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FTIR analysis of bacterial mediated chemical changes in Polystyrene foam

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ABSTRACT

The accumulation of polystyrene waste is of environmental concern. Although land filling, incineration are in practice, it leads to the evolution of secondary pollutants. Biodegradation could be a sustainable approach in managing the disposal of these wastes. Microorganisms through their enzymatic activity could degrade polystyrene. With this view, the present study was designed to evaluate the potential of bacteria harbouring in polystyrene waste dumped soil to degrade polystyrene through FTIR technique. Dominant bacteria identified were Klebsiella sp., Micrococcus sp. and Pseudomonas sp. These bacteria were inoculated in MSM (Minimal salt medium) containing Polystyrene for a period of one month. FTIR analysis revealed chemical changes depicted by the evolution of new absorption peaks representing C = O, C - H, C- O on exposure of Polystyrene to Micrococcus sp., and O-H, C-C, C-H to Pseudomonas sp., Yet, further studies have to be conducted to trace the degraded products.

Key words: Polystyrene, Biodegradation, Bacteria, FTIR

INTRODUCTION

Styrene molecules polymerise to form long chain hydrocarbon molecule known as polystyrene (PS) which is a synthetic, brittle, high glass transition temperature polymer, thermoplastic, non- biodegradable plastic and has preeminent feature like high impact resistance, food expectance, flame retardant, fast moulding capacity [1-4]. Today styrenic polymers, principally general purpose and impact grades of polystyrene (PS -GP and PS- HI, respectively), comprise a sixth of the total thermoplastics market. Polystyrene and expanded polystyrene (EPS) are commodities used in packaging, insulation materials in the form of a foam or bead and construction sectors [4,5]. One of the option for reducing polystyrene waste load in the environment is microbial degradation since microorganisms are capable of utilising organic and inorganic molecules [5]. Since microorganisms are capable of degrading most of the organic and inorganic materials including lignin, starch, cellulose, hemicellulose there is a lot of interest in microbial degradation of synthetic polymer waste material [6,7]. In our previous study, we have observed that fungal species (*Aspergillus sp., Penicillium sp.*, and *Fusarium sp.*) were able to degrade PET (Polyethylene terephthalate and PS foam [8]. FTIR is useful to elucidate chemical and physical structure, hydrogen bonding, and end group detection, degradation reactions, cross linking behaviour of molecules and copolymer composition in liquid and solid form of chemicals and polymers. FTIR technique is employed in the degradation studies of polymers to assess the chemical changes due to microbial activity [8,9]. The objective of this study was to evaluate the potential of bacteria in polystyrene waste dumped soil to degrade polystyrene through FTIR technique.

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 Determinative Bacteriology [10].

Preparation of PS samples:

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

Degradation of PS by bacteria in MSM

Three dominant bacterial isolates (*Klebsiella sp., Micrococcus sp.,Pseudomonas sp*.,) were used for PS degradation studies. 10 µl of the broth culture of each bacteria were inoculated each 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.

