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Biodegradation and Bioremediation of Polymer by Microbial Assisted Novel Process

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Abstract: The degradation of polystyrene (PS) and polyurethane (PU), both are foams, is a budding challenge due to increasing white pollution. The present investigation has focused on the microbial assisted biodegradation. Various indigenous microorganisms were isolated from sewage soil. PS – PU foam and bioplastic was used to screen the soil bacteria with biodegradation potential. The screened bacteria were subjected to biodegradation assay such as soil burial and liquid culture in the presence of PS – PU foams and bioplastic in a growth medium. Two microorganism were isolated-- *Pseudomonas aeruginosa* and *Aspergillus niger*. The degradation rate based on weight loss is conducted by *in vitro* assay for 10 and 35 days. The maximum degraded foam was analyzed through Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy and PS – PU foam and bioplastic act as a control without microorganisms. Results of the present study showed that the maximum degradation and weight loss in the polyurethane foam occurred in the soil burial method by *Pseudomonas aeruginosa* compared with *Aspergillus niger*. The soil burial method is more significant than liquid culture method. There is no weight reduction in the polystyrene foam in both organism and methods. For the degradation of synthetic polymers the isolated *Pseudomonas aeruginosa* and *Aspergillus niger* were encapsulated for future studies.

Keywords: Biodegradation, Bioremediation, *Pseudomonas aeruginosa*, *Aspergillus niger*, Polymer, Polyurethane

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Introduction

Plastics are extensively used in today's globe, which has improved the quality of human existence via convenient packaging of groceries and other items (Cosgrove *et al.*, 2007; Andrady and Neal, 2009). After the Metal Age, we now

choose the Polymer Age. No polymer, no world for a decade. Polymer means that huge molecules are made of tiny parts. In recent years, bacteria that breakdown carbon-carbon polymers have been identified. *Bacillus* sp. degrades Polyethelene or

polythene (PE). After primary and secondary screening, *Bacillus* sp. demonstrates highest degradation. Ninety species of microorganisms and fungi breakdown plastic, including *Bacillus megatherium*, *Pseudomonas* sp. *Azotobacter*, *Ralstonia eutropha*, *Halomonas* sp. (Chatterjee *et al.*, 2010). Hydrolysis and glycolysis with alcohols split polyesters (Wu *et al.*, 2003).

Current bacteria-mediated plastic degradation procedures for plastic film systems need 40–60 days to verify biofilms growth and plastic breakdown by electron microscopy and chemical structural changes (Yang *et al.*, 2020). Biodegradation was done via soil burial and liquid culture techniques to breakdown polyurethane foam, polystyrene foam, and bioplastic. The biodegradation was screened by FTIR (Fourier transform infrared spectroscopy) and SEM (Scanned Electron Microscopy) analysis. Fourier Transform-Infrared Spectroscopy (FTIR) identifies polymers and assesses plastic quality. When plastic absorbs infrared light, often in the mid-infrared range, the resultant spectrum (absorbance or transmittance) offers a unique "fingerprint" that may be used to screen and test materials.

The present investigation has focused on the microbial assisted biodegradation. Various indigenous microorganisms were isolated from sewage soil. PS – PU foam and bioplastic was used to screen the soil bacteria with biodegradation potential. The screened bacteria were subjected to biodegradation assay such as soil burial and liquid culture in the presence of PS-PU foams and bioplastic in a growth medium.

Materials and Methods

Collection of Test Sample and Sampling of Soil:

Polystyrene foam was collected from landfill litter. Polyurethane foam and bioplastic were obtained from the garbage in Appaspettai, Manapparai, Tiruchirappalli district, Tamil Nadu. These materials were used for degradation studies.

The sewage soil sample was taken in Thillai Nagar, Tiruchirappalli, Tamil Nadu, India. 3-5cm

soil samples were collected in sterile containers for analysis. Sewage soil is physicochemically characterised. This study was aimed to discover the soil organism that causes biodegradation.

pH Testing:

pH measures soil acidity or alkalinity. Negative logarithm of hydronium ion activity was measured in solution. In soil, it is measured in slurry. A pH metre was used for measuring soil pH.

Analyzing moisture:

One gram of sewage soil was cooked overnight at 130 °C in a crucible. Then the soil sample was weighed.

Material properties:

In 100 ml sterile beaker containing 20 ml of gasoline, added Polystyrene foam and polyurethane foam separately. Polystyrene foam dissolved in gasoline whereas polyurethane does not.

