GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

Check for updates

Biodegradation of Low-Density Polyethylene (LDPE) by *Aspergillus niger*

Tahani Gatea Obaid and Ihsan Flayyih Hasan AL-Jawhari *

College of Education for Pure Sciences, University of Thiqar, Iraq.

GSC Advanced Research and Reviews, 2023, 17(03), 101–108

Publication history: Received on 03 November 2023; revised on 15 December 2023; accepted on 18 December 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.17.3.0468

Abstract

The current study focused on the ability of *Aspergillus niger* to biodegrade low-density polyethylene, while studying some of the factors affecting the biodegradation process, including pH and temperature. Samples were collected from the soil of the waste landfill (sanitary landfill) of Dhi Qar Governorate, located 20 kilometers south of the center of the governorate, and using the dilution method. The genus *Aspergillus niger* had the highest appearance rate, reaching 100%, as it appeared in all samples, and the colony diameters in the solid medium were calculated. The fungus showed the ability to adapt and grow in the medium containing low-density polyethylene, with the a concentration of (0.1, 0.5, 1)%, and temperature of (15, 25, 35) ˚C, also pH (2, 4, 6), and the addition of a carbon source of glucose, maltose and a nitrogen source (NH_4Cl, $NaNo_3$), the decomposition metabolites and the remaining concentration were identified by using Fourier – transform Infrared Spectroscopy and Gas – Mass Spectroscopy, and later a confirmatory test was conducted to determine the ability of *Aspergillus niger* to biodegrade low-density polyethylene in the medium of mineral salts.

Keywords: Biodegradation; Polyethylene; Medium mineral salt; Solid medium.

1. Introduction

Polyethylene is kind of a plastic which is a stable and thermoplastic polymer. It consists of long chains of ethylene monomers and it can't easily degrade by microorganisms.

The polyethylene is one of the major sources of environmental pollution, which is a polymer made of long chain monomers of ethylene with different densities. The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tones of synthetic polymers are produced each year at international level (Shimao, 2001) With such huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue. Because of its very slow natural degradation, thus accumulates in environment in huge amount. It posses the main environmental pollution problems. The biodegradation is a promising method of solving this environmental issue among other physical and chemical degradation method. Our study illustrated the bio degradation of LDPE with the help of certain fungal species. isolated from polythene pollute . Microorganisms utilizing this organic complex polymer as carbon and biologically transforming to simpler one. The microorganisms secrete several LDPE degrading enzymes in different quantities, which expressed its degradation efficiency of the microorganism. (Bhardwaj *et al*, 2012).

In several studies, fungi were considered favorable for the degradation of LDPE due to their higher ability to form hydrophobic enzyme proteins, which helped the fungal species. in attachment to the polymer surface (Seneviratne, *et al*., 2006; Kershaw,1998). Kim and Rhee, 2003) also recorded several bacterial species. as biodegrading agents but the faster growth of fungal biomass was observed when compared to the bacterial species. (Shah, 2008). (Frazer,1994) concluded that the extra cellular enzymes were responsible for such degradation process, also recorded that these

* Corresponding author: Ihsan Flayyih Hasan AL-Jawhari

microbes attached to the inert surface of polyethylene with the help of enzymes secreted by them and grow on film by utilizing the LDPE, and the polymers are depolymerized and are degraded by the process of mineralization into the carbon dioxide (CO_2), water (H_2O) or methane (CH_4). The aim of this study was to isolate the soil fungi which were native to the site of polyethylene disposal sites and showing degradability in natural conditions. The LDPE degradation efficiency of isolates in laboratory condition was determined.

2. Materials and Methods

2.1. Chemicals and media

- All chemicals used in the current study were sourced from BDH and Merch .
- The low-density polyethylene powder was obtained from Nanjing duly biotech Company.

Potato Dextrose Agar (PDA): It consists of 200 gm of potatoes, 20 gm of dextrose and 20 gm of agar dissolved in a liter of distilled water then it is sterilized in an autoclave at 120 °C for 20 minutes and pressure 15 pounds.