RESULTS AND DISCUSSION

The FTIR spectra of control polystyrene is illustrated in fig 1.The signature peaks of PS include 3354 cm^{-1}, 2979 cm^{-1},2917 cm^{-1}, 2148 cm^{-1},1487 cm^{-1}, 1324 cm^{-1}, 1042 cm^{-1}, 877 cm^{-1}, 748 cm^{-1} (C-H bond stretching), 1645 cm^{-1} (C = C bond stretching), 1445 cm^{-1} (CH_2 + C=C bond stretching), 1152 cm^{-1} (C- O bond stretching), 1083 cm^{-1} (C-O-C bond stretching) (table1, fig 1). Naima Atiq *et al*., [9] have demonstrated the growth of *Microbacterium sp*. NA23, *Paenibacillus urinalis* NA26, *Bacillus sp*. NB6, *Pseudomonas aeruginosa* NB26 on polystyrene film. They have reported that there was no increase of area of absorption peaks in the bacterial treated and control films and have reasoned out that it may be due to no significant surface changes during 4 weeks of incubation with bacterial isolates. Shift in absorption peaks were evinced on inoculation of PS with *Klebsiella sp*., from 3354 cm^{-1} to 3350 cm^{-1} , 2917 to 2920 cm^{-1},2148 to 2144 cm^{-1}, 1645 to 1640 cm^{-1}, 1487 to 1490 cm^{-1} 1445 to 1447 cm^{-1}, 1083 to 1081 cm^{-1}, 1042 to 1040 cm^{-1}, 877 to 876 cm^{-1}, 748 to 749 cm^{-1} (table 2, 5, fig2). On the other hand, some of the peaks disappeared in the FTIR spectra of PS on exposure to *Klebsiella sp.*,(2917 cm^{-1},1324 cm^{-1} (C-H bond stretching) and 1152 cm^{-1} (C-O bond stretching) (Fig2). Further, increase in the intensity of peak was observed at 1640 cm^{-1}. On the other hand, decrease in the intensity of peak was evinced at 1081 cm^{-1}, 1040 cm^{-1}, 876 cm^{-1} and 749 cm^{-1} when compared to the control. Emergence of new peaks in the FTIR peak (1727 cm^{-1},(C = O), 1381 cm^{-1},(C-H),1280 cm^{-1},(C-O), 1183 cm^{-1},(C-O) was evident on exposure of PS to *Micrococcus sp* (fig 3). In addition, shift in absorption peaks were observed (from 3354 cm^{-1} to 3338 cm^{-1} , 2979 cm^{-1} to 2980 cm^{-1} , 2917 cm^{-1} to 2921 cm^{-1} , 2148 cm^{-1} to 2136 cm^{-1}, 1645 cm^{-1} to 1643 cm^{-1} , 1487 cm^{-1} to 1491 cm^{-1} , 1445 cm^{-1} to 1448 cm^{-1}, 1083 cm^{-1} to 1081 cm^{-1} , 1042 cm^{-1} to 1043 cm^{-1}) (table 3, fig 3). Decrease in the intensity of the peak was noticed at 1081 cm^{-1}, 1043 cm^{-1}, 877 cm^{-1}, 748 cm^{-1} when compared to the control. Disappearance of Absorption peaks were noticed in PS treated with *Micrococcus* 1324 cm^{-1},(C-H bond stretching) and 1152 cm^{-1} (C-O). From fig 4 it is observed that new absorption bands evolved at 3280 cm^{-1} (O-H), 1542 cm^{-1} (C-C) and 1395 cm^{-1} (C-H) in PS exposed to *Pseudomonas sp.* in MSM. On the other hand, certain peaks disappeared (3354 cm^{-1} (C-H), 1487 cm^{-1} (C - H), 1324 cm^{-1}(C-H bond stretching) and 1152 cm^{-1}(C- O). Shift in absorption peaks were observed (from 2979 cm^{-1} to 2977 cm^{-1} , 2917 cm^{-1} to 2922 cm^{-1} , 2148 cm^{-1} to 2133 cm^{-1} , 1645 cm^{-1} to 1636 cm^{-1} , 1445 cm^{-1} to 1447 cm^{-1}, 1083 cm^{-1} to 1081 cm^{-1},748 cm^{-1} to 749 cm^{-1}). Increase in the intensity of the peak was observed at 1636 cm^{-1} ,whereas, and decline in the intensity of the peaks were noticed at 1081 cm^{-1}, 1042 cm^{-1}, 877 cm^{-1} and 749 cm^{-1} when compared to the control (table 3, fig 3).

