detritus | Multidisciplinary Journal for Circular Economy and Sustainable Management of Residues

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BIODEGRADATION OF EXPANDED POLYSTYRENE USING *PSEUDOMONAS AERUGINOSA* VITARK5

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Article Info:
Received:
5 November 2024
Revised:
25 February 2025
Accepted:
27 March 2025
Available online:
31 March 2025

Keywords:
Polystyrene
Biodegradation
Biofilm
Biosurfactant
Valorization
Circular bio-economy

ABSTRACT

Polystyrene, a widely used thermoplastic, polluting the environment in the form of micro/nanoplastics. Though traditional methods are commonly employed for plastic management, microbial degradation remains a more promising and eco-friendly approach. The present study focused on the biodegradation of expanded polystyrene (EPS) using microbes isolated from plastic-contaminated sites and assessing the degradants for their industrial importance. An isolate, VITARK5, was able to grow well in Bushnell Haas agar containing EPS as the only carbon source and was chosen for biodegradation studies. With robust glycolipid biosurfactant synthesis and biofilm formation, VITARK5 was identified to be *Pseudomonas aeruginosa*. In order to study biodegradation, VITARK5 was inoculated in Bushnell Haas broth containing thin EPS film and incubated at 37°C and 120 rpm for two weeks. After biodegradation, gravimetric analysis showed a 14.53% weight reduction of EPS film compared to the control. Formation of cracks and rough surfaces was observed on the film under scanning electron microscopy. GCMS analysis showed the presence of industrially important chemicals such as valerenol, 3-hydroxyl, 4-methoxy benzaldehyde, oxalic acid, dodecane, azacyclododecane and hexadecane. FTIR spectroscopy confirmed the presence of functional groups associated with the EPS and its additives. Biofilm formation and biosurfactant production synergistically would have promoted biodegradation. The findings of the present study suggest that the isolate *P. aeruginosa* sp.VITARK5 may be used in bioremediation for polystyrene degradation and valorisation to achieve circular bio-economy.

1. INTRODUCTION

Polystyrene (PS), consists of styrene monomer, are rigid and amorphous thermoplastic polymer containing aromatic rings. It has a cross-linked carbon structure that is highly recalcitrant, and hydrophobic, making it difficult to breakdown (Wang et al. 2024). PS, one of the most commonly used synthetic plastics, is a pertinent packaging and insulation material due to its lightweight and thermo stability (Ho et al. 2018). Expanded polystyrene (thermocol) is used for making food containers and disposable cups that turn out to be single-use plastic wastes contributing to 'white pollution' (Savoldelli et al. 2017). The toxicity and extension of pollution increase with decrease in the size and increase in the concentration of plastic particles produced by several environmental factors. Micro and nano polystyrene particles are more toxic to the aquatic and terrestrial organisms than their parent compounds present in macro polystyrene plastics. Various hazards of micro/nano polystyrene reported include morphological abnormalities, decreased photosynthetic activity and growth inhibition in microalgae; stress, cytotoxicity and histological changes in both aquatic and terrestrial invertebrates; neurotoxicity, genotoxicity, immunotoxicity, developmental toxicity, reproduction toxicity in fish; damage on leaves and roots, alteration in metabolites, changes in chlorophyll content and genotoxicity in plants. Furthermore, additives of PS such as bisphenol A, phthalate plasticizers and brominated flame-retardants are potential endocrine disruptors (Qiao et al. 2022). Furthermore, micro/nano polystyrene enter into food chain of aquatic and terrestrial ecosystem causing biomagnification and affect the public health (Siddiqui et al. 2023).

Several efforts were made to degrade plastics in an eco-friendly method using microorganisms and their associated products such as biofilm, biosurfactants and enzymes. *Pseudomonas* sp. is the most commonly searched bacterium for environmental clean-ups, involved in biodegradation of recalcitrant polymers (Gambarini et al. 2021). *Pseudomonas* sp. AKS2 and *Pseudomonas* sp.

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Detritus / Volume 30 - 2025 / pages 99-108
https://doi.org/10.31025/2611-4135/2025.19475
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E4 were reported for degrading polyethylene (PE) using hydrolase and alkane hydroxylase respectively (Wilkes and Aristilde 2017). Polyurethane (PU) was found to be degraded by *P. aeruginosa*, *P. protegens*, *P. cepacia*, and *P. chlororaphis* and *P. fluorescens* (Cregut et al. 2013). There are only few reports on degradation of polypropylene (PP) and polyvinyl chloride (PVC) using *Pseudomonas* sp. because of their high hydrophobicity. However, *P. azotoformans* and *P. stutzeri* could use polypropylene as a sole carbon source after treated with ultraviolet radiation (Arkatkar et al. 2010). Gosh et al. 2013 mentioned *Pseudomonas putida* AJ and *Pseudomonas fluorescens B* – 22 as PVC degrading microbes. Though there are many reports on microbial degradation of PET, *Pseudomonas* sp. was not found to be effective candidates for PET degradation (Wilkes and Aristilde 2017).

Among the petroleum-derived plastics, PS is a popular choice for food packaging industries, despite their biodegradability issues and effect on the environment. However, various research findings showed that PS can be biodegraded by several microbes using their enzymes and biofilms. For instance, *Pseudomonas* sp. isolated from plastic dump yard was able to degrade PS more than 10% after 30 days of incubation (Mohan et al. 2016). Similarly, *Rhodococcus ruber* and *Bacillus paralicheniformis* G1 were able to reduce weight of PS about 0.8% after 56 days and 34% after two months of incubation respectively (Roy and Chakraborty, 2024; Choi et al. 2024). Recently, PS biodegradation by the microbes associated with guts of insects such as *Tenebrio molitor* (Machano et al. 2022) and *Plesiophthalmus davidis* (Woo et al., 2020) is paid more attention. In particular, *Enterobacter hormaechei* LG3, *Klebsiella* sp. WJ2020 and *Cellulosimicrobium* sp. WJ2025 were some potential PS degraders isolated from *Tenebrio molitor* (Lin et al. 2024; Kang et al. 2023). The present study aimed to isolate and identify PS degrading microorganisms from plastic contaminated sites, examine their ability to produce biofilm and biosurfactants that facilitate biodegradation, assess their potential for PS biodegradation, and identify biodegraded metabolites and assess their industrial relevance for valorisation.