Mineral salt medium : It is used for the growth of fungi (Shirling and Gott , 1966) and consists of (K_2HPO_4 , 1.71 g ; KH_2PO_4 , 1.32g ; $NaNo_3$, 0.42g ; MgSo4 . $7H_2O$, 0.42g ; $CaCl_2$, 0.02g) the contents are dissolved in a liter of distilled water then it is sterilized in an autoclave at 120°C for 20 minutes and pressure 15 pounds .

2.2. Isolation of fungus

One technique is applied to isolated fungi from the surface of healthy landfill soil (15cm) depth by using dilution plate method (Gupta *et al*., 2012). Two media were used in this study, Potato dextrose agar (PDA), and Mineral salts medium (MSM). Each media was supplemented with 250 mg chloramphenicol to kill bacteria. One loopful was transferred from the plates and examined under a dissecting microscope. General and specific taxonomic references were used for the identification for fungal species (Klich and Pitt 1992).

2.3. Ability of *A. niger* to grow on solid medium supplemented with low density polyethylene powder

The growth examined to find the resistant isolated fungi to low density polyethylene powder in solid medium, and the growth were comparing with control. The low-density polyethylene at a concentration of (0.1, 0.5, 1)% was added to warm PDA before solid in all plates. Dishes without added low density polyethylene powder were used in control plates. This experiment was done duplicate. All dishes were inoculated with 5mm from 7-day old colonies. The dishes were transferred to incubator with (15, 25, 35) ^oC. The colony diameter of all fungi under study were calculated after 30 days and compared with control.

2.4. Ability of *A. Niger* to grow in Mineral salts medium supplemented with low density polyethylene powder

The growth examined to find the resistant isolated fungi to low density polyethylene powder in liquid medium. The growth was comparing with control, as a weight of mycelium in mineral salts medium 250 ml containing low density polyethylene powder at a concentration of (0.1, 0.5, 1) % added as a source of carbon. The liquid mineral salts medium was inoculated with 5mm disk from the mycelial of the old 7 days. The control flasks were not inoculated with mycelial of fungi colony isolated. All flasks were covered with non- absorbent cotton wool and incubated 30 days in (15, 25, 35) ^oC, pH (2 ,4, 6). The flasks were shaken with orbital shaker (150 rpm) to mixed content. The mycelial dry weight of isolated fungi was calculated by using sensitive balance after filtration through Whattman NO.1 filter paper. Hydrogen ion concentration was calculated by using pH meter.

2.5. Biodegradation of Low-density polyethylene by *A. Niger*

The process of (Pramila and Ramesh ,2011) was followed to prepare LDPE powder from sheets. Immerse the powder in xylene and boil it for 15 minutes. To remove xylene from the solution, the LDPE solution was treated with ethyl alcohol. The xylene-ethyl alcohol was evaporated and then the LDPE powder was washed with ethanol to remove xylene residue and was again left to evaporate. The powder was dried in a hot air oven at 40-50°C for overnight.

2.6. Statistical Analysis

All applications were analysis by using (ANOVA) in program SPSS (version 23.0).

3. Results and Discussion

3.1. Ability of *A. Niger* to grow in solid media with low density polyethylene powder

The results in Table (1) indicate that *A. Niger* grow well in PDA medium to which low density polyethylene (powder). It was also found that there is a daily increase in the colony diameters of the isolated fungi, and this indicates that this fungus can grow in the presence of low-density polyethylene.

The results showed the ability of the *A. Niger* to degradation low-density polyethylene when adding polyethylene to the medium. These results are consistent with Sindujaa *et al*., 2011, who isolated the *A. Niger* and demonstrated its ability to degrade low-density polyethylene in solid culture medium.

Table 1 Ability the growth of *A. Niger* in solid medium supplemented with Low density polyethylene

Fungi	Concentration	The colong diameter	Control
A. Niger	0.1%	8.1	7.3
A. Niger	0.5%	7.0	6.6
A. Niger	1%	6.8	6.5
L.S.D = 0.036			

3.2. Ability of *A. Niger* to grow in mineral salts medium with low density polyethylene (powder)

Table (2) showed that the dry weight of *A. Niger* in a concentration 0.1%, 15°C, pH = 2, glucose, and $NaNo_3$ in the amount of 0.1 g was reached to (5.40) compared to the control (0.59), and the dry weight of *A. Niger* in a concentration (0.5)%, 25°C, pH = 4, glucose and a NH_4Cl reached to (7.16) compared with control (1.11).

