

Fungal Biodegradation of Polyvinyl Chloride in Aqueous Environments

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ABSTRACT

The recalcitrant nature of polyvinyl chloride causes significant environmental challenges during production and disposal. This study aimed to assess the ability of fungi isolated from the washing tank in a plastic production factory to biodegrade polyvinyl chloride (PVC). Over 60 days, fungi isolated were incubated with plastic pieces in Bushnell Haas medium. These strains were identified as *Corioloropsis gallica* (F1), *Aspergillus niger* (F2) and *Aspergillus flavus* (F3). After incubation, three methods were selected: Fourier transform infrared (FTIR) analysis, gas chromatography-mass spectrometry (GC-MS), and weight loss experiments, to determine the biodegradation of PVC. The FTIR analysis demonstrated changing peaks, disappearance, and formation of new bonds of the PVC treated compared with the control. A GC-MS analysis revealed the formation of carboxylic acid, alcohol, nitrile and a new compounds during the decomposition of PVC. The results of the weight loss experiment for microbial strains F1, F2, F3 and fungi consortium (FC) were 19, 25.3, 23.6, and 52.6%, respectively. The FC was prepared by combining all three fungi isolates. The present study concluded that these isolated fungal strains have potential for partial biodegradation of PVC plastics. Nevertheless, the results suggest that fungi consortium plays a significant role in the degradation of PVC in aqueous environment.

INTRODUCTION

Plastic is a wonderful material that is very easy to shape into different shapes. The amount of plastic in the world is seriously endangering society (Brandon & Criddle 2019; MacLeod *et al.* 2021). There have been many perspectives recently raised about plastic-related environmental hazards and crises, despite plastics' long-lasting usefulness (Barrowclough & Birkbeck, 2020). People are more inclined to use plastics for things they want since they are less expensive (Moshood *et al.*, 2022). Plastic can partially degrade through cracking and fractures, which leads to the formation of micro- and meso-plastics (Giacomucci *et al.*, 2019). The term micro-plastics (MPs) refers to particles of plastic with a diameter of 5mm or smaller (Vincent & Hoellein, 2021). It has been found

worldwide in freshwater, surface water, soil, coastal regions, and even the polar regions (Ma *et al.*, 2022). Since MPs are small, they can easily enter the food chain and have a detrimental effect on humans and other organisms (Yan *et al.*, 2019). Plastic waste must now be removed from the environment due to its hazardous materials and slow decomposition (Waring *et al.*, 2018). The management of plastic products at their end-of-life is currently inefficient, with the majority of plastic wastes going into the environment, and existing solutions like incineration do not meet the economic and environmental needs (Magnin *et al.*, 2019). Numerous polymer types, including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and polyamide (PA), are used to make particle plastic (Al-hussayni *et al.*, 2023). As reported in Whelton and Nguyen (2013), the most common polymer in freshwater was PVC. According to a study by Sultan *et al.* (2023), PVC is the most often used polymer in drinking water in Iraq because it is extensively used in water networks. In the world, polyvinyl chloride (PVC) ranks third behind polyethylene (PE) and polypropylene (PP). In 2018, its production reached 45 million tons, and it is used in food packaging, electronics coatings, medical devices, and other applications (Plastics Europe, 2021; Zichittella *et al.*, 2022). Consequently, the present study's micro-scale biodegradation treatment method for plastic was conducted using PVC as the model. Micro-plastics can be treated using a variety of techniques, including physical, chemical, and biological treatments. The following were some of the drawbacks of physical and chemical processes: they were costly, produced a lot of sludge, required a lot of chemicals, and required high energy costs (Hazirah *et al.*, 2014). The biological process has been the most appropriate method for dealing with MPs due to its relative efficiency and environmental friendliness (Cai *et al.*, 2023). More research showed that microbes are crucial to the breakdown of plastics (Tabatabaei *et al.*, 2023). Microbial organisms can use polymers as sources of carbon and energy for growth through a process called biodegradation (Mohanani *et al.*, 2020). Microorganisms degrade plastics by certain enzymes into oligomers and monomers through chain cleavage (Magnin *et al.*, 2019). In addition to enzymatically cleaving the products, the microbial cells absorb these water-soluble products and metabolize them to produce carbon dioxide and water during aerobic condition (Das & Kumar, 2015). Previous studies on the biodegradation of plastic revealed that a wide variety of fungi are capable of breaking down plastic materials (Veerappapillai & Muthukumar, 2015). For instance, Temporiti *et al.* (2022) reported that fungi that produce ligninolytic enzymes, such as laccases and peroxidases, were effective in partially breaking down Polyvinyl chloride and polyethylene. Ultimately, the integration of fungal biodegradation into waste management strategies could significantly reduce plastic waste, contributing to a more sustainable future. In this study, strains of fungi from the washing tank in the PVC pipe factory are isolated and identified. Moreover, a biodegradation study is conducted on micro-plastic (PVC) to

