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Biodegradation of Low Density Polyethylene by Fungi Isolated from Red Sea Water

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A B S T R A C T

Polyethylene is kind of plastic which is a stable and thermoplastic polymer. It consists of long chains of ethylene monomers and it can't easily degrade by microorganisms. In the current study, ten fungal strains were isolated from Red Sea water; Jeddah, Saudi Arabia. These isolates were screened to examine their ability in biodegradation of Low density polyethylene, selected fungi which related to *Aspergillus* and *Penicillium* showed ability to degrade polyethylene films and powder. *Penicillium sp* showed the highest percentage in reduction of polyethylene weight with (43.4%). Detection of morphological changes by SEM demonstrated that fungal growth was observed clearly on the treated film. Mycelia and conidia of *Aspergillus sp* and *Penicillium sp* have seen physically associated with the surface.

Introduction

Plastics can degrade via different mechanisms such as thermal, chemical, photo and biological degradation. The degradation of plastics is a physical or chemical change in polymers that occurs as a result of environmental factors, like light, heat, moisture, chemical conditions or biological activity (Tokiwa *et al.,* 2009) and (Nanda *et al.,* 2010). Biodegradation of microorganism can occur in aerobic conditions in nature and in an aerobic conditions in some environments such as sediments, landfills, compost and soil (Ishigaki *et al.,* 2004).

Aerobic microbes use oxygen as an electron acceptor, and break down organic chemicals into smaller organic compounds. CO_2 and water are the by-products of this process. C plastic + O_2 → CO_2 + H_2O + C residual +Biomass

While anaerobic biodegradation produces carbon dioxide, water and methane as a follow equation (Ishigaki *et al.,* 2004):

C plastic → CH_4 + CO_2 + H_2O + C residual +Biomass

Polyethylene is totally linear and available with varying range of densities from 0.91 to 0.97 g/cm^3. Low density PE has branching at random places to low packing of the polymer chains, whereas the high density polyethylene is more linear with minimal branching leading

to high packing density (Arutchelvi *et al.*, 2008; Abraham *et al.*, 2016).

Generally, the biodegradation of PE is a very slow process. A wide variety of Actinomycetes like Streptomyces strain and fungi like *Aspergillus* and *Penicillium* have been used in research to facilitate this process (Gu, 2003).

(Hussein *et al.*, 2015) mentioned that microorganisms over 90 genera from bacteria and fungi can degrade plastic such as *Bacillus megaterium, Pseudomonas sp., Azotobacter, Ralstonia eutropha, Halomonas sp*., etc.

El-Shafei *et al.*, (1998) investigated the ability of fungi and Streptomyces strains to attack degradable polyethylene that consisted of disposed-of polyethylene bags containing 6% starch. They isolated eight different strains of Streptomyces and two fungi *Mucorrouxii* NRRL 1835 and *Aspergillus flavus.*

Yamada-Onodera *et al.*, (2001) studied a strain of fungus, *Penicillium simplicissimum* YK that can biodegrade polyethylene without additives.

(Mahalakshmi and Andrew, 2012) mentioned that fungi are widely used in bioremediation due to their robust nature and for their great source of diverse enzymes.One of the widely reported fungi, *Phanerochaete chrysosporium,* commonly known as white-rot fungus, is able to degrade broad range of persistent pollutants and xenobiotics under nutrient limited conditions because of its robustenzyme machinery.

(Sowmya *et al.*, 2015) investigated the biodegradation of polyethylene by fungal consortium (*Curvularia lunata, Alternaria alternata, Penicillium simplicissimum* and *Fusarium* sp) and examine their efficiency in biodegradation of polyethylene by using FTIR and SEM.

The aim of this study is to isolate different fungal isolates capable to degrade low density polyethylene by using measurement of dry weight.

Materials and Methods

Materials

Low density polyethylene (Sheets and powder) obtained from Tasnee national company for petrochemicals, AlJubayl, Saudi Arabia.