Sample preparation:

Polystyrene, polyurethane foam, and bioplastic were cut and cleaned with 70% ethanol and

distilled water. Each sample (soil and liquid) was aseptically moved to the field and deposited in sterile medium in the lab (Fig. 1).

Aspergillus niger isolation:

By serial dilution, *A. niger* was isolated from sewage soil. Dissolving 1 g of dirt in 100 ml of sterilised distilled water gave the soil suspension. 10 min of orbital shaking at 200 rpm homogenised the dirt suspension. From this stock solution, 10⁻⁴-10⁻⁶ dilutions were made in sterile distilled water (Fig. 2).

PDA Medium and Broth:

Dissolved 20 g of malt extract in 900 ml of distilled water for making malt extract agar media. 0.1 N HCl or 0.1 N NaOH kept the medium pH at 4.8. Added 20 g agar. After making it 1000 ml, the medium was heated for 10 min and homogenised. Rotating the Petri plates clockwise and counter-clock wise helped disseminate soil

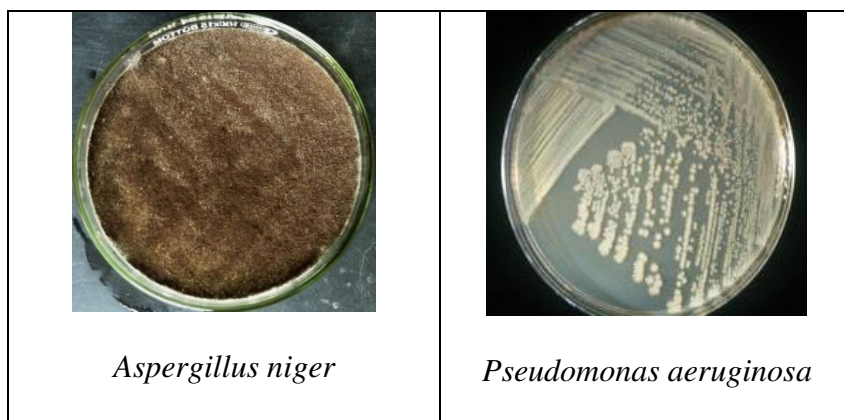


Polyurethane

Polystyrene

Bioplastic

Fig. 1: Samples.



Aspergillus niger

Pseudomonas aeruginosa

Fig. 2: Microbial Plates.

suspension evenly. Petri plates were cultured for 3-4 days at 30 °C. Young *A. niger* colonies were transplanted to PDA medium. 1 pinch of fungal spores was added to the potato dextrose broth, and it was cultured for 3-5 days at 37°C.

Aeruginosa isolation:

One gram of dirt was plated via serial dilution on nutrient media. Peptone, NaCl, yeast extract, and beef extract were dissolved in 100 ml distilled water and incubated at 37°C. Using a sterilised L-shaped glass rod, 0.1 ml of 10⁻⁵ dilution of soil suspension was placed over nutritional agar plates. 100 ml of microorganisms were put to broth in a centrifuge tube and incubated after mixing with microbes.

Bacteriology:

Using morphological and biochemical testing, bacteria were identified. After 24 h of growth, colony shape, motility, features, surface, and pigmentation were studied. Method of biochemical

characterizations by Tambuwal *et al.* (2018) was used.

Bacterial biochemistry:

Gram stains:

Pure 24 h culture was spread on a clean, grease-free slide and heated gently. The smear was stained with 2 drops of crystal violet solution for 60 sec, then iodine for 30 sec, then water and 70% alcohol for 15 sec. After counterstaining with saffranine solution for 1 min, it was rinsed and allowed to dry. The slide was viewed with a high-power objective. Gram-negative cells were pink or red whereas gram-positive were purple.

Motility:

The hanging drop method measured motility. A drop of culture was placed on a coverslip, vasoline was used, and the slide was inverted and observed under a microscope.

Catalase:

The 24 h culture was transferred onto a clean slide with a sterile inoculating loop. White froth indicated catalase enzyme.

Oxidase:

In the oxidase test, filter paper was placed in the reagent. The sterile loop was used to spread an organism's colony on filter paper. The organisms generated oxidase, which turned phenylene-diamine purple.

Citrate:

This test determined whether an organism can get all its carbon and energy from citrate. In 100 ml distilled water, 2.4 g citrate agar was dissolved. 10 ml of citrate medium was dispensed, covered, sterilised, and slanted-cooled. The 24 h culture organisms were streaked across the tubes' surfaces. Green to blue implied citrate use.