The dry weight of *A. Niger* in 1%, 35°C, pH = 6, maltose, and $NaNo_3$ reached to (1.60) compared to the control (1.22).

The results showed an increase in the dry weight of *A. Niger* in all concentration. This study agrees with (Ojha et al.,2017), as it is considered an optimal temperature for the growth of fungal hyphae and for the fungus to increase in the breakdown of low-density polyethylene.

The results showed that the pH = 4 is considered optimal, and this is consistent with the study of (Hamzah *et al*., 2017), in which that the optimal growth of mushrooms was between (4.5-6.5), depending on the type of mushroom studied.

Table 2 Ability of *A. Niger* to grow in in mineral salts medium supplemented with Low density polyethylene

Fungi	Concentration	temperature	pH	Carbon source	Nitrogen source	Dry weight	control
A. Niger	0.1%	15	2	Glucose	$NaNo_3$	5.40	0.59
A. Niger	0.5%	25	4	Glucose	$NaNo_3$	7.16	1.11
A. Niger	1%	35	6	Maltose	NH4Cl	1.60	1.22
L.S.D = 0.032							

3.3. Biodegradation of low density polyethylene powder by *A. Niger*

The results of the current study showed that the ability of fungi isolated from sanitary landfill soil in the study area to degrade low-density polyethylene at a concentration of (0.1, 0.5, 1)% in mineral salts medium at a temperature of (15, 25, 35) and at a pH (2, 4, 6).) respectively, with the addition of glucose, NH_4Cl and $NaNo_3$, after 30 days of incubation. Figure (1, 2, 3) showed the biodegradation of low – density polyethylene with a comparison with Stander by *A. Niger* after 30 days incubation. These figures showed that disappearance four peaks in a region 500-1000, 1000- 1500, and appear one a large peak in 3343 region, these region indicates the presence of an OH group, which means the presence of acid, and these results are consistent with (Zhang, 2020).

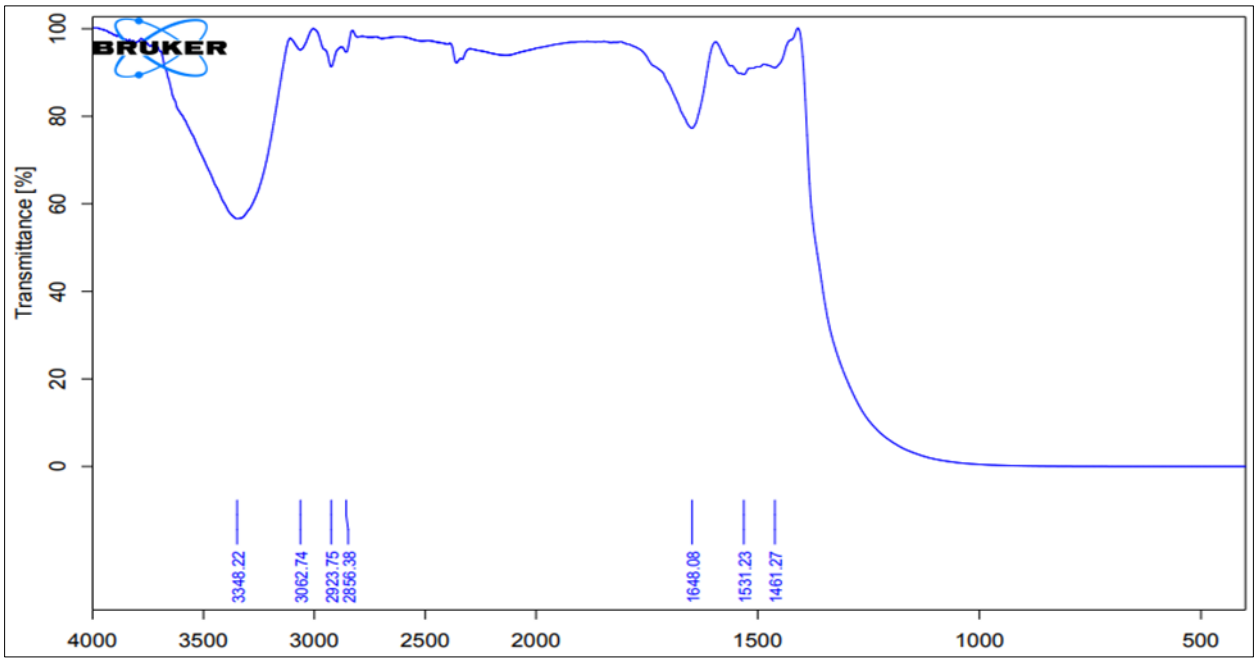

Figure 1 Biodegradation of low density polyethylene powder by *A. Niger* in 0.1% concentration after 30 days incubation by using FTIR spectroscopy

