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Research Article

A Study On The High Density Polyethylene Degrading Ability Of Fungal Isolates From The Garbage Dumping Site At Thanjavur

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Abstract

Plastic is a broad name given to different polymers having high molecular weight and that can be degraded by various processes. However, degradation by physical and chemical means leads to innumerable environmental hazards. On the other hand, degradation of plastics by microorganisms seems to be more effective, considering their abundance in the environment, their specificity in attacking plastics and has very less environmental hazards. Plastic and polythene waste accumulating in the environment are posing an ever increasing ecological threat. Municipal solid waste contains high amounts of cellulose, which is an ideal organic waste for the growth of most of microorganism as well as composting by potential microbes. The observed results presented that *A. flavus* could be used as a potential biodegradable agent for plastic materials.

Keywords: Biodegradation, Polyethylene, Plastic, *A.flavus*

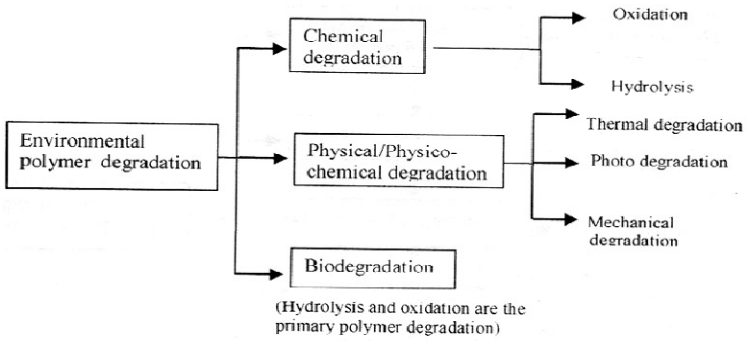
Introduction

Plastic is the general term for a wide range of synthetic or semi- synthetic polymerized products. Plastics are composed of petroleum based materials called resins (eg. Polythene & Polypropylene). During the past three decades, plastic materials have been used widely in food, clothing, shelter, transportation, construction, medical and leisure industries because of their light weight, low cost, relative stability and extreme durability[1]. Among various types of plastic polymers, the most popular and convenient plastic polymers include low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyproplene (PP), polyvinyl chloride (PVC), polystyrene (PS), nylon, polyethlene terephthalate (PET), polyurethanes, etc.

High Density Polyethylene (HDPE) is one of the synthetic polymers of high molecular weight and is hydrophobic in nature. It is a polythene thermoplastic reared by catalytic process, containing carbon, hydrogen as backbone elements and has little branching, giving it stronger inner molecular forces and tensile strength (4550 psi approx.). Thus they have wide industrial and day to day application. The versatility and resilience of these materials has lead to a great increase in their use and have rapidly moved in all types of utility. About one third of this material is used in the manufacture of disposable items such as bags, cups, wraps, trays and other packaging materials. A very general estimate of worldwide plastic waste generation is annually about 57 million tons [2].

Polythene being xenobiotic is the most commonly found non-degradable solid waste that has been recognised as a major threat to land and aquatic life both. Polythene sometimes could cause blockage in fish, birds, mammals and choke to death. An estimate of one million birds and ten thousand mammals die each year as a result of ingestion of or trapping of plastics in oceans [3,4]. Recent alarm on ecological threat is dumping of plastic waste into the ecosystem by anthropogenic activity. They do not break down easily in the environment because of their resistant to microbial attack, due to their high number of aromatic rings, unusual bonds or halogen substitutions [5]. As a result they remain in the environment for a very long period without any deterioration. The voluminous accumulation of waste plastics in the biosphere has given rise to the problem of severe environmental pollution. These problems have made waste focus in the management of solid waste. Recycling of plastics is not always economically possible. The threat associated with all ecosystems makes their degradation and deterioration necessary. These are highly resistant to biodegradation, leading to pollution and harmful to the natural environment. Municipal solid waste contains high amounts of cellulose, which is an ideal organic waste for the growth of most of microorganism as well as composting by potential microbes.

