JOURNAL OF AGRICULTURE AND BASIC SCIENCES
ISSN Online: 2518-4210
http://www.jabsjournal.com/

Full Length Research Article

Isolation and screening of polyethylene degrading fungi from solid waste material

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Abstract

Polyethylene is the material used in manufacturing different plastics due to its light weight and having excellent weight carrying ability. However, this material is resistant to degradation as it has high molecular weight. Due to their tremendous use, the accumulating plastic waste material is causing an ever increasing ecological danger. This problem can be resolved by manufacturing biodegradable plastics or by using natural ability of microorganisms to degrade plastic material. The current study has been designed to test the ability of fungi to degrade this non reducible material. Multiple soil samples were taken from municipal waste material in the suburb areas of Faisalabad and from University of Agriculture, Faisalabad. Isolated fungal species were screened for the biodegradability of plastic material. Among them, *Aspergillus* and *Penicillium species* exhibited biodegradability of plastic material. *Aspergillus species* were observed more effective in degrading polyethylene plastics. Additionally, effect on rate of biodegradation was tested on the basis of different parameters like starch blending, UV light, and time of incubation. UV light irradiation was found more effective parameter that greatly enhanced the rate of biodegradation of polyethylene strips observed by scanning electron microscope.

Keywords: *Aspergillus*, *Penicillium*, Biodegradation, Rate of biodegradation, Scanning Electron Microscope.
Received: 17-February-2016, Accepted: 25-April-2016

Introduction

Plastics are fabricated materials that contain small repeating subunits joined together by chemical bonds. They are extensively used in packaging of various sorts of useful products such as food, cosmetics, chemicals, pharmaceuticals and detergents (Mueller, 2006). The materials used in plastic packaging are economical, strong, light-weight, energy efficient and can be processed easily. Due to these extraordinary characteristics plastic products have become part and parcel of our routine life and industries (Zahra *et al.*, 2010).

However, majority of plastic materials such as polyethylene, polypropylene and polystyrene are resistant to degradation. Their enhancing demands augment their quantity in the environment, which has become a danger to the globe (Tokiwa *et al.*, 2009). The ability of these materials to sustain in the environment is on the behalf of their water repellency and high molecular weight (Chiellini *et al.*, 2003). Waste plastics pose huge stress on the environment. They deposit in the soil and become the source of water-clogging, thus leading to demolish the soil for agricultural farming (Gyung *et al.*, 2012). Combustion of polythene bags produces poisonous gases. Breathing in of these gases causes lung ailments and cancer (Pramila, 2011; Rustagi *et al.*, 2011).

Hazards by polythene plastics can be reduced by two ways. One is to establish biodegrading microbes having inherent capability to degrade plastics and second is to produce artificial polymers easily degradable (El-Shafei *et al.*, 1998). Biodegradation of polyethylene depends upon a number of factors such as morphology, molecular weight and density. It can also be affected by additives, antioxidants and initial showering from UV radiation. In order to make plastics easily degradable they can be integrated with natural polymers (e.g. starch and cellulose). Manufactured polymers containing esters or ether groups can also make them degradable by microbes (Nowak *et al.*, 2011).

In the process of degradation, depolymerases liberated from microorganisms convert complicated polymers into small molecules (e.g. monomers). These monomers can readily pass through the selectively permeable membrane of microbes and then can be used as carbon and energy sources (Gu, 2003).

Fungal species which have inherent ability to degrade polyethylene plastics are *Aspergillus fumigatus*, *Aspergillus terius*, *Fusarium solani*, *Penicillium notatum*, *Mucor rouxii*, *Mortierella polycephala* (Nowak *et al.*, 2011). As polyethylene is a dominant part of solid waste and cause environmental pollution leading towards health issues (Raut *et al.*, 2015). Therefore, study was designed with the objectives to isolate polyethylene degrading

fungi, to assess biodegradability by comparing the potential of isolated species and to optimize the different parameters for enhanced biodegradation of polyethylene. The findings of the study will help to suggest possible treatments of the polyethylene strips for rapid degradation.