Medium

Mineral salt medium (MSM) containing:1L distilled water (K_2HPO_4 (1g); KH_2PO_4 (0.2g); NaCl (1g); $CaCl_2.2H_2O$ (0.002g); $(NH_4)_2SO$(1g); $MgSO_4.7H_2O$ (0.5g); $CuSO_4.5H_2O$ (0.001g); $ZnSO_4.7H_2O$ (0.001 g); $MnSO_4.H_2O$ (0.001g) and $FeSO_4.7H_2O$ (0.01g.) polyethylene as a sole source of carbon (Sindujaa *et al.*, 2011).

Sea water samples collecting

Sea water samples were collected from red sea coast nearby Jeddah province, Saudi Arabia. Samples collect in sterilize bottle and transfer to lab.

Serial dilution method was used to isolate the fungi. Suspensions up to 10^{-6} were transferred to Cazpek'sdox ager and incubated at 28 °C for 7 days.

Identification of the fungi

Isolated fungi were identified based on their cultural and morphological characteristics. Microscopic characteristics of fungi were studied by staining them with lactophenol and examine them under microscope. Identification was done by following the keys of Raper and Fennell (1987).

Screening of polyethylene degrading fungi on solid medium

LDPE powder was added to synthetic medium at a concentration of 0.1% (w/v) and the culture was kept in shaker oven for 30 days at 28°C.

Fungal isolated were inoculated on LDPE powder containing Czapekdox agar plates and incubated at 28ºC for 7 days. Fungal isolates which gave maximum diameter were selected for further screening of biodegradation rates.

Fungal colonizing studies

LDPE sheets were cut into small pieces (2x2) cm of similar weight, disinfected with 70% ethanol for 30 min and transferred to sterile water for 20 min. Four LDPE sheets of similar weight were placed in Petri plates containing the Synthetic medium.

These sheets were inoculated with fungi using the cork borer. The Petri plates were incubated at 28°C and results were determined after one month based on the increasing of the dry mycelium weight.

Detection of biodegradation of polyethylene

Weight reduction

Before measuring of dry weight of the residual polyethylene, fungal colonization was washed from the film by using sodium dodecyl sulphate solution 2% (v/v) for four hours and washed it again by distilled water. The washed polymer film was placed on a filter paper and dried for one night at 60°C.

Weight loss=initial weight- final weight

%Weight loss = initial weight- final weight/initial weight ×100

Morphological changes

Untreated polyethylene films were cut into small pieces and added to flasks 250 ml containing 100ml of Mineral Salt Medium. Flasks were inoculated by selected fungal isolates separately and incubated for two months. After two months polyethylene films were sterilized by ethanol 70% for two hours and washed by distilled water. Sterilized films were prepared for analysis by scanning electron microscope to check the changes on the surface of polymer.

Results and Discussion

Ten fungal isolates were isolated from sea water and maintained on Cazpek's dox ager for 7 days at 28°C. These isolates were screened to examine their activity in degrading polyethylene on synthetic mineral medium. Marine fungi usually tolerated with strict conditions in water. Table 1 shows fungi which showed maximum growth on synthetic medium. *Aspergillus niger* (F2), *Aspergillus flavus* (F5), *Aspergillus terreus* (F7), *Aspergillus fumigatus* (F9) and *Penicillium sp* (F10*)* were selected based on a colony diameter growth. Other isolates showed moderate activity in a growth on medium supplement with polyethylene as a sole source of carbon. This result agrees with (Sindujaa *et al.,* 2011) who isolated species of *Aspergillus* from marine water, which was showed capability of degrading polyethylene.

Results in table 2 show the reduction of dry weight of polyethylene after 30 days of incubation with selected fungi. *Penicillium sp* showed the highest percentage in biodegradation of polyethylene film with (43.4 %). Other *Aspergilli* showed moderate activity in degradation of polyethylene films after one month of incubation. This result agrees with (Deepika and Madhuri, 2015) who found a significant difference in weight

