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# Potential of Soil Microbes in Degrading Polystyrene Foam

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**Abstract**

Persistence of polystyrene foam in the environment is known to cause detrimental effects on the biota. The present study reports the role of bacteria and fungi as potential candidates for biodegradation of polystyrene foam. Pretreatment of polystyrene foam with UV, heat, $HNO_3$, resulted in the formation of linear and crescentric fracture patterns as evinced by SEM microscopic images .It was interesting to observe that UV, heat, and $HNO_3$ treated polystyrene foam on inoculation with *Pseudomonas sp, Actinomycetes, sp.* and *Penicillium sp*. lead to crystal formation. Further, the polystyrene degradation was confirmed by FTIR studies, which indicated the formation of ester group as evinced in heat ; heat and $HNO_3$ ; UV, heat , and $HNO_3$ treated polystyrene foam.

**Key words**: Polystyrene foam, *Actinomycetes sp., Pseudomonas sp., Penicillium sp.*, FTIR, SEM .

## 1. Introduction

Polystyrene is used in packaging, electronics, construction, house and medical ware and disposable food services [1]. Its products are discarded in dumps, landfills or simple litter after their useful application [2]. Its hardness, hydrophobic nature and chemical composition cause it to persist in nature without any decomposition for longer period of time to marine life and natural ecosystems [3] . Styrene exposure for a short time can result in eye and mucous membrane irritation and gastrointestinal problems in humans. Styrene and its metabolites are known to cause serious negative effects on human health (4). Polymers weather due to environmental factors like light and temperature. Few studies have been conducted focusing on polystyrene biodegradation by fungi and bacteria [5,6,7,8,9]. In our prior work, we visualised electron microscopically the morphological changes on the surfaces PET (Polyethylene terephthalate) inoculated with *Pseudomonas sp* .[*10*]. It was not until the past few years that there is evidence of microbial attack on aromatic polyesters [11,12, 13]. In the present investigation, the potential of soil microbes *Pseudomonas sp, Actinomycetes sp and Penicililum sp* .to degrade polystyrene foam has been evaluated electron microscopically and through FTIR studies.

## 2. Materials and methods

### A. Isolation of Polystyrene foam degrading microorganisms from Polystyrene foam waste.

The collected Polystyrene foam waste from soil in different areas were scrapped several times with care to remove the soil, after that Polystyrene foam samples were cut into small pieces . Further, polystyrene foam were washed with distilled water and inoculated into Nutrient Broth medium at room temperature for 24 hour. The identification of bacteria was performed on the basis of microscopic examination and biochemical test according to Bergey's manual of determinative bacteriology[ 14]. The fungus was identified after staining them with cotton blue by following keys Raper and Fennell [15 ]. The phenotypic and chemotaxonomic characteristics of the *Actinomycetes* were determined by the method described by Shirling and Gottlieb [16].

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## B. Sample preparation

Polystyrene foam was collected from shop and was cut into small flakes about 0.5×0.5 cm size and were washed with distilled water. Further, they were treated with chemical ($HNO_3$ for 24 hour in room temperature), UV radiation (10 days in UV chamber ) and heat treated ($100^0$C for approximately 5 minutes) as shown in table 1. Finally they were treated with above three factors and treated Polystyrene foam were washed thoroughly with 70% ethanol and finally washed with distilled water thereafter kept in oven at $50^0$C for one hour [17]. Further, they were inoculated with bacteria (*Pseudomonas sp*) *Actinomycetes sp*. and fungi (*Penicillium sp*., ) for a period of one month.

Table-1: Treatment of Polystyrene foam with UV, heat, $HNO_3$ and inoculation with *Pseudomonas sp, Actinomycetes sp. and Penicillium sp.*

| **Treatment factors** | **Control** | **Treated Polystyrene foam inoculated with *Pseudomonas sp., Penicillium sp and Actinomycetes sp*** |
|---|---|---|
| UV | - | + |
| $HNO_3$ | - | + |
| Heat | - | + |
| Heat + $HNO_3$ | - | + |
| Heat + UV | - | + |
| UV + $HNO_3$ | - | + |
| Heat+ $HNO_3$ +UV | - | + |

_ Not exposed to treatment factors
+ Exposed to treatment factors

## C. Scanning Electron Microscopy (SEM)

The scanning electron microscopy analysis of fractured surface of PET film was carried out using Scanning electron microscope (VEGA3 TESCAN). The surfaces of the treated PET samples were coated with conductive heavy metals such as gold/ palladium.

