FUNGAL DEGRADATION OF POLYETHYLENE BAG ISOLATED FROM COASTAL ENVIRONMENT

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ABSTRACT

Fungi, isolated from coastal water of Karachi were subject to grow in the medium containing polyethylene bags only carbon source. Weight loss, strum test, Fourier transforms infrared spectroscopy spectroscope and scanning electron microscope were performed for, the evidence that the *Aspergillus nigar* and *Penicillium* spp. utilized polyethylene as the sole source of carbon. *Penicillium* spp. was able to degrade polyethylene (30%) more effectively than *Aspergillus niger* (19%). Further confirmation of plastic utilization was monitored by strum production, 0.833 and 0.985 g/L of CO_2 by *Aspergillus niger* and *Penicillium* spp., respectively. The results of attenuated total reflectance fourier transform infrared analysis showed a major observance bands at various frequencies after cultivation with fungal stains indicates some of the bond chains are degraded. Scanning electron microscopy images of surface cracking, attachment of microbes and hole formation can be clearly seen, Strum test and Fourier transform infrared spectroscopy provides the solid evidence of degradation. These findings showed that the fungal species can be used as a solution for polyethylene degradation.

Keywords: *Aspergillus niger*, *Penicillium* spp., Polyethylene, Strum test.

ABBREVIATIONS

%	Percentage
°C	Degree Celsius
ASTM	American society of Testing and materials
ATR	Attenuated total reflectance
$Ba(OH)_2$	Barium hydrooxide
$BaCo_3$	Barium carbonate
$BaCO_3$	Barium carbonate
cm	centimeter
CO_2	Carbondioxide
eq	equation
$FeSO_4$	ferrous sulfate
FTIR	Fourier transforms infrared spectroscopy
g/L	Gram per liter
h	Hour
H_2O	Water
HDPE	High-density polyethylene
K_2HPO_4	Dipotassium phosphate
KH_2PO_4	Monopotassium phosphate
LDPE	Low-density polyethylene
M	Molarity
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
min	Minute
mL	Milliliters
MSM	Minimal salt media
NaCl	Sodium chloride
NH_4NO_3	Ammonium nitrate
PE	Polyethylene
rpm	Revolution per minute
SEM	Scanning electron microscope
UV	Ultraviolent

INTRODUCTION

Plastic is a synthetic recalcitrant polymer, it is persistent in the environment for many years, one of the huge contributor of solid waste, especially in developing countries. Approximately more than 30% of the plastic is used as a packaging material which accounts annually more than 140 million tons, which is introduced into the environment as solid waste. Untreated and conventional disposing of large scale waste plastic material possesses a serious threat to the environment. (Shimao *et al.,* 2001). Due to larger molecular weight, halogenated substitutions, highly bonded rings, mostly these plastics are highly resistant to microbes. China, Indonesia and Philippine are the largest contributor of plastic solid waste, respectively. In 1940 mass production of plastic started and in the year 1988 exponential increase in the production of plastic was observed. Previously, United states produced 30 million tons of plasticper year (Neelam *et al.*, 2018). Approximately 140 million tonnes of synthetic polymers are produced worldwide each year (Roy *et al.,* 2008; Vatseldutt and Anbuselvi, 2014; Indumathi and Gayathri, 2016). In Pakistan plastic manufacturing industry grows 15% annually and approximately 600-700 processing units have emerged yearly (Sabir *et al.,* 2004).

