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PII: S0304-3894(16)30629-X
DOI: http://dx.doi.org/doi:10.1016/j.jhazmat.2016.07.008
Reference: HAZMAT 17864

To appear in: *Journal of Hazardous Materials*

Received date: 3-3-2016
Revised date: 2-7-2016
Accepted date: 5-7-2016

Please cite this article as: Vini C Sekhar, KMadhavan Nampoothiri, Arya J Mohan, Nimisha R Nair, Thallada Bhaskar, Ashok Pandey, Microbial degradation of High Impact Polystyrene (HIPS), an e-plastic with decabromodiphenyl oxide and antimony trioxide, Journal of Hazardous Materials http://dx.doi.org/10.1016/j.jhazmat.2016.07.008

# Microbial degradation of High Impact Polystyrene (HIPS), an e-plastic with decabromodiphenyl oxide and antimony trioxide

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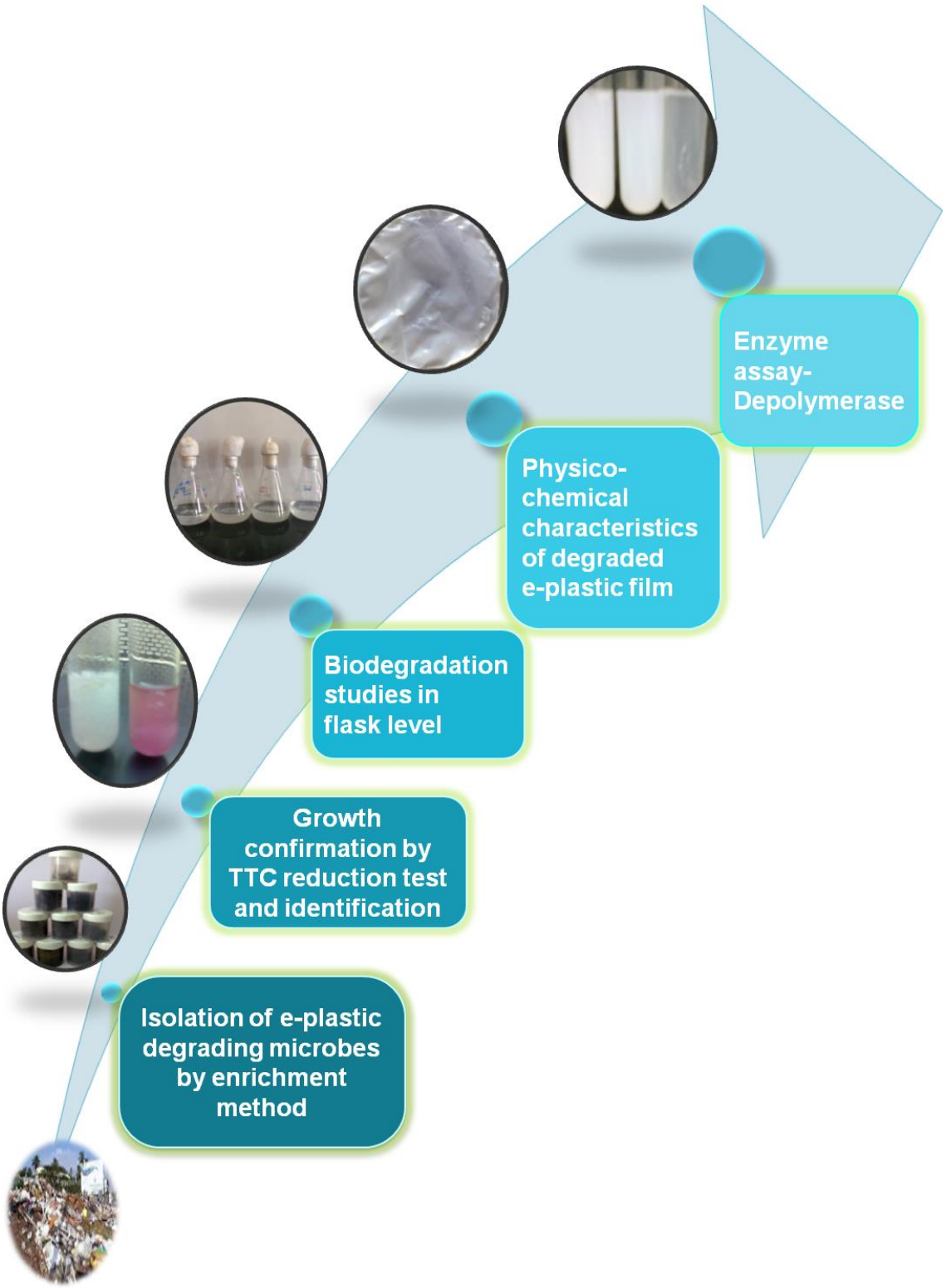
**Graphical Abstract**



**HIGHLIGHTS**

- Biodegradation of a High impact polystyrene e - plastic
- 12.4% (w/w) e plastic film lost using an isolate of Enterobacter *sp.*
- Noted changes in the physico-chemical characteristics of degraded e-plastic film
- Polystyrene intermediates were detected in the degradation medium.
- e-plastic degrading microbes displayed extracellular depolymerase activity.

**Abstract**

Accumulation of electronic waste has increased catastrophically and out of that various plastic resins constitute one of the leading thrown out materials in the electronic machinery. Enrichment medium, containing high impact polystyrene (HIPS) with decabromodiphenyl oxide and antimony trioxide as sole carbon source, was used to isolate microbial cultures. The viability of these cultures in the e-plastic containing mineral medium was further confirmed by triphenyl tetrazolium chloride (TTC) reduction test. Four cultures were identified by 16S rRNA sequencing as *Enterobacter sp., Citrobacter sedlakii, Alcaligenes sp.* and *Brevundimonas diminuta.* Biodegradation experiments were carried out in flask level and gelatin supplementation (0.1% w/v) along with HIPS had increased the degradation rate to a maximum of 12.4% (w/w) within 30 days. This is the first report for this kind of material. The comparison of FTIR, NMR, and TGA analysis of original and degraded e-plastic films revealed structural changes under microbial treatment. Polystyrene degradation intermediates in the culture supernatant were also detected using HPLC analysis. The gravity of biodegradation was validated by morphological changes under scanning electron microscope. All isolates displayed depolymerase activity to substantiate enzymatic degradation of e-plastic.

