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Assessment of Physicochemically treated plastic by fungi

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ABSTRACT

The most common polymer in plastics is polyethylene (PE), which is made from ethylene monomers (CH2=CH2). In natural form it is not biodegradable. To enhance the biodegradation of polyethylene, pretreatment strategies were followed. Three different pretreatment strategies were employed for the present study. In the first, PE films were thermally treated at 100ºc in an atmosphere of air for 30 days to induce oxidation and in the second they were subjected to UV light (UV-C,>300nm wavelength) for 10 days. Thirdly, they were suspended in concentrated nitric acid to enhance percent elongation for 10 days. These pretreated samples along with the untreated PE films were used as the sole carbon source for isolation of PE degrading strains. Submerged cultures with PE as the sole carbon source were inoculated with the isolated fungal strains and assessed for polymer biodegradation by weight loss, estimation of total carbohydrates and total protein in the culture supernatant.

Key words: Fungal degradation of plastics, physicochemically treated polyethylene.

INTRODUCTION

Each year more than 140 million tones of plastics are produced worldwide. In many countries, plastics are disposed off through open, uncontrolled burning and land filling. Open burning releases pollutants into the air that could cause various health problems. In addition, the burning of polyvinylchloride (PVC) plastics produces persistent organic pollutants and has been associated with a number of adverse effects in humans, including immune and enzyme disorders and chloracne, and they are classified as possible human carcinogens. Health may be affected by the polymer itself, by chemicals added to the plastic to make it more flexible, stable or flame retardant, or by colouring agents. These substances may be released to the air when the plastics are heated. When plastics are heated to form final products, monomers, additives and degradation products can be released. Small amounts of these may also be present in the resins before heating, they can affect the health of workers who use, clean or maintain the processing equipment [14].

Degradation of plastics:
Degradation of plastics is defined as any physical or chemical change in the polymer as a result of environmental factors, such as light, heat, moisture, chemical conditions or biological activity. Processes inducing changes in

polymer properties (deterioration of functionality) due to chemical, physical or biological reactions resulting in bond scission and subsequent chemical transformations (formation of structural in homogeneities) have been categorized as polymer degradation. Degradation has been reflected in changes of material properties such as mechanical, optical or electrical characteristics, in crazing, cracking, erosion, discoloration, phase separation or delamination. The changes include bond scission, chemical transformation and formation of new functional groups [19].

Sensitivity of polymers to photo degradation is related to the ability to absorb the harmful part of the tropospheric solar radiation. This includes the UV-B terrestrial radiation (~295-3-5 nm) and UV-A radiation (~315-400) responsible for the direct photo degradation (photolysis, initiated photo oxidation. Visible part of sunlight (400-760 nm) accelerates polymeric degradation by heating. Infra-red radiation (760-2500 nm) accelerates thermal oxidation [11];[19].

Thermal degradation of polymers is 'molecular deterioration as a result of overheating'. At high temperature the components of the long chain backbone of the polymer can begin to separate (molecular scission) and react with one another to change the properties of the polymer.

The chemical reactions involved in thermal degradation lead to physical and optical property changes relative to the initially specified properties. Thermal degradation generally involves changes in the molecular weight (and molecular weight distribution) of the polymer and typical property changes include reduced ductility and embrittlement, chalking, color changes and general reduction in most other desirable properties [18].

Biodegradation of plastics

Microorganisms such as bacteria, fungi and actinomycetes are involved in the degradation of both natural and synthetic plastics [10]. The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms responsible for the degradation differ from each other and they have their own optimal growth conditions [8].

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. While not many reports are available on fungal mediated degradation of polycarbonate, a *Geotrichum*-like fungus isolated from a biodeteriorated compact disk made of polycarbonate was able to degrade its components.[20]. The biodegradation of bisphenol, a monomer of PC, by fungi has also been reported. [16].

MATERIALS AND METHODS

Materials: High density polyethylene (HDPE) and Low density polyethylene (LDPE) which is the major cause of environmental pollution were used for the study. HDPE is used in the manufacture of milk jugs, butter tubs, detergent bottles, motor oil bottles, etc., LDPE is used in the manufacture of dispensable bottles, containers, wash bottles and various molded lab equipments. Polyethylene films (PE) were cut into 60x10mm strips for the invitro studies.