1.1 Nomenclature

- FTIR (fourier transform infrared spectroscopy)
- GCMS (gas chromatography mass spectrometry)
- PS (polystyrene)
- PE (polyethylene)
- PU (polyurethane)
- PP (polypropylene)
- PVC (polyvinyl chloride)
- PET (polyethylene trepthalate)
- CV (crystal violet)
- CTAB (cetyl trimethyl ammonium bromide)
- MB (methylene blue)
- THF (tetrahydrofuran)
- PCR (polymerase chain reaction)
- BLAST (basic local alignment search tool)
- NCBI (National Centre of Biotechnology Information)
- MEGA (molecular evolutionary genetics analysis)
- OD (optical density)
- LDPE (low density polyethylene)
- BHET (bis (2-hydroxyethyl) terephthalic acid)

2. MATERIALS AND METHODS

2.1 Polymer sample

Polystyrene samples in the form of Styrofoam (also known as expanded polystyrene or thermocol) used for packaging and insulation were used in this study.

2.2 Chemicals

Agar agar, phosphate buffer saline, crystal violet (CV), K_2HPO_4, KH_2PO_4, $(NH_4)_2SO_4$, $MgSO_4$, cetyl trimethyl ammonium bromide (CTAB), methylene blue (MB), and sodium dodecyl sulphate were obtained from Himedia Laboratories Pvt. Ltd., Mumbai. India. Arginine was obtained Loba Chemie Pvt. Ltd., Mumbai, India. Tetrahydrofuran (THF) was obtained from Avra Synthesis Pvt. Ltd., Hyderabad, India. Triton X-100 was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Nutrient broth (peptic digest of animal tissue 5 g/L; sodium chloride 5 g/L; beef extract 1.5 g/L; yeast extract 1.5 g/L) and Bushnell Has medium (K_2HPO_4 1 g/L, KH_2PO_4 1 g/L, $MgSO_4$ 0.2 g/L, $CaCl_2$ 0.02 g/L, NH_4NO_3 1 g/L and $FeCl_3$ 0.05 g/L) were obtained from Hi Media, Mumbai, India. PS agar medium (modified Charnock, 2021) contained two layers. The bottom layer was 50% nutrient strength of nutrient agar. The top layer was PS infused Bushnell Has agar medium. M63 minimal medium (O'Toole, 2011) used was with the composition of K_2HPO_4 7 g/L, KH_2PO_4 3 g/L, $(NH_4)_2SO_4$ 2 g/L, $MgSO_4$ 1 mM and arginine 0.4 g/L. CTAB-MB agar (modified Liu et al. 2013 and Hamzah et al. 2020) was nutrient agar containing 0.2 g/L CTAB and 0.005 g/L MB.

2.3 Sample collection

The soil samples and polystyrene samples that were either partially or completely buried were collected from plastic contaminated area in Villupuram, Tamil Nadu, India. They were collected in sterile sample container, transferred to laboratory within 24 hours and refrigerated at 4°C until further use.

2.4 Enrichment of PS degrading microbes

The collected polystyrene samples were cut and washed gently with distilled water thrice. One gram each of polystyrene and soil sample was added to Erlenmeyer flask (250 mL) containing 100 mL nutrient broth and the flask was incubated at 37°C and 120 rpm for 48 hours. After incubation, the nutrient broth served as the inoculum for the enrichment culture. A volume of 5 mL nutrient broth culture was inoculated into Erlenmeyer flask (250 mL) containing 100 mL Bushnell Haas medium with 1 g of PS samples and the flask was incubated at 37°C and 120 rpm for 14 days. After 14 days, a volume of 5 mL from the enrichment culture was used as inoculum for the successive culture. The procedure was repeated thrice. The culture was observed for turbidity throughout the enrichment period.

2.5 Isolation of PS degrading microbes

2.5.1 PS agar media preparation

The media contain two layers such as nutrient agar layer in the bottom and PS containing agar layer on the top. Nutrient agar of 50% nutrient strength was autoclaved and poured on petri plates covering quarter volume and allowed to solidify. PS solution was prepared by dissolving 0.1 g of PS in 25 mL THF. In 100 mL 1X PBS, 2.5% agar and 15 µL of Triton X-100 was added and autoclaved. Then, it was stirred for 5 to 10 minutes using magnetic stirrer and kept in sonicator wherein PS solution was added into the agar gradually using Pasteur pipette. The agar was poured on previously casted nutrient agar layer and allowed to solidify (Patil and Bagde, 2012 and Charnock, 2021).

2.5.2 Isolation and screening

The culture after triple enrichment was used for isolating PS degrading microbes. An appropriate volume of the culture was serially diluted upto 10^{-6} and spread on the petriplates containing PS agar medium and incubated at 37°C for a week. The plates were observed for growth of microbial colonies. The distinct colonies were subcultured on nutrient agar until pure single colonies were obtained. The isolates were again streaked on the PS agar media, incubated and observed for the duration of microbial growth. An isolate coded as VITARK5 grew well before 48 hours was chosen as the potential isolate.