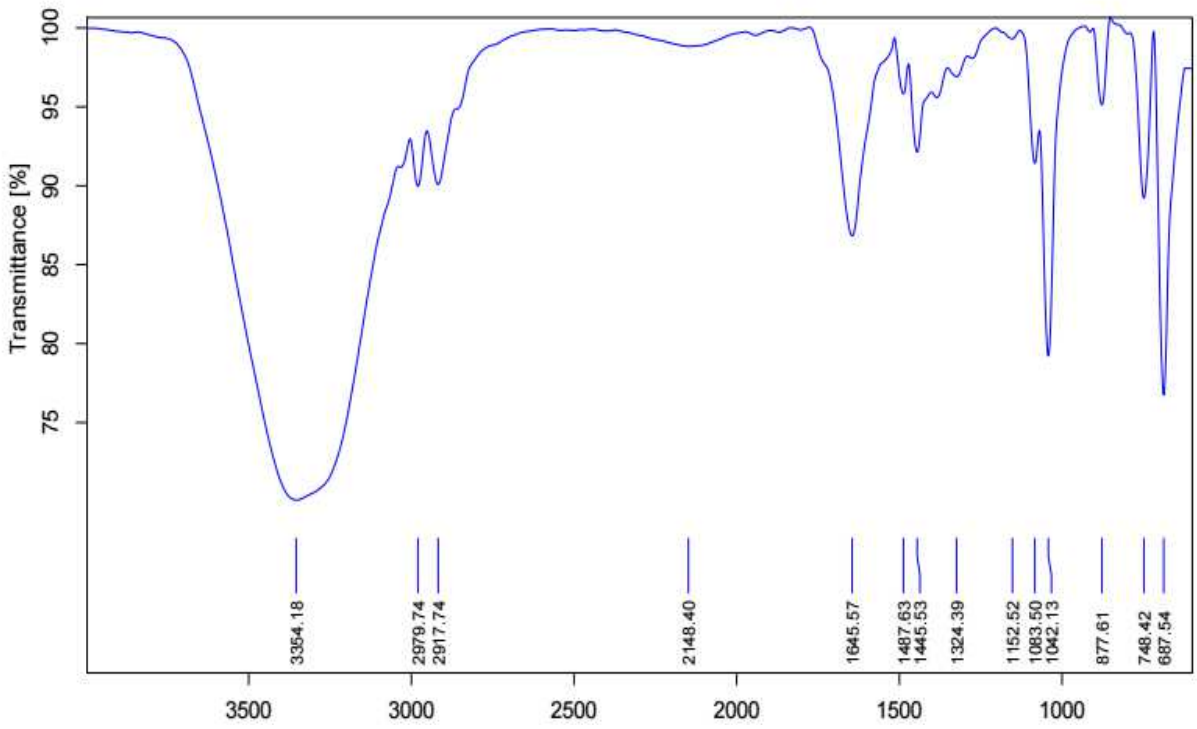

Fig 1. FTIR Spectrum of polystyrene foam control

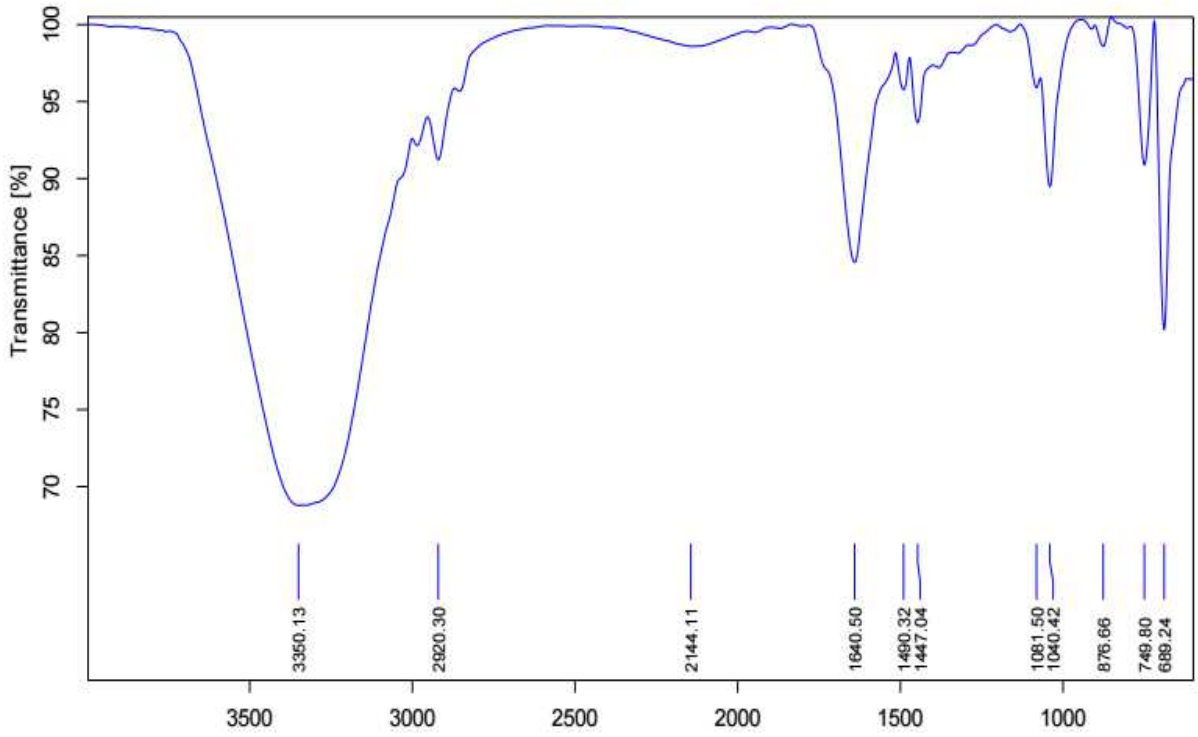

Fig 2. FTIR Spectrum of polystyrene