MR Test:

5 ml of glucose (1 g glucose, 0.5% KH_2PO_4 , 0.5% peptone, and 100 ml distilled water) phosphate broth were placed into clean test tubes, and the organisms were inoculated and cultured for 48 h at 37°C, after which methyl red was added and the colour was examined. Red means a good response.

VP Test:

The organisms were inoculated into 5 ml of glucose phosphate broth (1 g glucose, 0.5% KH_2PO_4 , 0.5% peptone, and 100 ml distilled water) and incubated at 37°C for 48 h. After incubation, naphthol and sodium hydroxide were added. A 30 min-old red colour suggested a favourable reaction.

Polymers and bioplastics biodegradation:

Following the method, microorganisms degrade artificial polymer foams and bioplastics. Polystyrene, polyurethane, and Bioplastic were cut into little pieces. The material was rinsed with water (to remove dirt) and sterilised with 70% ethanol. Each test and control utilised 1cm (0.5 g). Shade-dried material was weighed. The continual operation lasted 35 days for treated samples.

Liquid culture:

Pre-weighted plastics of 1 cm diameter (0.5 g) were infected in 25 ml of nutrition broth and potato dextrose broth containing *Pseudomonas aeruginosa* and *Aspergillus niger* media. Polyurethane foam, polystyrene foam, and bioplastics were incubated in a microbe-free media for 35 days. All samples were weighed at 10-day intervals to detect weight reduction. After incubation, the materials were collected, rinsed with distilled water, shade-dried, and weighed. Finally, polyurethane foam, polystyrene foam, and bioplastic weight loss were compared to the control.

Geoburial:

In a sterile container, 1500 g of sewage dirt was collected. 0.5 g of polyurethane foam, polystyrene foam, and bioplastic materials were implanted in 25 ml of *Pseudomonas aeruginosa* and *Aspergillus niger* (potato dextrose broth) media. Polyurethane foam, polystyrene foam, and bioplastics were incubated in microbe-free media for 35 days.

The container was kept at room temperature for 35 days. Polyurethane foam, polystyrene foam, and bioplastic samples were weighed every 10 days to assess weight reduction. After incubation, the materials were collected, rinsed with distilled water, shade-dried, and weighed. Finally, polyurethane foam, polystyrene foam, and bioplastic weight loss were compared to control.

Total per cent weight loss after 1 month was calculated as:

% Wt. Loss = $\frac{\text{Initial wt. in the beginning} - \text{Final wt. after ten days}}{\text{Initial wt. at beginning}} \times 100$

% Wt. Loss (after every 10 days) =

$\frac{\text{Initial wt. before ten days} - \text{Final wt. after ten days}}{\text{Initial wt. before ten days}} \times 100$

Nitrification:

Sewage is the major source of nitrates. Nitrates may leak from soil and contaminate groundwater. The phenoldisulfonic technique measures them. Nitrates react with phenol sulfonic acid to form a yellow nitrate derivative in alkaline solution. The sample's nitrate content determines its hue. The

sample was pipetted onto a porcelain dish and evaporated in a hot water bath. By constant stirring with a glass rod dissolved 2 ml of phenol sulphonic acid. Stirring in sodium hydroxide or ammonium hydroxide makes it alkaline. Filtered into a Nessler's tube and filled with pure water. After colour development, absorbance was measured at 410 nm. Concentration is shown along X-axis and absorbance along Y-axis. Comparing the sample absorbance to the standard curve yields the nitrate concentration in mg/l.

$$\text{Nitrates (mg/l)} = \frac{\text{Absorbance of sample} \times \text{conc. of Std.} \times 100}{\text{Absorbance of Std.} \times \text{Sample taken}}$$

FTIR analysis:

FTIR analysis was used to study structural and functional groups of polyurethane foams. Polyurethane foams were chopped into tiny circular pieces for FTIR analysis with a 4 cm¹ resolution.

SEM analysis:

JSM 500 SEM evaluated foam samples. Thin layers of gold were sputtered onto foam samples to make them conductive. Secondary electron detector was utilised to acquire topological pictures. SEM focuses further than an optical microscope. SEM can generate a fair depiction of the sample's high-magnification three-dimensional picture. SEM pictures were utilised to compare centre and side samples treated at various temperatures and magnifications.