determine which fungi types are capable of degrading micro- PVC. Additionally, our research lays the groundwork for lowering the pollution that micro-plastics cause.

MATERIALS AND METHODS

1. Materials and chemicals used in experiments

PVC plastic, with the chemical formula C_2H_3Cl , density of $1.38g/cm^3$ and molecular weight of $62.5g/mol$, was purchased from the Al-Azhar/Iraq factory for use in the biodegradation experiments. Zinc chloride ($ZnCl_2$) and the Bushnell Haas medium (BHM) were obtained from the University of Mosul's College of Environmental Sciences Laboratory. While the $0.45\mu m$ filter paper (CHMLAB GROUP, Spain), sodium dodecyl sulfate (SDS), and mini gDNA Fungi Kit (Geneaid/GBB100) were obtained from Earth Laboratory in Mosul, Iraq.

2. Micro-scale PVC plastic preparation

The PVC material was grated into different sizes using scissors and a bastard-cut hand file to create PVC particles. In order to eliminate larger debris and obtain particles within the micro-plastic scale, the plastic particles were filtered through sieves measuring between 0.3 and 1mm (Auta, 2018). This size scale was selected based on studies concerning the availability of MPs in aqueous environment (Warrier *et al.*, 2022; Yusuf *et al.*, 2023). Moreover, the PVC particles were weighed, sterilized for 30 minutes with 70% ethanol, and then left in sterile water for 20 minutes (Das & Kumar, 2015).

3. Sampling sites and isolate strain from water sample

The water samples were collected using glass bottles (250ml) sterilized with 2% sodium dodecyl sulfate (SDS) from the washing tank of the PVC pipe factory, Al-Azhar, in Mosul City (latitude: 36.2054N, longitude: 43.1224E) (Park *et al.*, 2021). After that, the samples were transported to the lab and sealed in light-blocking plastic packaging. The serial dilution method was conducted to screen strain from water sample as follows: Nine milliliters of sterile distilled water were used to dilute one milliliter of water sample. The mixture was then gently shaken to ensure homogeneity. Next, one milliliter of the 10^{-1} diluted solution was taken and put into sterile tubes that already held nine milliliters of distilled water for a 10^{-2} dilution. This process was repeated until a 10^{-4} dilution was achieved (Fig. 1). After that, one milliliter of each diluted sample was inoculated with potato dextrose agar, and every plate was incubated for two days at $37^\circ C$ (Mohammad *et al.*, 2022). Finally, colonies with different morphological characteristics were isolated (Khushboo *et al.*, 2023).