2.6 Phylogenetic analysis of VITARK5

The isolate VITARK5 was sub-cultured, centrifuged and the pellet obtained was used for DNA isolation using High pure PCR template preparation kit, Roche. A set of universal primers 27F and 1492R were used for PCR and 518F and 800R were used for sequencing. The reaction mixture (25 µL) for PCR contained 0.5 µL (10 µM each) of forward and reverse primers, 0.5 µL template DNA, 2.5 µL of dNTP mix (2 mM) and *taq* DNA polymerase buffer (10x) and 0.2 µL *taq* DNA polymerase enzyme (5 U/µL). PCR thermal cycler was programmed for initial denaturation (94°C for 5 min), cycle denaturation (94°C for 45 s), annealing (55°C for 45 s), extension (72°C for 1 min) followed by 34 cycles and a final extension (72°C for 5 min). The PCR products were resolved onto 1% agarose gel, purified and sequenced. The retrieved sequences were aligned using basic local alignment search tool (BLAST) for similarity search and were submitted to National Centre of Biotechnology Information (NCBI) GenBank database. A phylogenetic tree was constructed using molecular evolutionary genetics analysis (MEGA) software to identify the closely related microorganisms.

2.7 Biosurfactant production

2.7.1 Oil displacement test

It is one of the qualitative tests for detecting the presence of biosurfactants. An overnight culture of VITARK5 in nutrient broth was centrifuged and the supernatant was tested for the presence of biosurfactant. The test was performed on a petri dish containing 20 µL of crude oil forming film on the surface of 20 mL distilled water. A volume of 10 µL supernatant, distilled water and tritonX100 was added on the oil surface of test, negative control and positive control plates respectively. They were observed for formation of zone of oil clearance (Hamzah et al. 2020).

2.7.2 CTAB-MB agar test

It is a semi-quantitative test to identify anionic biosurfactants such as glycolipids (Thavasi et al. 2011). The assay was performed by modifying the medium composition in Liu et al. 2013 and Hamzah et al. 2020. Nutrient agar containing 0.2 g/L CTAB and 0.005 g/L methylene blue was used as the medium for the test. Wells were made in the agar medium into which a volume of 50 µL supernatant, obtained for previous test, was inoculated and incubated at 37°C. The dark blue halo formation around the wells would be observed for anionic biosurfactants (Hamzah et al. 2020).

2.8 Biofilm formation

Microtiter plate method was used to test the biofilm forming ability of the isolate VITARK5. It was grown overnight in nutrient broth and diluted to 1:100 with fresh M63 minimal medium containing arginine. A volume of 100 µl was added to 8 wells (D1 to D8) of 96 well plate and incubated at 37°C overnight. The culture was removed carefully and the wells were washed with phosphate buffer. A volume of 125 µl of a 0.1% CV solution was added and kept at room temperature for 15 min followed by washing the excess stain with phosphate buffer. The plate was turned upside down on tissue paper and allowed to dry overnight. Then, 125 µl of 30% acetic acid solution was added to each well to solubilize the CV and incubated at room temperature for 15 min. All the solutions were transferred to new wells and absorbance (OD_{550}) was measured in a plate reader using 30% acetic acid as a blank (O'Toole, 2011). Based on the average and standard deviation (SD) of OD values of control and test, the cut-off OD (ODc) and final OD values were calculated by the formula given below. The isolate was categorised as weak, moderate and strong for biofilm production by comparing the OD values as shown in Table 1 (Stepanovic et al. 2007).

$$Cut\ off\ value\ (ODc) = (Average\ OD\ of\ control) + (3*SD)$$
$$Final\ OD = Average\ OD\ of\ test - ODc$$

2.9 PS film biodegradation

2.9.1 PS film preparation

PS solution (0.1 g/mL) was prepared by dissolving 5 g of PS sample in 50 mL of THF. PS film was prepared from PS solution. A volume of 10 mL PS solution was poured and casted onto petri plates and kept under fume hood for two days at room temperature. The film thus obtained was cut into appropriate pieces, washed with 75% ethanol, dried with tissue paper and weighed (Patil and Bagde, 2012).

2.9.2 PS film biodegradation assay

Bushnell Has broth supplemented with 0.01% yeast extract was used as the medium for biodegradation

TABLE 1: Biofilm forming ability of VITARK5 based on absorbance.

S. No	OD value	Biofilm production
1.	OD ≤ ODc	Nil
2.	ODc < OD ≤ 2*ODc	Weak
3.	2*ODc < OD ≤ 4*ODc	Moderate
4.	4*ODc < OD	Strong

test. Erlenmeyer flask (250 mL) containing 100 mL of aforementioned medium and pre-weighed PS film inoculated with 1% VITARK5 was used as test whereas uninoculated culture medium served as control. Seed culture of VITARK5 showing OD_{600} range from 0.1 to 0.6 was used for inoculation (Ganesh Kumar et al. 2021). The test and control flasks in triplicates were incubated at 37°C and 120 rpm for two weeks. Plastic films were sampled using sterile tweezers on the last day (Day 14) of incubation in order to assess their biodegradation.

2.10 Assessment of PS film biodegradation

2.10.1 Gravimetric analysis

Plastic films in the test and control culture were washed once with 2% sodium dodecyl sulphate solution to remove bacterial colonies and twice with distilled water on the day of sampling (Khandare et al. 2021). The films were dried gently with tissue paper followed by drying in hot air oven overnight at 50°C. The films were weighed and checked for weight loss. Degradation (%) of plastic samples in the control and test was calculated as weight reduction (%). Biodegradation (%) was calculated by correcting degradation (%) of the test with control. The formulae for weight reduction (%) and biodegradation (%) are given below.