Fig. 1. Overview of degradation of polymers



Biodegradation of polymers primarily focuses on increasing the surface hydrophobicity, thereby enhancing microbial attachment. Hence most of the researchers recommend for the pre-treatment [6] for efficient microbial adherence. There is a growing interest in synthetic polymer biodegradation using effective microorganisms. Development of microbial communities on the surface of the polymer is found to be a powerful degrading agent. Aerobic metabolism results on carbon dioxide and water and anaerobic metabolism results in the production of carbon dioxide, methane and water [7,8]. The degradation leads to breaking of polymers to monomers creating an ease of accumulation by the microbial cells for further degradation. Much focus of attention was given for fungal isolates, since the fungal hyphae can adhere to HDPE strips in wet condition. Studies on fungal mediated degradation of polyethylene has previously been reported using Aspergillus sp. [9,10,11].

Most of the studies on the biodegradation of polyethylene are based on the biotic environment, but some studies have used axenic strains amended with polythene. This study is aimed to isolate the indigenous HDPE degrading fungi from garbage dumped sites describing the efficacy of degradation and functional group analysis by FTIR.

Sources of the Polythene Degrading Microbes

Following sites were reported to be rich source of polythene degrading microbes:

a. Rhizosphere soil of mangroves, b. Polythene buried in the soil, c. Plastic and soil at the dumping sites and d. Marine water.

Materials and Methods

Estimation of heterotrogenous fungi

The soil samples were collected in sterile zip lock bags from plastic contaminated places in Thanjavur, Tamil Nadu. The samples were serially diluted

and pour plated in sterile Potato Dextrose Agar to estimate and isolate heterotrophic fungi respectively[12]. 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 heterotrogenous 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 by Lacto phenol Cotton blue staining technique. Among all the soil samples collected *Aspergillus flavus* is found to be the dominant fungi and was further tested for the ability for degradation of HDPE in laboratory conditions.

Degradation of plastic strips by *Aspergillus flavus*

About 1g weighed strips were used for the degradation process. The strips were tested for degradation by burying in soil pits. Sterile soil pits were prepared and the strips were placed in layers with soil alternatively. The pits were then enriched with the inocula of A. *flavus* in intervals. The strips were allowed to degrade for three months. The degradation by the fungus was analysed by determining the dry weight at a regular interval of days. The plastic strips were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the plastics was calculated.

Optimization of fungal degradation

In order to elucidate the enhancement of degradation optimized with different pH, temperature, carbon and nitrogen sources on supplement were given to fungal inoculums amended with HDPE strips.

Effect of pH on HDPE degradation

To determine optimal pH, fungus cultures were cultivated in a 150ml flask containing 40ml optimized medium with different pH ranges from 7.0 to 9.0.The pH of the medium was adjusted by using 1N HCl or 1N NaOH. The flasks were kept in stationary stage at 28°C for 5days of cultivation.

Effect of Temperature on HDPE degradation

In order to determine the effective temperature for HDPE degradation by the fungal species, Fermentation was carried out 5ºC intervals in the range of 25ºC,30ºC,35ºC and 40ºC.

Effect of Carbon source on HDPE degradation

To determine the effective carbon sources for HDPE degradating by the specific fungal species, fermentation was carried out at different carbon sources for glucose, fructose, sucrose and starch were kept in 28ºC for 5 days of cultivation.

Effect of Nitrogen source on HDPE degradation

To determine the effective nitrogen sources for HDPE degradating by the specific fungal species, fermentation was carried out at different nitrogen sources for ammonium sulphate, potassium nitrate and ammonium nitrate.

Analysis by Fourier Transform Infra Red spectroscopy (FTIR)

The changes in the HDPE structure following different duration and subsequent incubation with fungal strain was analyzed by FTIR spectroscopy. HDPE samples degraded by *A. flavus* were collected after different intervals of incubation. The HDPE residue was air dried and used for FTIR analysis. HDPE samples were milled with potassium's bromide (KBr) to form a very fine powder. This powder was then compressed into a thin pellet which can be analyzed. KBr is also transparent in the IR.

Results & Discussion

The present study deals with the isolation, identification and ability of HDPE degrading fungi from soil. Synthetic plastic and soil sample was collected from the garbage dumped soils at Thanjavur was used in this study.

Microbial degradation of a solid polymer like polyethylene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities. The development of multicellular microbial communities known as biofilm, attached to the surface of synthetic wastes has been found to be powerful degrading agents in nature. When the total biodegradation process of

any organic substrate is considered the formation of microbial colony is critical to the initiation of biodegradation. Thus, the duration of the microbial colonization is an important factor that effects total degradation period. The Fungal species found associated predominantly with the degrading materials were identified as *A. niger,A. flavus, and Odium caricae* (Table 1 & Fig 2).