Polyethylene (PE) is the synthetic polymer of high molecular weight, having high hydrophobic structure, three dimensional arrangement and resistant to the microbial attack. It has a wide range of applications in food packaging, plastic bags, milk, water, motor oil bottles and toys etc, due to its ease carrying and moving properties it was widely used (Byuntae *et al.*1991; Kwpp and Jewell, 1992; Scott 1999; Tribedi and Sil 2013). Rapidly increased use uncontrolled plastic caused serious environmental threat to different sphere of the world, due to its persistent nature , the researchers focused on biodegradable plastics and biodegradation of plastic wastes in the last few years (Shah *et al.,* 2008). The word Degradation reflects the deterioration of a material properties i.e any erosion, cracking, optical or mechanical change in polymer (Pospisil and Nespurek, 1997). Microorganisms can play a significant role in the degradation of plastic, they secrets certain enzymes, which can cleavage long polymer chain and break it into monomers which eventually enough in size to consume by the microorganism. However hydrophobic nature and lack of functional group can resist microbial attack (Lau *et al.,* 2009; Esmaeili *et al.*, 2013). Many studies have been done for the degradation of LDPE by fungal species which produce degrading enzyme (Shah *et al.,* 2008) and extracellular polymers, such as polysaccharides, which facilitates the fungal colonies to develop on the polymer superficial (Gu 2003; Volke *et al*., 2009), however the fungal hyphae has an advantage of penetration ability. Apart from other environmental factors such as temperature, sunlight, pH and availability of oxygen are the key factors effecting the enzymatic degradation. Stabilizer and additives used for improvement of plastic strength can also be a barrier in degradation by microorganism (Kale *et al.,* 2015). The aim of the present work is to investigate the biodegradation of polyethylene (PE) by using curtain species of fungi isolated from coastal environment. The morphology and chemical changes of the structure on film were analyzed by FTIR and Scanning electron microscope (SEM) before and after degradation. This study has been carried out in Karachi, Pakistan during 2017.

MATERIALS AND METHODS

Isolation of fungus

Sea water samples were collected from the Karachi Coast, Clifton 3 m from the shore. Samples were incubated in nutrient broth and then transferred into the Sabouraud Dextrose Agar (mainly composed of dextrose, pentone and agar) dextrose media. The plates were incubated at 28°C for one week. On the basis of morphological and culture characteristics *Aspergillus niger* and *pencillium* spp. were isolated and identified.

Polyethylene film and its pretreatment

The polyethylene (PE) film was collected from the local market of Karachi, Pakistan used as a plastic bag. The film was cut into 2 X 2 cm pieces, washed with distilled water several times and soaked in 70% ethanol for 30 min., the process of washing and air drying was repeated in Laminar air flow chamber in sterile Petri plate. The PE films were also subjected in the UV chamber for 3 h.

Polyethylene degradation test

Culture plate technique

The isolated strains of fungus were subjected to the degradation test on SDA petri plates. The plates were incubated with the culture of *Aspergillus nigar* and *Penicillium* spp. with PE stripe. After incubation at 28°C for one week. The polyethylene stripe (PE) containing grown fungi was aseptically transferred into the minimal salt media plates ($MgSO_4.7H_2O$ (1 g/L), $FeSO_4$ (0.002 g/L), NaCl (0.2 g/L), K_2HPO_4 10.5 g/L), KH_2PO_4 (0.08 g/L), NH_4NO_3 (2.0 g/L) and Agar (0.7%) and Incubated at 20°C for one month.

Biodegradation in liquid media

The Polymer degradation ability of *Aspergillus nigar* and *Penicillium* spp. were determined by using synthetic media containing $MgSO_4.7H_2O$ (1 g/L), FeSO4 (0.002 g/L), NaCl (0.2 g/L), K_2HPO_4 (10.5 g/L), KH_2PO_4 (0.08 g/L) and NH_4NO_3 (2.0 g/L) at pH of 6.0. In each 500 mL of flask MSM was fortified with PE film with full loop of isolated fungus. The polyethylene film is the only source of carbon. The Flasks were incubated at 20 °C in shaking incubator at 120 rpm.

Strum test

Strum test (OECD 301B: ASTM D5209) was used for the evaluation of biodegradability of polymer material. The sterile piece of film was added to 300 mL basal salt medium as the only carbon source. Spore suspension of *Aspergillus niger* and *Penicillium* spp. were used for the degradation of polyethylene. Control bottles were prepared without any plastic. degradation test was performed at room temperature for the duration of four weeks. Before setting the system Ba $(OH)_2$ filtered and stored in the airtight bottles to prevent atmospheric contamination of CO_2 absorption in the system. Evolution of carbon dioxide, which was trapped in absorption bottle containing 0.01M, $Ba(OH)_2$ was monitored every week, The amount of CO_2 evolved during the test was measured by gravimetric method. $BaCO_3$ is insoluble in water and forms precipitates (Equation 1).