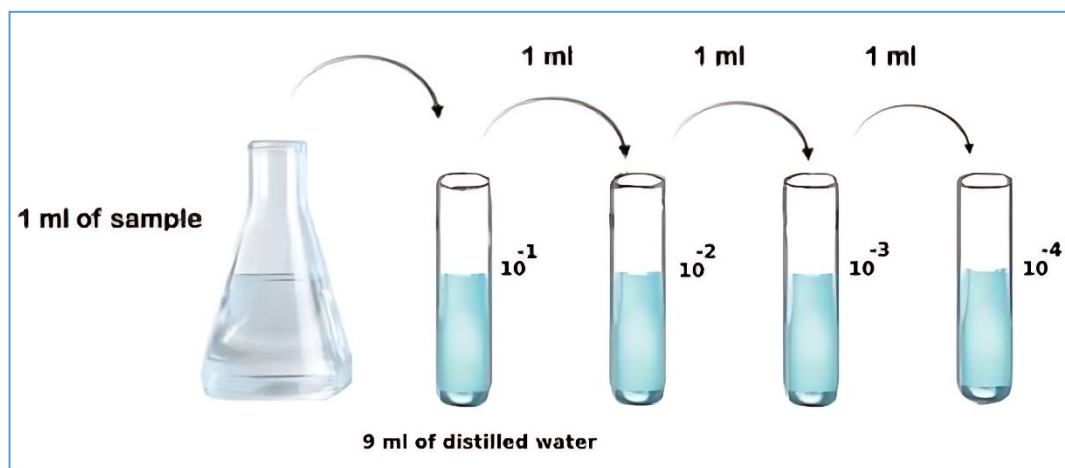


Fig. 1. Serial dilution method

4. Molecular identification of the purified fungus

As a first step, each fungi culture was transferred into a 1.5ml micro-centrifuge and centrifuged for 10 minutes at 5,000x g, and then the supernatant was discarded. Furthermore, the genomic DNA was extracted using the protocol of Presto™ Mini gDNA kit for yeast (Geneaid) (Magdalena *et al.*, 2024).

While in the second step, universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') were used to amplify fungus ITS gene through polymerase chain reaction (PCR). The reaction mixtures used in DNA amplification consisted of 0.5µl of each primer, 4µl of DNA template, and 14µl of nuclease-free water; they were added to Bioneer tubes (AccuPower®PCR PreMix, Korea) to make a total reaction volume of 20µl. The PCR protocol included an initial denaturation at 95°C for 5min, 35 cycles of 1min at 94°C, annealing at 53°C for 45sec, 72°C for 1min, and a final extension at 72°C for 5min. Subsequently, the finished products were visualized on 1% agarose gel (Seprianto *et al.*, 2023).

All samples were sequenced using ITS gene sequences, and it was found that *Corioloopsis gallica* (F1), *Aspergillus niger* (F2) and *Aspergillus flavus* (F3) were existing strains in the water sample, as described in the next section (Identification of bacterial isolates).

5. Biodegradation experiments of micro-plastics PVC and culture condition

Bushnell Haas medium (BHM) was prepared by mixing 1g of KH₂PO₄, 1g of K₂HPO₄, 1g NH₄NO₃, 0.2g of MgSO₄, 0.02 CaCL₂.2H₂O and 0.2 Fecl₃ in 1000ml of distilled water. In this investigation, Bushnell Haas medium was used to incubate both treated and untreated micro-plastics. The BHM medium used in this work meant that plastic particles were the only carbon source for the fungus (Khandare *et al.*, 2021). PVC (60mg) was added to four flasks that contain 100ml of medium BHM. Each flask was inoculated with 10ml of active cultures of F1, F2, F3 and fungus consortium (FC), respectively. The FC was prepared by combining all four fungi isolates. Regardless, the

experiments of plastic particles in flasks (lab scale) were as follows: BHM + F1 + PVC, BHM + F2 + PVC, BHM + F3 + PVC, BHM + FC + PVC, and PVC + BHM were used as a control (without fungi) (Maheswaran *et al.*, 2023). The pH of the medium was at 5.5. After that, the solution in flasks was continuously shaken at 180rpm at 28°C, using a shaker incubator (Labnet 211DS). Moreover, a pH meter (NOYafa EZ9901) was used to monitor pH values. All experiments were carried out in triplicates.

6. Separation of remaining MPs

Following 60 days of incubation, each sample was prepared to examine micro-plastic residual after biodegradation. Due to the greater density of the PVC than water, plastic particles that remain in solutions were challenging to be separated. As a result, the precipitate PVC was separated from the BHM medium using the separation density method, which involved PVC flotation with a saturated salt solution (ZnCl₂) that had a greater density (2.65 grams cm⁻³) (Dehghani *et al.*, 2017; Schrank *et al.*, 2022). For a minimum of five minutes, each sample was mixed with 100 milliliters of the prepared ZnCl₂ solution and vigorously stirred to separate any particles that adhered together. Then, the samples were kept untouched overnight. After that, the mixture was placed in a centrifuge at 6500rpm for 10min to remove cell debris and then, the supernatant containing floating micro-plastic particles was carefully filtered onto a 0.45µm filter paper (CHMLAB GROUP, Spain) to calculate weight loss %.

