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Biodegradation of Polyvinyl Chloride Film by Bacterial Consortium isolated from various contaminated sites of Gwalior

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ABSTRACT
Plastic Pollution in all over the world is the emergent in this present-day scenario. Material is demolished and generated all the time, But if there is anything that goes against this rule, it can create disaster to the entire universe.one such matter is plastic, Plastic are characteristically inert and resist to microbial attack and therefore they remain in the nature without any deformation for very long time. PVC is the record toxic plastic for our health and it's not good for the environment either. Biodegradation of plastic waste by using microbial strain could offer an eco-friendly solution to this problem due to their diverse metabolic capability, adaptability to different environment conditions. In the present study poly vinyl chloride was treated in the presence of developed bacterial consortium in laboratory .The consortium was developed using three bacteria, selected on the basis of utilization of PVC as primary carbon source, namely Pseudomonas spp., Bacillus spp. and Paenarthrobacter spp. isolated from the plastic waste disposal sites of Gwalior. The progressive treatments of PVC were conducted for six months in liquid culture medium under control condition. For this purpose, bio formulation of consortium was prepared and characterized for the viability up to 45 days of storage at 30°C .The consortium treated polymer samples were monitored through SEM and FT-IR spectroscopy. Analytical data revealed the bio deterioration potential of the developed consortium for PVC which could help in disposing the plastic waste. It will be helpful in the deduction of such kind of hazardous substances from the environment that leads to the accomplishment of sustainable and plastic free environment.
Keywords- Plastic pollution, Biodegradation, Polyvinyl chloride and Bacterial Consortium

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INTRODUCTION

In the year 2018, worldwide plastic production reached 359 million tonnes, while India creates roughly 3.4 million tonnes of plastic garbage each year, of which 10,376 tonnes are left uncollected on Day 1 (1). Plastics are a resistant substance that takes decades to breakdown, resulting in a worldwide build-up of 6.3 billion tonnes (2). Similarly, wastes generated as a result of plastic use are widely distributed in the maritime environment, with a total estimated weight of 5 trillion tonnes (3). Plastic is one of the most important and widely used materials on the planet, thanks to desirable properties such as light weight, low maintenance requirements, weathering resistance, low toxicity, transparency, and low cost, which make it ideal for use in a variety of industrial, commercial, and agricultural applications (4,5). Polyvinyl chloride (PVC) ranks third in plastic manufacturing from petroleum-based goods in India (5 million tonnes in 2018), behind polyethylene and polypropylene, to fulfil the growing demand (1).

Due to its widespread residential and commercial uses, polyvinyl chloride (PVC) is the third most manufactured plastic in the world. PVC's great strength and pliability have led to its widespread use in packaging materials, toys, furniture, plumbing, flooring, and construction components (6). When PVC is released into the environment, it is difficult to manage due to its persistence. Traditional PVC and associated product management strategies include landfilling, recycling, and combustion. These procedures are not environment friendly since they emit harmful by products, such as CO_2, chlorofluorocarbon (CFC), vinyl monomers, and dioxins, which pollute the atmosphere or the terrestrial environment (7).Biodegradation of plastic waste by using microbial strain could offer an ecofriendly solution to this problem due to their diverse metabolic capability, adaptability to different environment conditions. The presence of more than one bacterial strain in comparison to single isolates may enhance the degradation efficiency of the bacterial culture due to their co-operative activity.

Scientists around the world are looking for eco-friendly techniques for the safe handling and management of the plastic waste addition, two more consortiums have been reported for the degradation and use of polyvinyl chloride (PVC) as a carbon and energy source (8). Earlier describes the development of a talc-based formulation for long-term bacterial consortium storage, as well as how to degrade polymer (LDPE and PVC) by introducing a bacterial consortium consisting of *Microbacterium sp., Pseudomonas putida*, *Pseudomonas aeruginosa, Pseudomonas otitidis, Bacterium* Te(9). Similarily(worked on a bacterial consortium comprised of four bacterial species, *Pusdomonasotitidis, Bacillusaerius, Bacilus cereus, and A. pedis*, which used PVC as their primary carbon source (10).