**Key words:** e-plastic, High Impact Polystyrene, biodegradation, triphenyl tetrazolium chloride, depolymerase

**1. Introduction**

Tremendous technology growth has resulted in the global accumulation of electronic waste. The Waste Electrical and Electronic Equipment (WEEE), often known as e-waste implies old, end-of-life or discarded

electronic appliances. The e-waste is an exponentially increasing environmental pollutant, contains both valuable and toxic materials that require special handling and recycling methods [1].

High-impact polystyrene (HIPS) is widely used as a plastic resin in the electronic production processes [2]. HIPS polymer is a multiphase system with polybutadiene dispersed in a rigid polystyrene (PS) matrix and has improved fracture resistance, reduced transparency, modulus and tensile strength. The current worldwide production of HIPS is about one million tons per annum. [3]. Polybrominated compounds such as decabromodiphenyl ether and Antimony Trioxide ($Sb_2O_3$) are synergistic flame retardant combinations frequently added to HIPS [4, 5].

Insufficient means of safe plastic waste disposal are serious concerns over terrestrial as well as marine wildlife. Exposure to Decabromodiphenyl ether (decaBDE) leads to neoplastic liver nodules and combined hepatocellular adenomas or carcinomas in rats, and U.S. EPA (Environmental Protection Agency) has classified decaBDE as a possible human carcinogen (Class C) [6]. Debromination processes generate other toxic BDE congeners similar to banned PentaBDE, and OctaBDE as intermediates. An evaluation of the IARC (International Agency for Research on Cancer) concluded that the e-plastic constituent Antimony trioxide is carcinogenic to humans [7]. Antimony trioxide exposure in occupational settings causes lung diseases, birth defects and female reproductive disorders [8, 9]. The conventional landfill is not an eco-friendly solution, and disposal process is also burdensome to meet EPA regulations [10].

Microbial degradation is the primary process in the natural decaying, and this involves adherence and subsequent colonization of microorganisms on the surfaces. Plastics contain complex polymeric bonds that resist normal deterioration. The microbial extracellular enzymes can bind to plastic substrates and catalyze hydrolytic cleavage. Polymers are converted into low molecular weight oligomers, dimers, and monomers and finally mineralized to $CO_2$ and $H_2O$ [11]. Proper selection of microorganisms with broad enzymatic capabilities can achieve extensive biodegradation of highly compact polymers [12]. HIPS degradation has been reported only through thermal decomposition, and biodegradation remains unexplored so far. Recently, our lab reported *Pseudomonas* and *Bacillus* strains as e-plastic degrading microorganisms.[13]. Some of the previous studies showed that *Rhodococcus ruber* and *Bacillus sp.* are capable of degrading polystyrene plastic [14, 15].

In this paper, we investigated the biodegradation potential of enriched bacterial cultures that can utilize e-plastic film as sole carbon source. Our monitoring strategies include detection of physical and chemical changes of

the e-plastic, identification of intermediary products released into the medium during polymer degradation and the role of microbial enzymes in the breakdown process.

## 2. Materials and Methods

### 2.1.e-Plastics

Indian Institute of petroleum (IIP), CSIR provided four types of e-plastic samples. These are virgin plastics samples used in electric and electronic appliance and are specially prepared for research purpose and not available commercially. The samples were coded as (1) AMS 01 (HIPS with decabromodiphenyl oxide or ether and antimony trioxide) (2) AMS 01S (HIPS with decabromodiphenyl oxide or ether) (3) AMS 02 (HIPS with decabromodiphenyl ethane and antimony trioxide) and (4) AMS 02S (HIPS with decabromodiphenyl ethane). For degradation experiments, e-plastic films were prepared by a conventional solvent casting technique. About 0.5 g of each e-plastic was weighed, dissolved in 30 mL chloroform and transferred to a glass petri dish that was used as the casting surface and allowed to evaporate to get a thin film.

### 2.2. Media

For the isolation process, routine culture media such as Nutrient agar, Potato dextrose agar and Actinomyces agar (Himedia, India) were used. The composition of the mineral medium was as follows (g $L^{-1}$) : $(NH_4)_2SO_4$ (1), $K_2HPO_4$ (1.6), $KH_2PO_4$ (0.2), $MgSO_4.7H_2O$ (0.2), $FeSO_4$ (0.005), $ZnSO_4$ (0.005), $MnSO_4$ (0.005), Yeast Extract (0.1) and pH 7. The mineral medium was then autoclaved at 121ºC for 20 min. Pre-weighed sterilized plastic films were added to the mineral medium as sole carbon source to monitor biodegradation.

### 2.3. Enrichment and isolation of e-plastic degrading microbes

About 10 g of partially degraded plastic waste samples from a local rural market were transferred to a conical flask containing 95 mL of 0.85% NaCl solution and thoroughly mixed for 15 minutes. An aliquot of 5 mL supernatant was then transferred into a 250 mL conical flask containing 95 mL autoclaved mineral salt medium with

pre-weighed (0.5g) e-plastic film as sole carbon source. The flask was kept in a shaker at 30ºC at a speed of 150 rpm. After two weeks of incubation, plastic films were transferred into another 100 mL fresh mineral medium without any other carbon source. This step was repeated twice to get surface attached microorganisms. Finally, each plastic film was transferred and fixed firmly on the nutrient agar plates to check the adsorption of any microorganism on the plastic film [16]. Cultures attached to the film surface were then started growing on the nutrient agar plates, and single colonies were picked up and purified by repetitive streaking on nutrient agar plates.