7. Determination of weight loss of degrade PVC

For three hours, the remaining plastic on the filter paper was treated with a 2% (w/v) solution of sodium dodecyl sulfate (SDS) to eradicate any fungi that were colonizing to the PVC surface. The residual weight was measured after washing with distilled water and drying at 45°C for 2 hours and compared with the control of the pre-incubator (initial weights = 60mg). The percentage of weight loss was measured using the formula (1) (Park *et al.*, 2021).

$$\text{Weight loss (\%)} = [(IW - RW)/WI] * 100 \dots \dots (1)$$

Where, the initial weight of PVC is IW (mg), and the residual weight is RW (mg).

However, IW and RW were measured by an analytical balance model (Sartorius TE214S).

8. GC-MS examination

Following the fungus treatment incubation period, the solution was centrifuged at 6500rpm for 10 minutes to extract cell debris and separate floating PVC particles. Following this, it was carefully filtered onto a 0.45m filter paper to isolate them. The solution passing through filter paper was subjected to a GC-MS analysis, to determine whether or not plastic was breaking down into different new compounds (Lou *et al.*, 2020).

9. Examination of PVC polymers using FTIR

Following an incubation period in a BHM medium containing fungal cultures, the PVC residue on the filter paper was removed with forceps for FTIR analysis (IRAffinity-1S, SHIMADUZ, Japan) and to obtain infrared spectra for PVC. For three seconds, an FTIR spectrometer was used to record infrared spectra in the frequency range of 4000 - 600cm⁻¹ (Park & Kim, 2019). The micro- PVC samples were placed beneath the sample presser equipped with a built-in pressure sensor for analysis, and the auto sample presser was controlled either through an APC program or an operation panel. Subsequently, the results obtained for degraded PVC plastics were compared with the corresponding control (uninoculated control) to determine the formation of possible functional groups on MP's surfaces, which could be attributed to biodegradation.

RESULTS AND DISCUSSION

1. Identification of fungal isolates

The extracted DNA was shown on a gel electrophoresis, and a 1kb ladder was used to display the sizes of the PCR products. It was discovered that the sample had a molecular weight of 350bp. The ITS gene sequences of F1, F2 and F3 showed homology with the ITS gene sequences of *Corioloropsis gallica* (F1) (GenBank accession no. MH855593.1) *Aspergillus niger* (F2) (GenBank accession no. KF638509.1), and *Aspergillus flavus* (F3) (GenBank accession no. MW084630.1). Fig. (2) exhibits the nucleotide sequence alignment with the available database on NCBI after the PCR product appeared.

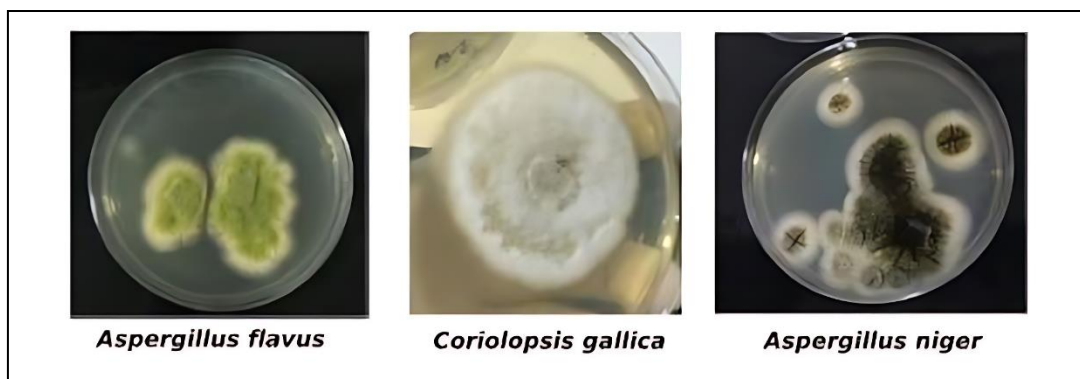


Fig. 2. Fungi that were isolated and identified using PCR technique

2. Weight loss measurement for PVC micro-plastic

The degradation of PVC plastic was measured depending on weight loss after 60 days of incubation with the individual and mixed isolates of fungi from the washing tank in a plastic production factory (F1, F2, F3, and FC). The percentage of weight loss for PVC was calculated as illustrated in Fig. (3). The comparison of the weight loss % of PVC by the different isolates (F1, F2, F3 and FC) after 60 days revealed, that there are

differences in weight loss % among all species, as follows: 19, 25.3, 23.6 and 52.6%, respectively. Moreover, it was observed that a substantial weight loss percentage of 52.6% was observed for PVC plastic incubated with strain FC. The biodegradation results indicate that most of the degradation of PVC was by the FC followed by the F2, which is attributed to the fact that plastics are complex substrates that require cooperation between microbial communities to achieve their degradation (Jiménez *et al.*, 2021). While, the low weight loss percentage (19%) was recorded by *Corioloopsis gallica*. Additionally, there was no weight loss in the control (uninoculated). The PVC plastic's decreased weight following the strain inoculation could be the result of enzymatic degradation breaking down the PVC carbon chain.