Various microorganisms, including *Pseudomonas aeruginosa, Aureobasidium pullalans, Rhodotorula aurantiaca, and Kluyveromyces sp.*, have been reported to cause in situ colonisation and substrate deterioration of plasticized PVC (11). In addition, the colonisation of *A. pullulans* causes significant substratum damage to the plasticized PVC, followed by *R. aurantiacai Kluyveromyces sp.,* indicating that microbial succession occurs during the colonisation process and that *A. pullulans* has been essential to the establishment of a microbial community on the polymers (12). It has been reported that the plasticizer content of PVC degrades in the presence of *P. fluorescens*, *Coryneform bacterium, and Mycobacterium sp* (13).Similar worked on the isolation and characterization of bacteria that degrade polyvinyl chloride (PVC) from plastic-contaminated soil(14).As PVC degrading bacteria, *E. coli, Staphylococcus*, *Pseudomonas,* and *Klebsiella* were identified. *Bacillus flexus* and *Pseudomonas citronellolis* species were used in the degradation of PVC by (15). However, degradation of thermoplastic in natural conditions is currently a global problem (16). Consequently, there is an urgent need to develop efficient consortia and their products in order to solve the pressing problem, namely the management of plastic waste in nature.The current work focuses on the isolation and identification of indigenously isolated bacterial species in order to assess their PVC biodegradation capacities. In this study, a variety of techniques were employed to characterise the degradability of the PVC films, including visual inspection, PVC weight loss, scanning electron microscopy (SEM) to observe surface changes, the Sturm test to assess CO_2 production, and Fourier transform infrared spectroscopy (FT-IR) to analyse structural changes in the polymer .The differential reaction of indigenously created bacterial consortium in polyvinyl chloride degradation was investigated in this research to preserve the environment healthy, dynamic, and secure.

MATERIAL AND METHOD

Polymer samples -The Polyvinyl chloride film were obtained from Gwalior Plastic Industry, Pinto Park, Industrial Area, Gwalior, M.P., and the powered (resin) form of Polyvinyl chloride were provided by Supreme Pvt. Ltd.,Malanpur, Industrial Area, Gwalior (M.P.).

Collection of soil samples-Plastic materials were observed polluting the locations either openly or partially covered in the soil. Soil samples were gathered from several municipal trash dumping sites in Gwalior and its surrounding disposal sites in the Gwalior city of Madhya Pradesh, India, where garbage has been disposed of extensively for many years.

Soil Burial Experiment- Soil was obtained from Botanical Garden area of Jiwaji University Campus. The soil was then screened to remove large clumps, plant debris, and macro-organisms. The experiment's soil was kept moist by regular water spraying. The container was kept at room temperature in the lab. Long strips of 15x3 cm PVC film were buried 4 inches deep. After two months, strips were removed and washed to be used for enrichment culture (Fig. 1).

Isolation of Polyvinyl Chloride Degrading Bacteria by Enrichment Method.

In 10 ml of sterile distilled water, 1 g of soil sample and liquid suspension were mixed. 5ml of soil suspension was added to 95 mL of sterile Minimal broth Davis w/o dextrose (5 ml) in distilled water with concentrations (g/L): K2HPO4 (7.0), KH2PO4 (2.0), sodium citrate (0.50), MgSO4.7H2O (0.10), and (NH4)2SO4 (1.0). The medium's pH was adjusted to 7.2, and 0.1 percent PVC powder was used as the only carbon and energy source. Enrichment was performed with slightly modification according to previously used method (17). All flasks were incubated at 120 rpm and 30^0C for one week. 1 ml of supernatant was added to fresh MSM medium after 1 week. Third and fourth enrichment cultures used the same method. The medium's PVC content increased from 0.1 to 0.5 percent with each enrichment culture and rise in culture broth turbidity indicates growth. 100 µl (0.1 ml) of the fourth enrichment culture was plated on nutrient agar and incubated at 30°C for 2-7 days.

Individual colonies grown on nutrient agar were evaluated for their ability to grow on solid MSM medium containing 0.1% emulsified PVC. Solid MSM plate containing emulsified PVC was formed by modifying earlier used method (18). Dissolving 0.1g PVC in 25 ml tetra-hydrofuran made the MSM agar plate (THF). The solution was added to molten MSM agar medium and immediately poured into plates. The plate lids were left slightly open for 30 minutes to allow complete solvent evaporation. Then, plate lids were closed.

Sub-culturing isolated colonies on the same medium produced pure PVC-degrading bacteria cultures. Individual colonies were streaked on Nutrient Agar plates for future studies.

Identification-The bacterial strains were identified macroscopically by examining colonymorphology, surface pigment, shape and size of nutrient plate. Microscopic examination included Gram's staining to study the staining behaviour, shape and cell arrangement. Further characterization was done by performing the following biochemical test. (19).

Screening of Polyvinyl Chloride Degrading Bacteria

Polyvinyl chloride powder were added to mineral salt medium(MSM) at a final concentration of 0.1 percent (w/v), and the mixture were sonicated in a shaker for 1 hour at 120 rpm, then the medium were sterilized for 20 mins at 121°C and 15 lbs/inch^{2}pressure. Sterilized MSM were poured to each plate before cooling. The isolated organisms were inoculated on polymer-containing agar plates and cultured for 2-3 weeks at 30°C. The bacteria that producing a zone of clearance surrounding their colonies were chosen for further study.