of LDPE compared to initial weight. *A. niger* reduced the weight of LDPE strip up to 26.17±0.05% while *A. flavus* the reduction was 16.45±0.01% after 6 months of incubation. In the study of (Singh and Gupta, 2014) biodegradation was measured in terms of weight loss, which was nearly 16 to 36 %after a period of 4 weeks. Fungal strain *Aspergillus japonicas* F3 (36%), *Fusarium* sp F6 (32%), *Aspergillus flavus* F1 (30%) showed effective degradation results in 4 weeks as compare to *Penicillium* sp F5 (24%), *Aspergillus niger* F2 (20%), *Mucor* sp F4 (16%). (Rani and singh, 2017) reported that *Fusarium* shows the best degradation with (77.668 %) for LDPE after one month of incubation (Das and Kumar, 2014) mentioned that microbial isolates were responsible for the decreasing weight of LDPE films by adhering on this inert surface and also utilizing it as the only carbon and energy source which was evident by increase in the fungal growth. Kavitha *et al.*, (2014) reported that the percentage of weight reduction of Low density polyethylene films which incubated with bacterial isolates was not as a result of chemicals in the mineral salt medium, but because of a biological process (Singh and Gupta, 2014; Dineshraj and Ganesh, 2016) confirmed that fungi are responsible for decreasing the weight of LDPE films by adhering on its inert surface.

Detection of morphological changes by SEM

Figure 1 shows five images of polyethylene treated with different fungi. Fungal growth was observed clearly on the treated film. Mycelia and conidia of (*Aspergillus niger*, *A. flavus*, *A. terreus*, *A. fumigatus* and *Penicillium sp*) could be seen physically associated with the surface.

In the study of (Raaman *et al.*, 2012) SEM analysis confirmed that the degradation by revealing the presence of porosity and fragility of the fungal degraded polythene surface. *Aspergillus* species were also grown on LDPE film (Das and Kumar, 2014) reported that the microbial colonization of a polymer surface is the first requirement for its biodegradation. Scanning electron micrograph showed the attachment of fungi on LDPE surface and formation of various holes and irregularities whereas the control film was appeared with smooth surface having no any pits, cracks or any particles attached on its surface.

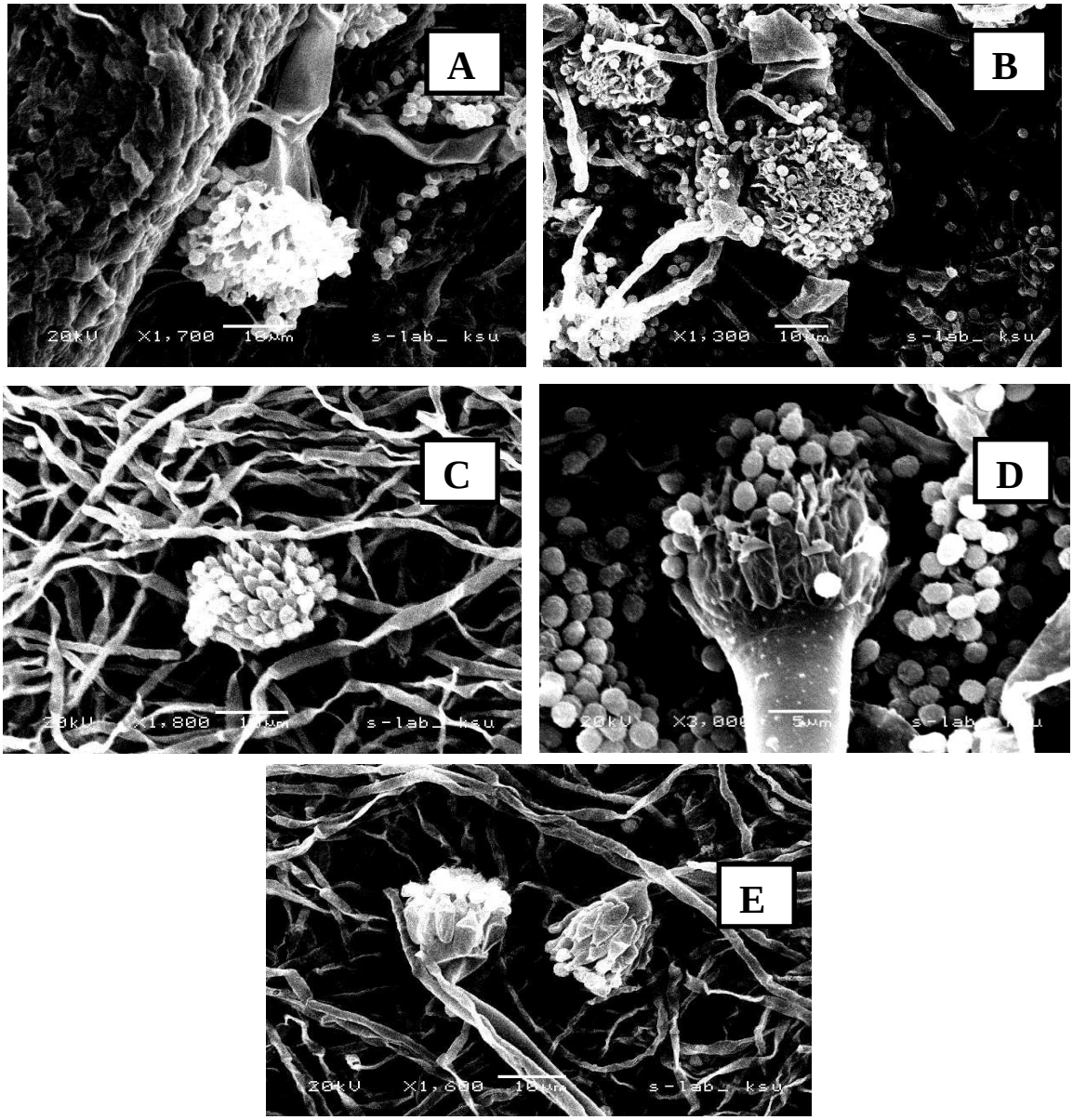
Table.1 Screening of polyethylene degrading fungi on solid medium