Results and Discussion

Soil physicochemical parameters:

Sewage soil is physic-chemically characterised. This research was aimed to identify the soil organism that causes biodegradation. The soil's moisture, pH, and temperature were characterised (Table 1). Imam *et al.* (1999) found that important biodegradation only happened when plastic formed, a metric linked with resident microbe numbers. Therefore, microorganism burden corresponds with polymer degradation. Kathiresan and Bingham (2001) revealed that plastic materials in mangrove soil have rich total

heterotrophic microbe counts of up to 79.67×10^4 and plant counts of up to 55.33×10^2 and are inhabited by 5 species of bacteria and 2 species of fungi.

Table 1: Physicochemical Parameters of Sewage Soil Sample

pH	6.8
Temperature (°C)	29.8
Moisture content	4.872

Isolation and Identification of Microorganism from the Sewage Soil:

Many colonies are obtained from sewage soil cultures. *Pseudomonas* sp. and *Aspergillus* sp. biodegrade polymers effectively. These two microorganisms were isolated from accessible colonies and recognised biochemically and morphologically. Purified isolates were tested for plastic degradation in 35 days on a growth medium at 37°C. Table 2 illustrates bacterial and fungal biochemistry. Figure 2 exhibits *Pseudomonas aeruginosa* and *Aspergillus niger* colonies. *Pseudomonas aeruginosa* degrades polymers.

Table 2: Biochemical Parameters of isolated microbes

Characteristics	<i>Pseudomonas aeruginosa</i>
Gram Staining	Negative
Shape	Rods
Motility	Motile
Catalase	Positive
Oxidase	Positive
MR	Negative
VP	Negative

Aspergillus niger has a dark brown hue, lengthy conidiophores, and globule vesicles. Tambuwal *et al.* (2018) reported a quality morphological strain. *Aspergillus niger* also degrades polymers effectively. *Pseudomonas* sp. degrades polystyrene. These polystyrene samples

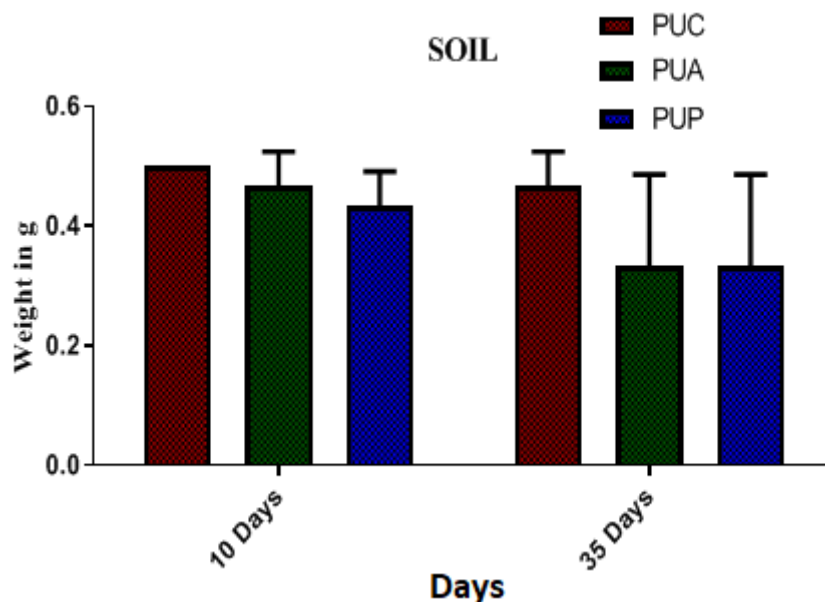


Fig. 3: Weight Loss of treated Polyurethane foam impregnated with microorganisms by burial process.

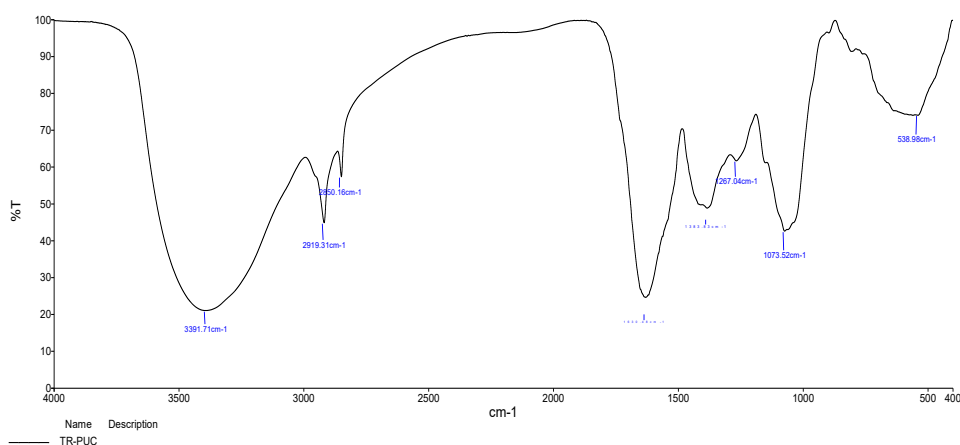


Fig. 4: FT-IR Spectra of Soil Buried Polyurethane Control.

