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GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF FUNGAL BASED LOW DENSITY POLYETHYLENE DEGRADATION BYPRODUCTS

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ABSTRACT

Polyethylene biodegradation results in the production of various by products. Polyethylene degradation byproducts were produced due to action of two potential fungal strains *Aspergillus fumigates* MF-14 and *Aspergillus flavus* MF-16) after sixty days of incubation with continuous shaking. Gas Chromatography-Mass Spectrometry analysis revealed a broad spectrum of polyethylene degraded byproduct produced by fungal strains during the degradation of polyethylene. Due to action of fungal strains *Aspergillus fumigates* MF-14 major polyethylene degrading product produced were heneicosane, 2-1-Hexacosanol (RT-32.250), 3-Methyltriacontane (RT-33.333) and *Octatriacontyl trifluroacetate* (RT-34.419) respectively. By-products of polyethylene film produced by *Aspergillus flavus* MF-16 were 2-methylpentacosane (RT-26.907), cyclohexylmehyl octadecyl ester (RT-33.127) and Oxalic acid.

KEY WORDS : Biodegradation, Gas Chromatography-Mass spectrometry, Low density polyethylene, *Aspergillus*

INTRODUCTION

Plastic was first developed in England in the 1850's, and is now widely used in all aspects of life and have gradually become an indispensable part of our life (Zhuang, 2022). Billions of virgin plastic products produced to date that are eventually discarded in the natural environment (Nielsen *et al.*, 2020). About 30 percent of plastic are used in packaging of food, cosmetics, and chemicals that is still expanding at a rate of around 12% annual growth (Premraj and Doble, 2005). Plastic's enduring nature arises from its high molecular weight, intricate three-dimensional structure, and hydrophobic qualities, all of which obstruct its accessibility to microorganisms (Hadad *et al.*, 2005). Only a small portion of this waste polythene is recycled; the remainder ends up in landfills where hundreds of years are taken by plastic to degrade completely (Moore 2008). Smaller plastic particles can actually be more ecotoxic since they can damage ecosystems at different trophic levels and are more easily digested by a wider range of organisms (Prata *et al.*, 2019). When plastic waste is disposed of in the ocean, harmful substances like bisphenol A (BPA), polycyclic aromatic hydrocarbons (PAH), and polybrominated diphenyl esters (PBDE) accumulate and cause major problems for marine life (Bryant *et al.*, 2016). Polyethylene biodegradation is the process by which living things, including bacteria, fungus, algae, and invertebrates, depolymerize polyethylene and use it as a food source. Microbial communities have possibilities for the degradation of polyethylene because of their and capacity to utilize a wide range of substances as substrates (Yao *et al.* 2022). The degradation of polyethylene involves several biochemical pathways that can be categorized into four main steps: bio-fragmentation,

biodegradation, assimilation, and mineralization. These processes rely on the actions of various active enzymes (Pathak, 2017). *Enterobacter*, *Bacillus* (Yang *et al.,* 2014), *Pseudomonas* (Muhonja *et al.,* 2018), *Stenotrophomonas*, and *Delftia* (Peixoto *et al.,* 2017) are some of the bacterial taxa that have been shown to be able to biodegrade polyethylene. Fungal species that are well-known for their ability to biodegrade LDPEs and HDPEs include *Aspergillus* (Muhonja, 2018), *Zalerion* (Raddadi, 2019), *Fusarium* (Sivan, 2011), *Chaetomium*, and *Pullularia* (Sudhakar *et al.,* 2008).

In the current investigation, in order to recognize harmful nature of by-products released due to the action of polyethylene degrading fungal isolates, we tried to identify and studying various polyethylene degrading by products by employing GC-MS tools.

MATERIALS AND METHODS

Low density polyethylene degrading fungal strains

Two most potential polyethylene degrading fungal strains *Aspergillus fumigates* MF-14 and *Aspergillus flavus* MF-16 based on previous study were used in the present study.

Production of polyethylene degradation by-products

Two potential fungal strains *Aspergillus fumigates* MF-14 and *Aspergillus flavus* MF-16 capable of biodegrading polyethylene were inoculated with polyethylene films as carbon source potato dextrose broth medium for sixty days with regular shaking at 140 ± 10 rpm at 25 °C. The degradation by-products of low density polyethylene films were investigated by means of gas chromatography-mass spectrometry (Shahnawaz *et al.,* 2016 and Sangale *et al*., 2019).