Genomic DNA from the isolates were purified as per the protocol of the manufacturers (Sigma NA2110-1KT, Gen EluteTM Bacterial Genomic DNA Kits) and 16S rRNA gene amplification was done by PCR using the set of primers 27F (Escherichia coli position 8-27, 5′-AGA GTT TGATCC TGG CTC AG-3′) and 1492R (Escherichia coli position 1510–1492, 5′-GGC TAC CTT GTT ACGACT T-3′). The PCR conditions ( 37 cycles of 3 min at 94ºC, 1 min at 55ºC, and 1 min at 72ºC ) were performed using a thermal cycler (Eppendorf-epgradient Mastercycler, Germany). The PCR products were purified and sequenced (Applied Biosystem ABI 3500 Series Genetic Analyzer, USA). Amplified DNA sequence from 16S rRNA fragments was compared using BLASTN at sequence data bank maintained by the National Center for Biotechnology Information (NCBI).

2.4. Triphenyltetrazolium chloride (TTC) reduction test

Bacterial isolates were assayed to detect their ability to utilize e-plastic as the sole source of carbon and energy. Individual strains were grown in Bushnell–Haas medium having a composition as follows ($gL^{-1}$): $NH_4NO_3$(1), $MgSO_4.7H_2O$(0.2), $K_2HPO_4$ (1), $CaCl_2.2H_2O$(0.1) and KCl (0.15). Aliquots of 20 µl of 1% TTC solution was added to 5 mL of the medium as an indicator of viability. Growth can be confirmed by the colour change of the medium as bacterial electron transport chain enzymes reduce colourless TTC to red-colored triphenyl formazan (TPF).

2.5. Evaluation of bacterial hydrophobicity

Bacterial cell surface hydrophobicity was determined using bacterial adhesion to hydrocarbon (BATH) test [17]. For performing BATH test, bacteria were cultured in nutrient agar medium until the mid-logarithmic phase, centrifuged, and washed (twice) with PUM buffer (pH 7.1) containing components such as (in g/L): $K_2HPO_4$(17),

$KH_2PO_4$(7.26), urea(1.8) and $MgSO_4.7H_2O$(0.2). The washed cells were resuspended in PUM buffer to an optical density of 0.4 at 600 nm (OD600). Aliquots of 1.2 mL each of the cell suspension were transferred to a set of test tubes to which 0.2 mL of hexadecane was added. The test tubes were vortexed for 2 min and allowed to stand for 15 min to facilitate phase separation. The OD600 of the aqueous suspension was measured. Cell-free buffer served as blank. Adherence (%) to hexadecane was calculated as the ratio of ODs after and before mixing with hexadecane multiplied by 100.

2.6. Quantitative estimation of bacterial biomass in the biofilm

The population density of biofilm on the e-plastic surface was estimated by determination of protein concentration according to Sedmak and Grossberg [18] with slight modifications. The e-plastic films of an equal area of 1 $cm^2$ were supplied to the mineral medium. The culture broth having cfu $2x10^8$/mL was inoculated to the medium. The e-plastic films colonized by the isolates were monitored at regular intervals up to 15 days. The e-plastic films were washed briefly in water and then boiled for 30 min in 5 mL of 0.5 N NaOH. The suspension was centrifuged, and the supernatant was saved, and the pellet was subjected to the same procedure once again. The two supernatants were combined, and the protein concentration was determined using Bradford assay.

2.7. Biodegradation experiment

All degradation experiments were carried out in triplicates for every 5 days interval of time. The pure cultures having cfu $2x10^8$/mL cells approximately were inoculated into the 100 mL mineral medium broth containing 0.5 % e-plastic film (AMS01) as sole carbon source. In another trial, gelatin (0.1% w/v) was also added to the mineral medium as an initial growth inducer. Inoculated media were incubated at 30ºC, 150 rpm for 30 days. The samples were withdrawn to assess the degradation on every 5 days. The assay was performed along with the control (mineral medium + plastic film). Once the strain had attained its stationary growth phase, the medium was centrifuged at 2,348 ×g (5,000 rpm) for 15 min to remove bacterial biomass. The degraded film samples were recovered from the broth after filtration and subsequent evaporation of the filtrate. Plastic film samples were washed using 70% ethanol and dried. Residual film weight was determined to measure the extent of degradation.

The degradation of these films was evaluated in terms of percentage of weight loss using the following formula

$$\text{Percentage weight loss of film} = \left[ \frac{\text{Weight of initial film - Weight of film after degradation}}{\text{Weight of initial film}} \right] \times 100$$

2.8. FTIR Spectroscopy

The degraded e-plastic film samples were analyzed by FTIR Spectroscopy (Nexus-870 FT-IR, Thermo Nicolet Corporation,Madison,WI,USA) and different peaks relative to C-O stretch, =C-H Bend, C-Br Stretch,$CH_2$ deformation, $CH_2$ bending (symmetrical), $CH_2$ bending (asymmetrical), $CH_2$ stretching were compared taking the same non-degraded plastic as a reference. The absorbance was taken in the mid-IR region of 400-4000 $cm^{-1}$ wave number.

2.9. TGA Analysis

The Thermogravimetric analysis (TGA) was performed and corresponding film weight loss was measured using TG-DTA 6200 (SII Nano-technology Inc., Japan) over a temperature range of between 50-700 ºC. The Nitrogen flow was set to 50 ml $min^{-1}$ in order to avoid corrosion, caused by the diffusion of HBr released during the decomposition of flame retarded HIPS. The differential thermal analysis (DTA) was also done to measure the thermal stability of e-plastic before and after biodegradation.

2.10. NMR analysis

$^1$H-NMR spectroscopy was done at 500 MHz (Bruker AVANCE II 500, Switzerland). E-plastic samples were dissolved in deuterated chloroform to obtain a concentration of 0.01 g/mL (w/v). The solution was filled up to 5 cm in NMR tubes. Both control and degradation samples were studied using $^1$H-NMR spectroscopy.