Development of Consortium

For the preparation of bacterial consortia, each selected strain was inoculated individually in a flask containing Polyvinyl chloride enriched medium, incubated for 2-4 days, and then checked their growth by taking optical density (O.D.)at 600nm at regular intervals of 24h using a spectrophotometer. Those bacteria showing higher growth individually were then randomly chosen to make different combinations of two and three strains, inoculated with the determined quantity into PVC-enriched medium, and monitored for 45 days. Better-growing combinations are chosen as consortiums, while those with less growth are left behind. A single colony of each culture was inoculated into 100-ml flasks containing 50 ml nutrition broth (pH7.0±0.02) and incubated at 37 °C with 120 rpm shaking for 12 hours. The calculated (CFU ml^{-1}) of each strain was combined to develop consortia (20).

MICROBIAL DEGRADATION STUDIES

Preparation of microbial cell suspension for various degradation studies

Nutrient broth was used to culture microbial cells in degradation studies. The pure bacterial culture was streaked on nutrient agar and incubated for 24 to 48 hours at 37°C. The pure colonies were inoculated in a 5 ml nutrient broth tube and incubated at 37^{o}C overnight. The overnight-incubated cells were harvested by centrifuging at 4500 rpm for 15 minutes. After discarding the supernatant, the cell pellet was centrifuged at 4500 rpm in 0.85% normal saline. It was done twice. Cell pellets were washed and re-suspended in PVC degradation medium.

Microbial degradation Polyvinyl Chloride films under laboratory condition by Liquid culture method.

PVC film were cut into small pieces(1 cm^2), weighed, sterilized with 70% ethyl alcohol, rinsed with sterile distilled water 2-3 times, and then dried. 500mg (0.5gm) of dried film was aseptically transferred into 100 ml of sterile mineral salt media and inoculated with 300µl of active consortia II and IV separately. The medium's pH was adjusted to 7.0±3. Minimal broth + consortium and minimal broth +PVC were used as positive and negative controls. The flasks were incubated for 6 months at 30 °C in 120 rpm. After 2, 4, and 6 months samples were analysed for PVC film weight loss (21).

ANALYSIS OF BACTERIAL DEGRADATION

Calculation of dry weight of Residual polymer

All the PVC film were collected after 2, 4, and 6 months of incubation to allow for exact weight measurements of the remaining polymer. A biofilm were developed on the PVC film. To achieve a maximum probable removal of cells and debris, it was rinsed for 4 hours with a 2 percent (v/v) aqueous sodium dodecyl sulphate solution (using a shaker), then distilled water, and finally with 70 % ethanol. Before weighing the PVC films, they were washed and placed on filter paper to dry overnight at room temperature.

The weight loss of PVC film were calculated using the following formulae (22).

$$\textbf{Percentage of weight loss (\%)} = \frac{\text{Final weight} - \text{Initial weight} \quad X\ 100}{\text{Original weight}}$$

Estimation of Remineralisation of Polyvinyl Chloride by Strum Test

The titrimetric method was used to determine the amount of CO_2 released by the biodegradation of plastics (23,24). The 1% bacterial mixture was inoculated into 300 ml of sterile MSM for each film in triplicate, and sterile PVC films were added to the flasks. Simultaneously, a control treatment (MSM+ inoculum) was conducted without plastic films. Through a silicon septum and tubes, 20 ml of sterile 0.1 N sodium hydroxide was connected to each inoculated flask. The flask was changed every five days of incubation, and CO_2 production was measured by titration against 0.1 N HCl for up to 6 months (23,24).

The evolved CO_2 from remineralization of plastic films was calculated by subtracting the CO_2 from the control treatment and using the initial carbon content of the plastic films.

ANALYSIS OF BIODEGRADATION OF POLYMER

Scanning Electron Microscopy (SEM)

Polyvinyl chloride films treated with bacterial consortium in a flasks in MSM for 6 months were analysed by SEM and compared with control. Specimens were attached to stubs using Electrodag 915 (Acheson Industries, Reading, United Kingdom) and sputter coated (model S150 device; BOC Edwards) with gold before being examined using a Stereo scan 360 Scanning Electron Microscope (Cambridge Instruments, United Kingdom.