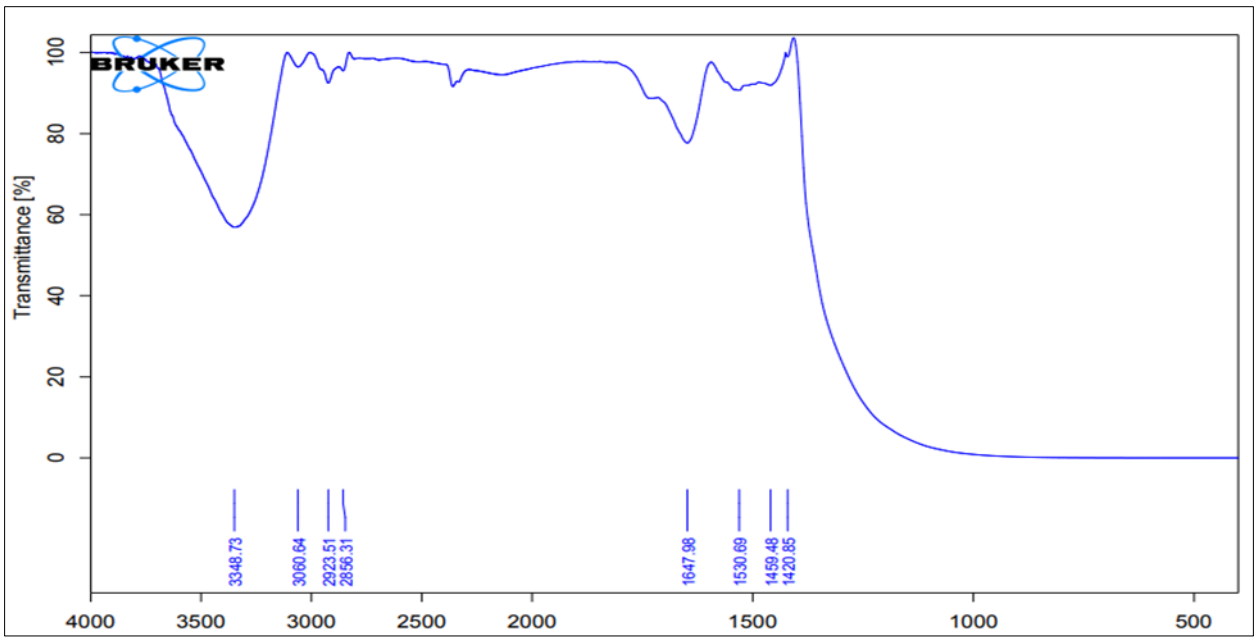

Figure 2 Biodegradation of low density polyethylene powder by *A. Niger* in 0.5% concentration after 30 days incubation by using FTIR spectroscopy