## D.FTIR spectrophotometer studies .

Fourier transform infrared (FT-IR) measurements were carried out with a FTIR spectrophotometer ( Nicolet model 8700 ) in the range of 4000—650 $cm^{-1}$. The spectra of the samples were recorded over a range of 4000 -400 $cm^{-1}$ with an average of 100 scans and a resolution of 4$cm^{-1}$ against an air background. [22].

## 3. Results

SEM images of untreated polystyrene foam exhibited porous surfaces nature of the closed type Polystyrene foam (fig 1).SEM images of heat treated Polystyrene foam illustrated complex linear and crescentric stress fracture patterns (fig 2). Further, the porous nature of closed type polystyrene foam disappeared. Adherence and biofilm formation by *Pseudomonas sp., Actinomycetes sp. and Penicillium sp*. was visualized in the SEM images of heat treated polystyrene foam inoculated with these microbes. (fig 3). Fig 4 shows SEM micrographs of the surface morphology of polystyrene foam treated with heat and nitric acid. A number of duckings and crescent structures appeared on the surface. In addition, adherence of *Pseudomonas sp., Actinomycetes sp. and Penicillium sp.* on the surface of heat and nitric acid treated polystyrene foam inoculated with the above species was evident (fig 5). In comparison to the untreated polystyrene foam, there was no change in the surface morphology of polystyrene foam exposed to UV (fig 6 ). Further inoculation with *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . did not elicit any remarkable change on the surface morphology of polystyrene foam (fig 7). UV and $HNO_3$ did not induce any detectable change on the surface morphology of polystyrene foam (Fig 8). Further on inoculation with *Actinomycetes sp., Pseudomonas sp*. and *Penicillium sp.,* growth of mycelium on polystyrene foam was evident. In addition, surface corrosion was evident on polystyrene foam (fig 9). SEM data for polystyrene foam treated with heat, $HNO_3$ and UV indicated pitting of the polystyrene foam surface (Fig-10). Further, inoculation with *Pseudomonas sp., Actinomycetes sp and Penicillium sp* . of the polystyrenes foam treated with heat , $HNO_3$ and UV radiation resulted in the formation of crystals, which was evident under the SEM images (fig 11). The SEM data for UV and heat treated and inoculation with bacteria could not be provided in the present finding due to melting of the samples while taking images under the SEM .