Fourier Transform Infra-red (FTIR) Spectrometric Analysis

Fourier transform infrared (FTIR) spectrum analysis were done to detect the degradation of different samples of Polyvinyl chloride after culturing in liquid media, on the basis of changes in the functional groups of PVC film treated with bacterial strain were recorded and compared with that of control films after 6 month of incubation .The polymer pieces were mixed with KBr and made into a tablet, which were fixed to the FTIR sample plate. Spectra were taken in triplicate at 400 to 4000 wave- numbers cm^{-1} for each sample.

RESULT AND DISCUSSION

PVC-degrading bacteria were obtained after incubation period and purified using the spread plate technique. Twenty-three bacteria were isolated from sampling sites, including four from the natural environment i.e. soil burial technique (Fig. 1). They were separated by colony shape, colour, and size on agar plates. Isolates possessed degrading capability. In the current study, no extra carbon and energy sources were added to bacterial growth media.

Figure no.1-Soil burial technique

Figure no.2.Clear zone formation byisolate 9B

Out of these 23 bacterial isolates, 7 isolates coded DS-1A, DS-3A,DS-4B, DS-5E,DS-14C, DS-7A and SB-C were selected based on size of clear zone on MSM agar plate (fig. 2) and out of these seven isolates, three isolates coded DS-9B, DS-4A and SB-C were further screened based on growth in liquid medium (spectrophotometer).

Table no. 1. Bacterial species used in consortium formulation

Consortium	Bacterial strains
I	*Bacillus spp.* (DS-9B)+ *Pseudomonas spp.* (SB-C)
II	*Bacillus spp.* (DS-9B)+*Paenarthrobacter spp.* (DS-4B)
III	*Paenarthrobacterspp,*(DS-4B *+Pseudomonas spp.* (SB-C)
IV	*Bacillus spp.* (DS-9B)*+Paenarthrobacter spp.* (DS-4B) + *Pseudomonas spp.* (SB-C)

All consortia were evaluated after 45 days of storage. Table. 1 describes bacterial combinations. After two days, the number of CFU/ml varied among consortiums. Consortium-IV numbers dropped from 284x108 to 275x108 after 45 days of storage.

Table no.2 Enumeration of total viable count of respective consortium under control ambient temperature

Consortium	Dilution Factor	CFU/ml at subsequent time intervals (days)							
		2nd	8th	14th	20th	28th	36th	40th	45th
I	10^8	177±1.1	173±1.4	133±1.4	126±1.2	115±1.7	65±1.1	47±1.7	36±0.8
II	10^8	271±1.5	267±1.7	261±2.0	254±1.7	240±1.7	188±1.7	130±2.0	107±2.0
III	10^8	198±1.7	184±2.0	173±1.7	156±1.7	133±2.0	120±1.7	91±2.0	55±1.4
IV	10^8	286±1.4	278±2.0	272±2.0	269±1.7	267±1.7	264±1.7	275±2.0	278±2.0

Consortium -I cell viability declined after 14 days (from 175x108 to 131x108), reaching 35x108 after 45 days. In consortium -II, cell viability declined slightly (from 269 x 108 to 237 x 108) up to the 28th day, then significantly (127 x 108) and continued to 104 x 108 CFU/ml. Consortium -III cell viability dropped to 53 x108 CFU/ml after 45 days, following the same trend as Consortium -II (Table 2). In the developed formulations, consortiums -IV and II were more viable than consortiums- I and III based on these data. Using consortium self-life results, consortiums II and IV were chosen for further degradation studies.
Identification of bacterial isolates -Bacterial isolates DS-2A, DS-4B, and SB-C were classified as members of *Bacillus* ,*Paenarthrobacter a*nd *Pseudomonas* based on their morphological and biochemical features (Table no.3).Microscopic examination included Gram's staining to study the staining behaviour, shape and cell arrangement. Further characterization was done by performing the following biochemical test. (19Holt *el al*., 1994).

Table No. 3 Morphology and biochemical characterization of selected Polyvinyl chloride degrading bacterial isolates