2.11. SEM analysis

The morphology of degraded film samples were monitored by scanning electron microscopy (Carl Zeiss EVO 18, Germany) after 30 days of incubation. The residual film samples were gently washed with distilled water

to remove excess medium and were dried. To examine the film surface erosion, samples were washed with 2% SDS followed by distilled water for the complete elimination of surface-adhered cells. Then, both samples were sputter-coated with a gold layer at 25 mA under Argon (Ar) atmosphere at 0.3 MPa and visualized [19, 20].

2.12. High-Pressure Liquid Chromatography (HPLC) Analysis

The intermediate compounds generated during plastic degradation were detected using high-pressure liquid chromatography (Shimadzu UFLC, Japan). The compounds used as standards were 2-phenyl ethanol, 1-phenyl-1, 2-ethanediol, Phenylacetaldehyde and Styrene oxide (Sigma, USA) [21]. The Prominence Liquid Chromatograph system consisted of SIL-20A HT Prominence Autosampler, DGU 20A3 Prominence Degasser, CTO-20A Prominence column oven and SPD M20A Prominence Diode Array Detector. The analysis was carried out by LC solution software. C18 column (Gemini NX 5u C18 110A, Particle size 5µ, Size 150×4.6 mm) was used for chromatographic analysis. The mobile phase was 50:50 acetonitrile and water respectively. Flow rate was adjusted to 0.6mL/min, and the injection volume was 10 µl. The UV 210 wavelength was used to detect metabolites. The culture supernatants were centrifuged and filtered through 0.2µm filter paper before analysis.

2.13. Depolymerase Assay

Plastic emulsion (0.2 mg/mL) in 20 mM phosphate buffer (pH 6.8) was used as the substrate. Enzyme solution of up to 1 mL was incubated with 3 mL of the substrate at 50º C for 20 min. The decrease in the turbidity of the plastic emulsion was measured at a wavelength of 650 nm. One unit of plastic degrading enzyme activity (depolymerase activity) is defined as a 0.001 O.D decrease in the absorbance per min [22].

**3. Results and Discussion**

3.1. Enrichment of e-plastic degrading bacterial consortium

Soil samples were collected from plastic dumping yard where the possibility of indigenous microbial plastic-survivors is high. The extreme environmental conditions provided by different compact polymers may

influence the inhabitant microbes to become polymer-degraders. Previous studies showed that enrichment screening is a very effective selection method for isolating bacteria that can degrade highly persistent environmental contaminants such as polyaromatic hydrocarbons (PAH) [16, 23]. After two-step enrichment screening, a mixture of bacteria capable of growing in the mineral medium containing e-plastic as the sole carbon source were obtained. The biofilm attached to the plastic film was allowed to grow on nutrient agar plates and four pure cultures (IS01, IS01S, IS011, and IS02) were isolated. All cultures were gram negative cocci.

3.2. Isolation and identification of bacterial strains

Based on 16S rRNA sequence homology, the isolates IS01 (NCBI accession no. KP861247), IS01S (NCBI accession no. KP861248) and IS02 (NCBI accession no. KP861245) were identified as *Enterobacter sp., Citrobacter sedlakii and Alcaligenes sp.* respectively with 99 % similarity and the isolate IS011 (NCBI accession no. KP861246) is identified as *Brevundimonas diminuta* with 100 % similarity. Reports indicate that *Alcaligenes sp*. is capable of degrading styrene dimers [24], and Enterobacter sp. contributes to polyhydroxyalkanoates (PHAs) biodegradation [25]. The e-plastic biodegradation is reported for the first time using any of these cultures.

3.3. Triphenyltetrazolium chloride reduction test

Previous studies showed that many marine bacteria capable of degrading low-density polyethylene were screened using triphenyltetrazolium chloride (TTC) reduction test [20]. Our results showed that it is the most rapid and effective method for checking the viability of e-plastic degrading bacteria. The colourless TTC was readily reduced by the bacterial electron transport system to red-coloured triphenyl formazan (TPF) as shown in the figure (Supplementary Fig.1). Viability experiment was carried out along with positive (mineral medium + glucose + isolate) and negative (mineral medium + e-plastic) controls. The growth was monitored on the basis of the intensity of red colour formation. All four isolates IS01, IS02, IS01S and IS011 exhibited good growth on e-plastic as sole carbon source. However, growth was significantly higher when 0.05% glucose was present. Isolate IS01 showed maximum growth as indicated by comparatively higher red pigment formation in the presence of e-plastic.

3.4. Evaluation of bacterial hydrophobicity

The BATH assay for bacterial hydrophobicity reveals the affinity of bacterial cells for an organic hydrocarbon such as hexadecane. The more hydrophobic the bacterial cells, the greater their affinity for the hydrocarbon, resulting in a transfer of cells from the aqueous suspension to the organic phase followed by a reduction in the turbidity of the culture. The cell suspension of IS01 showed highest hydrophobicity with adherence percentage of 72% followed by IS02 having 61% (Supplementary Fig.2). Previous studies suggest that cell surface hydrophobicity of an organism had a direct correlation with the ability to degrade non-polar polymers [26]. Addition of mineral oil could promote bacterial affinity and consequently leading to a higher bioavailability of plastic while less hydrophobic cultures were used [27].

3.5. Quantitative estimation of bacterial biomass in the biofilm

Microbial colonization and growth on the e-plastic film was monitored by quantifying extractable protein from the biodegraded film surface [15, 28]. Estimation of total protein content was done using Bradford assay. The figure (Supplementary Fig.3) showed a pattern of e-plastic film surface attachment by bacteria. The initial increase in the protein content denoted an increase in the surface-attachment of bacteria followed by constant protein level till the end of incubation period. Although bacterial growth is slow, it proceeds in a sustainable manner.