were not contaminated. *Aspergillus niger* biodegrades polystyrene faster than *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Micrococcus luteus*. PET and PS foam placed in soil, cow-dung, and trash will be destroyed by fungus by cleaving polymer bonds, according to Shalini and Sasikumar (2015).

Liquid culture:

The liquid culture technique showed no

substantial loss in polyurethane and polystyrene foams and bioplastic, which may be due to the pure culture of organisms that may not affect the biodegradation process.

Figures 3 and 4 illustrate the biodegradative impacts of microorganisms (*Pseudomonas aeruginosa* and *Aspergillus niger*) compared to biodegradation of all polymers. Usha *et al.* (2011) suggested that extracellular enzymes released by

the target organism breakdown polyethylene and plastic in liquid culture for two, four, and six months. In liquid culture, *Pseudomonas* species with increasing starch content degraded polythene in 150 days.

Geoburial:

Pure organism culture was put over the soil and bioplastics were implanted. The soil burial approach yields no substantial loss in polystyrene foam, perhaps due to the quicker biodegradation phase. Polyurethane foam biodegraded the best, followed by bioplastic. We exclusively analyse treated polyurethane foam. Degradation caused weight loss .

Microbiological Nitrification Test:

All treated polymer foams for liquid and soil organism cultures are nitrified. In soil burial culture, *Pseudomonas*-treated polystyrene and their control had greater nitrate concentrations than bioplastic, polyurethane control, and *Aspergillus*-treated bioplastic. Table 3 shows that *Pseudomonas* and *Aspergillus* treated polyurethane and bioplastic had the lowest nitrate concentrations. *Aspergillus*-treated polystyrene and bioplastic had more nitrate in liquid culture. Table 4 compares treated and untreated polyurethane, polystyrene, and bioplastics. High nitrate levels indicated pollution and the effect of nitrates are given in Table 5. Due to bacteria like *Pseudomonas aeruginosa* and *Aspergillus niger*, treated polyurethane foam in the soil must be less polluting. The nitrification test measured soil pollution and microbial biodegradability.

FTIR Analysis:

Fourier Transform Infrared Spectroscopy was used to discover molecular changes in soil-treated polyurethane foam. After 35 days in soil, PU foam was tested from 400 to 4000 cm^{-1} . Infrared spectrum of Polyurethane *Aspergillus niger* (PUA), Polyurethane *Pseudomonas aeruginosa* (PUP), and Polyurethane control (PUC) (Figs. 5, 6, 7). The peak at 3300 to 3500 cm^{-1} has been saturated to O-H bond stretching (hydroxyl groups), 2919 cm^{-1}

and 2924 cm^{-1} to C-H bond stretching (methylene groups), 2850 cm^{-1} , 2854 cm^{-1} , and 2857 cm^{-1} to N=H, C=H bond stretching (carbonyl and amine group), the medium, small peaks at 1630 cm^{-1} , 1631 cm^{-1} , and 1628 cm^{-1} to C=C bond stretching (alkene). New peaks at 1406 cm^{-1} and 1484 cm^{-1} are attributable to S-O bond stretching sulfoxide on both treated polyurethane foams.

Enzymes oxidise polyurethane to oligomers, dimers and monomers. Microorganisms convert them into CO_2 and water. FTIR study showed polyethylene generated hydroxyl and carbonyl groups, suggesting surface electron loss. Arutchelvi *et al.* (2008) reported polyethylene biodegradation.

SEM characterization of polyurethane foam:

The surface morphology of *Pseudomonas aeruginosa* and *Aspergillus niger* treated polyurethane after 35 days are analysed by SEM. Figure 8 and Figure 9 shows polyurethane *Pseudomonas aeruginosa*'s uneven surface after 35 days of culture (PUP). Bacterial deterioration destroyed plastic particles. This finding suggested that enzymes may be involved in PU biodegradation, which is consistent with earlier research (Yuan *et al.*, 2020). Control samples incubated without organisms and *Aspergillus niger*-treated polyurethane revealed comparable surface structures without harm. *Pseudomonas aeruginosa* absorbed on PU foam destroyed its surface due to *Pseudomonas aeruginosa*'s degradability.

