

THE ROLE OF ALTERNARIA ALTERNATE IN THE BIOACCUMULATION OF TWO TYPES OF POLYMERIC WASTE

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Abstract

The fungus (Alternaria alternate) was isolated from Abu Al-Khasib soil samples, and after obtaining pure isolates of the fungus, the fungus was grown with pieces of polymeric waste films of two types (PMMA & PS) and at different incubation periods (15 , 30, and 45 days). After each incubation period, the weight of the piece was measured, and the result was a decrease in weight as the incubation period increased. Thus, the role of the fungus was to decrease Weight because fungi play a major role in the bioaccumulation process

Keywords : PMMA, PS, Bioaccumulation, fungi.

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Introduction

Microbial disintegration of plastics is a relatively gradual process that is influenced by several factors, and suitable conditions are necessary in order to achieve a maximal degradation outcome. Common factors known to determine the speed at which fungal biodegradation occurs include polymer type, fungal species, temperature, pH, moisture, oxygen and CO₂ levels, as well as whether the plastic material has been pre-treated with ultraviolet irradiation and prooxidants (Fernandes et al., 2020; Muthukumar and Veerappapillai, 2015).

Biodegradation of synthetic plastics by fungi is deemed to be a result of metabolic evolution after prolonged exposure of fungi to xenobiotic polymers released into the environment from human activities (Alam et al., 2020; Meng et al., 2021). When exposed to high selective pressure, microorganisms tend to evolve very quickly due to their high genome plasticity, versatility, capacity to efficiently exchange genetic materials and to appropriately mix and match the desired catabolic pathways. Owing to the mass production and littering of plastics since the 1950 s, Jimenez et al. (2022) suggest that microbial enzymes that naturally degrade organic substrates evolved and adapted new strategies to act on plastics. Although many questions still exist pertaining to the evolution of plastic-degrading microbes and their enzymes, the majority of microbial geneticists support the concept of already existing enzymes having shifted and gained the capability to break down bonds in plastic polymers. Considering that microbes have also been shown to have exclusively acquired the ability to metabolize anthropogenic pollutants that are used in agriculture (such as pesticides and herbicides) through evolution, the idea of an evolutionary shift having aided microbial enzymes to utilize synthetic substrates has therefore been well adopted and is currently thought to offer an acceptable explanation to this phenomenon (Malhotra et al., 2021; Russell et al., 2011).

In biodegradation, the process is initiated by micro-organisms, i.e., bacteria and fungi. In general, this biodegradation of plastics involves the growth of fungi on the surface of plastic, where plastic is consumed as a food source by the fungi under the influence of environmental factors, such as temperature and pH. These fungi will secrete enzymes, such as cutinase, lipase, and proteases, carboxylesterases, esterases, lignocellulolytic enzymes, and some pro-oxidant ions which will degrade the plastics. By oxidation/hydrolysis enzyme improves the hydrophilicity of polymers and consequently degrade the high molecular weight polymer into low molecular weight. As high molecular weight is a large compound that cannot be transported across the cellular membrane of the fungi thus it primarily depolymerizes it into small monomers before they cross the cell membrane (Shah et al. 2008). The enzyme activity is mainly dependent on the solvent properties and the enzyme activity increases with polarity and decreases with the viscosity of the solvent in the biodegradation of polymers (Patel et al. 2013).

Several studies have been carried out on plastic-degrading fungi. For example, Lacerda et al. (Shimao,2001) investigated the fungi from the plastisphere within the aquatic environments of the western South Atlantic and Antarctic Peninsula. Microorganisms and enzymes that are able to degrade a variety of generally used synthetic plastics were comprehensively summarized. The microbial metabolic pathways for plastic depolymerization products and the current attempts toward utilization of such products as feedstocks for microbial production of chemicals with high value were highlighted by Ru et al. (Madigan, et.al.2003). Sánchez (Webb,et.al.2013) described the natural and unique ability of fungi to invade macro- and microplastic substrates by using enzymes that have the capacity to detoxify pollutants. Sáenz et al. (Wilkes, and Aristilde, 2017) experimented with biodegradation of low-density polyethylene (LDPE) by *Aspergillus niger* and *A. terreus* to increase the degradation rate without any co-substrate or photothermal treatment. Iram et al. [Pramila, and Ramesh,2011] reviewed some of the most common strategies for the degradation of various types of polymers, along with a list of potential microbes capable of feeding on them. The role of marine fungi was also recently reviewed by Zeghal et al. (Ojha,et.al.2017). Microbial degradation of plastics was reviewed by Kale et al. (Brodhagen,et.al.2015). However, a recent complete study on plastic-degrading fungi in all environments, including both aquatic and terrestrial environments, which addresses their detailed phylogenetic relationships, is lacking. As such, the information presented herein reviews plastic-degrading fungi reported thus far and their phylogenetic relationships.