Materials and methods

Multiple soil samples were collected from solid waste material dumps in University of Agriculture Faisalabad, Pakistan and from different places located in the vicinity of Faisalabad, Pakistan. These samples were taken by digging up the soil up to 3 cm and were used for the isolation of polyethylene degrading fungi (Gyung *et al.*, 2012).

Isolation of Fungi

Fungal species were isolated on potato dextrose agar at room temperature. After the maturation of colonies slide culture technique was applied for microscopic identification. The fungal isolates were first identified macroscopically by examining the colony characteristics. Furthermore, slide culture technique was performed for the identification of fungal isolates microscopically. They were identified on the basis of their hyphae structure and fruiting body appearance under the microscope (Ishii *et al.*, 2007).

Biodegradation Studies

Screening of Polyethylene Degrading Fungi

For testing the ability of fungi to degrade the plastic material, Plate Assay method was used. Those fungal species which have potential to degrade plastic were separated from other fungal isolates which did not have an ability to deteriorate polyethylene plastic (Usha *et al.*, 2011).

Plate Assay

In this technique the isolated species of fungi were tested for their ability to produce clear zones on the medium in which polyethylene powder was mixed along with different fungal isolates. First of all potato dextrose agar was prepared and polyethylene powder was mixed with it while it was in molten form. It was shaken in the shaker for one hour so that polyethylene powder got mixed properly in the fungal medium. Isolated species were inoculated into medium, after taking the medium from shaker containing polyethylene powder. The plates were then incubated for 3-5 days at room temperature to observe the results. The organisms producing clear zones around the colonies were selected for further analysis (Kim & Rhee, 2003; Usha *et al.*, 2011).

Optimization of Parameters to enhance the rate of Biodegradation

Different parameters were optimized for the biodegradation of polyethylene. These parameters include blending of starch as a raw material with the polyethylene and irradiation of polyethylene strips prior to take them into degradation study.

Starch Blending

On the basis of starch blending, polyethylene strips were divided in two groups.

1. Starch blended polyethylene strips
2. Non-starch blended polyethylene strips

Biodegradation of starch blended and intact polyethylene by isolated fungi was determined separately (Mao *et al.*, 2015).

UV light Irradiation

On the basis of UV light irradiation two groups were made.

1. Polyethylene irradiated strips with UV light at 340 nm wavelength for the duration of 2 days
2. Non- irradiated strips

Biodegradation of UV light irradiated and non-irradiated polyethylene by isolated fungi was determined separately (Ibiene *et al.*, 2013).

Scanning Electron Microscopy

The surface morphology of polyethylene strips was analyzed through Scanning Electron Microscopy to check for any structural changes after incubation. After thoroughly washing with sterilized distilled water, samples were mounted on stubs (silver painted). Silver coated stubs were carried out in vacuum to make the samples conducting. The images of the test samples were compared with those recorded on the original untreated samples for biodegradation (Suarez & Mano, 2001; Pramila, 2011).

Results

Aspergillus and *Penicillium* species were obtained after screening all the isolated species and tested for their ability to degrade polyethylene. This screening was done on the basis of formation of clear zone by the fungal species on polyethylene containing media. In total of 15 isolated fungal species were found involved in biodegradation of plastics. Among these isolates, 9 were from genus *Aspergillus* and 6 were from genus *Penicillium* (Figure 1-2).

Comparative study of Biodegradability

The ability of *Aspergillus* and *Penicillium* species was compared by measuring the clear zone in plate assay. It was observed that clear zone for *Aspergillus* species was wider (12mm) than that of *Penicillium* species (8mm) (Figure 1). Furthermore, the ability of *Aspergillus* species to degrade polyethylene was estimated to be 60 % while that of *Penicillium* species was about 40% as compared to that of each other. It was concluded that the *Aspergillus* species present in the soil of solid waste dumping areas has more potential to degrade the polyethylene than the *Penicillium* species isolated

from the same place. It was also observed that the rate of degradation by *Aspergillus* species was faster than the rate of degradation by *Penicillium*. It is due to the production of different enzymes which assist in the process of biodegradation. The ability of isolated *Aspergillus* species to release such type of enzymes was considerable.