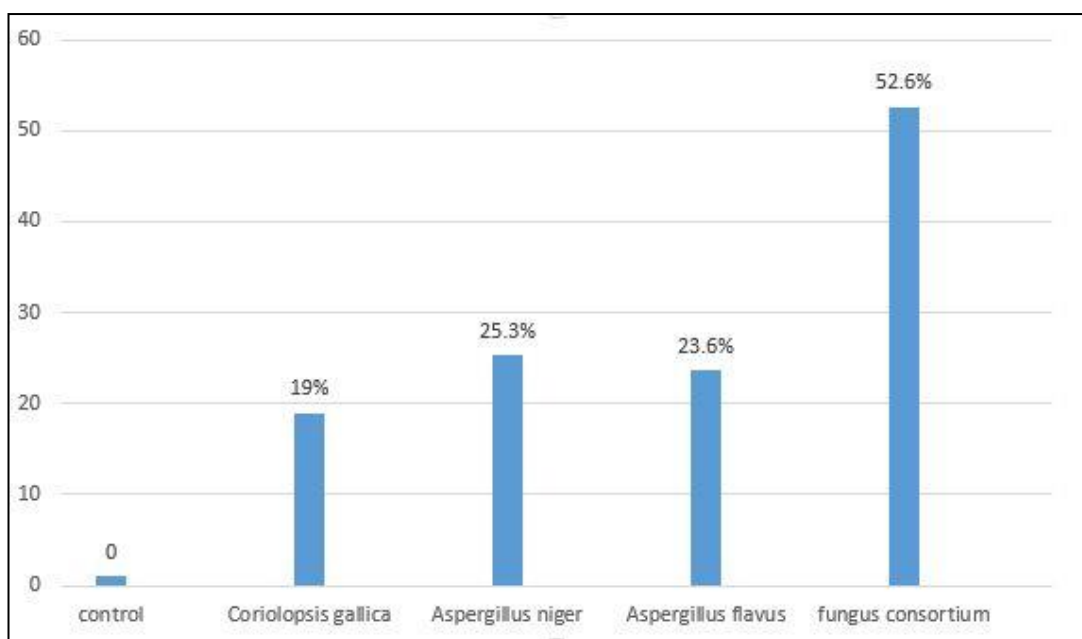


Fig. 3. Weight loss percentage for PVC with different culture after 60 days of incubation

Table (1) displays the studies on the degradation of PVC by fungus that have been published in the literature. *Trichoderma hamatum*, *Byssochlamys nivea* and *Trametes suaveolens* decreased the weight of PVC parts by 20, 15.5, and 18.3%, respectively, after 60 days of incubation at 28°C (Novotný *et al.*, 2022). Similarly, PVC samples that were incubated with *Aspergillus niger* and *Aspergillus glaucus* for 60 days demonstrated degradation rates of 10 and 32%, respectively (El-Dash *et al.*, 2023). Furthermore, after 28 days of inoculation with the fungal consortium consisting of *Trichoderma virens*, *Paecilomyces variotii*, *Penicillium funiculosum*, *Chaetomium globosum*, and *Aspergillus brasiliensis*, wrinkles and superficial deterioration were also seen on PVC film (Vivi *et*