Gas chromatography-mass spectrometry (GC-MS) analysis of the polyethylene degradation by-products

After sixty days, the cell isolates were centifuged at 8000 rpm for five minutes at room temperature in order to remove the cell debris and supernatant was collected. The low density polyethylene films were removed from the supernatant using whatmann No. 2 filter paper. The supernatant was transferred to fresh vials. The breakdown by-products of polyethylene were harvested using diethyl ether as a solvent. One millilitre of the dissolved degradation products of polyethylene films were injected in gas chromatography - mass spectrometry for being analyzed. 1ml/minute of helium was used as the carrier gas. The gas chromatography and mass spectrometry was equipped with a 30 m long, 0.25 mm ID, and 1.00 m df DB5 column. The quantifications, peak integration, and data processing were managed by the real-time analytic application gas chromatography-mass spectrometry solution. Using library search, we only identified significant peaks of the chromatogram (Shahnawaz *et al.,* 2016 and Sangale *et al*., 2019).

RESULTS

Gas chromatography-mass spectrometry analysis of polyethylene degradation by-products of potential and fungal strains

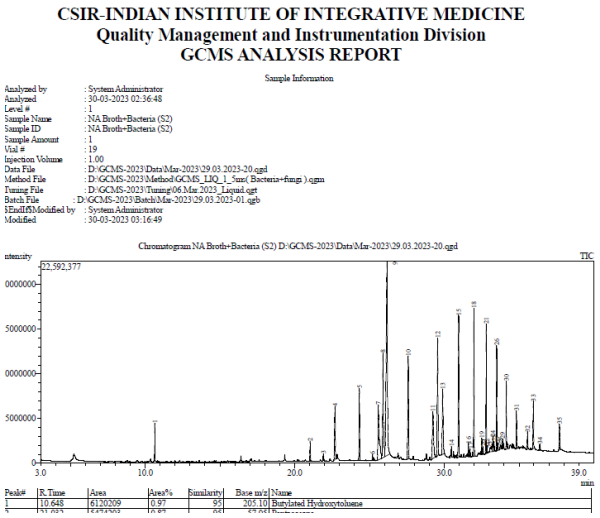
Due to the action of two potential fungal strains *Aspergillus fumigates* MF-14 and *Aspergillus flavus* MF-16 on low density polyethylene films were broken down into range of byproducts in a 250 ml flask containing potato dextrose broth. Using diethylether for gas chromatography and mass spectrometry analysis of these by-products, several chemicals were found at various retention times (RT) revealed different compounds at different retention time (RT). Five byproducts such as Butylated Hydroxytoluene (RT-10.648), Dotriacontane (RT-24.326), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (RT- 26.166), and Octadecanoic acid, was found in all the samples. In case of polyethylene degraded by products of the control total twelve, ten and eight by-products were recorded respectively. Among them six by-products were found common in all of the three controls. These by products were Butylated Hydroxytoluene (RT-10.648), Pentacosane (RT-21.031), Dotriacontane (RT-24.326), 2-hydroxy-1-(hydroxymethyl)ethyl ester (RT- 26.166), and Octadecanoic acid, 2,3-dihydroxypropyl ester (RT-29.894).

Production of polyethene degrading by-products by the action of fungal strains *Aspergillus fumigates* MF-14

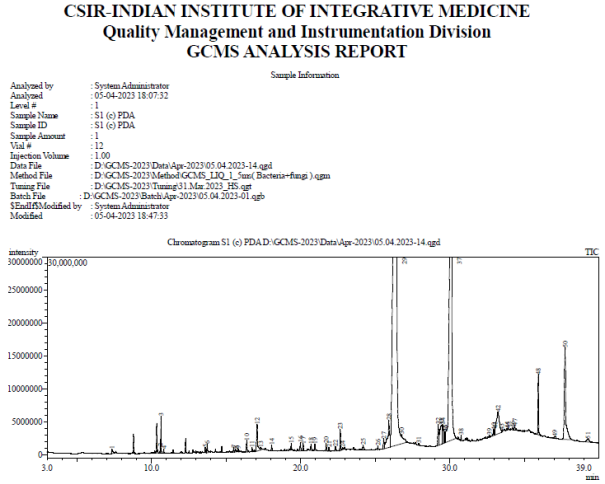
Due to the action of fungal strains *Aspergillus fumigates* MF-14 total eleven polyethylene degrading products were produced. Among them 8 were similar to control and only 3 were unique. These were heneicosane, 2-1-Hexacosanol (RT-32.250), 3-Methyltriacontane (RT-33.333) and Octatriacontyltrifluroacetate (RT-34.419) respectively (Table 1 and Figure 1).