After 6 weeks of co-culturing cyanobacteria and PE sheet, holes appeared on the surface. In contrast, after 30 days of incubation with *M. hydrolyticus* IRE-31, isolated from pulp mill effluent, surface fractures occur on LLDPE particles, indicating a possible better degrading capacity against PE with our bacterium strain.

SEM analysis of PU film sections after 4 weeks of culture with *P. aeruginosa* strain MZA-85 showed adhesion of strain MZA-85 to the surface with fractures spreading from the site of adherence, compared to where no such changes

Table 3: Effect of Concentration of Nitrate in Liquid Culture Method

S. No.	Name of the Microorganisms	OD Value	Concentration
1	PSC	0.04	2.26
2	PSA	0.11	2.01
3	PSP	0.04	2.26
4	PUC	0.19	2.20
5	PUP	0.14	1.91
6	PUA	0.54	0.47
7	BSA	0.19	2.20
8	BSC	0.05	2.23
9	BSP	0.22	1.62

PSC=Polystyrene control, PSA=Polystyrene *Aspergillus niger*, PSP=Polystyrene *Pseudomonas aeruginosa*, PUC = Polyurethane control, PUP = Polyurethane *Pseudomonas aeruginosa*, PUA = Polyurethane *Aspergillus niger*, BSC =Bioplastic control, BSA = Bioplastic *Aspergillus niger*, BSP =Bioplastic *Pseudomonas aeruginosa*.

Table 4: Effect of Concentration of Nitrate in Liquid Culture Method

S. No.	Name of the Microorganisms	OD Value	Concentration
1	PSA	0.19	2.20
2	PSC	0.16	1.83
3	PSP	0.25	1.51
4	PUA	0.23	1.58
5	PUC	0.31	1.30
6	PUP	0.21	1.65
7	BSC	0.23	1.58
8	BSA	0.19	2.20
9	BSP	0.28	1.40

PSC=Polystyrene control, PSA=Polystyrene *Aspergillus niger*, PSP=Polystyrene *Pseudomonas aeruginosa*, PUC = Polyurethane control, PUP = Polyurethane *Pseudomonas aeruginosa*, PUA = Polyurethane *Aspergillus niger*, BSC =Bioplastic control, BSA = Bioplastic *Aspergillus niger*, BSP =Bioplastic *Pseudomonas aeruginosa*

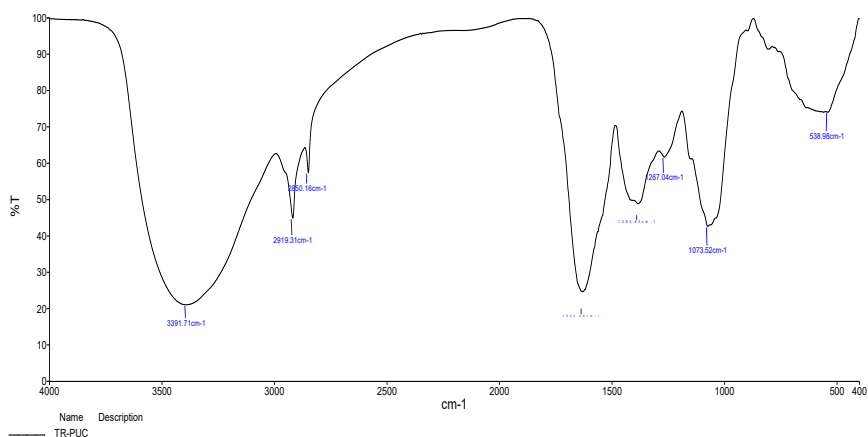


Fig. 5: FT-IR Spectra of soil buried polyurethane treated Polyurethane Control.