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Fig 1

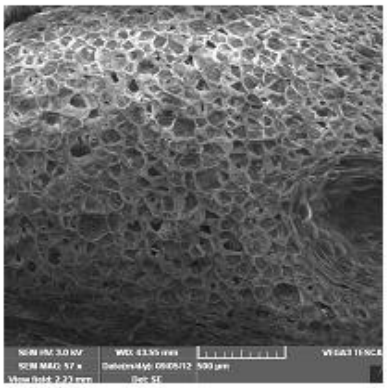

Fig 2

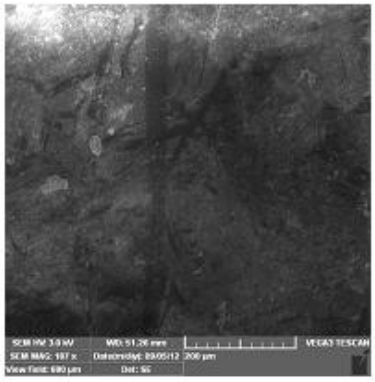

Fig 3

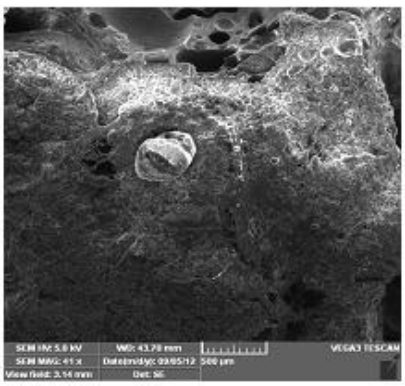

Fig 4

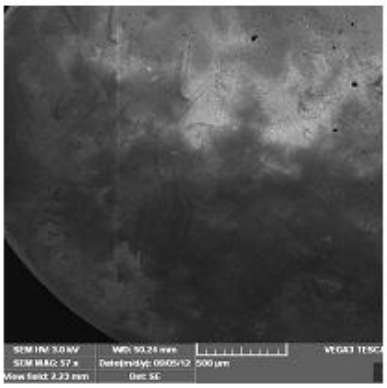

Fig 5

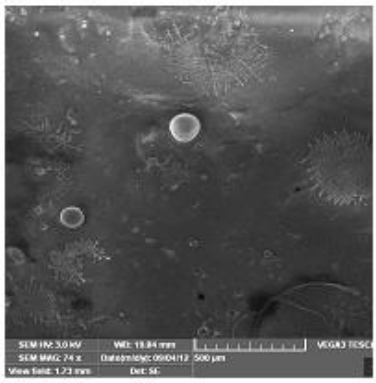

Fig 6

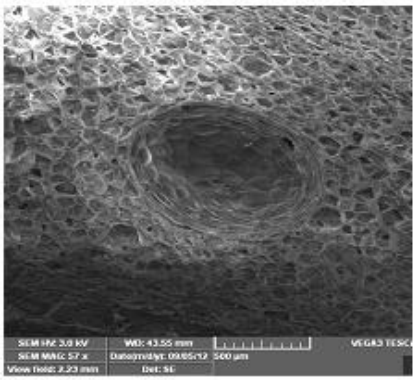

Fig-1 SEM Images of untreated PS foam
Fig-2 SEM Images of Heat treated PS foam
Fig-3 SEM Images of Heat treated PS foam inoculated with *Pseudomonas S*p., *Actinomycetes Sp*., *Penicillum Sp*.
Fig-4 SEM Images of Heat and Nitric acid treated PS foam
Fig-5 SEM Images of Heat and Nitric acid treated PS foam inoculated with *Pseudomonas S*p., *Actinomycetes Sp., and Penicillum Sp.*
Fig-6 SEM Images of UV treated PS foam