Physicochemical Treatment:

To enhance the biodegradation of polyethylene, pretreatment strategies were reported [13],[12]. Three different pretreatment strategies were employed for the present study. In the first, PE films were thermally treated at 100^{o}c in an atmosphere of air for 30 days to induce oxidation and in the second they were subjected to UV light (UV-C,>300nm wavelength) for 10 days. Thirdly, they were suspended in concentrated nitric acid to enhance percent elongation for 10 days. These pretreated samples along with the untreated PE films were used as the sole carbon source for isolation of PE degrading strains. [3][4].

Enrichment of PE degrading microorganisms

Soil samples were collected from a plastic dumpsite inside Madras Christian college campus, Chennai. A total of 1g of the soil sample was suspended in 10ml of sterile Milli-Q water and vortexed for 15 minutes. Nearly 100 µl of suspension was used as inoculums. Erlenmeyer flasks containing 100 ml of mineral salt medium, strips of untreated polyethylene, 0.01 %(w/v) glucose and 1 ml of inoculums were used for maintaining the first preculture. The later subcultures did not contain glucose but only the polymer as the sole carbon source. After three successive subculture, in which microorganisms were grown in presence of PE and without glucose pure, cultures were isolated on potato dextrose agar plates containing 50 mg of chloramphenicol to avoid bacterial contamination for fungal isolation.[2][3].

a)Identification and Characterization of PE degrading fungal Strains

Fungal strains were identified by the morphological features of their colony and conidia using microscopic examination.

Submerged Culture with Polyethylene as Sole Carbon Source

A mineral salt medium (100ml) was prepared, sterilized and to this pretreated PE films were suspended.[7] Fresh mycelium of the previously grown fungus on potato dextrose agar plates was scraped from the plate and suspended in 10ml of sterile water and vortexed. Around 1ml of this suspension was inoculated into the flasks and incubated at 30ºc and at 200rpm on an orbital shaker and were monitored for 6 months. Sampling was done every 2 months, in which the PE films were taken out under aseptic conditions, washed in sterile water and air-dried before further analysis.

a)Total Biomass

About 1ml of the culture was transferred into a 1.5ml micro centrifuge tube and pelleted down at 12000rpm at 4ºc for 25 minutes. The pellet was dried overnight at 50ºc and dry weight of the resulting biomass was calculated. Sampling was done in triplicates.

b)Total Protein and Reducing Sugars in the Culture Supernatant

The total protein concentration in the supernatant was determined by the method reported by Bradford [5]. Bovine serum albumin solutions were used as standards and absorbance was measured with a spectrophotometer at 595nm.The total carbohydrates were analyzed according to the method suggested by Dubois [6]. Glucose was used as the standard and the absorbance was measured at 495nm.

c) Physical analysis:

The polymer degradation was analyzed physically by measuring the weight loss

RESULTS

Characterization of the isolates: Based on colony morphology and lactophenol cotton blue staining the fungal isolates were identified as *Rhizopus arrhizus* (figure 1) and *Penicillium* sp.(figure 2)