$$Ba(OH)_2 + CO_2 \longrightarrow BaCO_3 + H_2O \quad (1)$$

Determination of percentage degradation

After exposing to the fungi for the period of one month the polyethylene film were washed throughly with Sodium dodecyl Sulphate and distilled water (Gilan *et al.,* 2012) the washed PE films were dried overnight. The formula used for the calculation of percentage degradation was described as Equation 2.

$$\text{Weight loss percentage} = \frac{Initial\ weight - Final\ weight}{Initail\ weight}\ X\ 100 \quad (2)$$

Scanning Electron Microscopy (SEM)

After an incubation period with fungal stains, the Polyethylene film (PE) was removed and dried in a Petri plate for 24 h. The sample was coated with 300°A gold and viewed under high resolution electron microscope (Jsm-6380 A, Japan).

Spectroscopic analysis

Fourier transform infrared spectroscopy (Thermo Scientific Nicolet TM iS10), recorded from a wave number of 400–4000 /cm under Attenuated Total Reflectance (ATR) mode.

RESULTS AND DISCUSSION

The microbial degradation of plastic is one of the most promissing opportunity to minimized plastic pollution by enhance degradation efficiency of certain enzymes (Umamaheswari and Murali, 2013). Biodegradation is excessively important for soluble as well as water immiscible polymers, because once the polymers entered the water bodies, it`s become harder to capture and recycle or incinerated (Sowmya *et al.,* 2014). In current study, *Aspergillus nigar* and *Penicillium* spp. were isolated and identified on the bases of morphological and culture characteristics. Biodegradability of these species was evaluated by the weight loss, strum test; Fourier transforms infrared spectroscopy (FTIR) and scanning microscopy. The growth of isolated fungi on synthetic media containing polyethylene as a sole source of carbon for degradation was monitored. The mass loss technique is highly applied in degradation test as the weight loss is the simple and quick way to measure the degradability of polymer. From the results of Table 1, it can be summarized that 19 and 30% degradation achieved with *Aspergillus niger* and *Pencillium* spp., respectively. These methods conform the degradation capacity of isolated fungi on the PE films assist in the further degradation test. Strum test was carried out to measure the metabolic carbon dioxide evolved during the growth period, which attribute as a good indicator of polymer degradation (Sharabi *et al.,* 1991; Pagga *et al.,* 2001).

Table 1. Weight loss percentage of degraded polyethylene (g).

Name of fungi	Initial weight	Final weight	Weight loss percentage
Aspergillus niger	0.0231 ± 0.002	0.0187 ± 0.001	19%
Pencillium sp.	0.0241 ± 0.0006	0.0168 ± 0.0008	30%

The results showed the difference in amount of carbon dioxide produced by utilizing PE film by both fungi (Table 2). However, *Pencillium* sp. (0.985 g/L) produce more CO_2 as compared to *Aspergillus niger* (0.833 g/L) during the incubation period of one month.

Table 2. Quantification of carbon dioxide after degradation (g/L).

Name of fungi	Amount of CO_2 (15 days)	Amount of CO_2 (30 days)
Aspergillus niger	0.484	0.833
Pencillium sp.	0.621	0.985