al., 2018). In a different study, it was found that fungi that generate ligninolytic enzymes, like laccases and peroxidases, were efficient at partially degrading polyethylene and polyvinyl chloride (Temporiti *et al.*, 2022). In a recent study, it was revealed that *Aspergillus niger* and *Aspergillus flavus* produce the greatest range of ligninolytic enzymes such manganese peroxidase, lignin peroxidase and laccases (Srikanth *et al.*, 2022). In another study, *Corioloropsis gallica*, a white-rot fungus that produces laccase, can analyze a significant portion of complex organic chemical groups in wastewater (Zainith *et al.*, 2020). On the other hand, in this work, the fungal community was isolated from the washing tank of the PVC pipe factory and identified their types. Then, it studied their efficiency in PVC removal from aqueous solution after 60 days. The strains *Corioloropsis gallica* (F1), *Aspergillus niger* (F2), *Aspergillus flavus* (F3), and fungus consortium (FC) were observed to survive on plastic, using it as a carbon source. F1, F2, F3, and FC showed 19, 25.3, 23.6, and 52.6%, respectively, weight loss from the initial weight of micro-plastic pieces for 60 days. Thus, Table (1) confirms that biodegradation mainly depends on the type of microorganism and microbiological activity. Generally, climate conditions such as temperature, moisture content, pressure, and microbiological activity all contribute to the degradation of synthetic plastics (Roohi *et al.*, 2017).

3. FTIR analysis

FTIR was used to study the appearance, disappearance, and changes in functional groups during the degradation of PVC. Following 60 days of incubation, an FTIR test demonstrated the change in spectra between the control and treated samples using FC fungi at different wavelengths, caused by the bending and stretching of bonds. Fig. (4) displays the FTIR spectra of PVC inoculated with fungus and PVC uninoculated (Fig. 4A, B). The peaks at 2852, 2920, and 2950 cm^{-1} in the control sample belong to the alkane groups (Suganya *et al.*, 2016), which are absent in the FTIR spectrum of PVC micro-plastic inoculated with FC. In addition, the peaks at 2357 and 1730 cm^{-1} for the C=O bond (carbonyl group) (Maheswaran *et al.*, 2023). The O-H stretching of carboxylic acid is responsible for the new bands observed at 3346 cm^{-1} for PVC inoculated with FC (Verdi *et al.*, 2021). In the FTIR spectra of PVC treated with the fungus consortium, new chemical peaks emerged, disappearances and changes of functional groups were observed. There is an elongation of the peak at 2362 cm^{-1} for the C=O bond (carbonyl

group) and a disappearance at 1730cm^{-1} for the ester carbonyl. Furthermore, several peaks disappeared, such as at 2960.73 , 2920.23 , and 2852.72cm^{-1} for alkanes, and at 1463.97cm^{-1} for C-H bending (alkanes). A clear change in functional groups was observed at 1072.42cm^{-1} for carbon-carbon bonds, possibly caused by the cleavage of the C-C bond (Maleki Rad *et al.*, 2022). Other functional groups shifted at $600\text{-}646\text{ cm}^{-1}$ for a carbon-chlorine bond. All of these changes can be attributed to the biodegradation of PVC by the fungus consortium, confirming that biodegradation of MPs by microbes occurred.

Table 1. Previous studies conducted on fungi capable of degrading plastics polyvinyl chloride

Microorganism	Time of exposure (days)	Weight loss (%)	Reference
<i>Trichoderma hamatum</i>		20	
<i>Byssochlamys nivea</i>	60	15.5	(Novotný <i>et al.</i> , 2022)
<i>Trametes suaveolens</i>		18.3	
<i>Aspergillus niger</i>	30	10	(El-Dash <i>et al.</i> , 2023)
<i>Aspergillus glaucus</i>		32	
<i>Corioloropsis gallica</i>		19.1	
<i>Aspergillus niger</i>	60	25.6	Present study
<i>Aspergillus flavus</i>		23.3	
Fungus consortium(FC)		52.6	

4. Analysis of biodegradation products

Following the plastic's 60-day biodegradation, we observed a drop in pH. This change is considered a strong evidence of the decomposition process of plastics. Numerous investigations have identified that the medium's pH decreases during biodegradation due to the increase in $-\text{COOH}$ concentration in the medium (Ojha *et al.* 2017; Maity *et al.* 2021). For instance, according to Kumar *et al.* (2019), there is a positive correlation between the weight loss of plastics and the pH reduction. It is also associated with the decreasing microbial population in the media. On the other hand, to determine the types of acids formed and another compounds, an examination was conducted using Gas Chromatography-Mass Spectrometry (GC-MS) technology of PVC

inoculated with a fungus consortium. GC-MS analysis was able to accurately identify these acids and another compounds, as shown in Fig. (5) and (Table 2) after 60 days of biodegradation. Polymer chains fragment into smaller molecules during biodegradation, which causes the cleavage of carbon bonds and the production of carboxylic acids and other compounds. Another study revealed that GC-MS analysis of biodegraded plastics detected various carboxylic acids (**Jeon *et al.*, 2021**). In addition to the formation of a carboxylic acid group, it has also been reported that when the carbon-carbon covalent bond is broken, ester, nitrile, and alcohol compounds are produced (**Gazi *et al.*, 2019**).