*Weight reduction % = [(Initial weight – Final weight)/ Initial weight]*100*
Biodegradation % = Degradation % of test – Degradation % of control

2.10.2 SEM analysis

The plastic films in the control and test culture were analysed using scanning electron microscope (ZEISS EVO 18). Their surfaces were observed for superficial changes such as roughness and cracks formation due to biodegradation.

2.10.3 FTIR analysis

The chemical modifications of plastic films due to biodegradation were detected by measuring the changes in functional groups on the surface of PS films through FTIR (IRA Shimadzu AVATAR 330, Japan) analysis in ATR mode. OMNIC Software was used for IR spectra analysis and OriginPro was used for constructing graph of the spectral values.

2.10.4 GCMS analysis

The biodegradation products of PS were analysed using Gas Chromatography Mass Spectrometry (GC-MS, Shimadzu, GC-2030, MS-QP2020). The major peaks of the chromatograms were identified using a library search.

2.11 Statistical analysis

MS Excel was used to perform statistical analysis. F-test and Welch's t-test was performed to determine statistical significance between control and treated samples of biofilm formation assay and biodegradation test. A paired t-test was performed to determine stastical difference between the data before and after biodegradation in control and treated samples. A significance level of $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Isolation and identification of VITARK5

The triple enrichment culture used for isolation of PS degrading microorganisms was turbid implying microbial growth. On isolation, six colonies showed distinguished morphology on PS agar plates and were named as VITARK1, VITARK2, VITARK3, VITARK4, VITARK5 and VITARK6. The isolate VITARK5 grew well among the other isolates within 48 hours and was chosen for further studies. It was subjected to molecular taxonomic identification and phylogenetic analysis. A BLAST search and phylogenetic tree of 16S rRNA sequence of VITARK5 showed the closest similarity to *Pseudomonas aeruginosa* (Figure 1) and therefore it was designated as *Pseudomonas aeruginosa* sp.VITARK5. The 16S rRNA sequence of the isolate was submitted to the GenBank under the accession number OQ875739.

3.2 Biosurfactant detection and identification

VITARK5 was able to develop a zone of clearance on the oil surface confirming the presence of biosurfactant (Figure 2) and also form a blue halo on CTAB-MB agar suggesting that the biosurfactant produced was anionic and glycolipid in nature (Figure 3).

3.3 Biofilm formation test

The biofilm produced by VITARK5 was stained with crystal violet and OD_{550} was measured (Figure 4). The cut off OD value (ODc) from the control was calculated as 0.044 and final OD value from the test was calculated as 0.202. The calculated values were compared with the standard table (Table 1) and VITARK5 was found to be strong biofilm producer (i.e) 4*ODc < OD.

3.4 PS film biodegradation

3.4.1 Gravimetric analysis

PS films in the control and test cultures were weighed before and after biodegradation. The weight of PS film in control and test culture was 0.167 g and 0.178 g before biodegradation and 0.165 g and 0.150 g after biodegradation respectively. The degradation of PS based on weight reduction was calculated as 1.2% and 15.73% for control and test respectively. The weight reduction in control might be due to leaching out of additives. Therefore, the degradation (%) of the test was corrected with degradation (%) of the control and calculated as 14.53%.