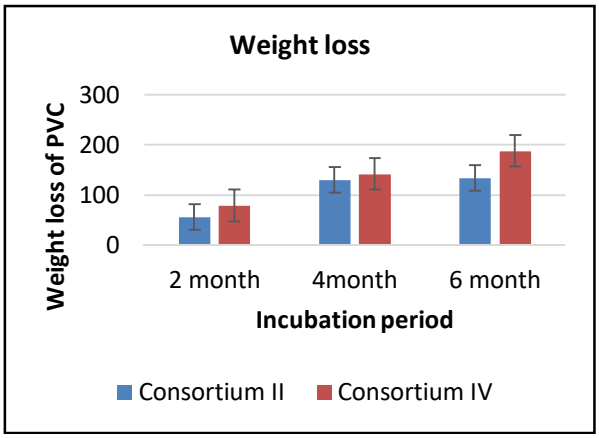
Characteristics	Isolate DS-2A	Isolate DS-4B	Isolate SB-C-
Colony Characteristics			
Shape	Round	circular	Round
Size	Small	Moderate	Large
Color	Cremish white	Light yellow	white
Surface	Granular	Convex	Convex
Margin	Entire	Undulate	Undulate
Morphology			
Straight rod	+	+	+
Cocci	-	-	-
Gram stain	+	+	-
Cell arrangement	Short rod, Single	Short chain, Single	Short Rod , Single
Spore	+	C	C
Motility	+	+	+
Enzyme production			
Amylase	+	-	-
Gelatinase	+	-	+
Carbohydrate Fermentation			
Glucose	A/-	+	+
Sucrose	A/-	-/-	-/-
Lactose	A/-	-/+	-/-
Mannitol	A/-	-/-	-/-
Urease	-	+	-
Nitrate reduction	+	+	+
Oxidase	+	+	+
Catalase	=	+	+
Indole production	-	-	-
Methyl Red	-	-	-
VogesProskauer	+	-	-
Citrate Utilization	+	+	+

+ positive ,- negative, A- acid

The selected bacterial consortium biodegraded PVC film in vitro. Every 2 months up to 6 months, weight loss was measured (Fig. 3). PVC film weight loss shows the efficiency of a soil bacterial consortium in biodegrading a highly persistent plastic structure when PVC is the only C-source. In both consortium II

and IV PVC weight loss (Fig.3) was 56±1.8, 130.3±2.5and 134.7±2.2after 60,120, and 180 days of incubation, and 79±1.7, 142±1.8and 142±1.5, after 180 days. Consortium II degrade PVC up to 10.5%, 25.6%, and 26.5%, likewise consortium IV degrade PVC 15.4%, 27.7%and 36.5% during 6 month of incubation. Unplasticized PVC, LDPE, and HDPE films were biodegraded in vitro using AIIW2. Weight loss was measured every 15 days for 3 months. The percent weight losses were 0.08± 0.02, 0.17± 0.04, 0.28± 0.02, 0.34 ±0.17, 0.32± 0.09, and 0.26 ±0.02 for PVC, 0.03 ±0, 0.21± 0.1, 0.29 ±0.07, 0.75 ±0.09, 1.26± 0.17, and 0.96± 0.02 for LDPE, and 0.22± 0.07, 0.43 ±0.08, 0.81 At the end of incubation, films dropped slightly less weight than on day 75; one possible reason is that bacteria used the released C compounds from plastic films during degradation (25). Similarly reported as, *Pseudomonas aeruginosa* and *Achromobacter sp*. degrading plasticized PVC up to 35%(26). In another study, PVC epoxidized with linseed oil in a 15/85 weight ratio lost 68% of its weight after 6 months in agricultural soil (27). In previous studies, degradation was linearly dependent on PVC epoxidation, but not intact PVC, indicating that the epoxy and ester groups provided by epoxidation processes induce faster degradation than in our study. No report exists on bacterial degradation of intact unplasticized.

The remineralization of plastic films was estimated using ISO 14855 2005 CO_2 evolution method. Conversions of PVC (Fig. 4) Cumulative CO_2 evolution by II and IV bacterial consortium during growth with PVC films is 27.40±1.1 and 29.69±1.3 mg CO_2 g^{-1} of C, on 2 month, which increased to 69.77±0.8, 78.44±1.6, and 79.69±1.1, 94.55±1.9 mg CO_2 g^{-1} of C on 4 and 6 month (Fig. 5). 0.051 cm3 and 0.046 cm^3 cumulative carbon dioxide production from cellulose filter paper and Novamont Mater-Bi after 5 days of incubation reported by(24).In another study, found the mineralization rates of starch-blended polyethylene and microcrystalline cellulose to be 1.00 and 1.23 per day, respectively, using CO_2 evolution method(28).



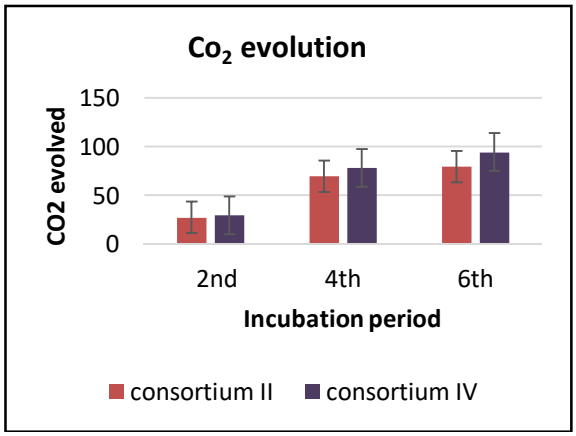


Fig..3- Average percent weight loss of PVC film Fig.4- CO_2 Evolution level Error bars indicate standard deviation (n = 3)