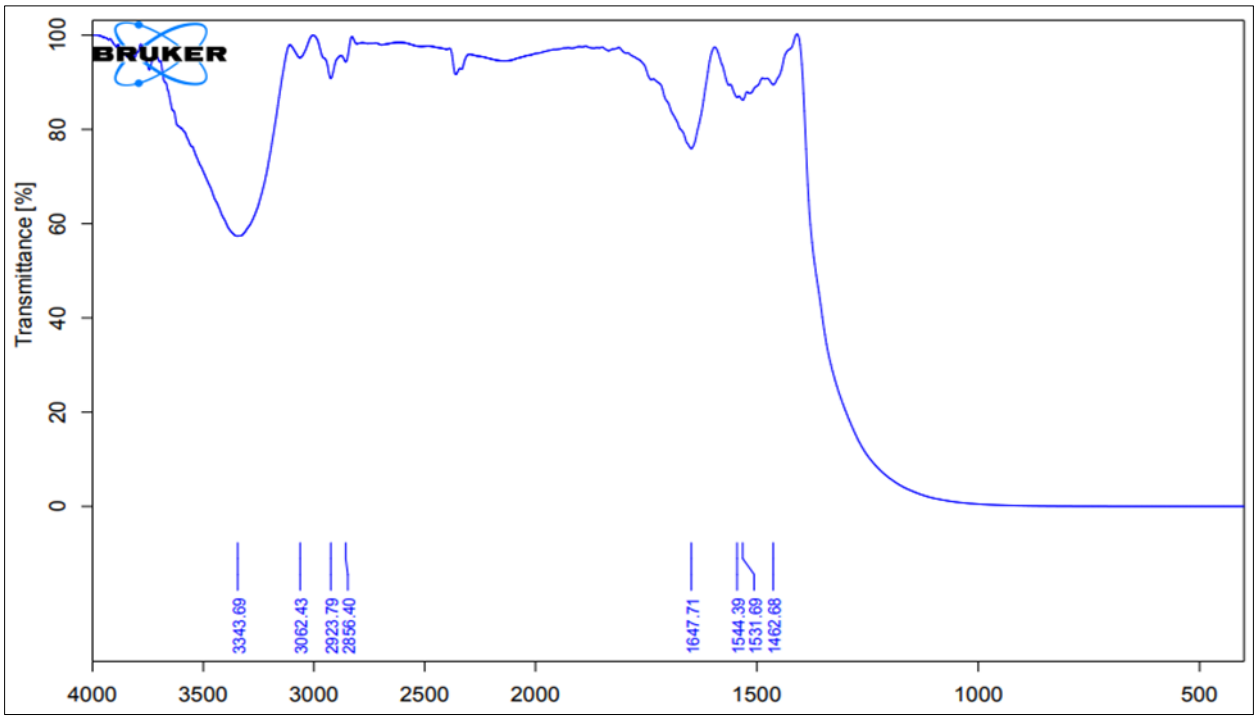

Figure 3 Biodegradation of low density polyethylene powder by *A. Niger* in 1.0% concentration after 30 days incubation by using FTIR spectroscopy