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Fig 7

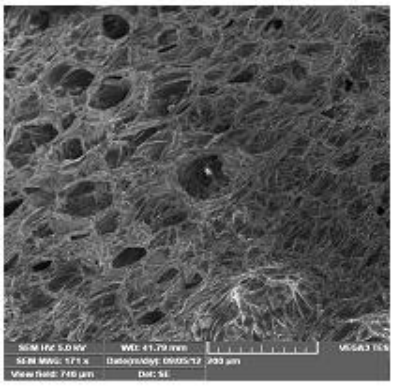

Fig 8

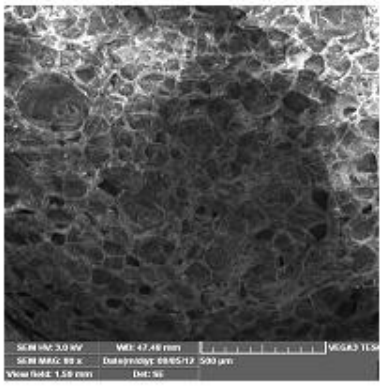

Fig 9

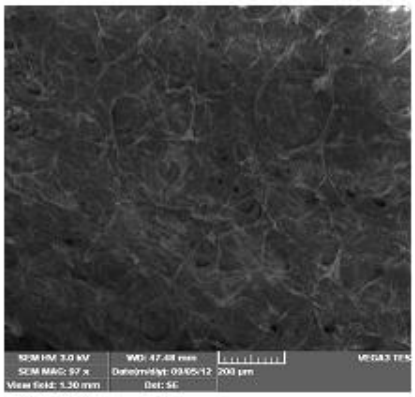

Fig 10

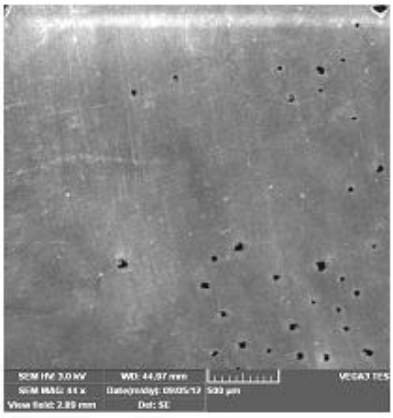

Fig 11

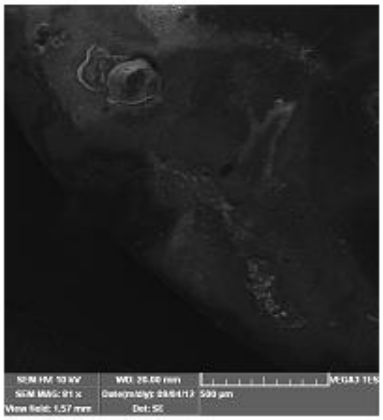

Fig-7 SEM Images of UV treated PS foam inoculated with *Pseudomonas S*p., *Actinomycetes Sp. and Penicillum Sp*.

Fig-8 SEM Images of UV and Nitric acid treated PS foam

Fig-9 SEM Images of UV and Nitric acid treated PS foam inoculated with *Pseudomonas Sp., Actinomycetes Sp. and Penicillum Sp,*

Fig 10 SEM Images of heat, UV and Nitric acid treated PS foam

Fig 11 SEM Images of heat, UV and Nitric acid treated PS foam inoculated with *Pseudomonas S*p., *Actinomycetes Sp. and Penicillum Sp*.

The FTIR spectra of polystyrene foam is shown in fig -12. The absorption bands at 3282 $cm^{-1}$ ,3068 $cm^{-1}$and 2913 $cm^{-1}$ have been attributed to aromatic and aliphatic C-H bond stretching , 2358 $cm^{-1}$ to O-C-O bond stretching , 1943 $cm^{-1}$to C=C=C bond stretching ,1602 $cm^{-1}$ and 1492 $cm^{-1}$ to –C-C aromatic bond stretching , 1451 $cm^{-1}$, 1028 $cm^{-1}$ ,905 $cm^{-1}$, 754 $cm^{-1}$, 695 $cm^{-1}$, 538 $cm^{-1}$to C-H bond stretching . It is inferred from fig -13 that polystyrene foam on exposure to heat elicited two characteristic absorption peak,1654 $cm^{-1}$ which has been assigned to C-C bond stretching and 1525 $cm^{-1}$ to C-H aromatic bond stretching . These two absorption peak did not appear in the FTIR spectra of untreated Polystyrene foam. On heating , peaks at 3068 $cm^{-1}$ decreased to 3066 $cm^{-1}$ , 2913 $cm^{-1}$ increased to 2924 $cm^{-1}$ , 2358 $cm^{-1}$ decreased to 2345 $cm^{-1}$ , 1602 $cm^{-1}$ increased to 1654 $cm^{-1}$ , 1028 $cm^{-1}$ decreased to 1027 $cm^{-1}$ ,754 $cm^{-1}$ increased to 771 $cm^{-1}$ , 695 $cm^{-1}$ increased to 696 $cm^{-1}$ , 538 $cm^{-1}$ increased to 546 $cm^{-1}$ . Sharp absorption peak was evident at 2345 $cm^{-1}$, which have been attributed to –O-C-O bond stretching and broad and bending absorption peaks at 1525 $cm^{-1}$ which has been assigned to C-H aromatic bond stretching . Further, the absorption peaks detected at 1492 $cm^{-1}$ and 1451 $cm^{-1}$ in the untreated polystyrene foam disappeared in the heat exposed polystyrene foam .Inoculation of

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*Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . with the heat treated polystyrene foam exhibited absorption peak at 3448 $cm^{-1}$, which has been assigned to C=O bond stretching , 1870 $cm^{-1}$ ester C=O bond stretching, 1458 $cm^{-1}$ to methylene group ,1181 $cm^{-1}$ to ether C-O bond stretching (Fig-14). Moreover , on inoculation with *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . peaks at 3447 $cm^{-1}$ increased to 3448 $cm^{-1}$ (C=O bond stretching ), 3066 $cm^{-1}$ decreased to 3025 $cm^{-1}$ (C-H bond stretching in indicated aromatic ring), 2364, $cm^{-1}$ increased to the 2369 $cm^{-1}$ (O-C-O bond stretching) , 771 $cm^{-1}$decreased to 766 $cm^{-1}$ (C-H bond stretching) and 696 $cm^{-1}$ increased to 697 $cm^{-1}$ (C-H bond stretching). Intensity of the absorption peak at 1944 $cm^{-1}$ increased in the *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . inoculated heat treated polystyrene foam .In addition, the absorption peaks observed at 1654 $cm^{-1}$ and 1525 $cm^{-1}$ in heat treated polystyrene foam disappeared on inoculation with *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . In comparison to the untreated polystyrene foam, the *Pseudomonas sp. Actinomycetes sp. and Penicillium sp* . inoculated heat treated polystyrene foam exhibited two characteristic peak at 1458 $cm^{-1}$ and 1068 $cm^{-1}$ ,which have been attributed to the methylene group and ether (C-O) bond stretching, respectively.