The SEM was used to detect changes in polymer film surface morphology after treatment with the consortia. SEM micrographs of the film (Fig.5A) as control showed a smooth surface. PVC film degraded by consortium IV (Fig. 5C) and consortium II (Fig. 5B) after 6 months of experimentation shows non-uniformly scattered whitened areas and erosion zones. Worn areas with random cracks and fissures disrupt PVC surface texture. However, biodegradation is identical to the initial efficiency documented by both consortia. Similar results of SEM micrographs showing surface disintegration and disruption of LDPE film samples in the presence of SPION and NBT were reported by (29).

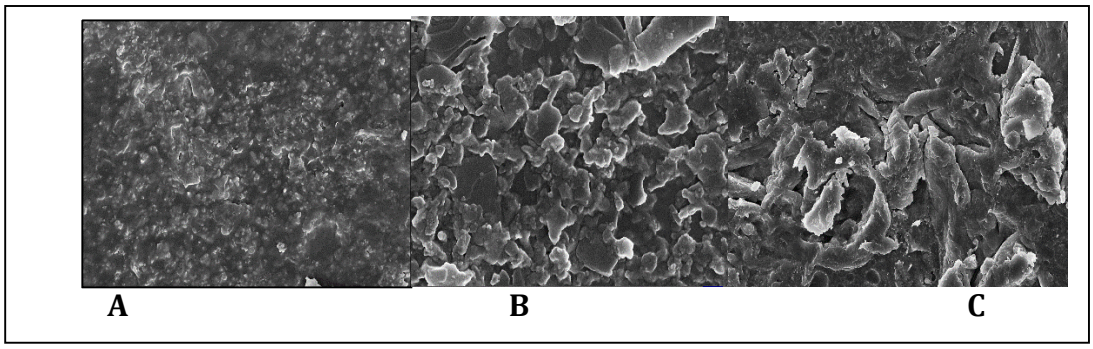


Figure no.4 Comparative SEM micrographs of PVC film degraded by consortium II and IV (b and c) by taking untreated PVC film as control (a). Scale bars=10µm; magnification=5.00 KX

The consortium's biodegradation potential was also reflected in the wave numbers (cm-1) compared to the untreated film (Fig. 6). Polymers incubated with consortium were monitored between ranges 4000 and 450 cm^{-1}. The control PVC film (Fig. 6C) and film by consortium II and IV (Fig. 6A and 6B). The characteristic wave numbers (cm-1) at 2962.8-2363.0 (ν CH2), 1469.6-1463.0 (δCH2), 1376.3-1371.8 (δCH), 1084.7-1073.2 (νC-O-C), and 745.9-721.5 (νC-Cl) for vinyl chloride–vinyl ether copolymers as reported by (30). Additional characteristic peaks reported at (cm-1)3092.99 (νOH), 2850.9 (ν CH2), 1599.06 (δOH), 614.35 (δC-C-C) and at 1726.36 (νC=O), 1255.71 (γCH), 1102.3 (C-C) represented to PVC film by consortium II and additional characteristic peaks reported at (cm-1) 3092.99 (ν OH), 2850.9 (ν CH2), 1599.06 (δ OH), 614.35 (δ C-C-C) and at 1726.36 (ν C=O), 1255.71 (γ CH), 1102.3 (δ C-C) (Fig 6B) represented to PVC film by consortium IV respectively. Laboratory-scale PVC powder treated with consortium had characteristic wave numbers of 3417 (OH), 1663 (OH), 1476 (CH2), 1094 (C-O-C), and 530 (C-C-C), found similar peaks in plasticized PVC(31).

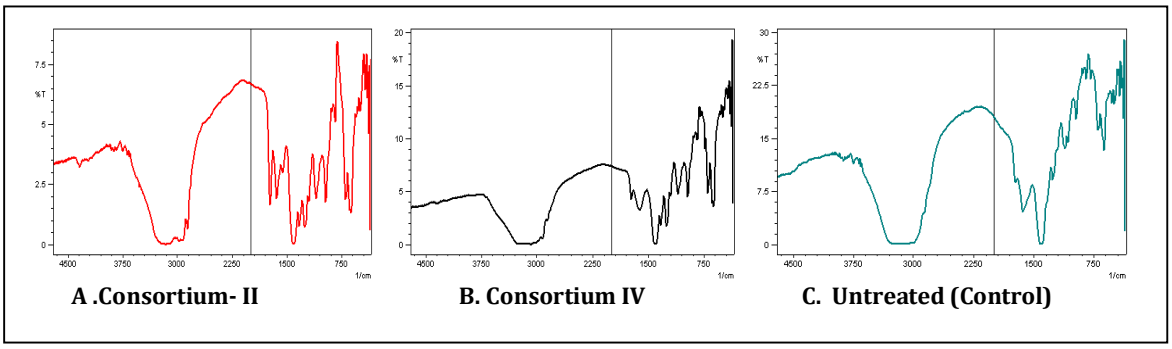


Figure no. 5 Comparative FT-IR spectra of consortium treated PVC (A and B) with reference to their controls(C).

