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# Fungi-agents of Plastic Biodegradation Report for ITR Course

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**Abstract—***Lack of degradability and the closing of landfill sites as well as growing water and land pollution problems have led to concern about plastics. With the excessive use of plastics and increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastics and biodegradation of plastic wastes has assumed increasing importance in the last few years. Awareness of the waste problem and its impact on the environment has awakened new interest in the area of degradable polymers. The interest in environmental issues is growing and there are increasing demands to develop material which do not burden the environment significantly. Biodegradation is necessary for water-soluble or water-immiscible polymers because they eventually enter streams which can neither be recycled nor incinerated. It is important to consider the microbial degradation of natural and synthetic polymers in order to understand what is necessary for biodegradation and the mechanisms involved. This requires understanding of the interactions between materials and microorganisms and the biochemical changes involved. Widespread studies on the biodegradation of plastics have been carried out in order to overcome the environmental problems associated with synthetic plastic waste. This paper reviews the current research on the biodegradation of biodegradable and also the conventional synthetic plastics and also use of various techniques for the analysis of degradation in vitro.*

## 1. INTRODUCTION

Due to plastic's resilience against degradation and its proliferation in industry, the issue of plastic pollution has evolved to become a threat to global ecology.

Plastic pollution arises from both terrestrial and marine sources.

The continual stream of pollutant plastic is maintained via two means that is purposfully, through illevergal or inappropriate dumping of domestic and industrial refuse, and in advertently, through poorly contained statics and transport waste. Directed by the elements, land waste plastics debris migrates to water waves, where it is further added to by the disposal or loss borne from marine vessels and offshore petroleum platforms. such pollution result in a number of deletrious repercussions.

## PLASTIC BIODEGRADATION

With more and more plastics being employed in human life an increasing pressure being placed on capacities available for plastic waste disposal, the need for biodegradable plastic and biodegradation of plastic waste has assumed increasing importance in the last few years. it concentrates on the biodegradation of plastics by microorganisms. Additives such as prooxydents and starch are applied in synthetic materials to modify and make plastic biodegradable. recent research has shown that thermoplastics derived from polyolefins, traditionally considered resistant to biodegradation are biodegraded by photo and chemical degradation. Thermostat plastics are easily attacked by microorganisms. Reviewing published and ongoing studies on plastic biodegradation are going on to reduce impacts of plastics waste on environment.

## DISADVANTAGES OF TRADITIONAL METHODS OF BIODEGARADTION

Under proper conditions, some biodegradable plastics can degrade to the point where microorganisms can completely metabolise them to carbon dioxide (and water). For example, starch based bioplastics produced from sustainable farming methods could be almost carbon neutral.

There are allegations that "Oxo Biodegradable (OBD)" plastic bags may release metals, and may require a great deal of time to degrade in certain circumstances and that OBD plastics may produce tiny fragments of plastic that do not continue to degrade at any appreciable rate regardless of the environment. The response of the Oxo-biodegradable Plastics Association (www.biodeg.org) is that OBD plastics do not contain metals. They contain salts of metals, which are not prohibited by legislation and are in fact necessary as trace-elements in the human diet. Oxo-biodegradation of polymer material has been studied in depth at the Technical Research Institute of Sweden and the Swedish University of Agricultural Sciences. A peer-reviewed report of the work was published in Vol 96 of the journal of Polymer Degradation & Stability (2011) at page 919-928, which shows 91% biodegradation in a soil environment within 24 months, when tested in accordance with ISO 17556.

## BIODEGRADATION OF PLASTICS BY USING DIFFERENT FUNGI

## MATERIALS AND METHODS

Estimation of heterotrophic fungi in the polythene samples: The polythene bags were collected in a sterile plastic box from the polluted areas of Chennai, Tamil Nadu. The samples were serially diluted and pour plated in sterile Potato Dextrose Agar to estimate and isolate heterotrophic fungi respectively. The plates were incubated at 37°C for 48 h. After incubation, plates with 30-300 colonies were chosen for counting and the total plate count for fungi was expressed as number of colony forming units per gram of soil.

### Characterization of the heterotrophic fungi

After counting and estimation of total, morphologically different colonies were picked up using sterile needle and forceps and aseptically transferred to sterile PDA agar slants for further characterization. Fungi were chosen for characterization and identified by macroscopic and microscopic observation (staining technique).