Figure: 1 Rhizopus arrhizus

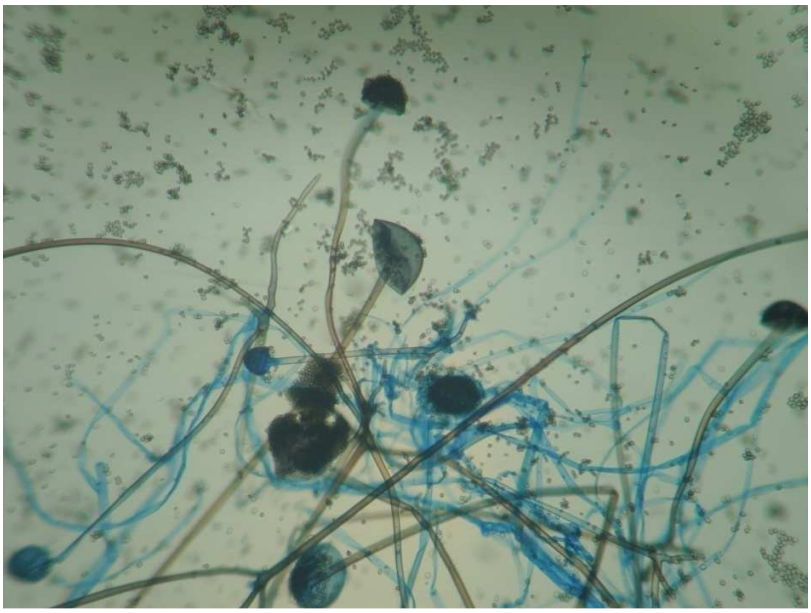
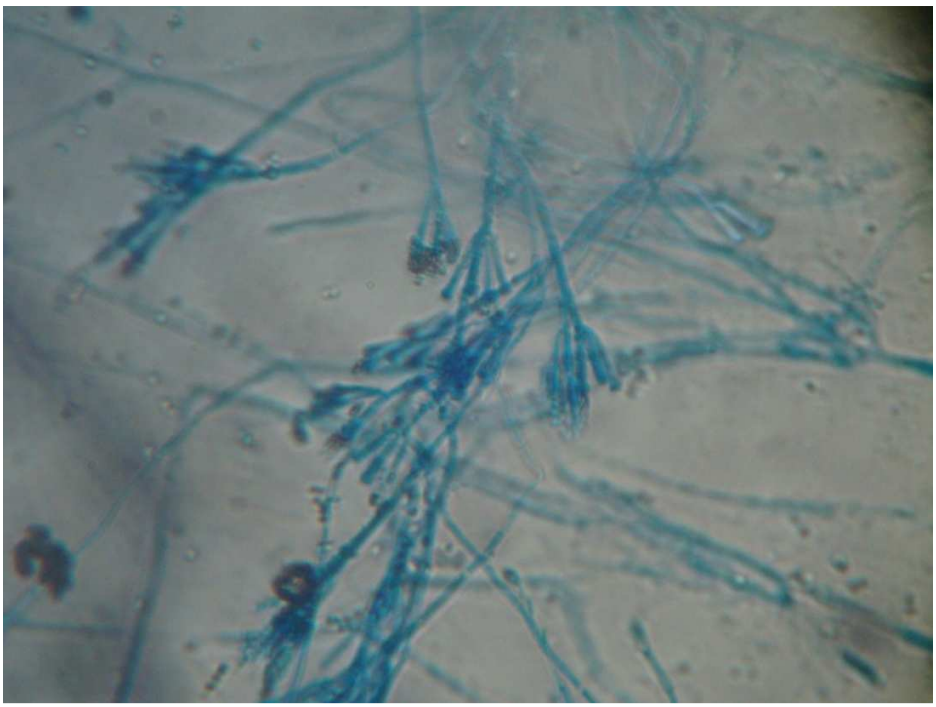


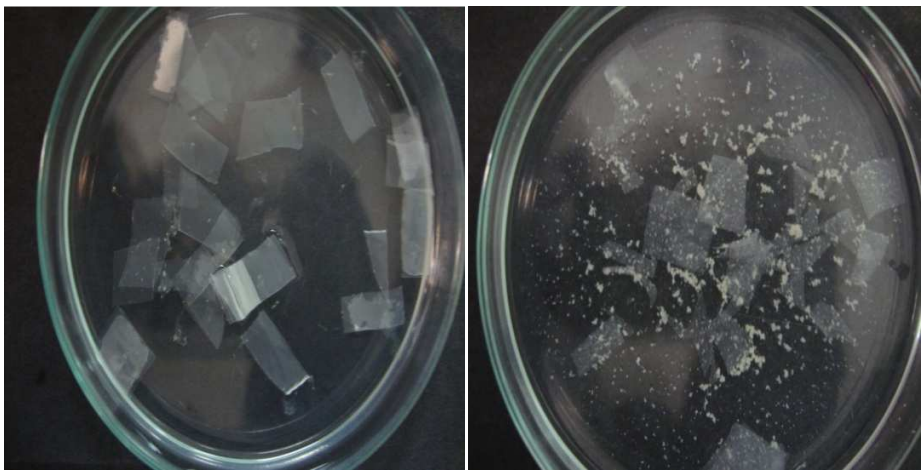
Figure: 2 *Penicillium* sp.



Physicochemically treated PE films were found to be effectively degraded by the fungal isolates than untreated films (figure 3)

