTER
17	24.847	394585	5.31	1-Cyclohexyldimethylsilyloxybutane
18	25.086	107312	1.44	9-Octadecenoicacid(Z)-,oxiranylmethylester
19	25.245	108742	1.46	CIS-15 TETRACOSENSAEURE,METHYLESTER
20	25.709	107802	1.45	3-Acetoxy-12-ursanol
21	25.854	203144	2.73	13-Docosenamide,(Z)-
22	26.179	848690	11.41	cis-13-Docosenoylchloride
23	26.548	923833	12.42	Glycidyl(Z)-9-nonadecenoate
24	27.513	61601	0.83	Verrucarol
25	28.191	128671	1.73	STIGMAST-5-EN-3-OL,OLEAT
26	28.620	301929	4.06	METHYL9-(ACETYLOXY)-3,6B,10,10,12A,12B,14A-HE
		7436902	100.00	

Table 3. GC-MS noticed a compound from PET plastic (SL-PETMP) inoculated media.

Peak	R. Time	Area	Area%	Name
1	6.715	382757	7.52	DODECANE
2	6.895	24041	0.47	DECANE, 2,5,9-TRIMETHYL-
3	7.023	25965	0.51	HEPTANE, 3,3,5-TRIMETHYL-
4	7.297	22498	0.44	DOCOSANE
5	7.381	14989	0.29	Acetyl valeryl
6	7.466	151879	2.98	Benzene, 1,3-bis(1,1-dimethylethyl)-
7	7.603	53045	1.04	Decane, 3-ethyl-3-methyl-
8	7.787	128760	2.53	DODECANE, 4,6-DIMETHYL-
9	8.441	29777	0.58	HEPTANE, 3,3-DIMETHYL-
10	9.120	27233	0.53	PENTADECANE
11	9.517	398119	7.82	TETRADECANE
12	9.712	70019	1.38	DECANEDIOIC ACID, DIDECYL ESTER
13	10.326	21858	0.43	UNDECANE, 4,4-DIMETHYL-
14	10.590	40076	0.79	OCTADECANE
15	10.646	124697	2.45	OCTADECANE
16	11.021	1343413	26.39	2,4-Di-tert-butylphenol
17	11.212	27788	0.55	1-IODOTETRADECANE
18	11.575	77116	1.51	(1R,2S,4R)-P-MENTH-8-ENE-2-OL
19	11.985	130870	2.57	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER
20	12.019	158530	3.11	Heptadecane
21	12.881	50418	0.99	1-(4-ISOPROPYLPHENYL)-2-METHYLPROPYL ACETAT
22	13.050	47126	0.93	2-PENTEN-1-OL, 2-METHYL-5-(2-METHYL-3-METHYL
23	13.101	24672	0.48	Sulfurous acid, hexyl octyl ester
24	13.156	59962	1.18	NONANE, 5-METHYL-5-PROPYL-
25	13.310	47647	0.94	Methanone, (1-hydroxycyclohexyl)phenyl-
26	13.455	31859	0.63	DECANOIC ACID, 8-METHYL-, METHYL ESTER
27	13.530	51300	1.01	2-PENTEN-1-OL, 5-(2,3-DIMETHYLTRICYCLO[2.2.1.0(2
28	13.948	40226	0.79	PENTADECANE
29	14.260	100269	1.97	TETRACOSANE
30	14.482	11660	0.23	PENTADECANAL
31	14.631	12092	0.24	DOCOSANE
32	15.390	10666	0.21	NONANE, 5-METHYL-5-PROPYL-
33	15.473	66586	1.31	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
34	15.523	75779	1.49	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
35	15.568	312829	6.14	Hexadecanoic acid, methyl ester
36	15.664	20615	0.40	METHYL ESTER OF 3-(3,5-DI-TERT-BUTYL-4-HYDROX
37	15.827	51495	1.01	(2R)-N-[3'-(METHOXYCARBONYL)PROPIONYL]BORN
38	15.935	115702	2.27	1,2-BENZENEDICARBOXYLIC ACID, DIBUTYL ESTER
39	16.287	41557	0.82	DOCOSANE
40	16.714	12074	0.24	1-(2-HYDROXYETHOXY)TRIDECANE
41	17.172	34202	0.67	1-OCTADECENE
42	17.416	13159	0.26	Sulfurous acid, 2-ethylhexyl hexyl ester
43	17.490	182690	3.59	Methyl stearate
44	18.130	6620	0.13	Oxalic acid, dineopentyl ester
45	18.611	18430	0.36	Nonane, 5-methyl-5-propyl-
46	19.011	53894	1.06	Myristic acid glycidyl ester
47	19.253	5209	0.10	METHYL-2,2-DIBUTYL BUTYRATE
48	19.298	5412	0.11	1H,5H-CYCLOPROPA[G][1,2,4]TRIAZOLO[1,2-A]CINNO

49	20.452	52520	1.03	1H-INDOLE-3-ETHANAMINE
50	20.605	95267	1.87	PREGNANE, SILANE DERIV.
51	20.683	25033	0.49	Glycidyl palmitate
52	20.946	8521	0.17	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYLESTER

Table 4. GC-MS noticed a compound from the PET plastic Inoculated (UV-PETMPs) media.