3.6. Biodegradation experiment

Initially, all four types of e-plastic samples were examined for biodegradation using the strains obtained from enrichment method. After 30 days of incubation, e-plastic films were recovered, and weight reduction was analyzed and the film weight loss studies were carried out in triplicates. The weight reduction was found maximum with AMS01 film. Thus, for subsequent characterization studies we took the more susceptible AMS01 films which is HIPS with decabromodiphenyl oxide or ether and antimony trioxide. Decabromodiphenyl ether or oxide is a brominated flame retardant which belongs to the group of polybrominated diphenyl ethers (PBDEs). The chemical is generally used in conjunction with antimony trioxide in polymers, mainly in HIPS to enhance their flame retardant efficiency (halogen-antimony synergistic effect) which is used in the television industry for cabinet backs. Interestingly, among all samples, this particular sample with antimony trioxide conjunction provided the best results.

Results represent the average weight loss percentage of the AMS01 film by the isolates with standard deviation (Fig.1). Isolates IS01 and IS02 having a high degree of hydrophobicity showed maximum percentage weight loss of e-plastic film. However, IS011 and IS01S also showed good degradation that reflects its ability to secrete extracellular plastic degrading enzymes into the medium even though it is less hydrophobic. Interestingly, gelatin supplementation (0.1% w/v) increased the rate of degradation of e-plastic to three-fold. Maximum weight loss (12.4%w/v) was obtained with IS01 treated AMS01 e-plastic film. Previous studies have demonstrated that gravimetric analysis of polystyrene film after eight weeks of incubation with Rhodococcus ruber showed weight reduction of 0.8% [15]

3.7. FTIR Spectroscopy

Biodegraded e-plastic film samples were analyzed using FTIR spectroscopy to check the changes in the chemical structure. The important absorbance peaks of polystyrene are $CH_2$ asymmetric and symmetric stretching around 2924 $cm^{-1}$ and 2852 $cm^{-1}$, 3026 $cm^{-1}$(aromatic C–H stretches), 756 $cm^{-1}$ (out-of-plane C–H bending mode of the aromatic ring), 698 $cm^{-1}$ (ring-bending vibration) and 1600 $cm^{-1}$ and 1491 $cm^{-1}$ (benzene ring) [29]. The e-plastic film treated with IS01 showed narrowing of absorption peaks in the range of 1320-1000 $cm^{-1}$ and 1000-650 $cm^{-1}$ that correspond to the C-O stretch and =C-H Bend respectively (Fig.2). Most importantly, absorption peak in the region between 690-515 $cm^{-1}$ which determines C-Br Stretch is decreased as a result of microbial degradation. Previous reports have showed that lower intensity of characteristic peaks corresponds to the degradation of the plastic film [30].

3.8. TGA Analysis

Supplementary Figure 4 shows the thermogram. The film control and degraded films were compared using weight loss measurement. It indicates that the initial degradation temperature was slightly reduced under microbial treatment. The thermal stability of degraded film using IS01 was decreased to a small extent and it was proved by the difference in the differential thermal analysis (DTA) plot. The phase transitions of the control and degraded polymer could be compared using DTA analysis. Previous reports recommend that TGA measurement can be used to compare the thermal stability of high impact polystyrene nanocomposites produced by different extrusion processes [31].

3.9. NMR analysis

The structure of polystyrene consists of an aliphatic chain with allied aromatic groups linked to every other carbon atom. The aliphatic and aromatic protons appeared in 1-2 ppm and 6-7 ppm signal region, respectively in the $^{1}$H-NMR spectrum control (Fig.3a). However, in the degraded sample a significant increase in the number of peaks in the aliphatic and aromatic signal region was observed (Fig.3b). But, no new additional peaks have appeared in the degraded film samples elsewhere. The observation might be attributed to slow depolymerization process, the formation of volatile compounds or dislocation of the polymer units into the surrounding medium. Oxidation by-products could be partially soluble in aqueous medium. Previous biodegradation studies also used $^{1}$H-NMR spectroscopy as an analytical tool for detecting degradation of polymers [32].

3.10. SEM analysis

The SEM images (Fig.4A) indicated that e-plastic films before biodegradation exhibited smooth surface with no characteristic surface defects. After 30 days of incubation, the film surface becomes rough with numerous pores and grooves which provide evidence for the deterioration of the film due to the action of the microbial plastic degrading enzymes (Fig.4B). Surface attachment and colonization of IS01 on e-plastic films were also seen clearly in SEM images (Fig.4C). Similar biodegradation studies utilized SEM micrographs as a tool to demonstrate cavities and grooves formed on the plastic film, which directly reflected the extent of microbial colonization and degradation [33].

3.11. High-Pressure Liquid Chromatography (HPLC) Analysis

To determine the intermediate products formed during e-plastic degradation supernatants from isolates grown in mineral medium for 120 h with AMS01 as sole carbon source were analysed by High-pressure liquid chromatography (HPLC). The investigation on the microbial metabolic pathway revealed that polystyrene degradation intermediates include predominantly 2-phenyl ethanol, 1-phenyl-1,2-ethanediol, phenylacetaldehyde and styrene oxide [34, 35]. Using these compounds as HPLC standards, culture supernatants after biodegradation were examined and compared. Interestingly, all four bacterial isolates showed the presence of 1-phenyl-1, 2-ethandiol. However, the extent of intermediate formation was not uniform and only isolate IS01 produced styrene

oxide in addition to 2-phenyl ethanol and 1-phenyl-1, 2-ethanediol. Samples incubated with IS01S and IS011 also contained 2-phenyl ethanol as degradation intermediate (Fig 5 a, b and Supplementary figure 5 a, b, c, d). The HPLC data confirm the e-plastic film breakdown process and also support the assumption that the degradation intermediates were mobile and remained in the medium. This may be one of the reasons behind the absence of new peaks in the NMR analysis of film. The correlation of HPLC data with FTIR spectroscopy results proves that the degradation of AMS01 is oxo-biodegradation.