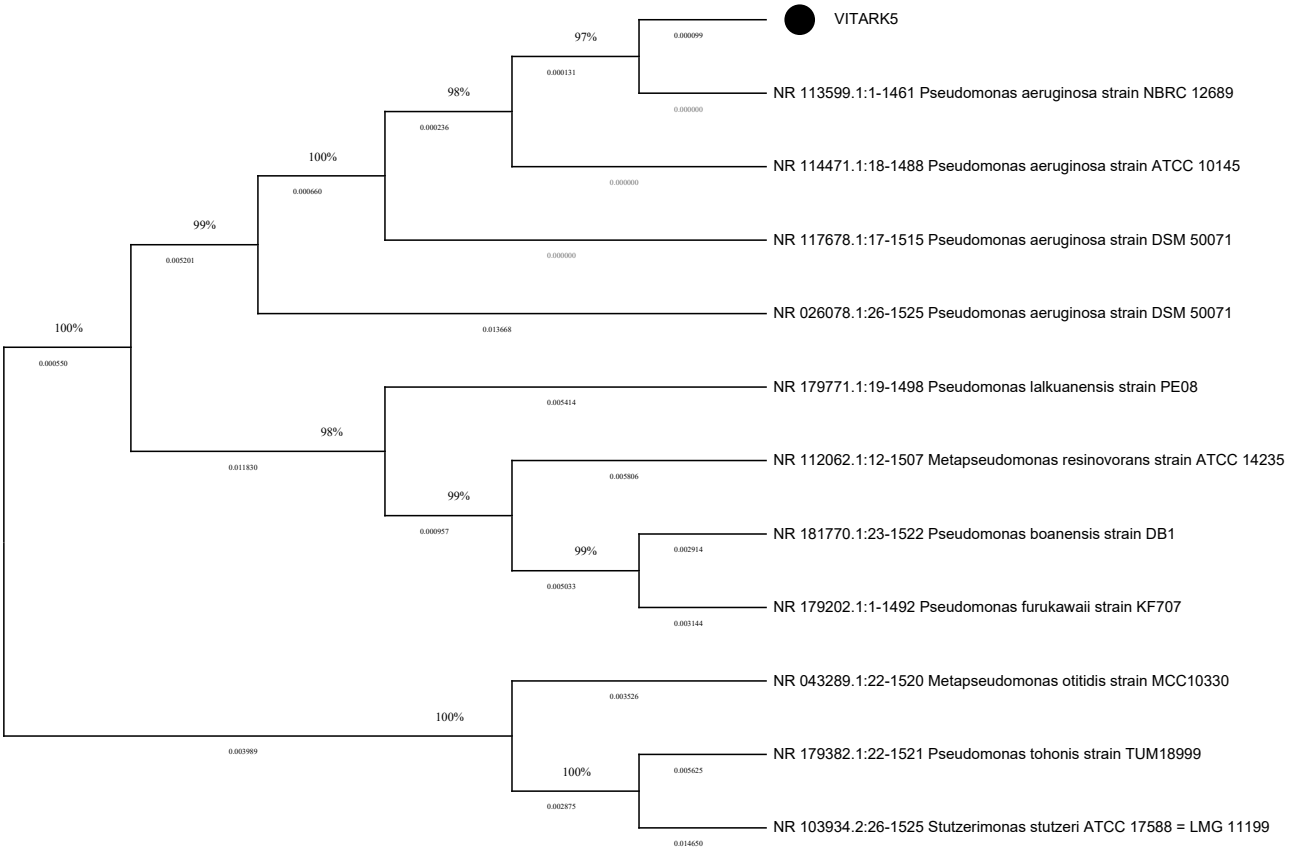

FIGURE 1: Phylogenetic analysis of VITARK5 showing closest relationship with *Pseudomonas aeruginosa*.

3.4.2 SEM analysis

Surface of PS films in the control and test cultures were observed for morphological changes after biodegradation. The surface was observed to be rough and uneven in nature. SEM analysis revealed the crack, pit formation, rupture, and rough surfaces on PS film due to biodegradation by VITARK5 (Figure 5 and Figure 6).

3.4.3 FTIR analysis

FTIR results confirmed the surface changes on the PS film of control and test cultures (Figure 7 and Figure 8). FTIR analysis of PS was well studied by Smith, 2021 and our results were compared with it for identifying significant peaks. The peak at 537.07 cm^{-1} in control and test indicated the presence of halogen compounds that would possibly

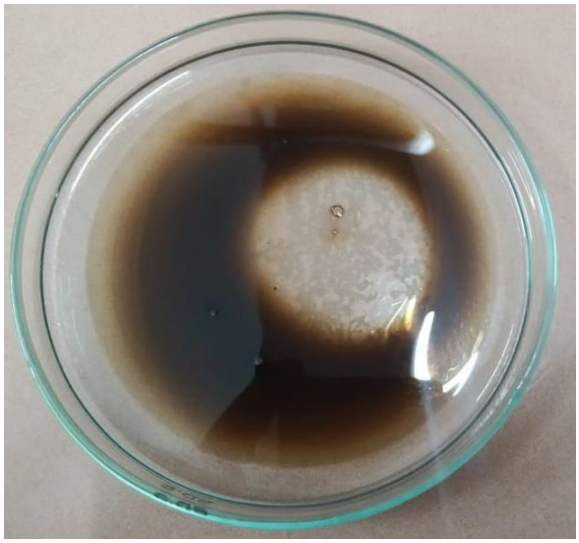

FIGURE 2: Zone of oil clearance by the supernatant of VITARK5 indicating the presence of biosurfactant.

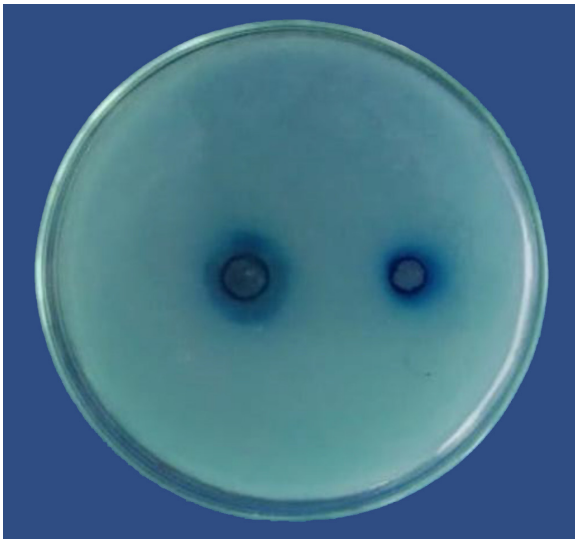

FIGURE 3: Blue halo formation by the supernatant of VITARK5 indicating the presence of glycolipids biosurfactant.

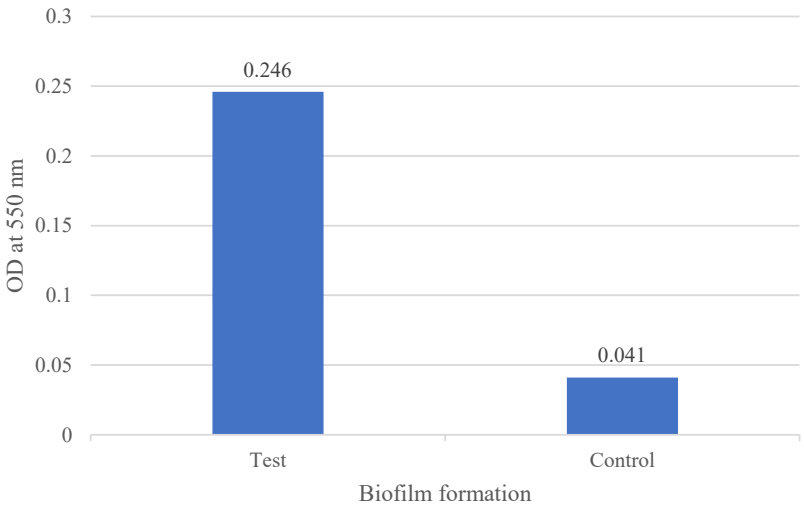

FIGURE 4: A graph showing the biofilm forming ability of VITARK5 compared to control.