Isolate Code	Colony diameter (mm)* ±SD
F1	22±0.3
F2	95±0.05
F3	63±0.1
F4	41±0.2
F5	78±0.5
F6	68±1
F7	85±0.1
F8	31±0.7
F9	82±0.4
F10	70±1.1

Table.2 Measurement of dry weight of polyethylene after 30 days of incubation with fungal isolates

Fungal isolates	Dry weight of polyethylene (g)			
	Initial weight	Weight after treatment	Weight loss	Percentage (%)
F2 (*Aspergillus niger*)	0.661	0.532	0.129	19.5
F5 (*Aspergillus flavus*)	0.701	0.587	0.114	16.2
F7 (*Aspergillus terreus*)	0.654	0.511	0.143	21.8
F9 (*Aspergillus fumigatus*)	0.602	0.478	0.124	20.5
F10 (*Penicillium sp.)*	0.511	0.289	0.222	43.4

Fig.1 SEM photograph of polyethylene treated with (A) *Aspergillus niger*, (B) *A. flavus* (C) *A. terreus* (D) *A. fumigatus*, (E) *Penicillium* sp



In the study of (Ojha *et al*., 2017) the FE-SEM images confirmed that the two fungal strains (*Penicillium oxalicum* and *Penicillium chrysogenum*) have been able to break down the complex polymer of polyethylene of both HDPE and LDPE into its monomeric forms. The grooves and cracks further confirm the fragility brought about to the plastic sheets on treating the sheets with fungal cultures. Only after the fungal isolates starts colonizing the plastic sheets by utilizing HDPE/LDPE as the sole source of carbon, the degradation starts.

(Pramila and Ramesh, 2011) noticed that structural changes such as formation of pits, cracks and minute holes, reproductive structures and spores grown through the LDPE films were observed under SEM.

Mahalakshmi *et al.,* (2012) Sowmya *et al.,* (2015) found structural changes and erosions on the surface of the PE films. Cavities were also observed on the polyethylene surface as a result of biodegradation by *Aspergillius* sp and *Penicillium* sp.

References

Abraham, J., Ghosh, E., Mukherjee, P. and Gajendiran, A. 2017. Microbial Degradation of Low Density Polyethylene. *Environ Progress & Sustainable Energy*, 36(1): 147-154.

Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhandari, S., Uppara, P.V. 2008. Biodegradation of Polyethylene and Polypropylene. *Indian J. Biotechnol.,* 7: 9–22.

Das, M.P. and Kumar, S. 2014. Microbial Deterioration of Low Density Polyethylene by *Aspergillus* and *Fusarium* sp. *Int. J. Chem. Tech. Res.,* 6(1): 299-305.

Deepika, S. and Madhuri, R.J. 2015. Biodegradation of low density polyethylene by micro-organisms from garbage soil. *J. Experimental Biol. Agri. Sci.,* 3(1): 15-21.