Table 5: Effect of Nitrate

S. No.	Standard mg/ml	Mean Value
1	10	3.102
2	5	2.533
3	2.5	1.987
4	1.25	0.944
5	0.625	0.884
6	0.312	0.762
7	0.156	0.557
8	0.078	0.191

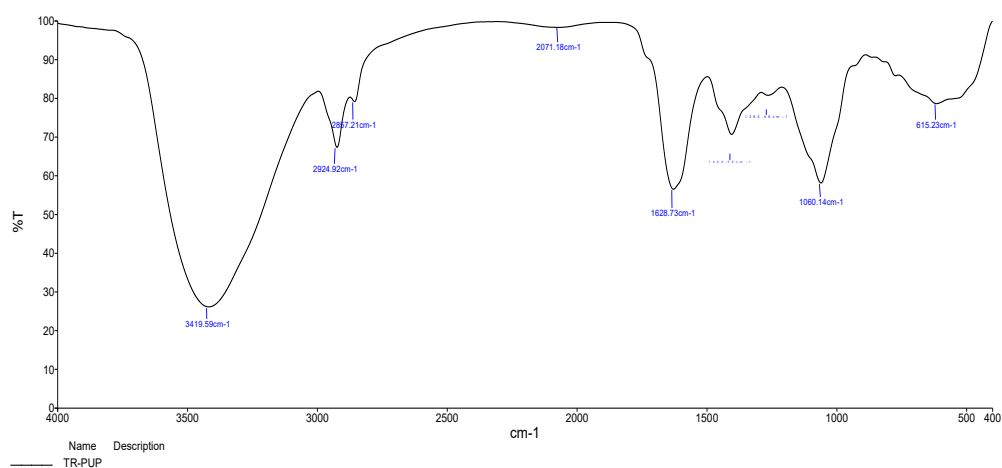


Fig. 6: FT-IR Spectra of soil buried polyurethane treated with *Aspergillus niger*.

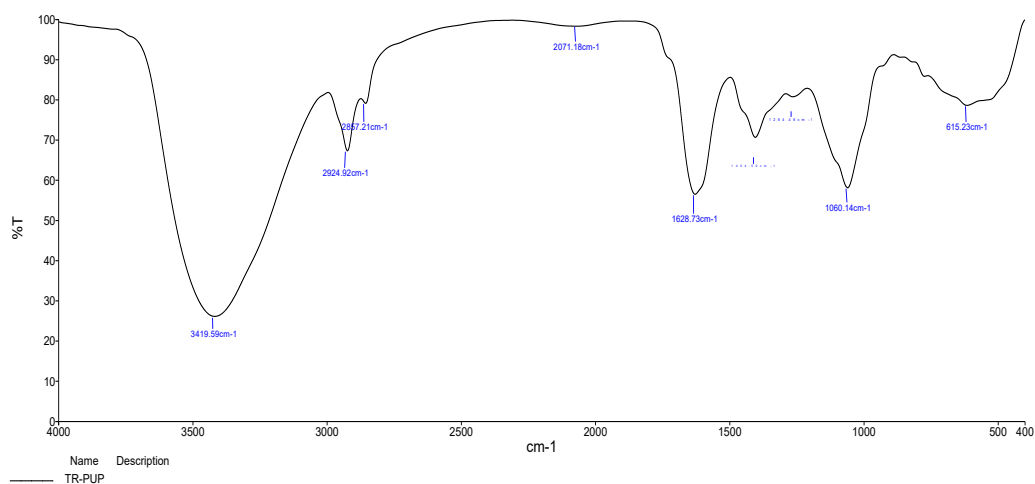
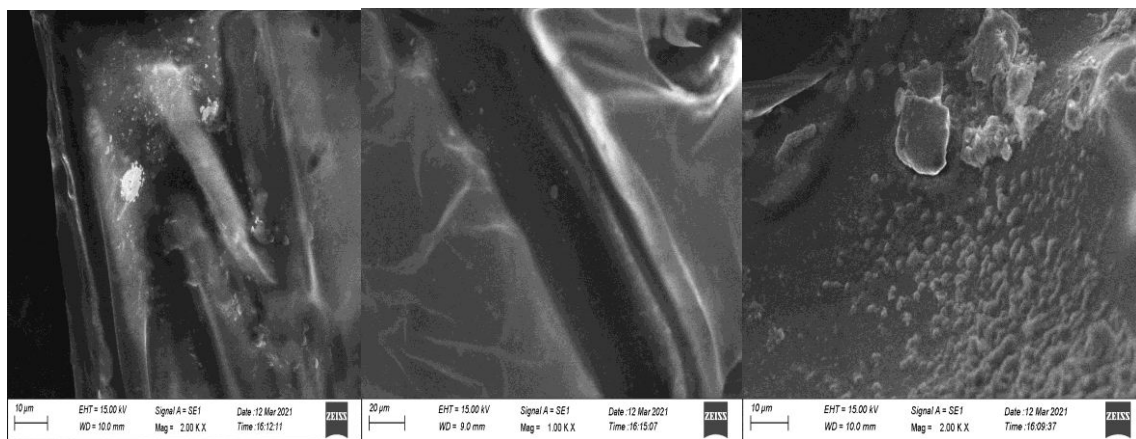


Fig. 7: FT-IR Spectra of soil buried polyurethane treated with *Pseudomonas aeruginosa*.