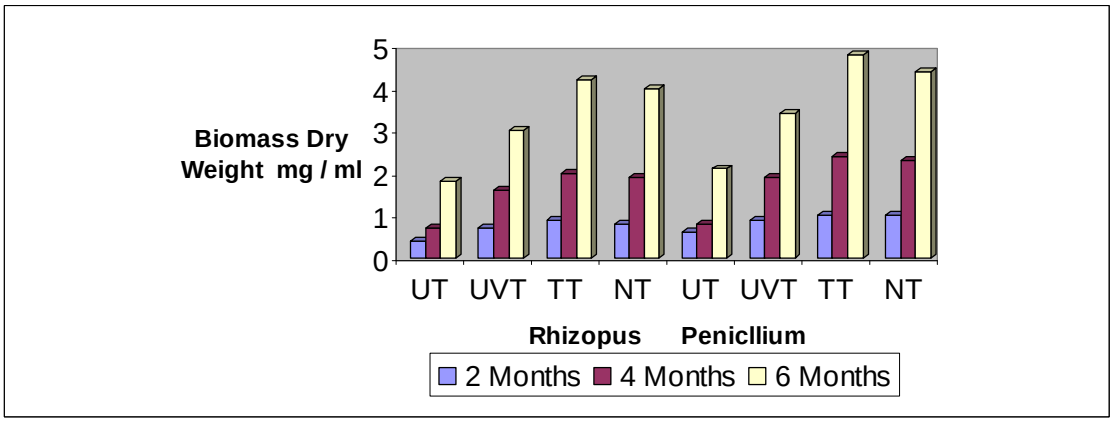
Figure: 3

Untreated PE films showing degradation Pretreated PE films showing degradation



Total Biomass

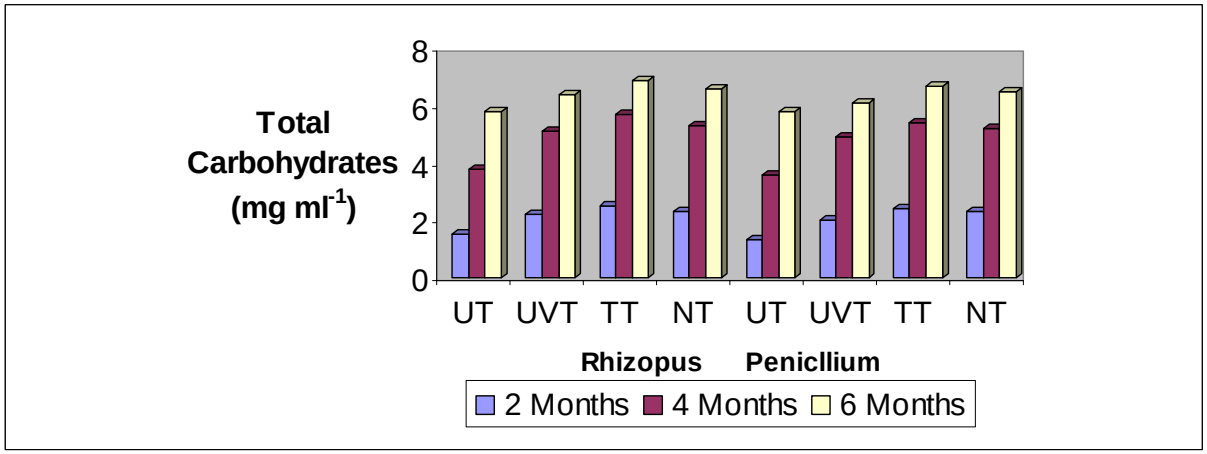
Fungal biomass is a direct measure of the growth of the culture in the medium utilizing PE as a Carbon source. Chart shows the variation in biomass during 2,4 and 6 months respectively.(chart 1)



UT – Untreated, UVT – Ultraviolet treated TT – Thermally treated, NT – Nitric acid treated

Total Carbohydrates in the culture supernatant :-

The amount of total carbohydrates in terms of reducing sugars (glucose in the present study) produced by two fungal strains during 6 months of exposure of PE was determined. (chart2)