The results showed the same pattern reported by Gajendiran *et al.* (2016) who studied the degradation of Low-density polyethylene (LDPE) using *Aspergillus clavatus* which produce 2.32 g/L $CO_{2.}$ Shah *et al.* (2009) reported the production of 1.8 g/L of CO_2 by using the fungal strain of *Fusarium* spp. Biodegradation of inert material such as polyethylene takes many years to degrade. FTIR spectroscopy was used to conform the mechanical biodegradation of PE films. Spectrum of PE film incubated with *Aspergillus niger* for 30 days exhibited many different changes. In control the peak at 2909/cm shifted to 2914/cm in treating spectrum. The peak at 2340/cm shifted to 2358.95/cm and was more prominent after degradation confirmed the degradation. Peak showed at 1368.24/cm was the characteristic peak of N=O bend, 1294.83/cm and 1114.68/cm were prominent structural change as the peaks are low or absent in treating sample (Figs. 1 and 2). The FTIR spectra of PE film incubated with *Pencillium* spp under the laboratory conditions shows number of new groups' formation after degradation (Fig. 3). New dominate peak observed at 2356.9/cm which attribute carbonyl C=O bond stretching. The absorption band at 2234.1/cm, 2193/cm, 2041.8/cm, 1982.8/cm, 1645.1/cm and 1543.8/cm all were dominated after degradation. The peaks at 1645.1/cm and 1543.8/cm were indicated as C-H stretching (methylene group) (Umamaheswari and Margandaw, 2013). After treatment a characteristic band which was attributed as C-H bond stretching 1472.61/ cm shifted to 1464.4/cm with low the peak intensity. The absorption peak noticed at 730.28/cm (C-H bond stretching) become totally absent after degradation (Fig. 3).

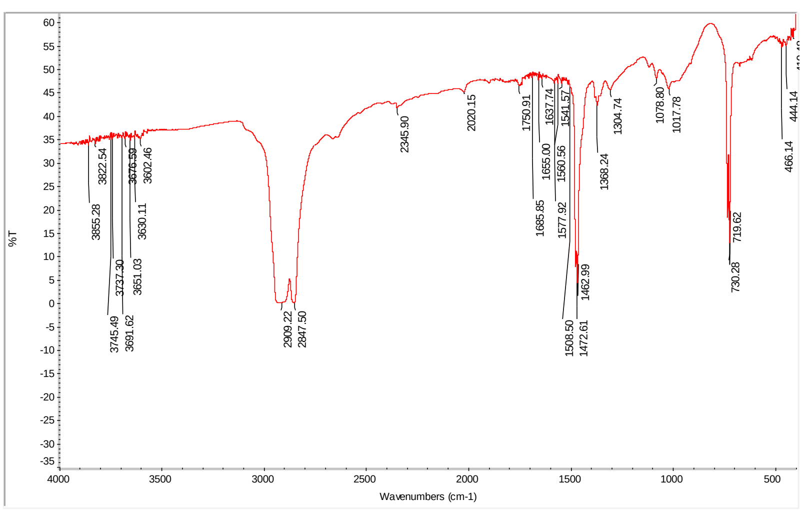

Fig. 1. FTIR spectra of untreated PE film.

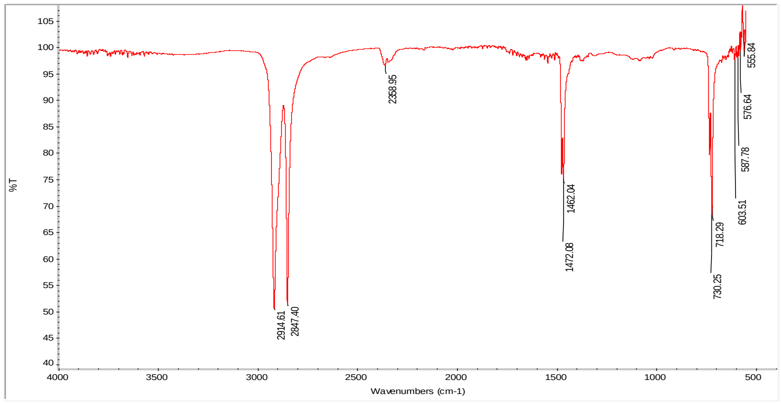

Fig. 2. FTIR spectra of polyethylene after 30 days incubation with *Aspergillus niger*.