Methods

Collecting samples from agricultural soil: -

A soil sample was collected from the agricultural area in Abu al-Khasib for the purpose of isolation, including a fungal isolation, for the purpose of experimenting with the growth of fungi on polymeric waste. and placed it in a tube containing sterile distilled water. The mixture was shaken well and five dilutions were made of it. Then, the culture dishes were prepared, containing PDA culture medium, for the purpose of isolating the

fungi from that soil. The method of diffusion was used for isolation, and after the dishes were incubated at a temperature of 25 °C for five days, after that they were examined. The dishes were microscopically determined and the most visible sex was purified and isolated on a culture plate alone until it was used for the experiment. The isolation was *Alternaria alternate* .

Growing fungus on polymeric waste: -

The agricultural soil was sterilized by the autoclave to ensure that it does not contain microorganisms. Then 3 grams of that sterile soil were weighed and placed in a sterile glass tube. This method was repeated with ten tubes for each type of polymeric waste, and since we have two types of polymeric waste represented by (PMMA and PS). After placing the sterile soil in all the tubes, drops of sterile distilled water were placed to form a moist soil, and then placed in all the tubes except for two tubes as a control. Since all the tubes contain that piece, then all the tubes were incubated in the incubator at a temperature of 25 °C for three incubation periods (15, 30 and 45) days After each incubation period, that piece is weighed and the value is recorded.

Preparation of polymeric samples

Recycled polystyrene of food dish and recycled PMMA were collected and crash to fine powders .

1g of the recycled polystyrene was dissolved in THF with stirring for 3hrs. then the sample was pour to glass petri dish to dry at oven for 24 h .at 45C 0 thin film were contain. the same process was done on the recycled PMMA .

The same method of preparation into polymeric litter PS

Results and Discussion

The results showed that the fungus *Alternaria alternate* had an effective role in growing on these polymeric wastes, as the results of the different incubation periods showed a decrease in the weight of the pieces of the polymeric waste, and the decrease was direct. The danger of polluting the environment with these wastes, and this is what was proven by this study and the two mentioned tables:

Table (1) Average weight of PMMA polymer waste pieces for three incubation periods

Incubation period	15 days	30 days	45 days
Weight gram	0.2	0.18	0.13

*For your information, the weight of the control sample is 0.2 grams.

Table (2) Average weight of PS polymer waste pieces for three incubation periods

Incubation period	15 days	30 days	45 days
Weight gram	0.2	0.15	0.10

After these results, our study demonstrated the ability of mushrooms to reduce environmental pollution by polymeric waste, and this agreed with other studies, including:

The biodegradation of synthetic polymers through composting provides a suitable alternative for assessing fungal activities under relatively natural conditions as opposed to lab-based conditions that are highly controlled (Amaral-Zettler et al., 2020; Sařsek et al., 2006). Since composting involves the integrated interaction of microbial consortia which collectively enhance the degradation process, compost conditions are therefore considered appropriate for metagenomic studies aimed at deciphering microbial community structure and for predicting the survivability of target species in situ (Kumar et al., 2021). Furthermore, metagenomic studies of fungi from composting environments may provide a rich resource of gene pool of fungal species responsible for plastic biodegradation and also make it feasible to explore unculturable microbial communities that might be involved in the degradation process as demonstrated by Basak and Meena (2022) and Purohit et al. (2020). In plastic composting, sterile plastic materials are buried together with plant residues in a compost pit or under a landfill or sludge where fungal species and organisms can colonize and start the decomposition process (Brodhagen et al., 2015). The buried polymers are left beneath the soil for a given period to allow gradual degradation by natural microbes. Some studies have even sought to isolate species colonizing the buried synthetic polymers and assess their capability to degrade various types of plastics (Mercier et al., 2017). Although in some instances, a group of fungi were introduced into compost that consists of synthetic materials to determine the combined effects and role of different species on the biodegradation process. This is often applicable in studies that carry out composting under controlled conditions whereby easily decomposable bio substrates such as starch, bran, and cellulose plant wastes are blended with plastic polymers to accelerate microbial growth and secretion of biodegradative enzymes (Brodhagen et al., 2015; Janczak et al., 2018).