Screening and identification of polyethylene degrading fungi:

Agar amended with substrates like 1% starch, 1% gelatin, 1% tween-80 served as the suitable medium for the action of enzymes present in culture extracts of fungi. Agar amended with each substrates were separately sterilized at 15 lbs for 15 min. About 5-20 mL of sterilized substrate was poured into sterile Petri-dish. The plates were surface dried over night and the wells were cut aseptically to load the culture filtrates of isolated fungi.The plates loaded with culture filtrates were incubated at 37°C for 3-4 h. After incubation, opacity was observed around the well surface which indicated the positive result for the respective substrates. Further quantitative assay was performed.

Microbial degradation of plastics: The pre-weighed low density polyethylene (LDPE) strips of 1 cm dia were aseptically transferred to the conical flask containing 50 mL of Rose Bengal broth medium, and separately inoculated with the selected fungal strains. Control was maintained with low density polyethylene (LDPE) strip in the microbe free medium. Four flasks were maintained for each treatment and left in a shaker. After one month of shaking, the polythene strips were collected, washed thoroughly using distilled water, shade-dried and weighed for final weight. From the data collected, weight loss of the polythene strip was calculated. Further the surface of degraded low density polyethylene (LDPE) was analyzed.

### Pestalotiopsis microspora-

It is a species of endophytic fungus capable of breaking down and digesting polyurethane.This fungus seceretes a small enzyme that is responsible for plastic degradation.Its polyurethane degradation activity was discovered in the Yasuni National Forest within the Ecuadorian Amazonian rainforest by a group of student researchers led by molecular biochemistry professor Scott Srobell as part of Yale's annual Rainforest Expedition and Laboratory. It's the first fungus species found to be able to subsist on polyurethane in anaerobic conditions. This makes the fungus a potential candidate for bioremediation projects involving large quantities of plastic.

The Yale students had discovered that Pestalotiopsis microspora fungus can break down plastic. It's a species of fungi that can be found in many regions of the world and can decompose polyurethane, a common plastic that is used to make things like insulation, synthetic fibers, plastic for electronics and sealants.

The fungus was 10 days old when the experiment started and in only a matter of days, he says, it had significantly decomposed about a quart size amount of the plastic.

The study found that several species of fungi were able to at least partially decompose polyurethane, but this type was the only fungus able to do it in water without oxygen, one of the most challenging environmental conditions. We found that two isolates of peslotiopsis microspora (E2712A and e3317B) were able to degrade PUR when grown anaerobicallyta.

Pestalotiopsis microspora that is responsible for PUR degradation appears to be a member of serine hydrolase family. Furthermore, activity extended throughout the medium at a distance well removed from the areas of fungal growth. This suggests that the enzyme responsible for degradation is extracellular, secreted and diffusible. In compaarision to an inactive cell – free filtrate from a fungal culture grown in rich medium, we found that the polyurethanase is inducible when pestalotiopsis microspora E2712A is grown in minimal PUR medium containing a suspension of impranil DLN. By using activity –based probes, the active enzymes were identified as a serine hydrolase with an approximate molecular mass 21 kDa. The protein was shown to be able to degrade PUR after subsequent purification, showing that activity is independent of other components of culture filtration.

**pestalotiopsis microspora**

## 2. RESULT

Fungi identified from polluted site were identified asAspergillus niger, A. japonicus, A. terreus, A. flavus and Mucor sp. Two predominant fungi Aspergillus niger and A. japonicas were selected for further studies.Aspergillus niger showed degradation of low density polyethylene up to 5.8% in one month while A. japonicas showed more capability to degrade low density polyethylene up to 11.11% in one month under laboratory conditions. For further confirmation, SEM analysis was done at different magnification. The control polythene strips displayed a normal surface view but the polythene strips treated with A. niger and A. japonicus showed appreciable surface corrosion, folding and cracks. This may be due to the fungal extracellular metabolites and fungal enzymes Both control and test fungi treated with polythene strips were heated to 0-200°C at the heating rate of 10°C/min. The melting point was reduced in the fungal treated polythene strips (161oC) when compared to control (162.2oC) polythene strips.