Scanning Electron Microscopy

The photomicrographs obtained through SEM showed significant physical and structural damage of polyethylene strips by fungal species. The degree of biodegradation was assessed by the extent of pits and cracks observed on the surface of strips. Each sample was compared with control to assess the degree of biodegradation. The strip of intact polyethylene revealed a minor damage as it is resistant to degradation (Figure 3-*a*-*b*). Polyethylene strips irradiated with UV light showed enhanced degradation due to formation of carbonyl subunits on exposure to UV light when compared with control (Figure 4-*a*-*b*). Starch blended polyethylene showed extraordinary changes in the structure and morphology as compared to that of other two parameters. It suggests that starch tends to increase the biodegradation of plastic more than UV light irradiation treatment (Figure 5-*a*-*b*).

Discussion

To investigate the structure of the surface and changes in the structure of plastic material during biodegradation various microscopy-based techniques have been employed by a number of researchers. Ishii *et al.* (2007) isolated twelve polyethylene degrading fungi from various soil environments. Isolated species were isolated and assessed for their ability to degrade polyethylene (Ishii *et al.*, 2007). In present study, the different fungi were isolated and identified by examining morphological, colonial and microscopic characteristics.

Usha *et al.* (2011) performed the screening of polyethylene degrading fungi by using clear zone method. In that study, *Aspergillus*, *Penicillium* and *Mucor* sp. were screened by observing the clear zone around the colonies after 15-20 days of incubation. In the present study, *Aspergillus* and *Penicillium* species were screened as these isolates produce the clear zones around their colonies. Nowak *et al.* (2011) isolated *Penicillium*, *Aspergillus*, *Mucor* and *Mortierella* sp. from soil and assessed their activity for the degradation of polyethylene. *Aspergillus* sp. had more ability to colonize the surface of polyethylene and same species was more able to degrade polyethylene as well as polyester (Nowak *et al.*, 2011). The results of the present study are also in accordance with the findings of Nowak *et al.* (2011). Clear zone method revealed that *Aspergillus* species has more ability to degrade polyethylene than that of *Penicillium* species, which indicates that *Aspergillus* species have more potential to degrade polyethylene than *Penicillium* species.

In the present study, SEM was performed for the identification of physical and structural changes. After analysis by using SEM it was observed that there were prominent changes in the surface morphology of polyethylene films which showed the mechanical destruction of the polyethylene films. Cracks, dark spots, surface corrosions, pits were correlated with the degree of polyethylene biodegradation. Suarez & Mano (2001) used scanning electron microscope to examine the surface deterioration and alterations in the mechanical properties of polyethylene films after biodegradation. The outer surface showed many cracks which depict the process of biodegradation. Cooper & Corcoran (2010) performed Scanning Electron Microscopy for the assessment of biodegradability of plastic. They examined various pits, cracks, fractures, grooves on the surface of plastic which revealed the surface damages due to biodegradation.

Conclusion

Among fungal species (*Penicillium* and *Aspergillus*) isolated from waste material, polyethylene strips are more effectively degraded by *Aspergillus* species. The results of the present study showed that the treatment of polyethylene strips with different parameters accelerated its degradation. It was observed that starch blending and UV light irradiation treatment of polyethylene strips increased the rate of degradation by fungi. Moreover, the results showed that starch blending is more effective treatment than UV light irradiation for rapid biodegradation, observed under scanning electron microscope. On the basis of the findings of the present study it is suggested that prior treatment of polyethylene with starch or UV light, should be followed in the industry so that the threat of pollution can be decreased otherwise it cannot be eliminated completely from the environment.