The mechanism of biodegradation involves the action of microbial enzymes on the surface of the plastics. The microbes such as bacteria and fungi attach to the plastic film and inert the enzymes and grow on it by utilizing it as substrate and source of nutrition. Therefore, the polymers slowly get depolymerized and degradation will be completed by mineralization process, where H₂O (water), CO₂ (carbon dioxide), CH₄ (methane) are end products (Frazer 1994; Montazer et al. 2019). The ability of fungi was they can invade substrates using enzymes that can detoxify pollutants. Fungi can also produce some surface-active proteins, i.e., hydrophobins to coat hyphae to hydrophobic substrates. The growth of many fungi can also cause small-scale swelling and bursting, as the fungi penetrate the polymer solids (Griffn 1980).

The rapid development of chemical industry in the last century has led to the production of approximately 140 million tons of various polymers annually (Shimao et al., 2001). Many of these are non-biodegradable and persist almost indefinitely in an environment, their accumulation has triggered research to develop more readily degradable materials and identify new methods for eliminating existing polymer waste (Madigan et al., 2003).

Compared with other degradations, the biodegradation method is mostly preferred due to its pollution-free mechanism and eco-friendly process. In biodegradation, the process is initiated by micro-organisms, i.e., bacteria and fungi. In general, this biodegradation of plastics involves the growth of fungi on the surface of plastic, where plastic is consumed as a food source by the fungi under the influence of environmental factors,

such as temperature and pH. These fungi will secrete enzymes, such as cutinase, lipase, and proteases, carboxylesterases, esterases, lignocellulolytic enzymes, and some pro-oxidant ions which will degrade the plastics. By oxidation/hydrolysis enzyme improves the hydrophilicity of polymers and consequently degrade the high molecular weight polymer into low molecular weight. As high molecular weight is a large compound that cannot be transported across the cellular membrane of the fungi thus it primarily depolymerizes it into small monomers before they cross the cell membrane (Shah et al. 2008). The enzyme activity is mainly dependent on the solvent properties and the enzyme activity increases with polarity and decreases with the viscosity of the solvent in the biodegradation of polymers (Patel et al. 2013).

The natural degradation rate of plastic is extremely slow, and that causes plastics wastes to accumulate in all components of the environment (Brodhagen, et.al.2015 and Mercier,et.al.2017). The long chain polymer structure, high molecular weight, and hydrophobicity cause plastics to be resistant to biodegradation (Janczak,et.al.2018). In fact, some plastics take up to 1000 years to degrade (Frazer,1994). These facts are the reason for the rapid plastic accumulation in natural environments. Hence, developing an efficient process to accelerate the plastic degradation rate is essential to avoid this annual accumulation. Several solutions have been provided in the scientific community and have been experimentally proved to some extent. Those methods include photodegradation (degraded by light), chemical degradation, thermal degradation (degraded by heat), irradiation using gamma rays, and biodegradation (degraded by biological additives or microorganisms) (Frazer,1994 and Grifn GJL,1980). However, a method with minimum harmful effects to nature but without or at most minimum toxic by-products is required (Grifn GJL, 1980). The processes such as photo-degradation, chemical degradation, thermal degradation, and irradiation using gamma rays cause many negative impacts on nature, such as accumulation of heavy metals in ecosystems and disturbance in natural ecosystem functioning. Moreover, those methods require high costs and energy levels to perform. Therefore, scientists across the globe have tended to investigate better biodegradation methods that do not result in harmful effects and represent an eco-friendly approach for managing plastics (Grifn GJL,1980). Furthermore, biodegradation is a proper solution since it is cost-effective and does not require much energy. More than 400 microbes have been recorded to be capable of plastic degradation (Shimao,2001). The present study was focused only on plastic-degrading fungi among plastic-degrading microbes.

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