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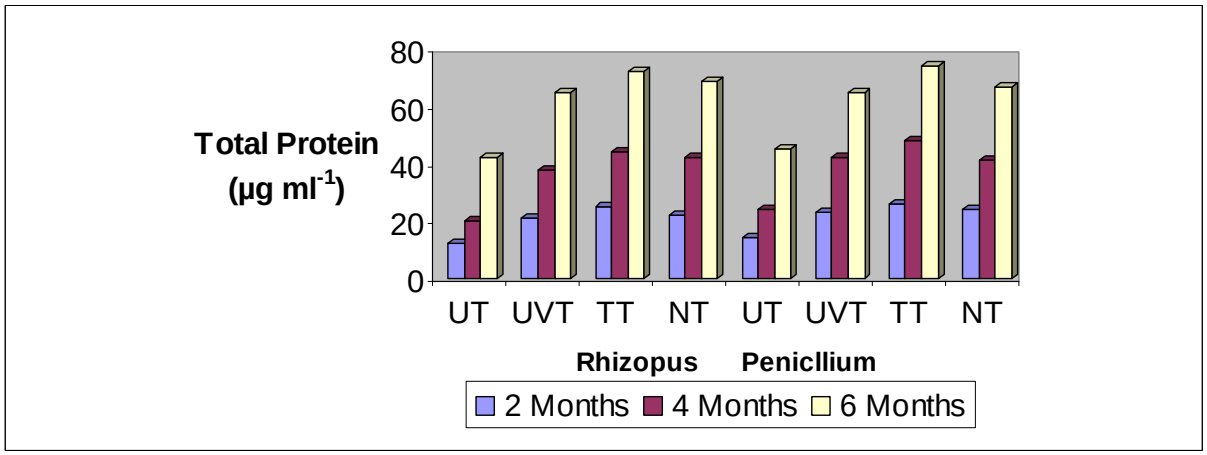
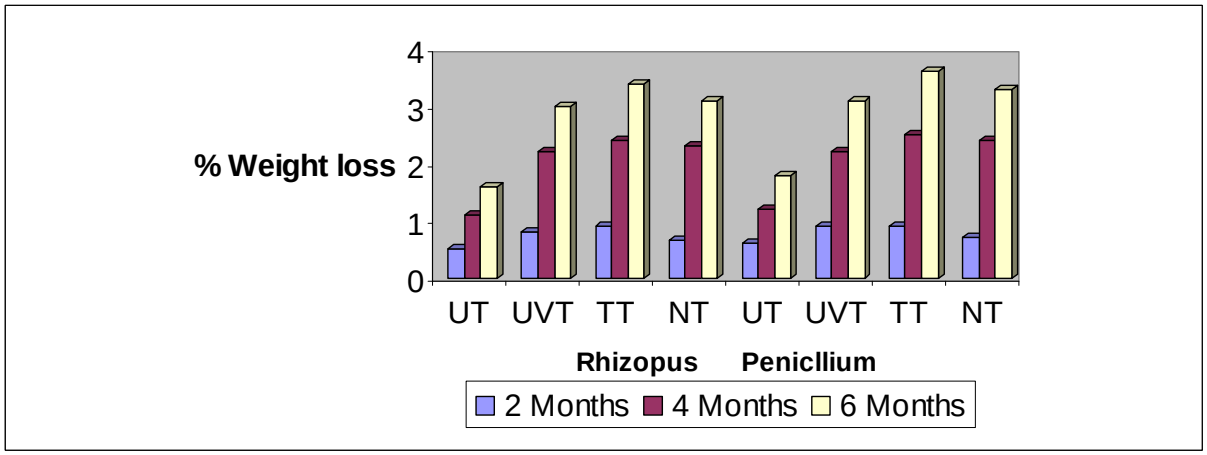
Total Proteins in the culture supernatant :-
Total proteins in the supernatant of cell – free culture was summarized in chart 3



UT – Untreated, UVT – Ultraviolet treated TT – Thermally treated, NT – Nitric acid treated

Physical analysis:-
Weight loss:-
Weight loss of pretreated & untreated PE over the study period of 6 months was shown in chart. 4.



UT – Untreated, UVT – Ultraviolet treated TT – Thermally treated, NT – Nitric acid treated

DISCUSSION

Physicochemically treated PE films were found to be effectively degraded by the fungal isolates than untreated films .The hypothesis is that physicochemical treatments of the polymer leads to its oxidation and subsequent breakdown assisting in the easy assimilation by the fungus and, hence, can be effectively used as a pretreatment strategy before subjecting the polymer to biodegradation.[1].The oxidized polymer helps in adhesion of microorganisms (due to probable changes in the hydrophobicity of the polymer surface), which is a prerequisite for biodegradation.[3].Similarly in the present study, a higher biomass was observed on the pretreated samples. Because carbohydrates in the medium constitute the main energy source for their growth and metabolism during the non-availability of readily assimilating carbon source, microorganisms adhere to the polymeric surface during the formation of the biofilm, which is essential for bringing about degradation. [9].The use of PE as a carbon source was well revealed in this study by the production of more amount of reducing sugars.

Microorganisms are unable to transport the polymeric material directly into the cell due to the lack of its solubility in water & its size. They excrete extra cellular enzymes which aid in the degradation of polymers outside the cells [21].Hence extracellular proteins were produced in more amounts by pretreated PE samples.

The superficial growth of hyphae on the polymer surface was a function of the oxidation levels of treated sample was observed [17]. Therefore pretreated samples showed greater weight loss than untreated samples.

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