Production of polyethylene degrading by-products by the action of fungal strains *Aspergillus flavus* MF-16

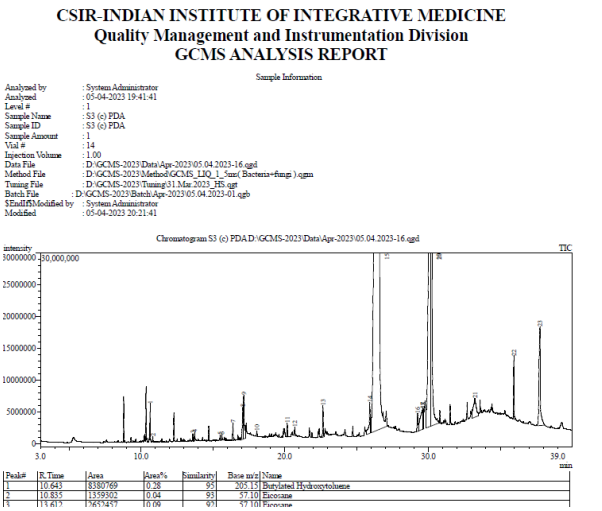
Total fifteen by-products of polyethylene film



a) control polyethylene



b) *Aspergillus fumigates* MF-14



c) *Aspergillus favus* MF-16

Fig. 1. Chromatogram of polyethylene degradation products produced due to action of **a)** control polyethylene **b)** *Aspergillus fumigates* MF-14, **c)** *Aspergillus favus* MF-16

degraded by *Aspergillus flavus* MF-16 were noticed and recorded. Among fifteen by-products, nine were common with that of control and six different by-products were recorded. These were Palmitoyl chloride (RT-21.922), Hexatriacontane (RT-22.695), Triacontyl heptafluorobutyrate (RT-33.740), Methyloctacosane (RT-31.729), and Oxalic acid, cyclohexylmehyl octadecyl ester (RT-33.127) (Table 1 and Figure 1).

DISCUSSION

The current study focused on monitoring the by-products generated from polyethylene biodegradation, utilizing gas chromatography-mass spectrometer (GC-MS) analysis. During this

Table 1. List of the identified compounds produced due to biodegradation of polyethylene using fungal strains *Aspergillus fumigates* MF-14 and *Aspergillus flavus* MF-16

S.No	Retention time (RT)	Name of the compound	Control	Fungal strains	
				Aspergillus fumigates MF-14	*Aspergillus flavus* MF-16
1.	10.650	Heneicosane	+	+	-
2.	32.250	2-1-Hexacosanol	-	+	-
3.	33.333	3-Methyltriacontane	-	+	-
4.	34.419	Octatriacontyltrifluroacetate	+	+	-
5.	21.922	Palmitoyl chloride	+	-	+
6.	22.695	Hexatriacontane 2-	-	-	+
7.	33.740	Triacontyl heptafluorobutyrate	-	+	+
8.	31.729	Methyloctacosane (RT-),	+	-	+

analysis, the emergence of new peaks and compounds was observed. In the current study, by the activity of two most potential low density polyethylene degrading fungal strains *Aspergillus fumigates* MF-14 and *Aspergillus flavus* three major by-products were generated. i.e, heneicosane, 2-1-Hexacosanol, 3-Methyltriacontane, and Octatriacontyltrifluroacetate (Table 1 and Figure 1). Gas chromatography mass spectrometry analysis detected 4,6-Octadiyn-3-one, 2-methyl which was categorized as a ketone. Additionally, 4,4-Dimethyl-2-pentene, an alkene produced by *Aspergillus* sp. as reported by Christabel *et al.,* (2018). The by-products recorded with MF-16 fungal culture among were Palmitoyl chloride, Triacontyl heptafluorobutyrate, and Oxalic acid and cyclohexylmehyl octadecyl ester. Potential polyethylene breakdown products from *Aspergillus terreus* isolate MANF1/WL and *Aspergillus sydowii* isolate PNPF15/TS biodegraded polythene were 1,2-Bis(trimethylsilyl) benzene, Hexasiloxane, 2-Cyclohexen-1, 1,2-Benzenedicarboxylic acid, 7-Methylenebicyclo [3.2.0]hept-3-en-2-one, Octadecanoic acid, Cyclohexane-1, and Dodecahydropyrido[1,2-b]isoquinolin-6-one (Sangle *et al.,* 2019).

CONCLUSION

The present work indicates that naturally occurring microorganisms in the landfills have a great efficiency to degrade polyethylene especially fungi. The fungi of the genus *Aspergillus* have highest capacity to degrade low density polyethylene under laboratory conditions. Based on the present study, it can also be concluded that the due to action of two potential fungal strains the major by products of the low density polyethylene in the culture supernatant extracted belongs to plastisizer, fatty acids and alcohols. The enzymes that causes breakdown of low density polyethylene should by isolated and characterised to improve the efficiency of polyethylene biodegradation process.

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