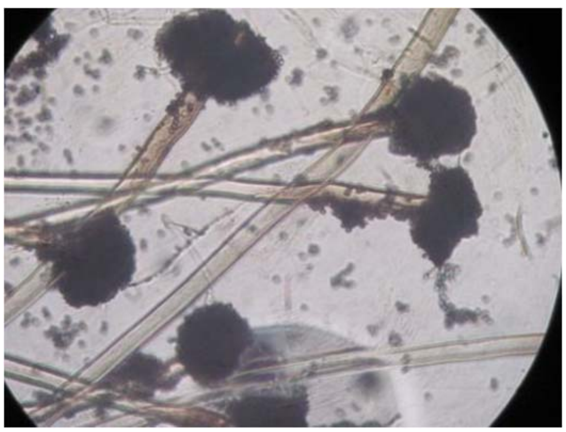

**Fig-B Isolated A.niger fungal culture under microscopic view**

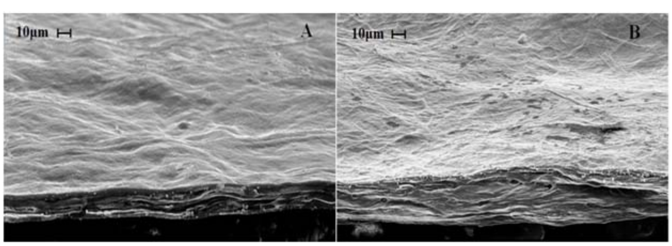


**Fig C SEM of polyethene film treated with A.japonicus**

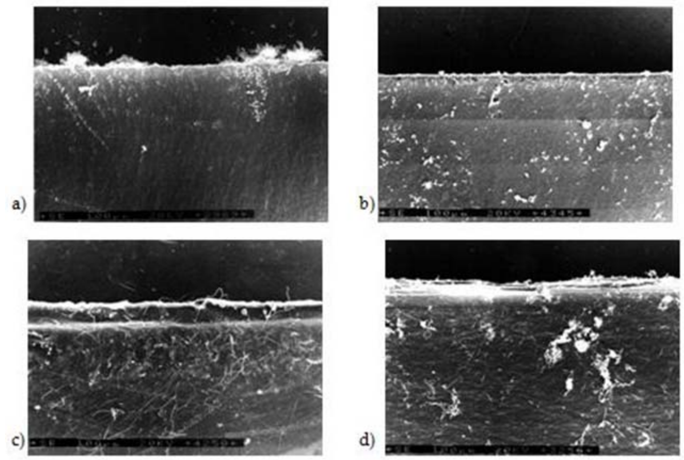


**Fig D SEM of polyethene film treated with A.niger**

## 3. DISCUSSION

Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environments. High-density and low-density polyethylenes are the most commonly used synthetic plastics and they are slow in degradability in natural environments, causing serious environmental problems. In this regard, there is a growing interest in non-degradable synthetic polymer biodegradation using effective microorganisms.There is no report on polythene degradation by Aspergillus japonicus so far. This is the first experimental report of low density polythene (LDPE) degradation under laboratory conditions by showing effective ability of A. japonicus. The potency of degradation by A. Japonicas is twice than that of A. niger i.e., A. japonicus degraded 11.11% per month while A. niger degraded 5.8% per month. The polythene bags in the soil of polluted sites have been degraded by the presence of fungi besides abiotic factors such as moisture, heat, temperature, etc of the soil (Anonymous, 1999). The mechanism of degradation is not known exactly and the surface of plastic material has turned from smooth to rough with cracking and the molecular weight reduction, increase in carbonyl double bond groups, erosion on the surface of polyethylene is due to the microorganisms (Weiland et al., 1995). In the process of depolymerization at least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerises. During degradation,exo-enzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, e.g.oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer membranes of the microbes, and then to be utilized as carbon and energy sources (Frazer, 1994; Hamilton et al., 1995). Hence, further study on microbial enzymes or organic acids in degradation of the polythene and plastic will pave way for finding technology for degrading these environmentally hazardous plastic materials.

## 4. CONCLUSION

Fungal strains Aspergillus niger and A. japonicus were selected for polythene degradation under laboratory conditions. Their effectiveness on the degradation of commercial polythene carry bags of low density polyethylene was studied over a period of 2 and 4 weeks.

Biodegradation was measured in terms of mean weight loss, which was nearly 8 to 12% after a period of 4 weeks. Further, SEM analysis confirmed the degradation revealing the presence of porosity and fragility of the fungal degraded polythene surface. Aspergillus japonicus showed 12% degradation potential when compared to A. niger of 8% degradation in one month period.

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