CONCLUSION

Plastics are rigid and persistent in the environment, and their widespread use led to plastic waste accumulation. Biodegradation is an eco-friendly and interesting way to recycle plastic. Soil bacteria can adhere to and degrade plastic and grow when plastic is the only source of carbon. The selected *Bacillus, Paenarthrobacter*, and *Pseudomonas* species can degrade PVC. Biodegradation of polymeric materials involved morphological changes in film surface revealed by SEM and FT-IR analysis, and mineralization of plastics into CO_2. Thus, comparative biodegradation studies showed that neither consortium's biodegradation potential was affected during or after storage in consortium formulation. The consortium manages to prolong the shelf life and maintain the bacteria's efficacy, suggesting it is stable. In formulations, consortiums IV and II were more viable than I and III. So, the consortium could be used for plastic-waste management. Therefore, the current investigation may be a step toward field application and commercialization as a carrier for long-term PVC-degrading consortia, which may minimise solid waste disposal in the environment.

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AUTHORS CONTRIBUTION-
At their respective stages, all of the authors contributed equally.

CONFLICT OF INTEREST-
The authors declare that there is no potential for a conflict of interest in their work.

REFERENCES
1. Central Pollution Control Board, 2018. Annual report. Material on plastic waste management, CPCB New Delhi India. https://cpcb.nic.in/uploads/plasticwaste/ Annual_Report_2018-19_PWM.pdf (accessed October 2019).
2. Geyer, R., Jambeck, J. R., & Law, K. L. (2017). Production, use, and fate of all plastics ever made. *Science advances*, *3*(7), e1700782.
3. Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PLoS One 9, e111913.
4. Jenkins, S., Quer, A. M. I., Fonseca, C., &Varrone, C. (2019). Microbial degradation of plastics:New plastic degraders, mixed cultures and engineering strategies. *Soil microenvironment for bioremediation and polymer production*, 213-238.
5. Amobonye, A., Bhagwat, P., Singh, S., & Pillai, S. (2021). Plastic biodegradation: frontline microbes and their enzymes. *Science of the Total Environment*, *759*, 143536.
6. Varma, A. J., Deshpande, S. V., &Kondapalli, P. (1999). A comparative study of the thermal behavior of PVC, a series of synthesized chlorinated polyethylenes and HDPE. *Polymer degradation and stability*, *63*(1), 1-3.
7. Jayasekara, R., Harding, I., Bowater, I., Lornergan, G., 2005. Biodegradability of selected range of polymers and polymer blends and standard methods for assessment of biodegradation. J. Polym. Environ, 13, 231–251.
8. Anwar, M. S., 2011. Biodegradation studies of Polyvinyl Chloride and Low Density Polyethylene using indigenously developed microbial consortia, Ph.D.thesis. GovindBallabh Pant University of Agriculture & Technology Pantnagar, P. 1–174.
9. Sah, A., Negi, H., Kapri, A., Anwar, S., & Goel, R. (2011). Comparative shelf life and efficacy of LDPE and PVC degrading bacterial consortia under bioformulation. *Ekologija*, *57*(2).
10. Anwar, M., Negi, H., Zaidi, M., Haider, G., Gupta, S., &Goel, R. (2013). Biodeterioration studies of thermoplastics in nature using indigenous bacterial consortium. *Brazilian Archives of Biology and Technology*, *56*(3), 475-484.
11. Booth, G. H., & Robb, J. A. (1968). Bacterial degradation of plasticised PVC-effect on some physical properties. *Journal of Applied Chemistry*, *18*(7), 194-197.
12. Webb, J. S., Nixon, M., Eastwood, I. M., Greenhalgh, M., Robson, G. D., & Handley, P. S. (2000). Fungal colonization and biodeterioration of plasticized polyvinyl chloride. *Applied and environmental microbiology*, *66*(8), 3194-3200.
13. Nakamiya, K., Hashimoto, S., Ito, H., Edmonds, J. S., Yasuhara, A., & Morita, M. (2005). Microbial treatment of bis (2-ethylhexyl) phthalate in polyvinyl chloride with isolated bacteria. *Journal of bioscience and bioengineering*, *99*(2), 115-119.
14. Kumar, S., Teotia, U. S., & Singh, Y. (2017). Screening of poly vinyl chloride degrading bacteria from plastic contaminated area of Baddi. *Journal of Applied Pharmaceutical Research*, *5*(3), 34-37.
15. Giacomucci, L., Raddadi, N., Soccio, M., Lotti, N., & Fava, F. (2019). Polyvinyl chloride biodegradation by Pseudomonas citronellolis and Bacillus flexus. *New biotechnology*, *52*, 35-41.
16. Nowak, B., Pająk, J., Drozd-Bratkowicz, M., &Rymarz, G. (2011). Microorganisms participating in the biodegradation of modified polyethylene films in different soils under laboratory conditions. *International Biodeterioration& Biodegradation*, *65*(6), 757-767.
17. Patil, R., &Bagde, U. S. (2012). Isolation of polyvinyl chloride degrading bacterial strains from environmental samples using enrichment culture technique. *African Journal of Biotechnology*, *11*(31), 7947-7956.
18. Ishigaki, T., Sugano, W., Ike, M., Kawagoshi, Y., Fukunaga, I., & Fujita, M. (2000). Abundance of polymers degrading microorganisms in a sea-based solid waste disposal site. *Journal of Basic Microbiology: An International Journal on Biochemistry, Physiology, Genetics, Morphology, and Ecology of Microorganisms*, *40*(3), 177-186.
19. Holt, J. G., Krieg, N. R., Sneath, P. H., Staley, J. T., & Williams, S. T. (1994). Bergey's Manual of determinate bacteriology.
20. Anwar, M. S., Kapri, A., Chaudhry, V., Mishra, A., Ansari, M. W., Souche, Y.,&Goel, R. (2016). Response of indigenously developed bacterial consortia in progressive degradation of polyvinyl chloride. *Protoplasma*, *253*(4), 1023-1032.
21. Roy, P. K., Titus, S., Surekha, P., Tulsi, E., Deshmukh, C., &Rajagopal, C. (2008). Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium. *Polymer degradation and stability*, *93*(10), 1917-1922.