foam inoculated with *Klebsiella sp.*, in MSM

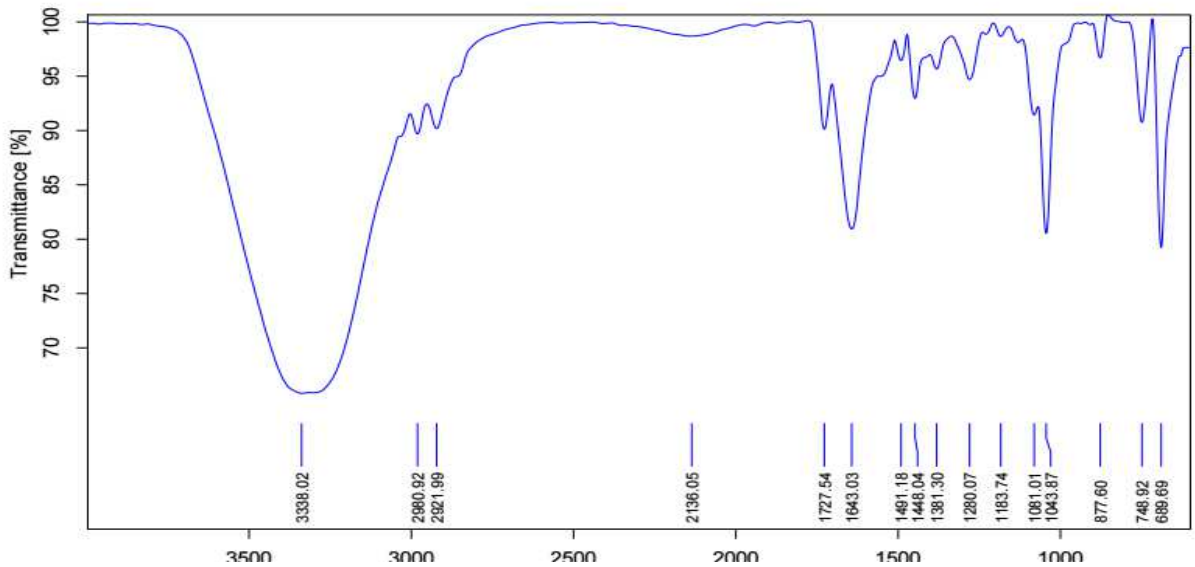

Fig 3 . FTIR Spectrum of polystyrene foam inoculated with *Micrococcus sp.*, in MSM

