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BIODEGRADATION OF PLASTIC POLYMERS USING LIGNINOLYTIC FUNGAL SPECIES

Biological Science

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ABSTRACT

Polyethylene and plastic are resistant to degradation and decay. Their accumulation in the environment produces environmental problems. So, the purpose of this research is to evaluate the ability of several fungal species to destroy plastic materials, choose the most active strain, and investigate some physical parameters influencing ligninolytic enzyme productivity.

KEYWORDS

Plastic degradation, Lignolytic fungi, Ganoderma, SEM, FTIR

INTRODUCTION

Mostly Papers starts with introduction. It contains the brief idea of work, requirement for this research work, problem statement, and Authors contribution towards their research. Sufficient recent reference citation [1] from last 2 years should be included for showing the existing challenges and importance of current work. This section should be succinct, with no subheadings unless unavoidable [2, 3]. State the objectives of the work and provide an adequate background related to your work, avoiding a detailed literature survey or a summary of the results.

As the world's population continues to grow, so does the amount of plastic wastes that people produce. Plastic is composed of major toxic pollutants and thus it has the potential to negatively impact the natural environment and create severe problems for plants, wildlife and even human populations [1]. The accumulation of these products has led to increasing the amounts of plastic pollution around the world. Plastic wastes are commonly accumulated in soil due to the very slow rate of degradation. As a consequence, the toxic chemicals that are included in the plastic structure (i.e. bisphenol A and phthalates) are released in the soil and affect negatively both soil ecosystem and human health [2]. Egypt generated more than 20 million tons of municipal solid waste (MSW) in 2013 and plastic wastes (mostly polyurethane) were approximately 13% of the total MSW [3]. There are three main mechanisms for plastic waste management in Egypt and worldwide; landfilling, recycling, and incineration [4]. However, those mechanisms produce toxic gases such as chlorofluorocarbon (CFC), vinyl monomers and dioxins [5]. According to the latest plastic waste estimation done by the Plastic Technology Center, 35% of the plastic waste in Egypt is recycled, 33% is buried in open dump sites, and 32% is burned [6]. Recently, bioremediation of plastic waste was considered as an economic and environment friendly strategy for plastic waste management that could totally reduce the severe pollution produced from the conventional plastic remediation methods [7]. Both fungi and bacteria have demonstrated their ability for plastic degradation has proved incorporation of microbial species in the degradation of both natural and synthetic plastics. However, fungi could play a remarkable role in degradation of polymers in soil due to their high ability to produce variety of enzymes such as Glucosidase, Cutinase, amylase, lipase, esterase, cellulase, pectinase and hemicellulase. A few previous studies have isolated fungi from plastic contaminated soils and investigated its ability in degrading plastic polymers

Research Methodology

This part should contain sufficient detail to reproduce reported data. It can be divided into subsections if several methods are described. Methods already published should be indicated by a reference [4], only relevant modifications should be described. Methodology should be written concisely in detail by maintaining continuity of the texts.

Collection And Isolation Of The Fungi

Fungal samples were collected from State Forest Research Institute and were bring to the laboratory. The fungal isolates were grown on Minimal media and Potato Dextrose Agar media (PDA), and the cultures were also maintained as per the standard protocols (Figure 1).

Figure 1: Pure Culture Of Ganoderma Lucidum

Characterization Of The Most Efficient Polythene Degrading Fungal Isolates

To most efficient fungal isolates with potential to degrade polythene (5 based on %WL and 5 based on % loss in TS) were characterized based on morphological keys109 and molecular tools. At morphological level, microphotographs of the selected fungal isolates were captured using Trinocular microscope (Leica DM3000, Germany) equipped with cooled CCD camera (Leica DFC450, Germany). Te captured photographs were processed by Leica Application Suite (Version 4.5.0). Cetyl Trimethyl Ammonium Bromide (CTAB) method110 was used to isolate genomic DNA from the 6 days old fungal cultures (100mg of fungal mycelium) to identify the elite polythene degrading fungi at molecular level. Te PCR reaction (25µl) was carried out using 1.5mM MgCl2, 0.25mM of each dNTPs, 1XTaq bufer, 1U/µl Taq DNA polymerase, 10 pmole of each primer of ITS gene93 (Supplementary Table S6) and 50ng DNA template. The PCR (Veriti, gradient thermocyler cycler, Applied Biosystem, USA) was programmed at initial denaturation 95°C 5min, 40 cycles with denaturation at 95°C 1min, annealing 59°C 1min, extension 72°C 1min followed by final extension at 72°C 10min. Te PCR products were separated on 1.2% Agarose gel (Invitrogen) prepared using 1X TAE buffer against negative control and 100bp DNA ladder (Invitrogen). All the amplified bands were eluted from the Agarose gel using Qiagen gel extraction kit (Cat. No. 28115) as per the instruction manual and were given to commercial lab along with primers for purification and sequencing. The sequences obtained from the commercial lab were viewed using chromas lite and were curate to make conting using MEGA 6 software.