22. Konduri, M. K., Anupam, K. S., Vivek, J. S., DB, R. K., &Narasu, M. L. (2010). Synergistic effect of chemical and photo treatment on the rate of biodegradation of high density polyethylene by indigenous fungal isolates. *International Journal of Biotechnology & Biochemistry*, *6*(2), 157-175.
23. ISO 14855 (2005) Determination of the ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions—method by analysis of evolved carbon dioxide.
24. Mohee, R., Unmar, G. D., Mudhoo, A., &Khadoo, P. (2008). Biodegradability of biodegradable/degradable plastic materials under aerobic and anaerobic conditions. *Waste Management*, *28*(9), 1624-1629.
25. Kumari, A., Chaudhary, D. R., &Jha, B. (2019). Destabilization of polyethylene and polyvinylchloride structure by marine bacterial strain. *Environmental Science and Pollution Research*, *26*(2), 1507-1516.
26. Das, G., Bordoloi, N. K., Rai, S. K., Mukherjee, A. K., &Karak, N. (2012). Biodegradable and biocompatible epoxidized vegetable oil modified thermostable poly (vinyl chloride): Thermal and performance characteristics post biodegradation with *Pseudomonas aeruginosa* and Achromobacter sp. *Journal of hazardous materials*, *209*, 434-442.
27. Riaz, U., Vashist, A., Ahmad, S. A., Ahmad, S., & Ashraf, S. M. (2010). Compatibility and biodegradability studies of linseed oil epoxy and PVC blends. *Biomass and bioenergy*, *34*(3), 396-401.
28. Leejarkpai, T., Suwanmanee, U., Rudeekit, Y., &Mungcharoen, T. (2011). Biodegradable kinetics of plastics under controlled composting conditions. *Waste management*, *31*(6), 1153-1161.
29. Kapri, A., Zaidi, M. G. H., &Goel, R. (2010). Implications of SPION and NBT nanoparticles upon In Vitro and In Situ biodegradation of LDPE film. *Journal of microbiology and biotechnology*, *20*(6), 1032-1041.
30. Mekki, H., &Belbachir, M. (2007). Preparation of vinyl chloridevinyl ether copolymers via partial etherification from PVC. *Express Polymer Lett*, *1*, 495-498.
31. Ramesh, S., & Yi, L. J. (2009). FTIR spectra of plasticized high molecular weight PVC–LiCF3SO3 electrolytes. *Ionics*, *15*(4), 413-420.

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