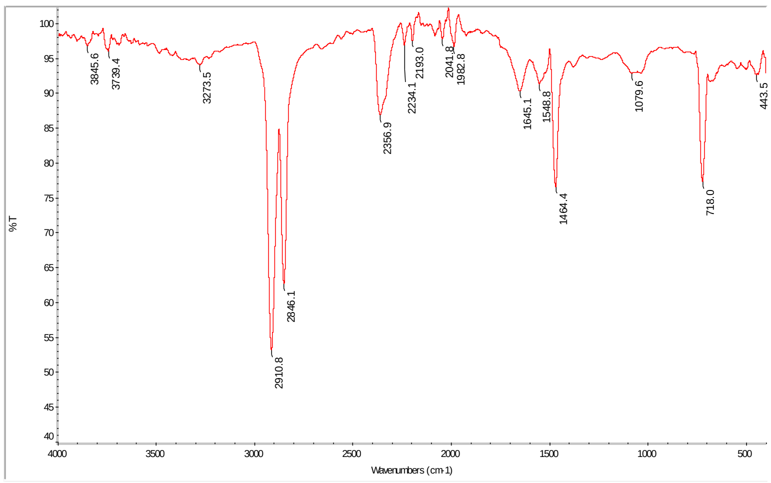

Fig. 3. FTIR spectra of polyethylene after 30 days incubation with *Penicillium* spp. isolate.

Cornell *et al.* (1984) and Albertsson *et al.* (1987) stated the formation of carbonyl groups is the main factors of degradation, the microorganism can easily degrade carbonyl groups hence shortened the PE chain. Scanning electron microscopy revealed the significant surface and structural changes of PE film. In the present work incubated film colonized by fungi shows the formation of cavities and erosions (Figs. 5, 6 and 7).

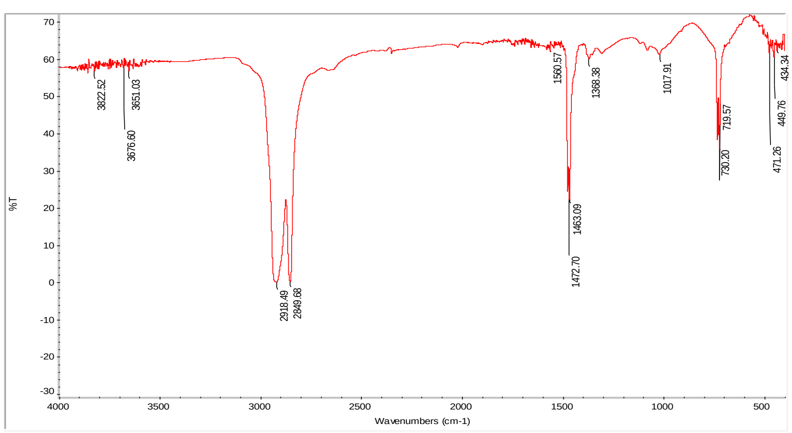

Fig. 4. FTIR spectra of UV exposed PE film under laboratory condition.