3.12. Enzyme profiling of the isolates

Some microorganisms have exhibited significant potential to metabolize highly stable polymers by means of their extracellular and intracellular proteins stimulated by the stressful environment. The extracellular enzyme production during e-plastic degradation was tested in this study and all isolates were found to secrete extracellular depolymerase enzyme. This observation is consistent with the data obtained by Zu et al. in which the extracellular and intracellular enzyme secreted by *Ochrobactrum sp.*T elevated during degradation of tetrabromobiphenol A [36]. Depolymerase assay clearly demonstrates the turbidity clearance of e-plastic emulsion due to the enzyme activity in the culture supernatant (Supplementary Figure 6). Heat killed crude enzyme was treated with e-plastic emulsion as a negative control. The lack of turbidity clearance shows that enzymes, not the acids in the medium, are solely responsible for the reduction in turbidity. Addition of gelatin to the medium enhanced the depolymerase activity significantly (Fig. 6) due to better microbial growth.

**4. Conclusion**

Nowadays, eco-friendly biodegradation of e-plastic waste is of significant relevance due to the deleterious effects of chemical and thermal degradation. In the present study, four non-pathogenic e-plastic eating bacterial strains were successfully isolated and identified. Reduction in the absorption of characteristic peaks of plastic films in FTIR results, detection of degradation intermediates in the culture supernatant, firm attachment of microbial cells on the plastic film surface and subsequent morphological changes in SEM images confirmed the process of biodegradation. The action of depolymerase is the contributing factor in the degradation process. Future aspects of this study include the development of a microbial consortium to enhance the rate of biodegradation and to unveil the mechanism in detail.

**Acknowledgements**

This work is financially supported by CSIR, New Delhi under the XII th Five year plan Net work project Waste to Wealth, (CSC 0120).

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**Legends to the Figures**

**Fig.1.** Weight loss of microbially treated AMS01 film after incubation for 30 days. Maximum weight loss (12.4%w/v) was obtained for IS01 (*Enterobacter sp.)* treated AMS01 e-plastic film.

**Fig.2.** FTIR analysis of biodegradation of AMS01 film - A) Control AMS01, B) Degraded AMS01 using IS01 (*Enterobacter sp.).*

**Fig.3a.** $^{1}$H-NMR analysis of control AMS01 film. The aliphatic and aromatic protons appeared in 1-2 ppm and 6-7 ppm signal region, respectively in the $^{1}$H-NMR spectrum control.

**Fig.3b.** $^{1}$H-NMR analysis of degraded AMS01 film using IS01 ( *Enterobacter sp.)* showing significant increase in the number of peaks in the aliphatic and aromatic signal regions

**Fig.4.** SEM analysis of biodegradation of e-plastic films using microorganism. A) Control film, B) Degraded film sample, C) IS01 (*Enterobacter sp.).* colonization and attachment to the e-plastic film surface.

**Fig.5.** a) HPLC analysis of standard mixture containing 125 ppm of each standard. **a** - 1-Phenyl-1,2-ethanediol, **b**-Phenyl ethanol, **c** – Phenyl acetaldehyde, **d**- Styrene oxide, b) HPLC analysis of biodegradation intermediates of e-plastic treated with the IS01 respectively.