The structural differences revealed by FTIR spectra of heat and $HNO_3$ treated Polystyrene foam is presented in fig-15. In comparison to the FTIR spectra of untreated polystyrene foam, appearance of new absorption peaks was evinced. Absorbance intensity at 2851$cm^{-1}$ elicited symmetrical stretching of C-H bond (methylene group). Absorption peak visualized at 1870 $cm^{-1}$ have been attributed to a strong C=O bond stretching. Broad absorption peak is evident at 1718 $cm^{-1}$, which has been assigned to C=O bond stretching . The peak assigned to the C-O stretching vibration is

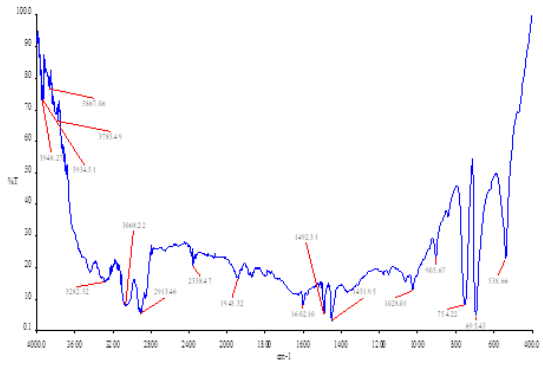

Fig 12 FTIR spectra of untreated PS foam

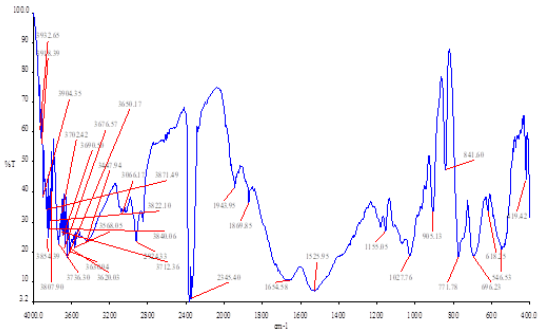

Fig 13 FTIR spectra of heat treated PS foam

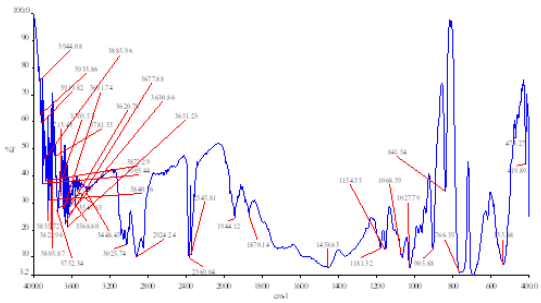

Fig 14 FTIR spectra of heat treated PS foam inoculated with *Pseudomonas sp., Actinomycetes sp. and Penicillium sp,*

found around 1181 $cm^{-1}$. Further, absorbance intensity at 1154 $cm^{-1}$ and 1068 $cm^{-1}$ indicates ester group ( C=O bond stretching ) and 841 $cm^{-1}$ reveals methylene group ( C-H bond stretching ) .However , after exposure of polystyrene foam to heat and $HNO_3$ ,the peak of C-H (aromatic ) stretching vibration was shifted from 3068 $cm^{-1}$ to 3059 $cm^{-1}$ and 3025 $cm^{-1}$. Absorption peaks at 2913 $cm^{-1}$ increased to 2922 $cm^{-1}$ ( C-H bond stretching), 2358 $cm^{-1}$ decreased to 2369 and 2345 $cm^{-1}$ (O-C-O stretching ). A polystyrene foam characteristic cumulative double bond system occurred in the C=C=C bond stretching at 1943 $cm^{-1}$ which was found to increase to 1944 $cm^{-1}$ . The C=C stretching vibration observed at 1602 $cm^{-1}$ in the untreated polystyrene foam disappeared in the FTIR spectra of heat and $HNO_3$ treated polystyrene foam. Characteristic absorption frequencies observed in the untreated polystyrene foam at 1492 $cm^{-1}$ has been attributed to asymmetric ring stretching in which C-C bond is stretching .