Peak	R. Time	Area	Area%	Name
1	6.715	382757	7.52	DODECANE
2	6.895	24041	0.47	DECANE, 2,5,9-TRIMETHYL-
3	7.023	25965	0.51	HEPTANE, 3,3,5-TRIMETHYL-
4	7.297	22498	0.44	DOCOSANE
5	7.381	14989	0.29	Acetyl valeryl
6	7.466	151879	2.98	Benzene, 1,3-bis(1,1-dimethylethyl)-
7	7.603	53045	1.04	Decane, 3-ethyl-3-methyl-
8	7.787	128760	2.53	DODECANE, 4,6-DIMETHYL-
9	8.441	29777	0.58	HEPTANE, 3,3-DIMETHYL-
10	9.120	27233	0.53	PENTADECANE
11	9.517	398119	7.82	TETRADECANE
12	9.712	70019	1.38	DECANEDIOIC ACID, DIDECYL ESTER
13	10.326	21858	0.43	UNDECANE, 4,4-DIMETHYL-
14	10.590	40076	0.79	OCTADECANE
15	10.646	124697	2.45	OCTADECANE
16	11.021	1343413	26.39	2,4-Di-tert-butylphenol
17	11.212	27788	0.55	1-IODOTETRADECANE
18	11.575	77116	1.51	(1R,2S,4R)-P-MENTH-8-ENE-2-OL
19	11.985	130870	2.57	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTER
20	12.019	158530	3.11	Heptadecane
21	12.881	50418	0.99	1-(4-ISOPROPYLPHENYL)-2-METHYLPROPYL ACETAT
22	13.050	47126	0.93	2-PENTEN-1-OL, 2-METHYL-5-(2-METHYL-3-METHYL
23	13.101	24672	0.48	Sulfurous acid, hexyl octyl ester
24	13.156	59962	1.18	NONANE, 5-METHYL-5-PROPYL-
25	13.310	47647	0.94	Methanone, (1-hydroxycyclohexyl)phenyl-
26	13.455	31859	0.63	DECANOIC ACID, 8-METHYL-, METHYL ESTER
27	13.530	51300	1.01	2-PENTEN-1-OL, 5-(2,3-DIMETHYLTRICYCLO[2.2.1.0(2
28	13.948	40226	0.79	PENTADECANE
29	14.260	100269	1.97	TETRACOSANE
30	14.482	11660	0.23	PENTADECANAL
31	14.631	12092	0.24	DOCOSANE
32	15.390	10666	0.21	NONANE, 5-METHYL-5-PROPYL-
33	15.473	66586	1.31	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
34	15.523	75779	1.49	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
35	15.568	312829	6.14	Hexadecanoic acid, methyl ester
36	15.664	20615	0.40	METHYL ESTER OF 3-(3,5-DI-TERT-BUTYL-4-HYDROX
37	15.827	51495	1.01	(2R)-N-[3'-(METHOXYCARBONYL)PROPIONYL]BORN
38	15.935	115702	2.27	1,2-BENZENEDICARBOXYLIC ACID, DIBUTYL ESTER
39	16.287	41557	0.82	DOCOSANE
40	16.714	12074	0.24	1-(2-HYDROXYETHOXY)TRIDECANE
41	17.172	34202	0.67	1-OCTADECENE

42	17.416	13159	0.26	Sulfurous acid, 2-ethylhexyl hexyl ester
43	17.490	182690	3.59	Methyl stearate
44	18.130	6620	0.13	Oxalic acid, dineopentyl ester
45	18.611	18430	0.36	Nonane, 5-methyl-5-propyl-
46	19.011	53894	1.06	Myristic acid glycidyl ester
47	19.253	5209	0.10	METHYL-2,2-DIBUTYL BUTYRATE
48	19.298	5412	0.11	1H,5H-CYCLOPROPA[G][1,2,4]TRIAZOLO[1,2-A]CINNO
49	20.452	52520	1.03	1H-INDOLE-3-ETHANAMINE
50	20.605	95267	1.87	PREGNANE, SILANE DERIV.
51	20.683	25033	0.49	Glycidyl palmitate
52	20.946	8521	0.17	1,2-BENZENEDICARBOXYLIC ACID, DIOCTYL ESTER