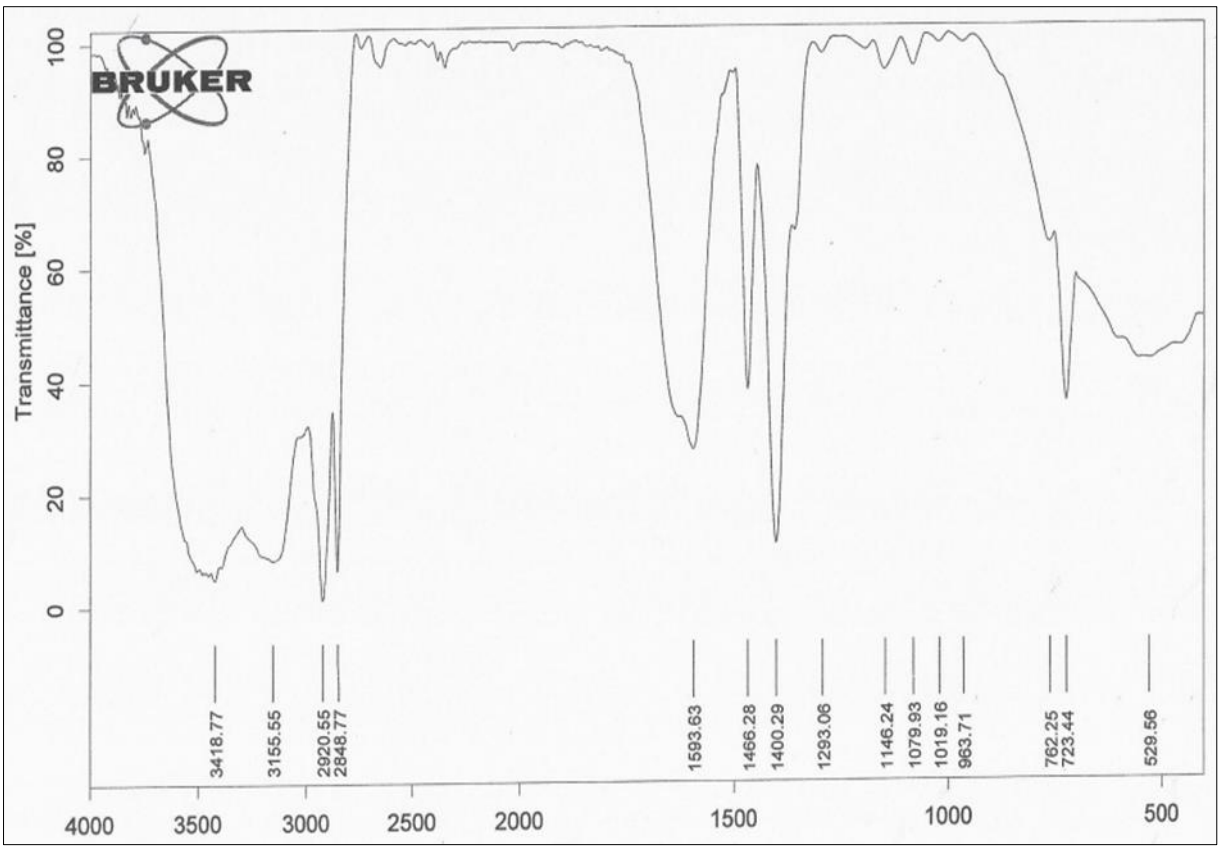

Figure 4 Low density polyethylene (Standard)

Figures (5, 6, 7) showed the ability of *A. Niger* to degradation low-density polyethylene powder Chromatographic peaks were disappear in 0.5% concentration Figures (6,7), and new peaks were appear in Figure(5), these peaks indicating the formation of decomposition products and the appearance of many metabolites and chemical compounds that differ in their retention time. These results agree with (Sanniyasi *et al*.,2021).

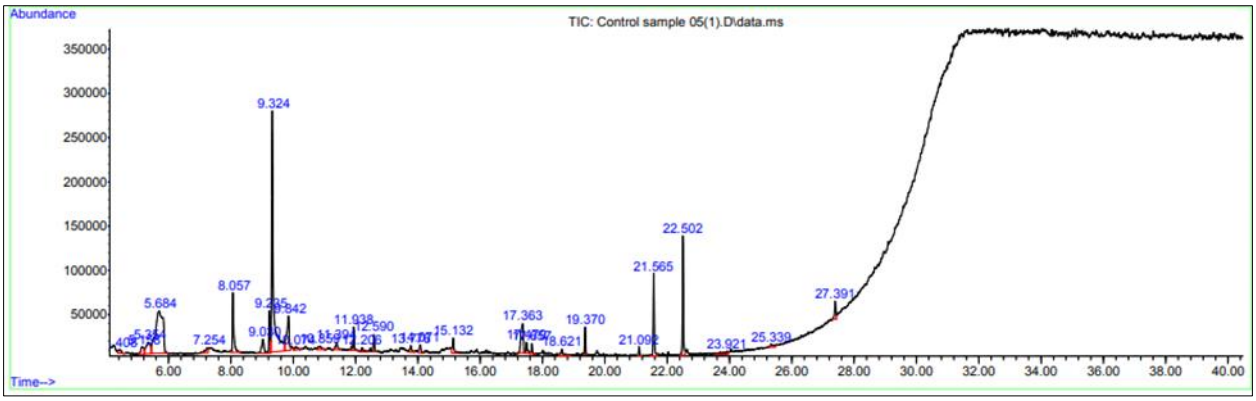

Figure 5 Biodegradation of low-density polyethylene powder using *A. niger* in 0.1% concentration by using GC-Ms spectroscopy