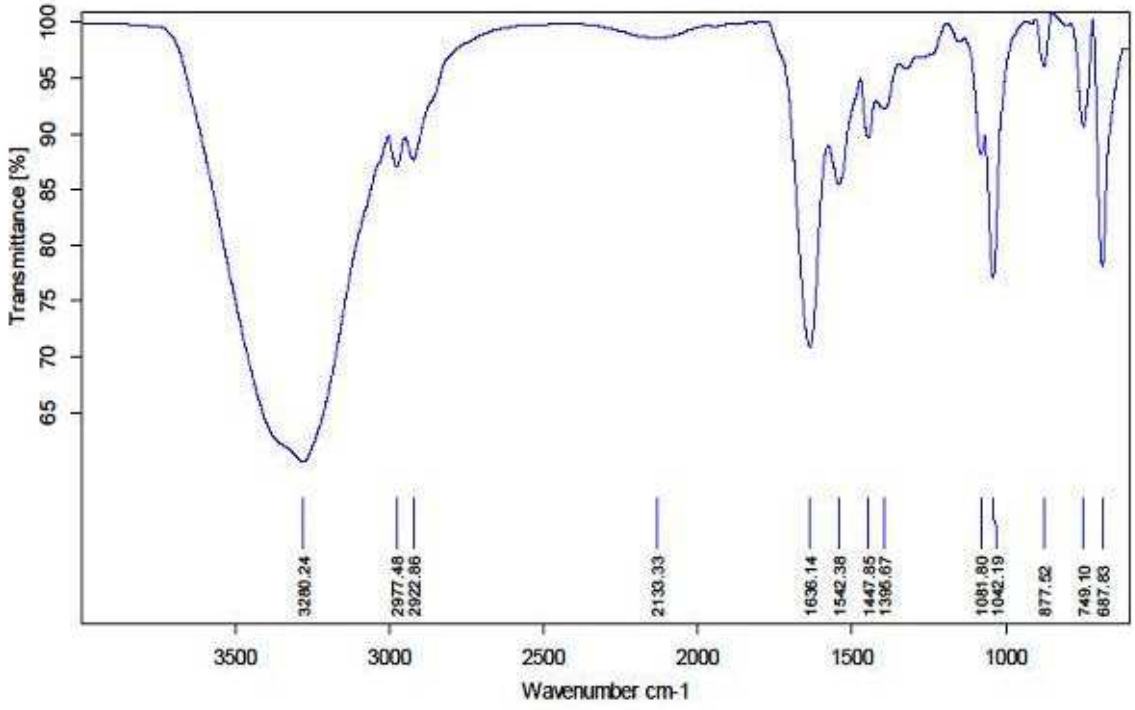

Fig 4. FTIR Spectrum of polystyrene foam inoculated with *Pseudomonas sp.*, in MSM

Table 1. Band assignment of polystyrene foam control

Wave number (cm^{-1})	Functional group	Relative intensity	References
3354	C-H	vs	[11]
2979	C-H	m	[12-14]
2917	C-H	m	[15-17]
2148	C-H	w	[12,18,19,20]
1645	C=C	vs	[8]
1487	C - H	m	[20,21]
1445	CH_2+ C=C	m	[16,17]
1324	C-H	w	[13,21,22]
1152	C-O	w	[11]
1083	C-O-C	m	[16,12]
1042	C-H	vs	[8,18,21]
877	C-H	m	[11,12.13,21]
748	C-H	vs	[13,24]

vs- very strong; w – weak; m-medium

Tabe 2 Band assignment of polystyrene foam inoculated with *Klebsiella sp.*, in MSM

Wave number (cm^{-1})	Functional group	Relative intensity	References
3350	C-H	vs	[11]
2920	C-H	w	[15-17]
2144	C-H	vw	[12,18,19,20]
1640	C=C	vs	[8]
1490	C - H	m	[20,21]
1447	CH_2+ C=C	m	[16,17]
1081	C-O-C	m	[16,12]
1040	C-H	s	[8,18,21]
876	C-H	m	[11,12.13,21]
749	C-H	s	[13,24]

vs- very strong; w – weak; vw – very weak; S- strong

Table 3 Band assignment of polystyrene foam inoculated with *Micrococcus sp.*,in MSM

Wave number (cm^{-1})	Functional group	Relative intensity	References
3338	C-H	vs	[11]
2980	C-H	w	[12-14]
2921	C-H	w	[15-17]
2136	C-H	w	[12,18,19,20]
1727	C=O	w	[4,8,12,13,17, 23, 25, 26,27,28,29,30,]
1643	C=C	vs	[8]
1491	C-H	v	[20,21]
1448	CH_2+ C=C	m	[16,17]
1381	C-H	w	[8,13,21,22]
1280	C-O	vs	[8]
1183	C-O	w	[14, 20]
1081	C-O-C	w	[16,12]
1043	C-H	vs	[8,18,21]
877	C-H	m	[11,12.13,21]
748	C-H	m	[13, 24]

vs- very strong; w – weak; vw-very weak; m-medium

Table 4: Band assignment of polystyrene foam inoculated with *Pseudomonas sp.*,in MSM