**Fig.6.** Depolymerase activity of IS01S, IS011, IS01 and IS02 with and without gelatin supplementation.

**Figure 1**

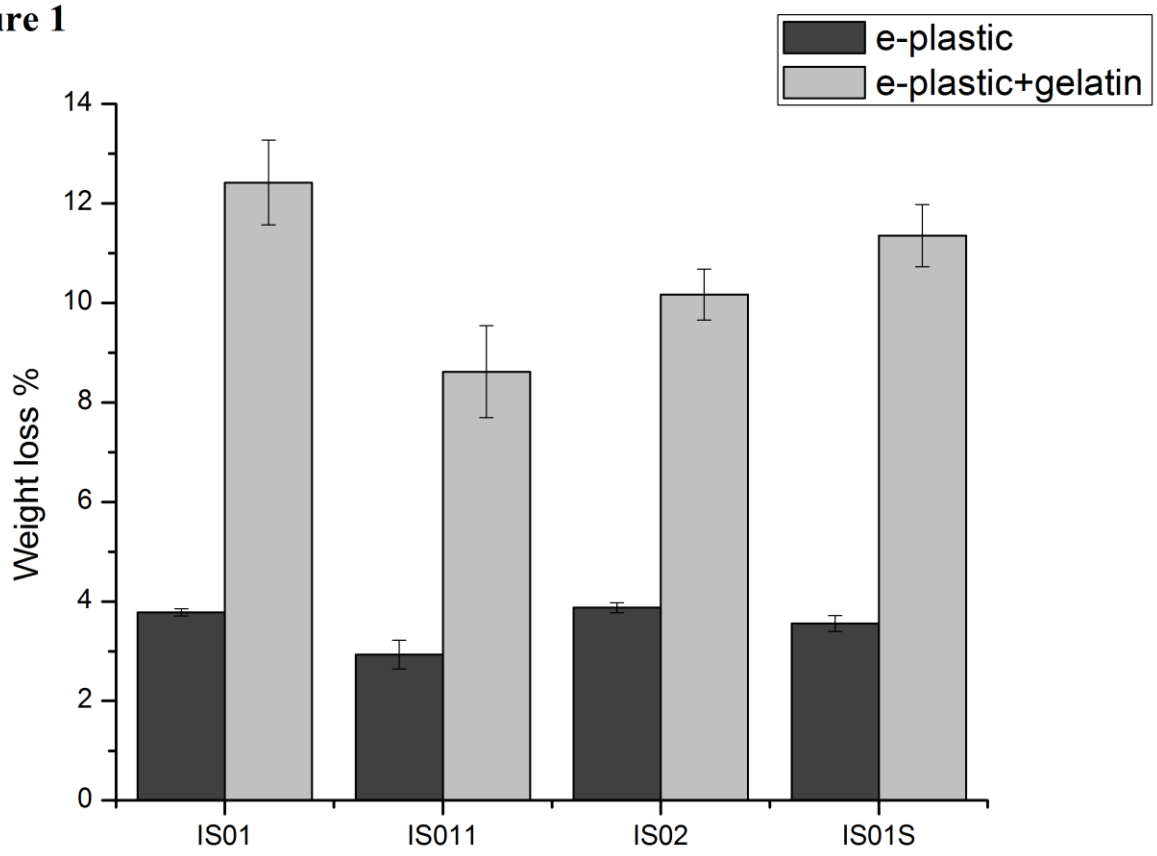

**Figure 2**

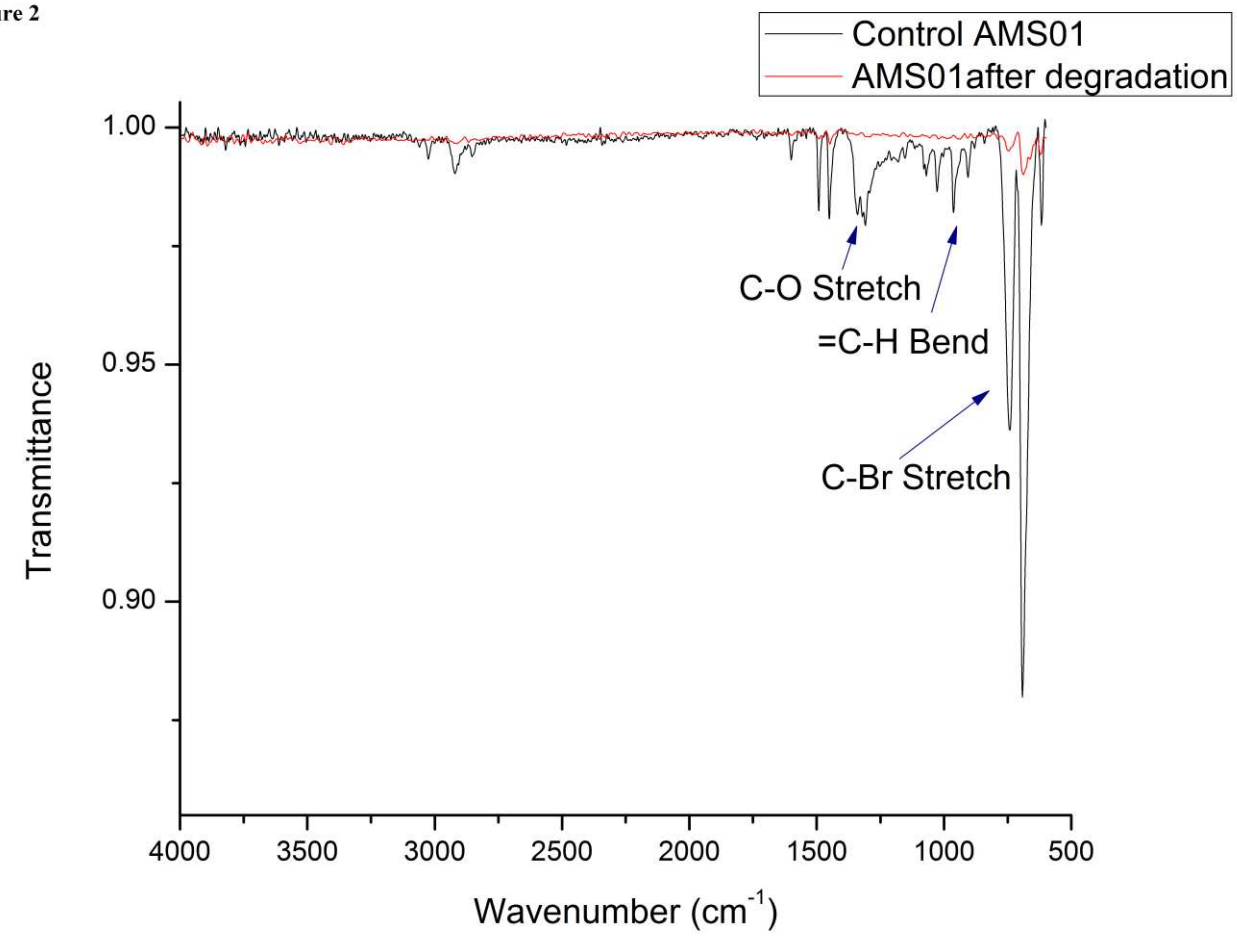