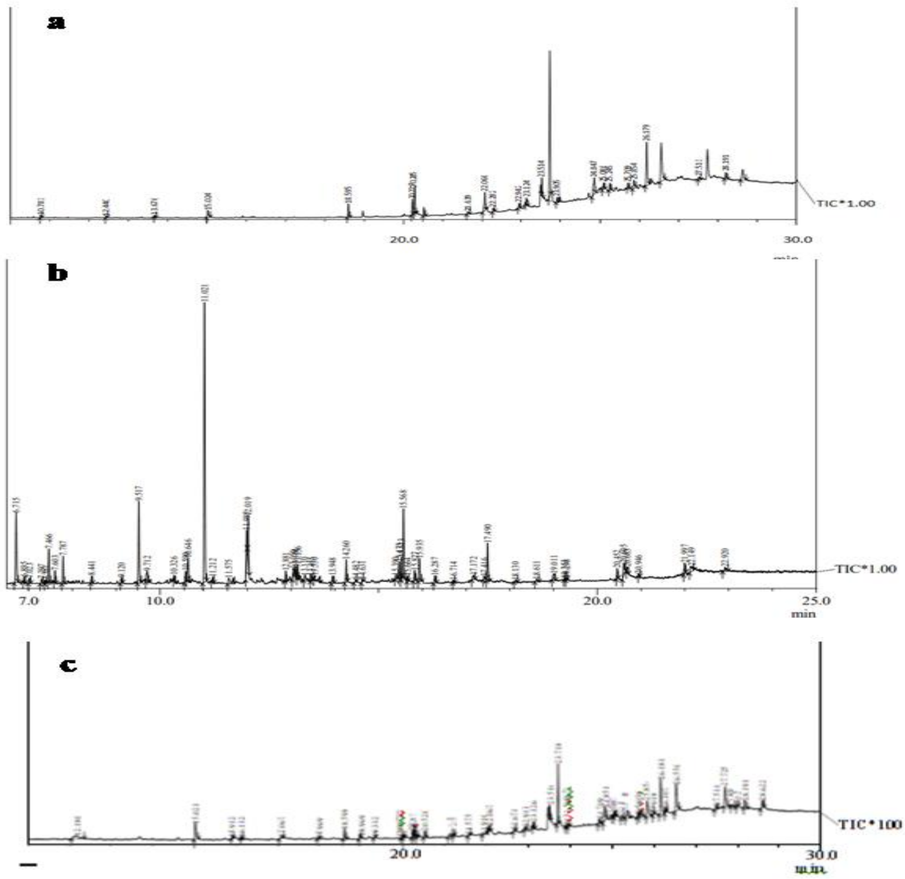

Plate 1. Chromatogram of PETMPs degradation products due to a) Untreated PETMP + *B. subtilis* b)UV-PETMPs + *B.subtils* c) SLPETMP+ *B. subtilis.*

The biodegradation of PETMP with *Bacillus subtilis* was confirmed after 6 months of incubation through FTIR. The new peaks formation was observed such as Alkyl aryl ether group, alkenes group and carboxylic acid in the bacterial treated PETMP. While studying the degradation of PET powder in soil Umamaheswari and Murali, reported that by FTIR, it was confirmed that PET powders were converted to alkene and methylene groups **(**Umamaheswari and Murali, (2023)**).** This result parallel with previous research, according to Gupta and Devi., (2020) an increase in the keto carbonyl bond index, ester carbonyl bond index, and vinyl bon index of FTIR spectra demonstrated polyethylene biodegradation (Gupta and Devi., 2020). In present study, in the chromatogram oxidized hydrocarbons were present, such as Dodecane, Docosane, Decane, Dodecane, 4,6-dimethyl, Pentadecane, Tetradecane, Decanedioic acid, didecyl ester, Undecane, 4,4-dimethyl, Octadecane, 1-iodotetradecane, Heptadecane, tetracosane and Pentadecanal. These findings were well supported by the work of Kyaw *et al*., (2012), who have reported that 18 different biodegraded products were identified from the polythene such as benzene, methyl, tetrachloroethylene, benzene,1,3-dimethyl, octadecane,7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, hexadecanoic acid, ethyl ester, eicosane, octadenoic acid,docosane,3-chloropropionic acid, heptadecyl ester, tricosane, octadecanoic aid, butyl ester,1-nonadecene,tetracosane,pentacosane,1,2-benxendicarboxylic acid, di-iso-ostyl ester, and hexacosane. Shahnawz *et al*., also reported the major by products in the PEDP in the culture supernatant of *L.fusiformis* strin VASB14/WL (1,2,3,4 tetra methyl benzene) and *B.cereus*strain VASB1/TS (1,2,3 trimethyl benzene,1ethyl 3,5-dimethyl benzene, 1,4 di methyl 2 ethyl benzene, and dibutyl phthalate) (Shahnawaz *et al*., 2016).Pramila and Ramesh used *A.baumannii* for degradation of polyethylene (LDPE) and with GCMS analysis recorded 2-butene,2-methyl,acetone,and ethane (Pramila *et al*.,2011). Mahalakshmi *et al*., who have analysed PEDP in culture supernatant extracted with distilled ether produced due to the action of Bacillus and *Pseudomonas* using GC-MS and reported octadecadienoic acid, octadecatrienoic acid, benzene dicarboxylic acid, and cyclopropanebutanoic acids as the main by products (Mahalakshmi *et al*.,2012).