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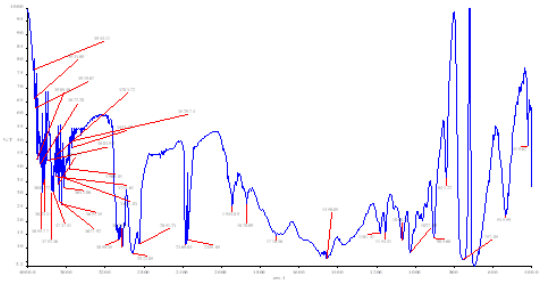

Fig- 15 FTIR spectra of heat and $HNO_3$ treated PS foam

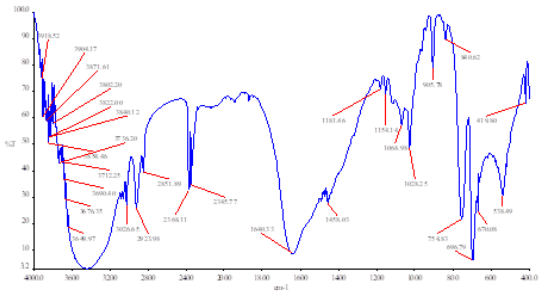

Fig -16 FTIR spectra of Heat and $HNO_3$ treated PS foam inoculated with *Pseudomonas sp., Actinomycetes sp. and Penicillium* sp.

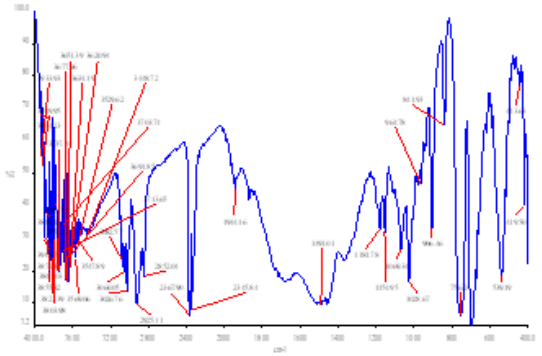

Fig -17 FTIR spectra of UV treated polystyrene foam

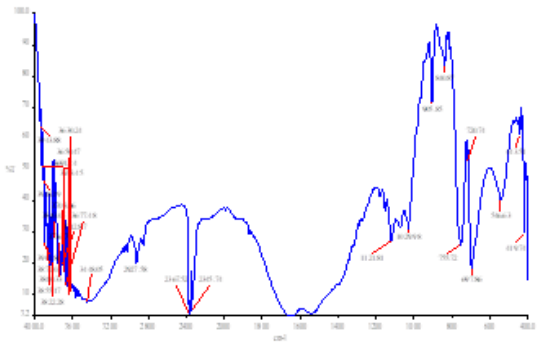

Fig 18 FTIR spectra of UV treated PS foam inoculated with *Pseudomonas sp., Actinomycetes sp. and Penicillium* sp.

FTIR spectroscopy of *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . inoculated polystyrene foam treated with heat and $HNO_3$ exhibited absorption peak at 3447 $cm^{-1}$(C-O bond stretching ), 2851 $cm^{-1}$(C-H bond stretching , which indicates metheylene group and 1640 $cm^{-1}$ ( C-C bond stretching ), which were not detected in the FTIR spectra of heat and $HNO_3$ polystyrene foam (fig 16) . Further , new absorption peaks at 3447 $cm^{-1}$ and 1640 $cm^{-1}$ peak in *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . inoculated heat and $HNO_3$ treated polystyrene foam were detected when compared to untreated polystyrene foam .