**Figure 3a**

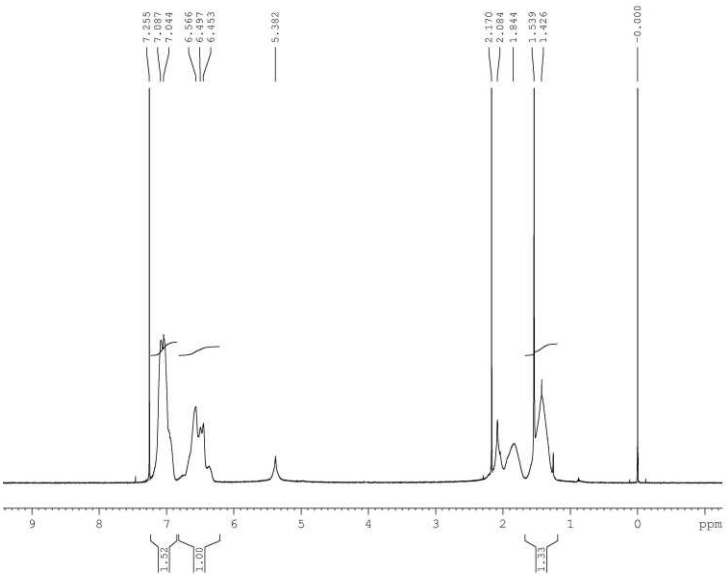


**Figure 3b**

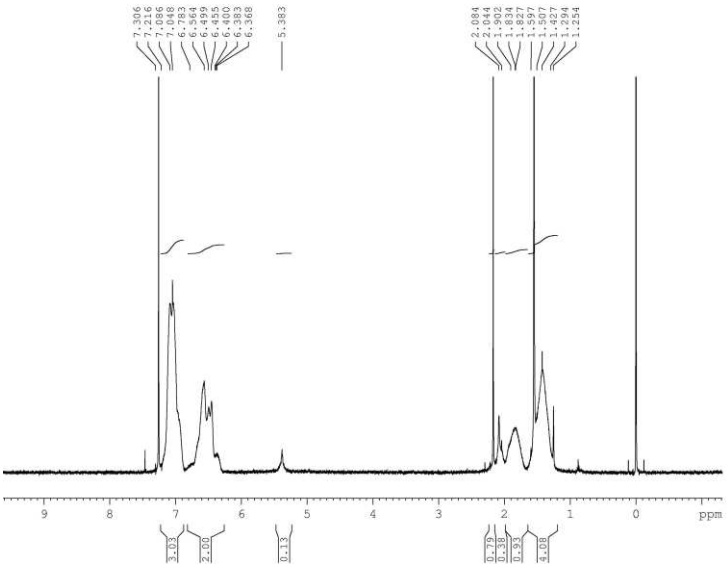

**Figure 4**

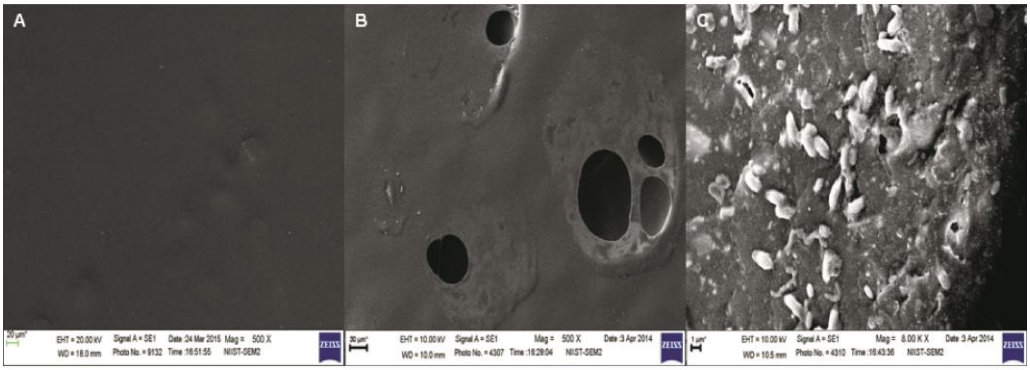

**Figure 5**

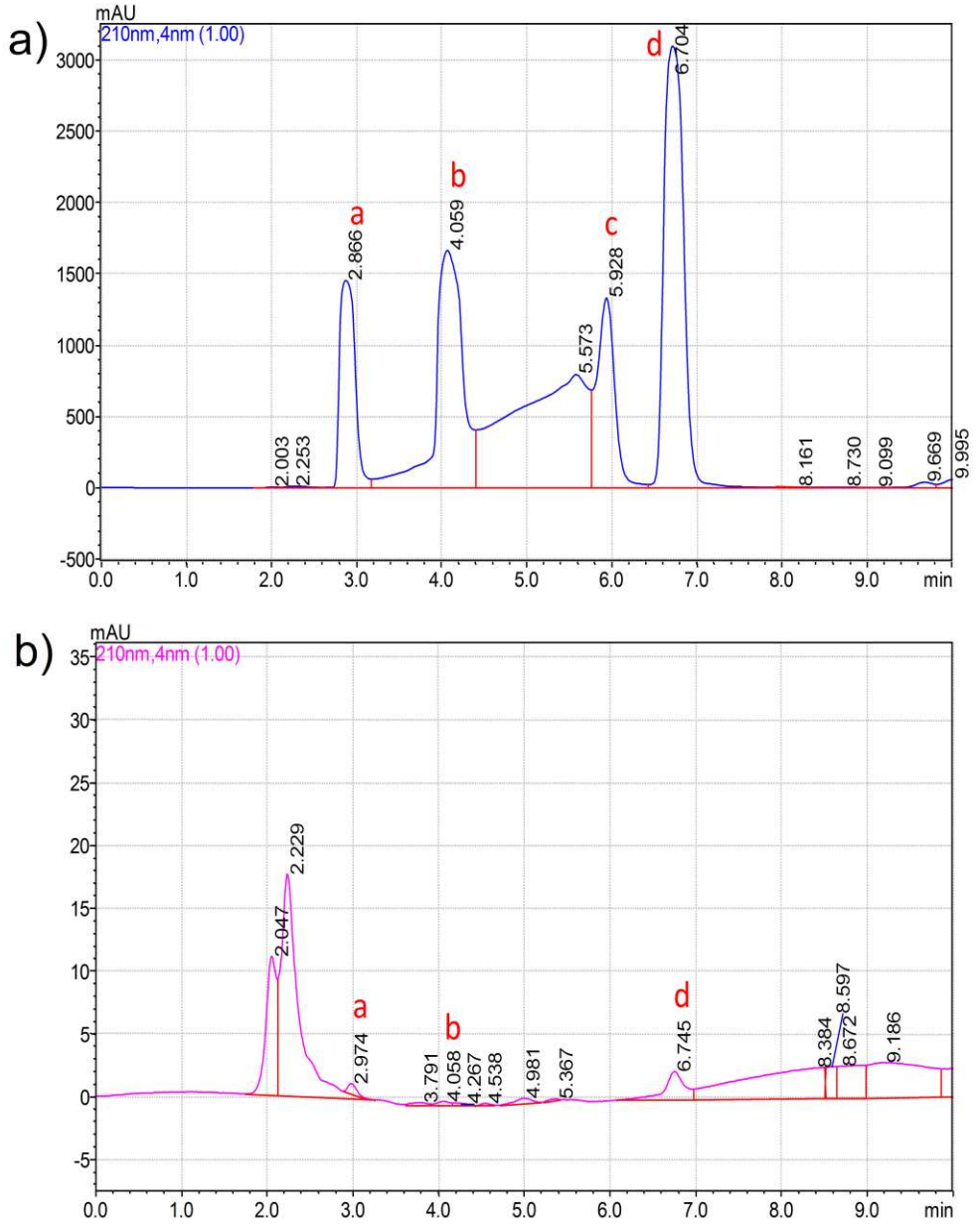

**Figure 6**