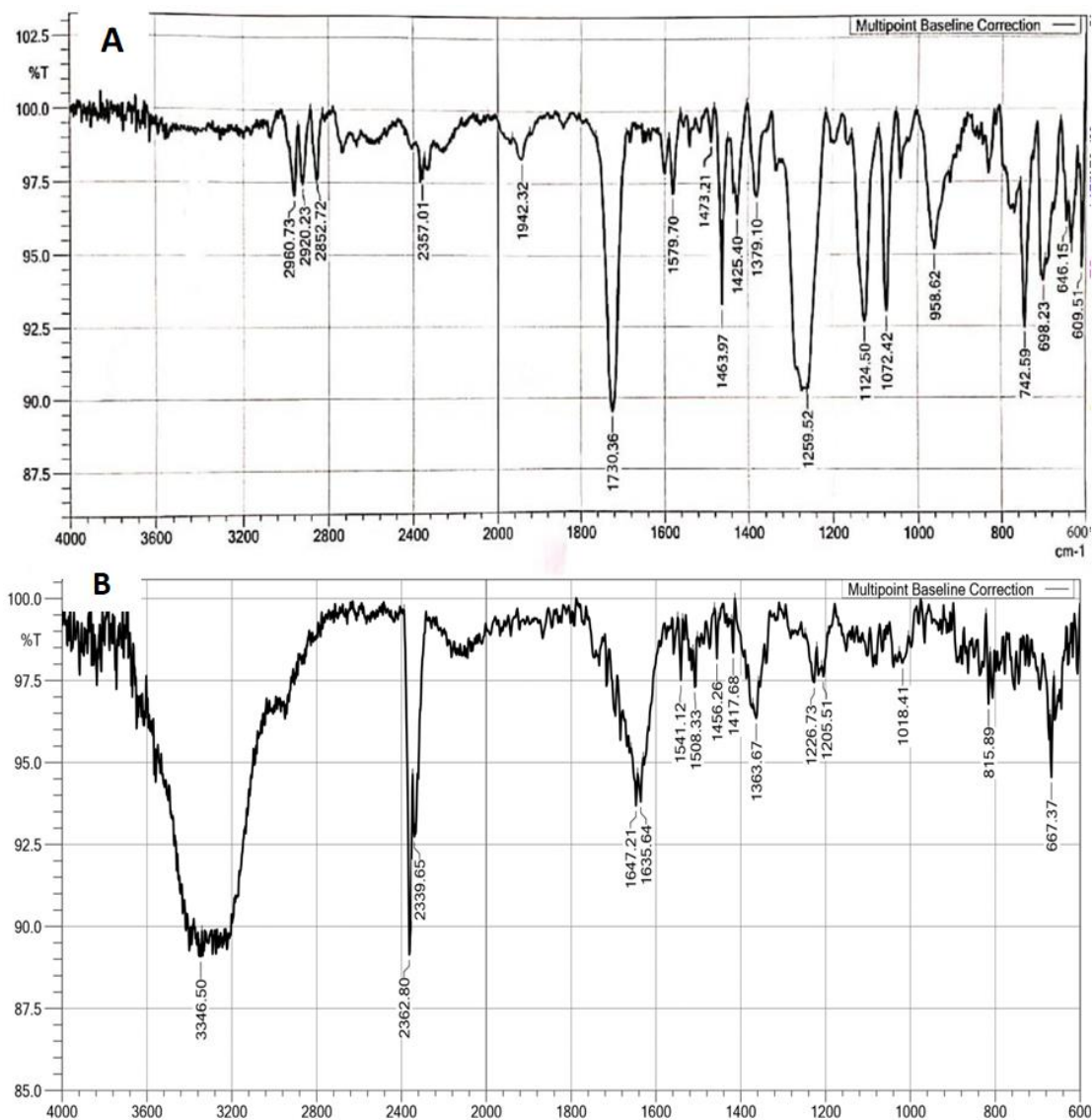


Fig. 4. The FTIR pattern of PVC, (A) control (uninoculated), and (B) treated with fungus consortium (FC)