It can be seen fig17 that , new sharp absorption peaks appeared at 2852 $cm^{-1}$, which has been attributed to methylene group (C-H bond stretching) , 1181$cm^{-1}$ , 1154$cm^{-1}$ and 1069$cm^{-1}$ which have been assigned to ether group ( C-O bond stretching ) and 906 $cm^{-1}$ to methylene group (C-H bond stretching) on exposure of polystyrene foam to UV. Other characteristics peaks were nearly unchanged. Further, on inoculation of UV exposed polystyrene foam with *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . appearance of two absorption frequencies were noticed at 1652 $cm^{-1}$ which has been assigned to C-C bond stretching and 1121 $cm^{-1}$ to ether group (C-O bond stretching) (fig 18). Appearances of many new spectral absorption frequencies were visualised in the FTIR spectra of heat ,UV, and $HNO_3$ treated polystyrene foam (Fig19). Absorption peaks at 3449 $cm^{-1}$ has been attributed to C=O bond stretching, 3025 $cm^{-1}$ to C-H bond stretching (aromatic ring), 2369 $cm^{-1}$ and 2345$cm^{-1}$ to (O-C-O bond stretching) , 1871 $cm^{-1}$ to ester group (C=O bond stretching ),1803$cm^{-1}$ to C-H bond stretching (ether group), 1355 $cm^{-1}$ to methylene group ( C-H bond stretching), 1181 $cm^{-1}$, 1154$cm^{-1}$ and 1068 $cm^{-1}$ to ester group (C- O bond stretching ) . On inoculation of the above treated polystyrene foam with *Pseudomonas sp.* , *Actinomycetes sp. and Penicillium sp* . C=O bond stretching was evident at absorption frequency of 1719 $cm^{-1}$ and presence of methylene group at absorption frequency of 1342 $cm^{-1}$ ( C-H bond stretching ) (Fig 20).

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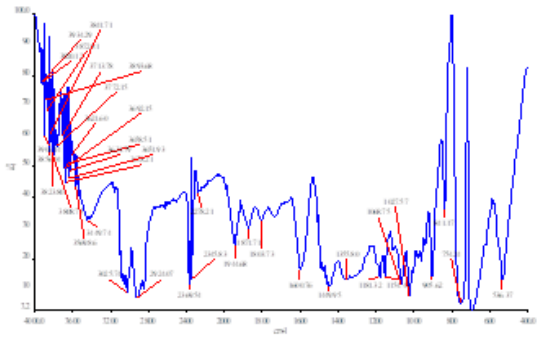

Fig -19 FTIR spectra of UV , $HNO_3$ and heat treated polystyrene foam

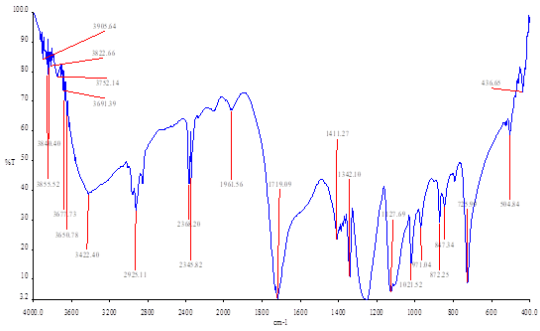

Fig 20 FTIR spectra of UV , $HNO_3$ and heat treated PS foam inoculated with polystyrene foam *Pseudomonas sp., Actinomycetes sp. and Penicillium* sp.,

## 4. **Discussion**

To visualise the existence of morphological changes as a result of polystyrene foam degradation , the treated polystyrene foam samples were evaluated through scanning electron microscope. Compared to the images of untreated polystyrene foam, a number of duckings and crescent structures appeared on the surfaces of heat and nitric acid treated polystyrene foam inoculated with *Pseudomonas sp.* Further, on inoculation of UV and $HNO_3$ treated polystyrene foam with *Pseudomonas sp.* resulted in surfaces corrosion. In addition, formation of crystals was evident on inoculation of heat, UV and $HNO_3$ treated polystyrene foam with *Pseudomonas sp.*, *Actinomyctes sp.* and *Penicillium sp.* UV treated polystyrene foam did not show any differences in surface morphology compared to the control . Therefore, it can be concluded that the surface structure is not damaged and mechanical stability is still maintained after treatment with UV. In fact, recently (21) referred that mineralization of high molecular weight requires different microorganisms able to break the polymer into small pieces and monomers and other capable of using those compounds . From a chemical point of view, polystyrene can be described as a material composed of vinyl monomers containing C=C bonds . Polystyrene molecules possess long hydrocarbon backbone, with a benzene ring linked to every other carbon atom.