be from the presence of brominated hydrocarbons such as hexabromocyclododecane and hexabromocyclohexane used as flame retardants. The absorption peaks ranging from 1620 to 1400 cm^{-1} could identify the ring formation due to C-C stretching in benzene (Smith, 2021). Likewise, peaks at 1451.17, 1492.63 and 1600.62 cm^{-1} were observed in test while at 1452.13 and 1492.63 cm^{-1} in control. The ring bending representing mono-substituted benzene was confirmed by peaks at 696.17 and 695.21 cm^{-1} in control and test respectively. Also, C-H wagging corresponding to the benzene was showed by peak at 751.13 cm^{-1} in both control and test. Furthermore, unsaturated C-H stretching of benzene was observed at 3024.80 cm^{-1} in test. The methylene group in the styrene showed absorption peaks at 2921.9 and 2848.6 cm^{-1} (Fang et al, 2016). Accordingly, there were peaks at 2850.27 and 2923.55 cm^{-1} in test and at 2923.59 cm^{-1} in control. The peaks at 1027.87 and 1069.33 cm^{-1} in test represent the functional group of vinyl ether and primary alcohol respectively. These peaks were seen only in the test implying that they were formed from the biochemical reactions of VITARK5 with PS film.

3.4.4 GCMS analysis

The control and test GCMS spectrum (not shown) was compared to identify the compounds that were degraded, converted and newly formed during biodegradation process. The compounds that were found commonly in test and control such as benzene, oxetane, octadecane, cyclododecane, disiloxane, butenedioic acid, diethyl mercaptal, pentamethyl benzaldehyde and bis(2-ethyl hexyl) phthalate were considered as non-degraded compounds. The compounds that were found only in control such as cyclopentane, 2,5, dimethylbenzophenone, o-cymene, triacontane, eicosane, octane, pentadecanol and furan were considered as degradable or convertible compounds. These compounds would have also leached out from PS film and converted into new compounds. The compounds that were found only in test samples such as benzene propanoyl bromide, dodecane, hexadecane, azacyclododecane, octanal, tridecanal, oxalic acid, valerenol, 3-hydroxyl,4-methoxy benzaldehyde and n-heptyl isocyanate were considered as newly formed or derived compounds.

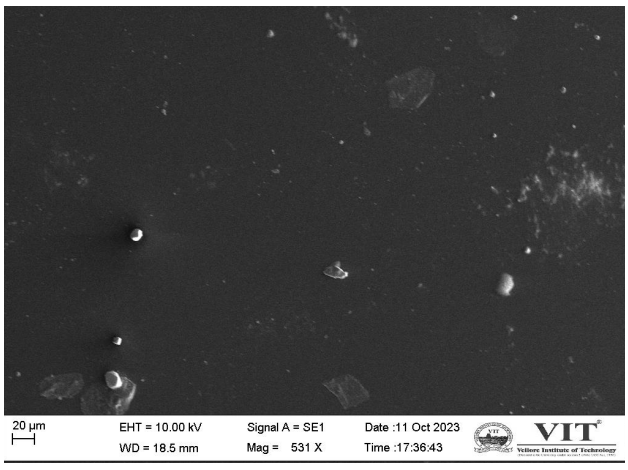

FIGURE 5: SEM image of PS film in control group with no pits or cracks.

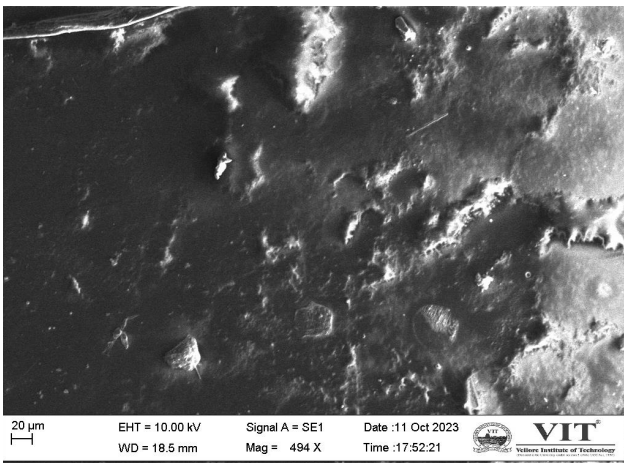

FIGURE 3: SEM image of PS film in test group with rough and cracked surface.

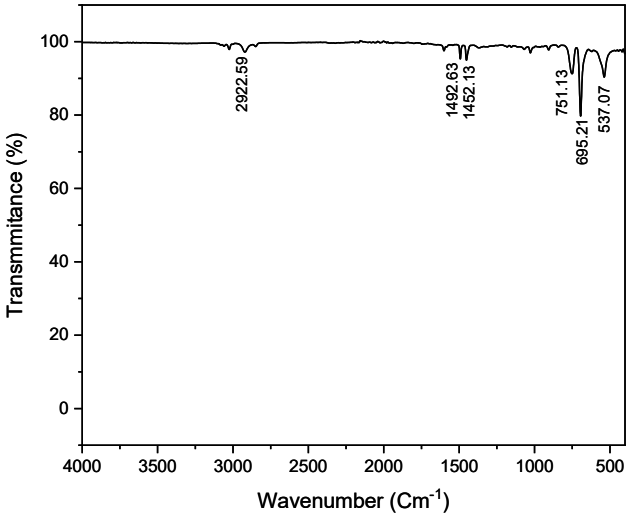

FIGURE 7: FTIR peaks of PS film in control group.