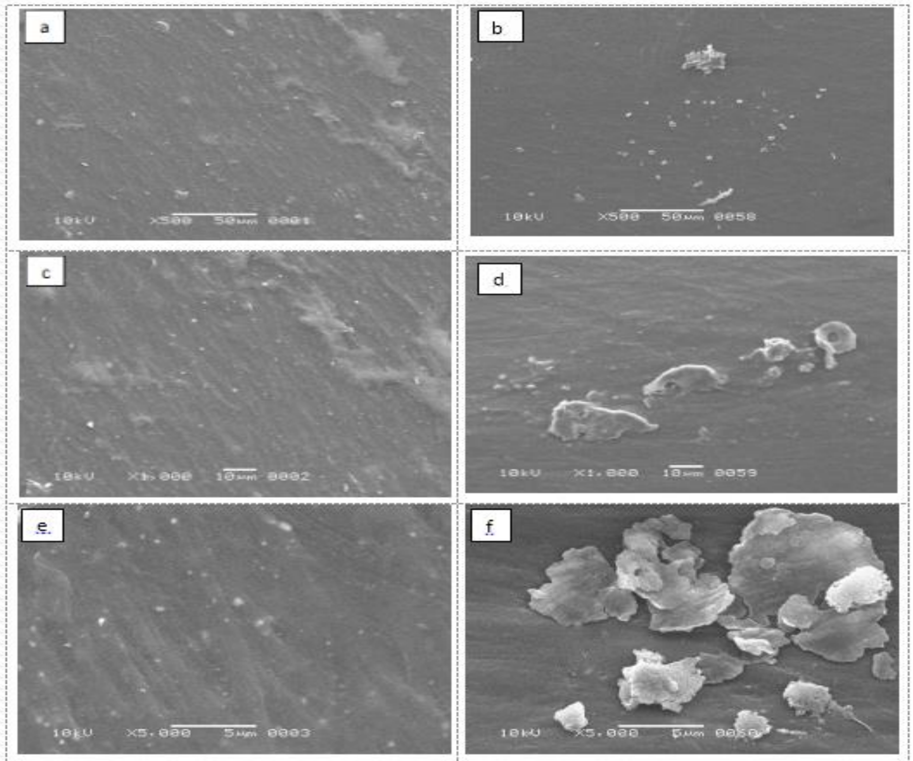

Fig. 5. Scanning electron micrograph of *Pencillium* spp. on the surface of polyethylene plastic.
(a, c, e) are the control at resolution of 500x,1000 x, and 5000x.
(b, d, f) is Scanning electron micrograph after treatment at the respective resolution.

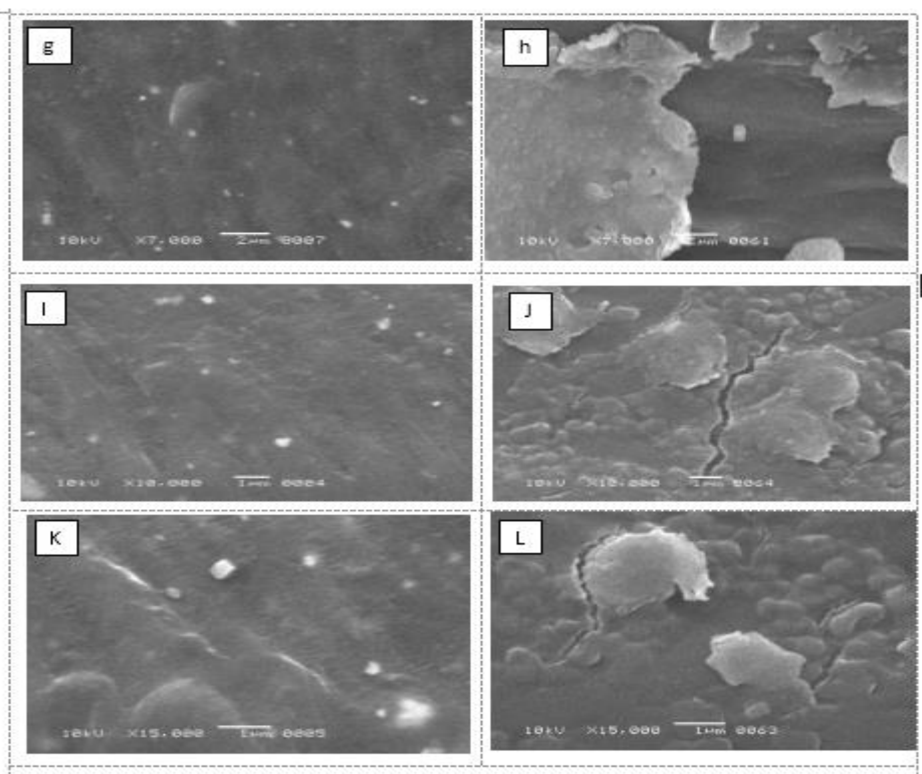

Fig. 6. Fig.6. Scanning electron micrograph of *Pencillium* sp. on the surface of polyethylene plastic.
(G, I, K) are the control at a resolution of,7000 x,10,000x and 15,000x. (h, J and L) are Scanning electron micrograph after treatment at the respective resolution.