Abiotic Degradation

The abiotic degradation of the plastic bags was the exposure to sunlight up to 30 days. This exposure was in the summer time in a green house. In this season, the sunlight is from 6:00 am to 5:00 pm.

Biotic Degradation

For the biotic degradation the plastic polymers without exposure to sunlight was used Ganoderma lucidum. These polymers were cut in fragments of 5 cm^2 (Figure 3) and placed in a glass flask (100 ml) containing plastic cover fragments (5–10 cm^2) and Mineral Medium.

RESULTS

This section may each be divided by subheadings or may be combined.

A combined Results and Discussion section is often appropriate. This should explore the significance of the results of the work, don't repeat them. Avoid extensive citations and discussion of published literature only, instead discuss recent literature for comparing your work to highlight novelty of the work in view of recent development and challenges in the field.

In each glass flask fours discs of agar (6–8 mm) containing the mycelium of Ganoderma lucidum were inoculated (Figure 4). This fungus was cultivated in 20 ml of potato dextrose lignin (0.1%) agar (PDLA) for 15 days. The mother culture is maintained on PDA at 4°C. After inoculation the culture were incubated at 25°C for 10, 20, 15 and 30 days.

The capacity of Ganoderma lucidum to produce mushrooms in plastics waste may be evaluated under the same growing conditions. However, for mushrooms formation it is need, after the mycelial growth (about 10 days), a thermal shock that can be performed by reducing the incubation temperature to 4°C for 24 h and returning to 25°C. During the mushrooms growth, the flasks should be kept in a place at 18 ± 2°C and a relative humidity of 80%.

A total of 140 days were the time applied to degradation of the plastic bags, being 120 days of exposure to sunlight and 120 days of fungal incubation. According to the manufacturer, depending on environmental conditions, for example, the exposure to oxygen and outdoor element, plastic bags decompose within a maximum period of 18 months after disposal.

They also add that in only 111 days the biodegradability index of plastics bags was 88.86%. Our time of abiotic degradation is the same those used for calculating the biodegradability index and corresponds to ¼ of the required time for the decomposition of these bags.

However, after 4 months of exposure to sunlight we did not observe any fragmentation of the plastic bags, only the appearance of small cracks and the bleaching of the film were observed (Figure 6).

According to them, this time of exposure is insufficient for other physical or chemical changes, concluding that the mechanical properties alterations, such as the reduction of breaking energy and elasticity facilitated the fungal colonization of plastic waste. The chemical and physical changes in the low-density polyethylene (LDPE) was observed after pretreated of the LDPE sheets with low discharge plasma (O_2, 3.0 × 10−2 mbar, 600 V) for 6 minutes.

In this experiment, we observed a no fragmentation of plastics bags after 3 months of exposure to sunlight (Figure 5). The control samples were cut with a scissors, but after that exposure to sunlight, it was no longer possible to cut the bags. This result shows that there are needed more than 7 months of exposure to sunlight to completely degradation of the plastics.

However, this result is promising, shows the ability of abiotic degradation of these bags and enables new testing using these bags with exposure to sunlight in a period equal to or greater than 5 months and inoculation of microorganisms to complete degradation of the remaining polymers.

In the plastic bags the presence of titanium was identified, a component of the pro-oxidant additive. This element presents a higher relative concentration than the other elements analyzed and it is uniformly distributed on the surface of the bags. This homogeneous distribution was also observed to manganese, iron and cobalt. Furthermore, with the exception of titanium and cadmium, the other elements analyzed are important for fungal metabolism. These micronutrients may be elicitors or enzyme cofactors. Thus, the presence of these elements may also have contributed to the Ganoderma lucidum growth on the surface of plastic bags.