According to the GC-MS study, plastic is broken down into a number of new molecules that lead to a pathway that produces enough energy to support bacterial growth **(**Shakir Ali *et al*., 2023**)**. *Pseudomonas sp.* SH5B produces, Fluoren-9-ol, 3,6-dimethoxy-9-(2, Tris(tertbutyldimethylsilyloxy), 2,5-Dihydroxybenzoic acid, 3TMS, 7HDibenzo(a,g)carbazole, 3-Hydroxymandelic acid, 3TMS der, 2,6- Dihydroxybenzoic acid (Fig. 4), 3TMS, 1,3,5-Benzetriol, 3TMS derivative, 1,3,5,7,9-Pentaethylcyclopentasi, Tetrasiloxane, 1,7- dially octadecyl, Benzo[h]quinoline, 2,4-dimethyl-, and 2,4-Dihydroxybenzaldehyde, 2TMS. At the same time arsenous acid, Tris(trimethylsilyl, Tris(tert-butyldimethylsilyloxy), 2,5- Dihydroxybenzaldehyde, 2TMS, Propenenitrile, 2-(2-benzothiazole, 5-(p-Aminophenyl)-4-phenyl-2-thi, 2,6-Dihydroxybenzoic acid, 3TMS, Salicylic acid, 2TMS derivative, Cyclotetrasiloxane, octamethyl, 1,1,3,3,5,5,7,7-Octamethyl-7-(2, 2,60 -Dimethoxy-20 -(trimethylsilyl, (1H)Indolo[2,1-a]isoquinoline, and 5,1,3,5,7,9-Pentaethylcyclopentasi during plastic degradation (Shakir Ali *et al*., 2023). In our present study, 3- Dodecen, 1-Nonadecene, 7- tetradecenal,9-cotadecen was identified by GC-MS methods in supernatant of Sun light treated PET MPs and *Bacillus subtilis* incubated MSM medium after 6-month incubation. Similar observation were also reported in the pyrolysis of mixed plastic by GC-MS method (Odigbo *et al*.,2023). Similarly, Nonanamide, Hexadecan amide (Bongekile Vilakati *et al*.,2021), Dibutyl phthalate was discovered in MSM-containing sun-treated PETMPs, which was compatible with the findings of Shahnawaz *et al*. (2016). Dibutyl phthalate was identified by GC-MS from *Bacillus cereus* biodegradation polythene culture supernatant. Benzene, 1,3-bis(1,1-dimethylethyl) was detected by GC-MS in the supernatant of UV light treated PETMPs incubated with *Bacillus subtilis* in MSM media in our research. There is a strong association with Udeme Dickson *et al*., who used benzene, 1,3-bis(1,1-dimethylethyl), and dodecane as biomarkers to identify total petroleum hydrocarbons (TPHs) in soil (Udeme Dickson *et al*.,2020).The research states, fatty acid and alkanes were discovered to be the breakdown products by GC-MS analysis after thirty days of incubation in Moraxella catarrhalis strain BMPPS3 polyurethane (PU) containing synthetic medium (Maheswaran,*et al*., 2024). Additionally, the biodegradation of PET by *Gordonia sp.,* CN2K is noted for the metabolites founded in spend medium compounds, bis(2-hydroxyethyl) terephthalate (BHET), mono(2-hydroxyethyl) terephthalate and terephthalate (MHET) (Chandramouli Swamy *et al*.,2024). Sustainable solutions that make responsible use of waste materials are needed to stop environmental degradation and protect our ecosystem. Only a few numbers of research have been published so far on the biodegradation of pre-treated PET microplastics by bacteria. The conclusion drawn from this study is that PET is used as a carbon source by the Bacillus subtilis strain.

CONCLUSION

The current investigation demonstrated the degradation of sun light pre-treated PETMPs, UV-exposed PETMPs, and non-pretreated MPs by *Bacillus subtilis*. The biodegraded products were identified in the supernatant of microplastics (sun light pre-treated PETMPs, UV-exposed PETMPs) and bacteria inoculated mediums by GCMS. The methods developed may help in future to degrade PET microplastics. We need to analysis by-products toxicity and asses complete degradation methods of by-products of PET.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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