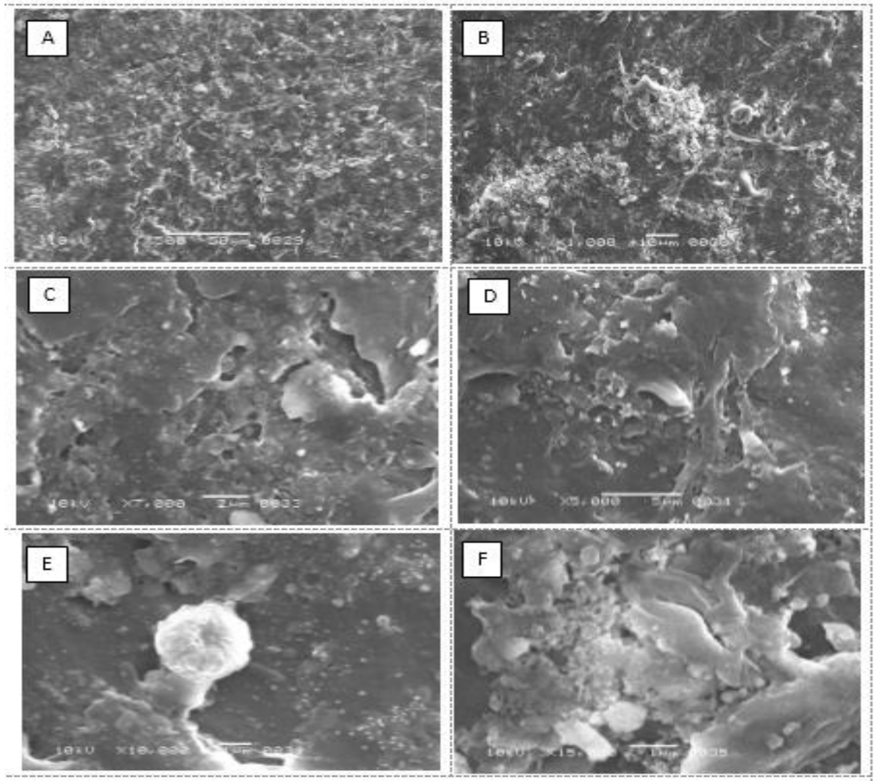

Fig. 7. Scanning electron micrograph of *Aspergillus nigar* on the surface of polyethylene plastic on different resolution after treatment (A, B, C, D, E and F) 500x,1000 x,7000 x, 5000 x,10,000x and 15,000x respectively.

The primary reason of the mass loss is surface erosion by fungal. SEM images of control polyethylene did not show any significant changes (Figs. 5 and 6). While the results of *Aspergillus niger* and *Pencillium* sp. showed the Cracks and holes appeared in film due degradation (Bonhomme *et al*., 2003; Khan *et al.*, 2017). Disruption of polyethylene structure can be seen in the SEM image of *Aspergillus nigar* (Fig. 7). The Nature of microorganisms and pre treatment is the important factor of degradation. According to the Griffin (1980), growth of fungi cause cracking, bursting and swelling of plastic as fungi penetrate on the polymer. SEM micrographs, Strum test and FTIR results showed that the specie of *Pencillium* sp. degrades better than *Aspergillus niger*. Various previous studies were conducted by using *Pencillium* spp. and *Aspergillus* spp. showed the degradation of plastic. *Pencillium simplicissium* shows 7.7% degradation by forming carboxlyic acid, easters (1018.43/cm) and alkanes (875.38/cm) (2865.19/cm) (Sowmya *et al*., 2015). However, according to Usha *et a*.,(2011) *Aspergillus glaucus* give better results than *Aspergillus niger* (28%). Khan *et al.*(2017) showed that the shifting and absence of band in the FTIR spectra indicated that during stress condition microbes produced certain enzymes which cause hydrolysis of a certain groups of polymer leads to the structural and chemical changes.

CONCLUSION

The current study focuses on the effective biodegradation of polyethylene. The isolated fungus species are the native species of the sites contaminated with plastic. As polyethylene accumulation can cause long environmental crisis. Although there are many works on LDPE and HDPE degradation there is not much chemical study present behind Fungal degradation. The data obtained from Strum, FTIR and SEM shows sufficient evidence that the *Aspergillus niger* and *Pencillium* spp. degrade PE and these microbes can be used in natural and artificial circumstance for biodegradation.

ACKNOWLEDGEMENT

Authors are thankful to the Institute of Environmental studies, University of Karachi and Pakistan Council of Science and Industrial Research for their support throughout the study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript. In addition, the ethical issues; including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy have been completely observed by the authors.

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(*Accepted for publication June 2019*)