Wave number (cm^{-1})	Functional group	Relative intensity	References
3280	C-H	vs	[11]
2977	C-H	w	[12-14]
2922	C-H	w	[15-17]
2133	C-H	w	[12,18,19,20]
1636	C=C	vs	[8]
1542	C-C	m	[14]
1447	CH_2+ C=C	w	[16,17]
1395	C-H	w	[8,13,21,22]
1081	C-O-C	w	[16,12]
1042	C-H	vs	[8,18,21]
877	C-H	m	[11,12.13,21]
749	C-H	m	[13,24]

vs- very strong; w – weak; m-medium

Table 5 Comparative band assignment of polystyrene foam in MSM inoculated with bacteria

Control polystyrene	PS +*Klebsiella sp.,*	PS +*Micrococcus sp.,*	PS + *Pseudomonas sp.,*
3354(C-H)	3350(C-H)	3338(C-H)	3280(C-H)
2979(C-H)	2920(C-H)	2980(C-H)	2977(C-H)
2917(C-H)	2144(C-H)	2921(C-H)	2922(C-H)
2148(C-H)	1640(C=C)	2136 (C-H)	2133(C-H)
1645(C=C)	1490(C-H)	1727(C=O)	1636(C= C)
1487(C- H)	1447(CH_2+C=C)	1643(C=C)	1542(C-C)
1445(CH_2+C=C)	1081(C- O- C)	1491(C-H)	1447(CH_2+C=C)
1324 (C- H)	1040(C-H)	1448(CH_2+C=C)	1395(C-H)
1152(C- O)	876(C-H)	1381(C-H)	1081(C- O- C)
1083(C- O- C)	749(C- O- C)	1280(C-O)	1042(C-H)
1042(C-H)		1183(C-O)	877(C-H)
877(C-H)		1081(C- O- C)	749(C-H)
748(C-H)		1043(C-H)	
		877(C-H)	
		748(C-H)	

PS- Polystyrene;Numbers in shaded boxes indicate new peaks

As evinced in this study, Jack Takahashi and Andrew Tsai [32] also have demonstrated that *Pseudomonas putida* S12 had the ability to utilise styrene as a sole carbon and energy source in its liquid form and have suggested that *P. putida* could be used for the bioremediation of styrene.The present result is in line with Kamble Asmita *et al* [5] who have demonstrated that PET and PS can be degraded by microorganisms Like *Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes* and *Aspergillus niger* harbouring different types of soil. Under areobic conditions, styrene is generally metabolised via oxidation of vinyl side chain [33,34]. However oxidation of the aromatic ring was reported [35].

Tero – Petri Ruoko [4] have reported that polystyrene on irradiation with UV-light resulted in changes in the IR spectra of PS which were limited to the formation of the hydrogen peroxide and hydroxyl groups (3600 -3200 cm^{-1}), carbonyl groups (1800- 1650 cm^{-1}) and the area associated with double bonds (1300- 1800 cm^{-1}). Achhammer *et al*., [30] have reasoned out that the formation of hydroxyl and carbonyl groups in polystyrene on exposure to UV radiant energy may be due to chain scission or a competitive cross- linking or by reaction at chain ends or by oxidation of the benzene ring. They have also stated that carbonyl groups are most likely in the form of ketones; however, by means of the infra red spectra it is not possible to differentiate between the carbonyls of the ketone , aldehyde, and acid, all of which might be present to some degree.

Jaleh *et al*., [29] have reported that FTIR spectra of UV irradiated PS elicited changes in the carbonyl and hydroxyl regions, which indicated photooxidation. They have attributed it to the formation of aromatic and aliphatic ketones of the acetophenone type, and OH/ OOH groups in the main chain. As evinced in this study, they have also registered two new absorption peaks at 1721 cm-1 and 1290 – 1320 cm-1, which are characteristic absorptions of carbonyl (C=O) and hydroxyl groups, respectively [27]

CONCLUSION

FTIR analysis of PS inoculated with bacteria isolated from PS waste dumped soil in MSM indicates that these bacteria were able to induce chemical changes in PS , which could be attributed to the potential of these bacteria to utilise PS as a sole souce of carbon and their ability to degrade PS.

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