Dineshraj, D. and Ganesh, P. 2016. Screening and Characterization of Isolate Fungi from Plastic Waste Dump Yard Sites. *Int. J. Scientific Res.,* 5(1): 301-302.

El-Shafei, H.A., Abd El-Nasser, N.H., Kansoh, A.L., Ali, A.M. 1998. Biodegradation of disposable polyethylene by fungi and Streptomyces species. *Polym. Degrad. Stab.,* 62: 361-365.

Gu, J.D. 2003. Microbiological deterioration and degradation of synthetic polymeric materials: recent research advances. *Int. Biodeterior. Biodegrad.,* 52: 69-91.

Hussein, A.A., Al-Mayaly, I.K. and Khudeir, S.H. 2015. Isolation, Screening and Identification of Low Density Polyethylene (LDPE) degrading bacteria from contaminated soil with plastic wastes. *Mesopotamia Environ. J.,* 1(4): 1- 14.

Ishigaki, T., Sugano, W., Nakanishi, A., Tateda, M., Ike, M., Fujita, M. 2004. The degradability of biodegradable plastics in aerobic and anaerobic waste landfill model reactors. *Chemosphere,* 54: 225-233.

Kavitha, R., Mohanan, A.K. and Bhuvaneswari, V. 2014. Biodegradation of low density polyethylene by bacteria isolated from oil contaminated soil. *Int. J. Plant, Animal and Environ. Sci.,* 4(3): 601-610.

Mahalakshmi, V. and Andrew, S.N. 2012. Assessment of Physicochemically treated plastic by fungi. *Annals of Biol. Res.,* 3(9): 4374-4381.

Mahalakshmi, V., Siddiq, A. and Niren, A.S. 2012. Analysis of Polyethylene Degrading Potentials of Microorganisms Isolated From Compost Soil. *Int. J. Pharmaceutical Biol. Arch.,* 3(5): 1190-1196.

Nanda, S., Snigdha, S.S., Abraham, J. 2010. Studies on the biodegradation of natural and synthetic polyethylene by Pseudomonas spp. *J. Appl. Sci. Environ. Manage.*, 14(2): 57–60.

Ojha, N., Pradhan, N., Singh, S., Barla, A., Shrivastava, A. Khatua, P., Rai, V. and Bose, R. 2017. Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization. *Scientific Reports*, PP: 1-13.

Onodera, K.Y., Mukomto, H., Katsuyaya, Y., Saiganji, A. and Tani, Yoshki. 2001. Degradation of polyethylene by a fungus, *Penicillium simplicissimum* YK. *Polymer Degradation and Stability*, 72(2): 323-327.

Pramila, R. and Ramesh, K.V. 2011. Biodegradation of low density polyethylene (LDPE) by fungi isolated from marine water– a SEM analysis. *African J. Microbiol. Res.*, 5(28): 5013-5018.

Raaman, N, Rajitha, N., Jayshree, A. and Jegadeesh, R. 2012. Biodegradation of plastic by *Aspergillus* spp. isolated from polythene polluted sites around Chennai. *J. Acad. Indus. Res.*, 1(6): 313- 316.

Rani, A. and Singh, P. 2017. Screening of Polyethylene Degrading Fungi from Polyethylene Dump Site. *Int. J. Chem. Tech. Res.*, 10(3): 699-704.

Raper, K.B. and Fennell, D.I. 1987. The genus Aspergillus, R.E. Krieger (ed. Huntington, NewYork, 686.

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.

Singh, J. and Gupta, K.C. 2014. Screening and Identification of Low density Polyethylene (LDPE) Degrading Soil Fungi Isolated from Polythene Polluted Sites around Gwalior city (M.P. *Int. J. Curr. Microbiol. App. Sci.*, 3(6): 443-448.

Sowmya, H.V., Ramalingappa, B., Nayanashree, G., Thippeswamy, B. and Krishnappa, M. 2015. Polyethylene Degradation by Fungal Consortium. *Int. J. Environ. Res.*, 9(3): 823-830.

Tokiwa, Y., Calabia, B.P., Ugwu, C.U., Aiba, S. 2009. Biodegradability of plastics. *Int. J. Mol. Sci.*, 10: 3722-3742.

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