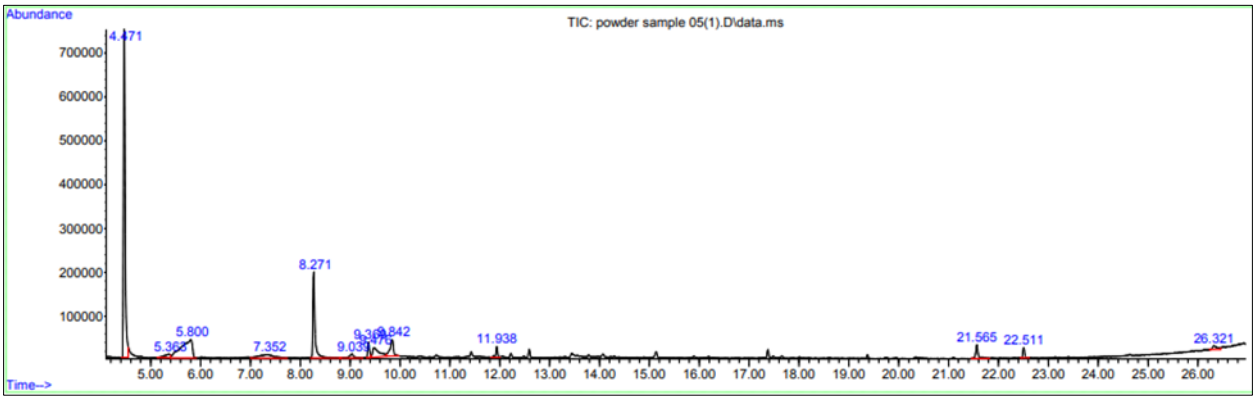

Figure 6 Biodegradation of low-density polyethylene powder using *A. niger* in 0.5% concentration by using GC-Ms spectroscopy

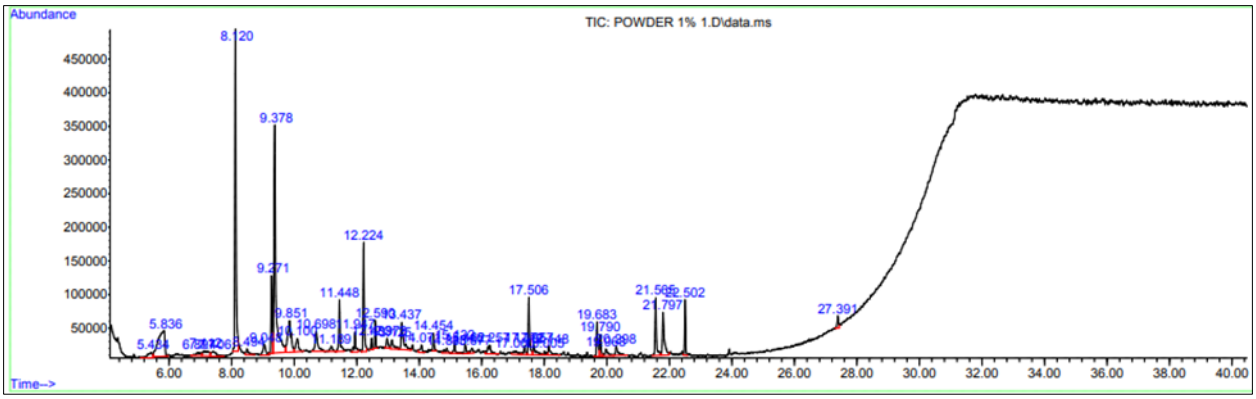

Figure 7 Biodegradation of low-density polyethylene powder using *A. niger* in 1.0% concentration by using GC-Ms spectroscopy

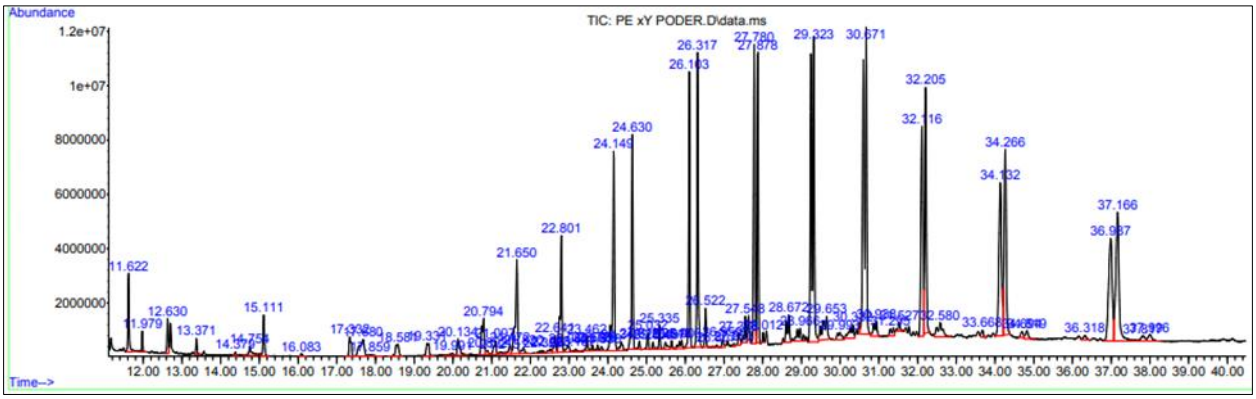

Figure 8 Low-density Polyethylene (Standard)