Table 1. Compounds produced through biodegradation by a fungus consortium (FC) determined using GC–MS analysis

Microorganism	Compound
Fungus consortium (FC)	Methanecarboxylic acid.
	Hydroxymethylcyclopropane.
	Carbonic acid, ethyl-, methyl ester.
	1,3,5,7 -Tetraoxoacane.
	2-Propenoic acid, 2-methyl-, ethenyl ester.
	Trifluoroacetic acid, 2-methylpropyl ester.
	2-Hydroxy-gamma-butyrolactone.
	Pyridine-3-carbonitrile, 1-ethyl-4, 6-dimethyl-2-oxo-1, 2-dihydro.
	Nitrous acid, 2-methylpropyl ester.
	1, 4-Epoxy-naphthalene-1(2H)-methanol, 4, 5, 7-tris (1, 1-dimethylethyl)-3, 4-dihydro.

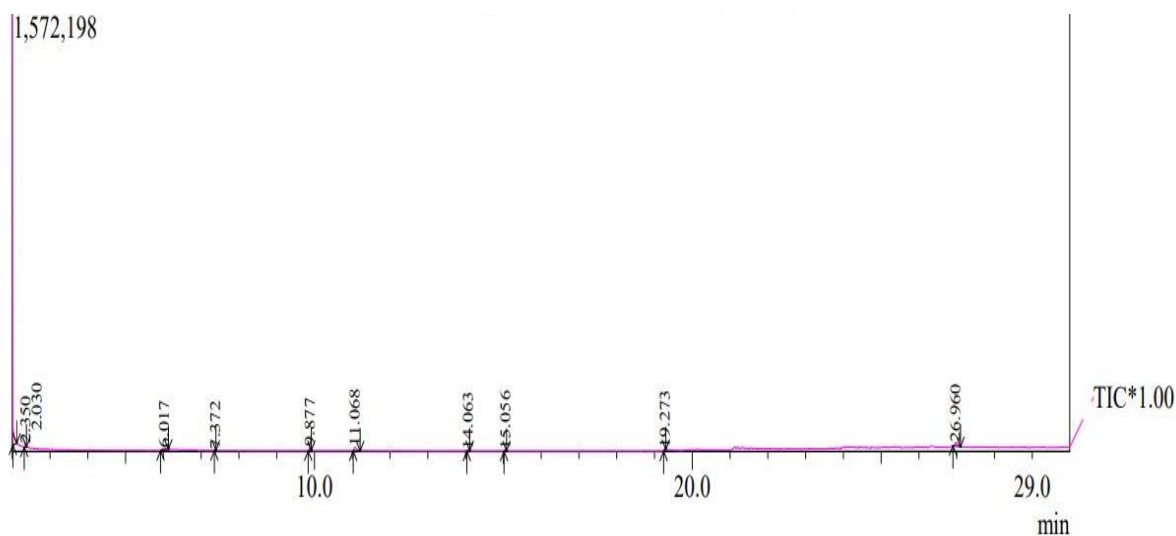


Fig. 5. Chromatograms represent the compounds produced from PVC degradation by fungus consortium activity

CONCLUSION

Three fungal strains isolated from the washing tank in a polyvinyl chloride (PVC) pipe factory were tested for their ability to degrade micro-PVC plastics. After 60 days of incubation, the PVC micro-plastics exhibited potential for biodegradation. The biodegradation process of micro-plastic PVC was studied in flasks with the presence of the strains *Corioloopsis gallica*, *Aspergillus niger*, *Aspergillus flavus*, and a fungal consortium (FC). Over the 60-day incubation period, each strain could partially degrade the micro-plastics. The combined action of all isolated fungi (fungal consortium) resulted in a more significant weight loss, approximately 52.6%. FTIR analysis indicated that bonds were broken, and new bonds were formed during biodegradation. GC–MS analysis showed that the plastic was degraded into several new compounds that provided energy for fungal growth. Further proteomics and metagenomics studies are needed to understand the mechanisms by which these fungi degrade plastic. Additionally, the results suggest that using fungal consortia in bioaugmentation studies could enhance the degradation of micro-polyvinyl chloride.

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