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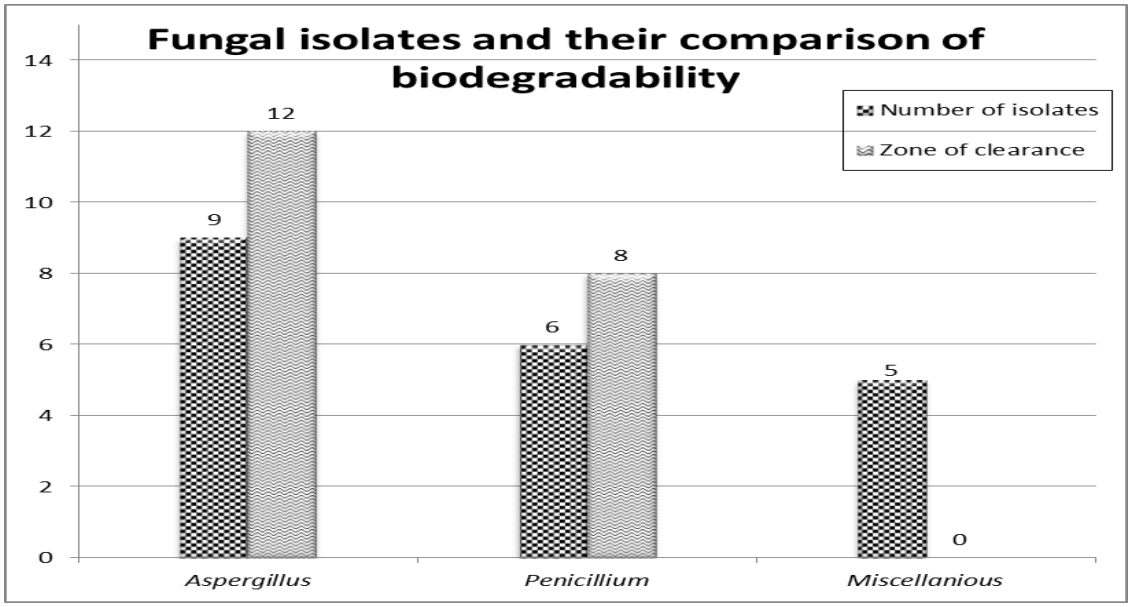


Figure-1: Isolation of fungal species and their comparison for biodegradability.

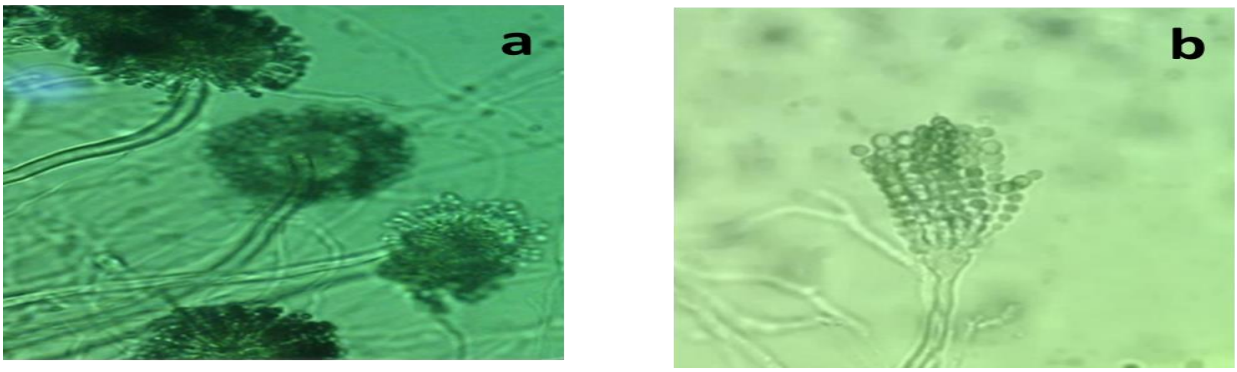
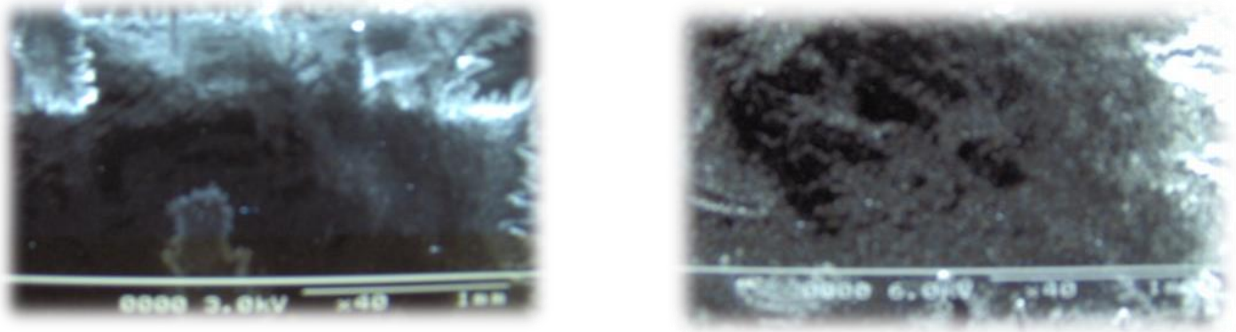
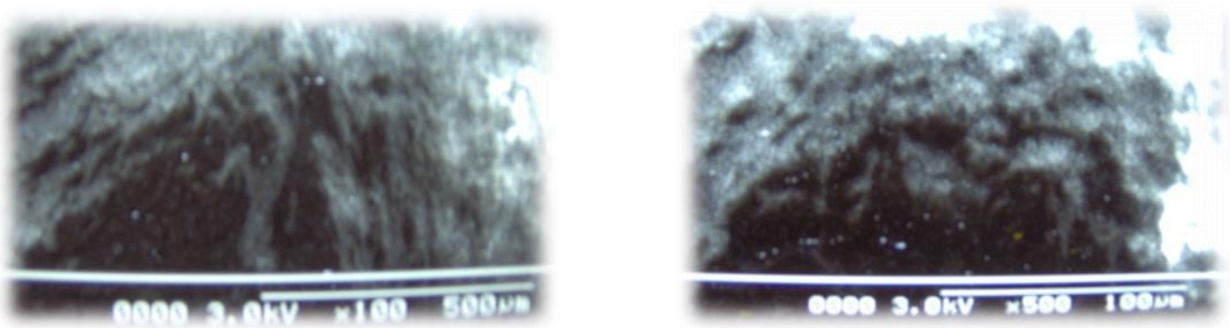


