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. CO₂ and water are the by-products of this process.

\[
\text{C plastic} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{C residual} + \text{Biomass}
\]

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

\[
\text{C plastic} \rightarrow \text{CH}_4 + \text{CO}_2 + \text{H}_2\text{O} + \text{C residual} + \text{Biomass}
\]

Polyethylene is totally linear and available with varying range of densities from 0.91 to 0.97 g/cm³. 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 Mucor rouxii 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 robust enzyme 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<sub>2</sub>HPO<sub>4</sub> (1g); KH<sub>2</sub>PO<sub>4</sub> (0.2g); NaCl (1g); CaCl<sub>2</sub>·2H<sub>2</sub>O (0.002g); (NH<sub>4</sub>)<sub>2</sub>SO(1g); MgSO<sub>4</sub>·7H<sub>2</sub>O (0.5g); CuSO<sub>4</sub>·5H<sub>2</sub>O (0.001g); ZnSO<sub>4</sub>·7H<sub>2</sub>O (0.001 g); MnSO<sub>4</sub>·H<sub>2</sub>O (0.001g) and FeSO<sub>4</sub>·7H<sub>2</sub>O (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<sup>-6</sup> 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 agar 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 (Raman 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.

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