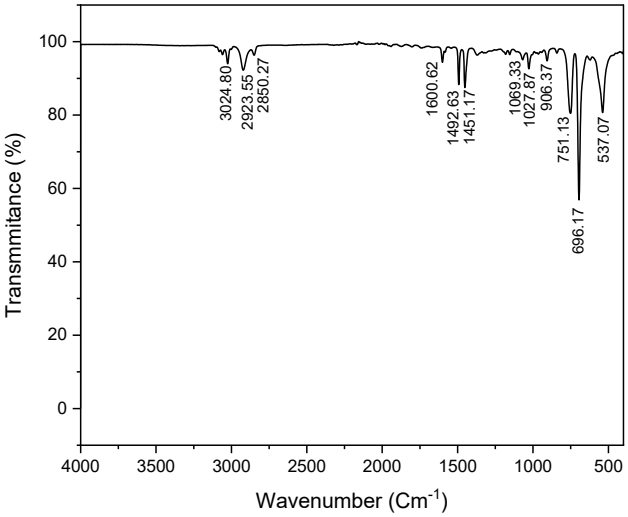

FIGURE 8: FTIR peaks of PS film in test group.

3.5 Statistical analysis

An F-test was performed with the values of control and test groups of biodegradation test and biofilm formation test in order to determine the significance of variance. The observed results showed that unequal variance ($p < 0.05$). The results of Welch's t-test showed that the biodegradation and biofilm formation was statistically significant ($p < 0.05$). The paired t-test was found to be non-significant ($p > 0.05$) for control and significant ($p < 0.05$) for test. These results showed that the degradation in the control was negligible and it was significant in the test.

4. DISCUSSION

Plastic contamination increases the environmental stress by limiting nutrient availability for microbial communities other than plastics. In order to survive, microbes adapt to utilise the plastics as carbon source and thus they are plastic degraders. Therefore, in this study, a plastic contaminated site was chosen for isolating PS degrading microbes. The major challenge in PS biodegradation is the surface properties of PS that hinder microbial interactions. Microbes with the ability to produce biosurfactants, amphiphilic molecules that bridge between hydrophobic and hydrophilic surfaces, and biofilm, an assemblage embedded in an extracellular matrix, are crucial to overcome these hindrances. Therefore, PS degrading isolate VITARK5 was tested for biosurfactant production and biofilm formation.

The inherent hydrophilic character of cell surfaces hinder their interaction with PS necessitating the attachment of hydrophilic functional group on PS surface. Such modifications enable improved adhesion of bacterial colonies and increased accessibility of secreted extracellular enzymes to the polymer surface (Wilkes and Aristilde 2017). These modifications can be carried out by the bio-surfactants that contain polar head attaching to hydrophilic surface while non-polar tail attaching to PS region. Ghosh et al. (2019) reported that biosurfactants secreted by *P. azotoformans* and *P. stutzeri* were able to make hydrophobic polypropylene films into slightly hydrophilic. It is well-known that *Pseudomonas* sp. commonly produce rhamnolipids that are anionic and glycolipid biosurfactants. Noordman and Janssen, (2002) found that rhamnolipids produced by *P. aeruginosa* were able to stimulate assimilation of hydrophobic compounds. Evidently, Taghavi et al. (2022) investigated the effect of rhamnolipid on microbial colonisation and biodegradation of thermoplastics and showed the feasibility of increasing the biodegradation efficiency of PS in the presence of rhamnolipid. Therefore, biosurfactant activity shown by VITARK5 during PS film biodegradation would be because of rhamnolipid production. On the other hand, rhamnolipids were reported for having various role in biofilm development especially microcolony formation (Pamp and Tolker-Nielsen, 2007).

Biofilm is one of the key strategies for the survival of species against unexpected changes of living conditions such as temperature and nutrient availability (Thi et al., 2020). It allows for better microbial and enzymatic interactions through direct contact with the polymer, which in turn, speeds up the biodegradation (McFall et al. 2024, Plakunov et al. 2020). In the study *Pseudomonas aeruginosa* sp.VITARK5 isolated from plastic contaminated site was capable of producing biofilm. *Pseudomonas aeruginosa* is indeed a good biofilm producer and a model organism for biofilm formation (Thi et al., 2020). Thus, VITARK5 could attach on the hydrophobic surface and would have aided in PS biodegradation. Consequently, *Pseudomonas* spp. AKS2 showed increased cell surface hydrophobicity and LDPE biodegradation when they were allowed to form biofilm. It was also found that exopolysaccharides produced by biofilm facilitated microbial adherence to the LDPE surface (Wilkes and Aristilde 2017). It is attested by previous studies on biofilm forming *Pseudomonas* species capable of degrading plastics such as polyethylene, polypropylene and polyvinyl chloride (Ghosh et al. 2019; Giacomucci et al. 2019). Moreover, *P. aeruginosa* employs a complex network of interconnected signal transduction pathways, referred

to as quorum sensing which facilitates communication among individual bacterial cells to form self-aggregated biofilm matrix effectively. Beyond the development of biofilms, quorum sensing has been associated with the regulation of various physiological processes essentially include tolerance to stress and adjustments in metabolism (Thi et al., 2020). This in turn makes *P. aeruginosa* to cope up with utilisation of hydrophobic substrate such as plastics for survival. Furthermore, it facilitates befriending other microorganisms of its kind to develop symbiotic consortium that are effective in degrading plastics. For instance, a co-culture of *Bacillus subtilis* MZA-75 and *P. aeruginosa* MZA-85, yielded the highest weight reduction of PU film, in addition to demonstrating the superior esterase activity when compared to the respective strains individually. (Shah et al. 2016). Also, Five distinct strains of *Pseudomonas* and *Bacillus* species as consortium isolated from petroleum contaminated site exhibited synergistic growth utilising PET and bis (2-hydroxyethyl) terephthalic acid (BHET) as their sole carbon sources (Roberts et al. 2020).