Mycelial growth of Ganoderma lucidum was observed on the surface of the plastic waste (Figure 6). This figure shows an example of mycelial growth in oxo-biodegradable plastics after 30 and 90 days. This paper was added in the culture medium to retain moisture and to be an inducer of fungal growth and stimulates the synthesis of lignocellulolytic enzymes that degrades the paper itself and the plastic.

Conformation Of PE Degradation By Spectral Analysis

Scanning Electron Microscopic (SEM) Analysis

The results of degradation of the polythene strips by the fungal isolate was confirmed by the wrinkles on the surface, formation of holes and cracks, crumbling and were visualized in Scanning electron microscopic (SEM) with a magnification of 50,000 photographs (Figure 7).

Fourier-transform Infrared Spectroscopy (FTIR) Analysis

Chemical changes by Fourier transform infrared spectroscopy (FTIR) were determined. These alterations were the disappearance or formation of new functional groups in spectrum of FTIR with scanning of 500 at 4000 cm−1 wave numbers.

To degradation of the plastic bags were also confirmed with FTIR analysis in terms of changes in Carbonyl Index. Maximum change in Carbonyl Index was recorded in untreated polythene (4.36) with the fungi (Ganoderma lucidum) compared to pre-treated plastic strips (2.56) (Figure 4).

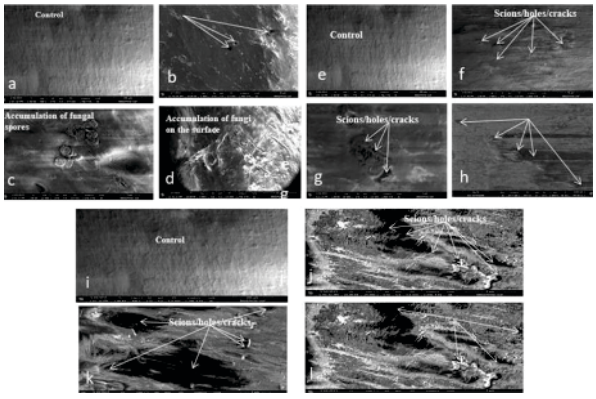

Figure 2: Plastic Fragments + Fungal Culture In Minimal Medium

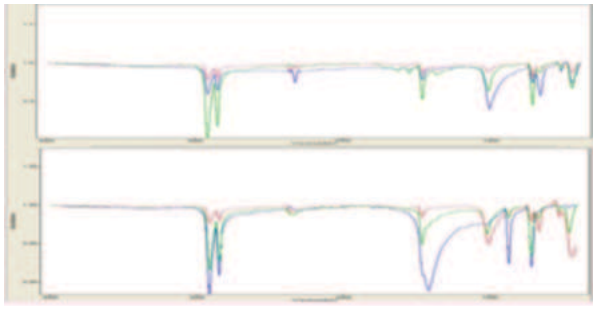

Figure 3: Photograph Of SEM

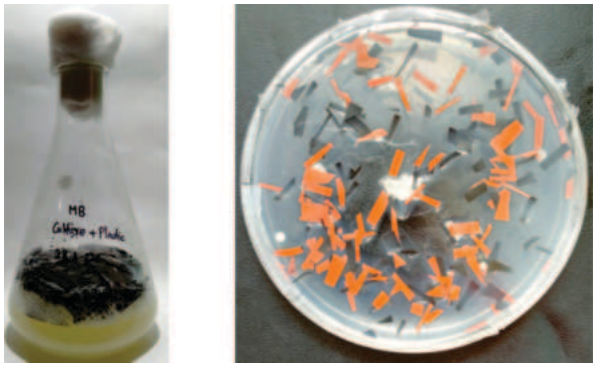

Figure 4: Ftir Spectrum Of Plastic Degradation

CONCLUSIONS

The fungal isolate Ganoderma lucidum strain is the most efficient and elite polythene deteriorating fungi based on reduction in weight, reduction in tensile strength, SEM and FTIR analysis. SEM analysis of the surface of the degraded polythene showed disturbances such as cracks, scions, fissures and holes which confirms corrosion. FTIR analysis shows formation of carbonyl group (1710–1740^{-1} cm) carboxylic group (1630-1840 cm^{-1}), CH strees (2915 cm $^{-1}$) and CH_2 group (720 cm^{-1}) after the UV and carboxylic group chemical treatment in control. These peaks were found to be reduced after fungal treatment. These decreasing peaks are due to the consumption of

carbonyl and carboxylic acid derivatives by fungi indicating the de-polymerization of the polythene chain.

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