Structural changes were evinced on exposure of polystyrene foam to physical, chemical and biological agents. On exposure of polystyrene foam to heat, formation of ester group was observed in the FTIR spectra. Further, on inoculation of the heat treated polystyrene foam with *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . resulted in ether group formation in addition to ester group .On the other hand, heat and $HNO_3$ synergistically resulted in formation of ester group .In addition, ester group was also formed on inoculation of polystyrene foam with *Pseudomonas sp* ., *Actinomycetes sp and Penicillium sp* . When polystyrene foam was treated with $HNO_3$ , formation of ether group was evident . Furthermore, on inoculation of $HNO_3$ treated polystyrene foam with *Pseudomonas sp., Actinomycetes sp and Penicillium sp.* the formation of ester group, in addition to ether group was observed. When polystyrene foam were exposed to UV, formation of ether group was evident. Further, on inoculation of UV treated polystyrene foam to *Pseudomonas sp., Actinomycetes sp. and Penicillium sp* . resulted in the formation of methylene group, Ester group formation was observed on treatment of polystyrene foam to heat, UV and $HNO_3$ . Similarly, on further inoculation of the heat, UV and $HNO_3$ treated polystyrene foam with *Pseudomonas sp.* resulted in the formation of ester group .This study provides the proof of concept that *Pseudomonas sp, Actinomycetes sp. and Penicillium sp.* are capable of degredating polystyrene foam. This finding is in good accord with the reports of Eya *et al* ., [18] who have stated that plastic degradation might result from attack by the soil microflora in soil. Kimura et al., [19] have emphasized that the degradation of plastic was mainly caused by bacteria and fungi and that different soil conditions affected the rate of degradation of plastics. Attack of *Pseudomonas sp.* on the aromatic polystyrene is well supported by Hooker *et al.*, [22] who have observed enzymatic hydrolysis of cyclic oligomers of aromatic polystyrene. Many researchers have proved that microorganisms attack aromatic polystyrene [11, 12, 13 ].

The present result is in good accord with Chetra and Madhuri, [23] , who have observed the growth of *Actinomycetes sp.* and bacteria inside as well as on the surfaces of PET crystals .It has been

reported that *Bacillus sp* is involved in initial degradation of dimethylphthalate by esterases [27,30].Modifications of surface chemistry of polyethylene terephthalate by many marine bacteria have also been observed [ 28].

Our finding agrees with Pramila and Vijay Ramesh [24] who have observed colonization of fungi *Aspergillus flavus* and *Mucor circinelloides* on the surface of LDPE ( Low density Polyethylene ) ,which has caused some physical changes. Microorganisms that are known to biodegrade polystyrene include, *Actinomycete sp ,Rhodococcus ruber* [5], *Curvularia species* (8) *Bacillus, Xanthomonas, Sphingobacterium* [29], *Serratia marcescens, Pseudomonas sp.* and *Bacillus sp.* [7], *Bacillus coagulans* [2], brown rot *Gleophyllum trabeum* and white rot *Basidiomycete, P. chrysosporium, Trametes versicolor* and *Pleurotus ostreatus* (9). Fungi are successfully used to degrade plastics and other xenobiotics [24]. *P. chrysosporium* is also reported to biodegrade polymeric materials [30]. Our finding gains support from the observations of Naima Atiq [19] who have reported that *Rhizopus oryzae* NAI, *Aspergillus terreus* NA2 Phanero chaete , Chryso sporium NA3 were able to colonies polystyrene foam surfaces for long period of time without any Carbon source .

The FTIR results demonstrated that biodegradation of Polystyrene foam was mainly caused by microorganisms which resulted in the formation of many low molecular weight polymers. In general, it can be concluded that the electron microscopic observations are in good agreement with those obtained in FTIR assays, showing a higher level of degradation in the microbe inoculated polystyrene foam when compared to the untreated ones.

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