Figure-2: Microscopic view of *Aspergillus* (a) and *Penicillium* (b) isolated from soil samples.



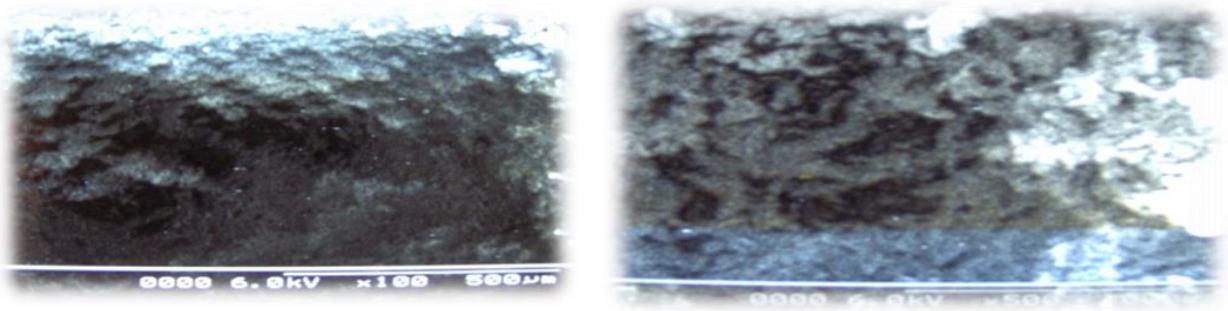
(a) **(b)**

Figure-3 (a & b): Scanning Electron Micrograph showing the structure and appearance of intact polyethylene (a) control film (b) film after the exposure of intact polyethylene strip to the fungal culture in potato dextrose broth.



(a) **(b)**

Figure-4 (a & b): Scanning electron micrograph showing the structure and appearance of UV irradiated polyethylene strip (a) control film (b) film after the exposure of intact polyethylene strip to fungal culture in potato dextrose broth.



(a) **(b)**

Figure-5 (a & b): Scanning electron micrograph showing the structure and appearance of starch blended polyethylene strip (a) control film (b) film after the exposure of polyethylene strip to the fungal culture in potato dextrose broth.