PS is generally poly(1-phenylethene-1,2-diyl) meaning that it can be converted to mono-, di- and oligomers of aliphatic or aromatic compounds with the help of enzymes such as oxidoreductases and hydrolases produced by VITARK5. Accordingly, valerenol, 3-hydroxyl,4-methoxy benzaldehyde, Azacyclodecan-2-one and oxalic acid were produced after biodegradation. The major biochemical reactions of reported PS degrading enzymes are PS hydroxylation (Alkane-1 monooxygenase (alkB) and Serine hydrolase), PS depolymerisation by free radicals (Laccase AbiLac1) and hydrolysis of phenyl acetic acid (Arylesterase) (Kim et al. 2024). Alkane hydroxylases and monooxygenases are capable of breaking C-C bonds whereas ring-hydroxylating dioxygenases are capable of breaking PS side chains producing aromatic ring compounds leading to PS depolymerisation (Hou et al. 2021). Choi et al. 2023 reported that styrene monooxygenase, styrene oxide isomerase, phenylacetaldehyde dehydrogenase, and phenylacetyl coenzyme A ligase are some of the key enzymes involved in PS degradation. Though various reports on PS degrading enzymes are available, it is quite difficult to find out the enzymes that degrade expanded PS completely because of its complex structure and composition.

Plastics are not only made up of its respective monomers but also of additives that make them super strong to withstand extreme environmental conditions and resist biodegradation. According to Rijkse and Metsaars 2008, the composition of expanded polystyrene may include but not limited to filler, styrene, blowing agents, flame retardants, nucleating agents, plasticisers, demoulding agents, free-radical polymer initiator, suspension stabiliser, chain-transfer agents and cross-linking agents. Plastics degrading microbes thus face dual challenge of degrading hydrophobic plastic polymers as well as their toxic additives. From GCMS results, it was apparent that VITARK5 degraded PS and additives present in the expanded PS. The degradants produced after biodegradation by VITARK5 were matched with their possible parent compounds for better understanding of PS biodegradation. For instance, butadiene, α-methyl styrene and divinyl benzene are some of the cross-linking agents from which butadienoic acid, pentamethyl benzaldehyde, cymene, dimethyl benzophenone, benzene would have derived. Flame-retardants such as hexabromocyclododecane and hexabromocyclohexane would have broken down to form cyclodecane and benzene propanoyl bromide. Mineral oil and paraffin waxes are used as plasticisers that might be responsible for production of several alkane compounds include octadecane, octane, triacontane, eicosane, hexadecane and oxetane. However, some of the compounds produced such as oxalic acids were the by-products of central metabolic pathway and found to enhance the biodegradation. For instance, oxalic acid produced by P. aeruginosa NY3 during n-hexadecane biodegradation was found to increase the degradation efficiency by acting as promoter (Nie et al. 2017).

Pseudomonas aeruginosa VITARK5 could not only degrade PS but also produce biosurfactants, showing that PS can be exploited as a substrate for biosurfactant synthesis. The PS biodegraded products such as hexadecane, dodecane, 3-hydroxyl,4-methoxy benzaldehyde and oxalic acid find their applications in fuel, pharmaceutical, textile and food sectors. For instance, oxalic acid was used for metal removal (Shathi et al. 2024) and for postharvest quality preservation of fresh fruit and vegetables (Hasan et al. 2023). Therefore, *P. aeruginosa* VITARK5 can be used for PS biodegradation and bio-valorisation that not only mitigates PS waste but also useful for producing economically significant products. However, the entire potential of this strategy will depend on future developments in biotechnology and sustainability regulations.

5. CONCLUSIONS

This study suggests that plastic contaminated soil samples can be used to isolate potential plastic degrading microorganisms. *Pseudomonas aeruginosa* sp.VITARK5 is a potential PS degrading microbe that produces glycolipid biosurfactants and strong biofilm facilitating microbial adherence and colonisation on PS surface. Thus, VITARK5 degrade PS with the help of biofilm, biosurfactant and enzymes. Further research on medium optimisation for biofilm and biosurfactant production using PS as substrate would enhance biodegradation. The degradants of biodegradation were found to be commercially important and therefore biovalorisation can be achieved.

Though various reports are available on microbial degradation PS, most of the reported microbes such as *Bacillus* sp. and *Pseudomonas* sp. are pathogenic. It obstructs their direct application on contaminated environment due to horizontal gene transfer of multi-drug resistance and other toxic traits. Therefore, PS needs to be segregated from contaminated area and treated under controlled environment using these microbes. However, segregation of plastics is a cumbersome and long-drawn-out process. Development of sensors to detect plastics would make the process easier. Though microbial degradation produce less toxic metabolites, their slow

degradation rate necessitate effective approaches that enhance rate of the reaction. Tandem approaches like combining biological degradation with physical or chemical pretreatment methods would enhance the degradation and can also be used for production of value added products. For istance, PS is converted to polyhydroxyalkanoate (PHA) by *Pseudomonas putida* CA-3 using a hybrid chemo-biological method (Ward et al., 2006). Savoldelli et al. (2017) used thermal degradation followed by biological degradation for the production of useful by-products such as benzene and naphthalene from PS using different microbes among which *P. putida* was found to be more productive. Furthermore, various tools and techniques of biotechnology such as genetic engineering, metabolic engineering, protein engineering and multi-omics would not only help in the discovery and engineering of many PS degrading microbes and enzymes but also facilitate optimisation of bioprocesses required for valorisation. Future research directions of the present work include increasing the degradation efficiency of *Pseudomonas aeruginosa* sp. VITARK5 by genetic modifications and medium optimisation, identification and characterisation of key enzymes involved in PS biodegradation for enzyme-mediated PS bioremediation, and scaling up the experiments for assessing the feasibility and integration of PS biodegradation for real time applications.

ACKNOWLEDGEMENTS

We would like to thank the management of VIT University for providing the necessary research facilities to carry out this work.

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