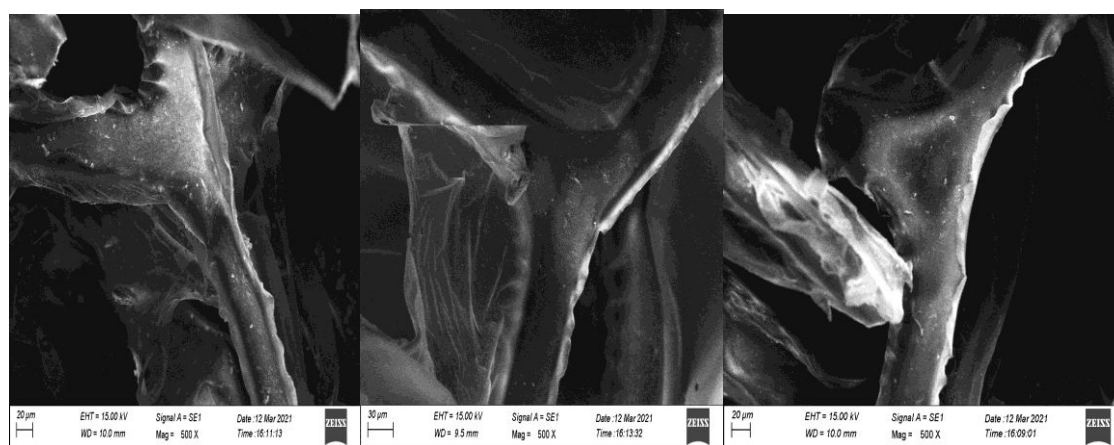


PUC

PUA

PUP

Fig. 8: Biodegradative effect of treated polyurethane foam by using SEM analysis with magnification 2.00KX. SEM captures of Polyurethane control (PUC), Polyurethane *Aspergillus niger*, (PUA) Polyurethane *Pseudomonas aeruginosa* (PUP), particles following 35 days of incubation or treatment under soil conditions.



PUC

PUA

PUP

Fig. 9: Biodegradative Effect of Treated Polyurethane Foam by Using SEM Analysis with magnification 500X. SEM captures of Polyurethane control (PUC), Polyurethane *Aspergillus niger* (PUA), Polyurethane *Pseudomonas aeruginosa* (PUP), particles following 35 days of incubation or treatment under soil conditions.

were observed (control). Strain MZA-85 cell attachment and surface fissures indicated PU film deterioration, which additional investigation verified.

Conclusion

Polystyrene, Polyurethane foams, and Bioplastic polymer were collected from the sewage soil. The biodegradation process using two microbes were *Pseudomonas aeruginosa* and *Aspergillus niger*. These are highly effective in the degradation of compounds. Soil burial and liquid culture methods were used for plastic degradation

because most of the plastics were thrown in the aquatic region and landfill. Polyurethane was buried in the soil and liquid cultured. Same as for the polystyrene and bioplastic was buried in the soil and used in liquid culture. After 10 days there was no change in the polystyrene and polyurethane, only in the bioplastic changes and weight loss was observed. After 35 days there was no changes in the polystyrene. Polyurethane had slight changes and weight loss has been observed.

Polyurethane compound is taken from the sources and washed with ethanol. FTIR technique

used for slightly degraded polyurethane to elucidate the structural characterization and to identify their functional groups. SEM analysis had shown the morphological changes in the degraded polyurethane. Polystyrene, polyurethane is a vast variety of plastics that take years to degrade both in liquid and soil culture. Bioplastic can also take a month to degrade. To consolidate these problems the isolated microorganisms were used to degrade in both soil and liquid culture. The microorganisms used were eco-friendly organisms. Within 35 days with the microorganisms, we can see the difference and changes in the plastic polyurethane foam. Bio-capsules can also be made with microorganisms which makes the increasing level of the microbial community and degrade quickly to make the plastic-free environment. This study may serve for the future development of effective bioremediation processes.

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