4. Conclusion

The ability to degradation low-density polyethylene powder by *A. Niger* in mineral salts medium after 30 days incubation. It has been noted that environmental factors play an important role through their impact on the ability of fungi to biodegrade low-density polyethylene because they contribute to biodegradation over time and the fungi need time to adapt to it in order to begin the biodegradation process.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Bhardwaj H., Gupta R. and Tiwari A., (2012) Microbial Population Associated with Plastic Degradation, doi:10.4172/ scientificreports., 1(5) 272.

[2] Frazer A.C., (1994) O-methylation and other transformations of aromatic compounds by acetogenic bacteriaIn: Acetogenes is Drake HL (edi). Chapman & Hall, New York.

[3] Gupta, V. C.and Neha, S. (2012). Batch biodegradation of phenol of paper and pulp effluent by Aspergillus niger. Int. J. Chem. Eng. Appl., 3 (3):21-28.

[4] Hamzah, A.; Manikan, V. and Abed Aziz, N. F. (2017). Biodegradation of tapiscrude oil using consortium of bacteria and fungi: optimization of crude oil concentration and duration of incubation by response surface methodology. Sains Malaysiana. 46(1): 43– 50.

[5] Kershaw M.L. and Talbot N.J., (1998), Hydrophobins and repellents: proteins with fundamental roles in fungal morphogenesis, Fungal. Genet. Biol., 23, 18 33.

[6] Kim D.Y. and Rhee Y.H., (2003), Biodegradation of microbial an polyesters by fungi. Appl. Micro. Biotech., 61, 300 30

[7] Klich M and Pitt J 1992. A laboratory guid to the common Aspergillus species and their teleomorphs. common. Sci. Indus Res Org. Australia.

[8] Ojha, N.; Pradhan, N.; Singh, S.; Barla, A.; Shrivastava, A.; Khatua, P.; Rai, V. and Bose, S. (2017). Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. Sci. Res., 39(7):1-13.

[9] Pramila R. and Vijaya Ramesh K. (2011) Biodegradation of low density polythene (LDPE) by fungi isolated from municipal landfill area, J.Microbial.Biotech.Res., ,1(4):131-136.

[10] Sanniyasi, E., Gopal, R. K., Gunasekar, D. K., & Raj, P. P. (2021). Biodegradation of low-density polyethylene (LDPE) sheet by microalga, Uronema africanum Borge. Scientific Reports, 11(1), 17233.

[11] Seneviratne G., Tennakoon N.S., Weerasekara M.L.M.A.W. and Nanadasena K.A.,(2006) LDPE biodegradation by a developed Penicillium Bacillus biofilm, Curr. Sci., , 90, 20 21.

[12] Shah A.A., Hassan F., Hameed A. and Ahmed S., (2008), Biological degradation of plastics: a comprehensive review, Biotechnol. Adv., 26, 246 265

[13] Shimao, M., (2001) biodegradation of plastics. Curr.Opin. Biotechnol., 12: 242-247

[14] Shirling, E.B. and Gottlieb, D. (1966). Methods for characterization of Streptomyces species. International Journal of Systematic Bacteriology, 16, 313-340 .

[15] Sindujaa, P., Padmapriya, M., Pramila, R. and Ramesh, V. 2011. Bio-degradation of low density polyethylene (LDPE) by fungi isolated from marine water. Res. J. Biol. Sci., 6(4): 141-145.

[16] Zhang J, Gao D, Li Q, Zhao Y, Li L, Lin H, Zhao Y (2020) Biodegradation of polyethylene microplastic particles by the fungus Aspergillus favus from the guts of wax moth Galleria mellonella. Sci Total Environ 704:135931.