TABLE.1. HDPE Polyethylene Degrading Fungal Isolates

Characteristics	*Aspergillus flavus*	*Aspergillus niger*	*Odium caricae*
Morphology	Yellowish to green color	Dark brown to Black spores on PDA	Dark white to dull white

Fig 2: Fungal Isolates From Garbage Dumping Site

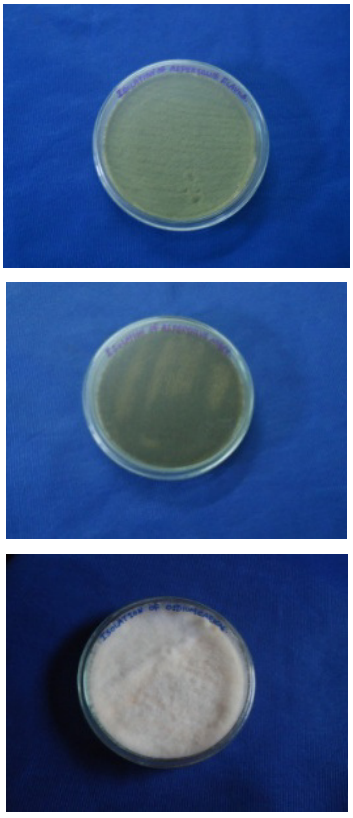


TABLE 2:Determination Of Weightloss Of HDPE By *A. flavus*

Days of Incubation	Before degradation in mg	After degradation in mg	Weight of polyethylene degradation	Percentage of polyethylene degradation
20	1mg	0.842	0.158	15.8%
40	1mg	0.782	0.218	21.8%
60	1mg	0.764	0.236	23.6%
Control	1mg	0.993	0.007	0.7%

Effect of pH on HDPE degradation

The activity was decreasing under both acidic conditions and at basic pH (Table3).The maximum HDPE degradation was observed at pH 8 (0.10U/ml) for *Aspergillus flavus*.The minimum amount of HDPE degradation was recorded at pH 10(0.02U/ml).

TABLE.3.Effect Of Ph For The Fungal Isolate *A. flavus*

S.No	Different pH	OD value at 660nm
1.	7	0.08
2.	8	0.10
3.	9	0.04
4.	10	0.02

Effect of Temperature on HDPE degradation:

The maximum HDPE degradation was obtained at 35ºC (1.30U/ml).On the otherhand, the minimum amount of HDPE degradation was observed at temperature 25ºC(O.12U/ml).The influence of temperature on the enzyme activity and stability was presented in Table.4.

Effect of Carbon source on HDPE degradation

Among the different carbon sources tested, the maximum HDPE degradation was recorded in starch(1.52U/ml) supplemented medium followed by glucose (0.64U/ml)and minimum HDPE degradation was recorded with sucrose and fruc-

tose(0.30U/ml&0.08U/ml) (Table.5).

Effect of Nitrogen source on HDPE degradation:

The maximum amount HDPE degradation was observed in potassiumnitrate (0.85U/ml) followed by ammonium disulphate (0.42U/ml) supplemented medium and minimum enzyme activity was observed in ammonium nitrite(0.20U/ml). potassium nitrate was found to be the desirable nitrogen source for enhancing the degradation of HDPE. The results were presented in Table.6

TABLE.4. Effect On Temperature For The Fungal Isolate *A. flavus*

S.No	Different Temperature	OD value at 660nm
1.	25ºC	0.12
2.	30ºC	0.16
3.	35ºC	1.30
4.	40ºC	0.86

TABLE.5. Effect On Carbon Source For The Fungal Isolate *A. flavus*

S.NO	DIFFERENT CARBON SOURCE	OD VALUE AT 660nm
1.	Glucose	0.64
2.	Fructose	0.08
3.	Sucrose	0.30
4.	Starch	1.52

Table.6. Effect on nitrogen source for the isolate fungal

S.No	Different Nitrogen source	OD value at 660nm
1.	Ammonium disulphate	0.42
2.	Potassium nitrate	O.85
3.	Ammonium nitrite	0.20

Analysis Of Biodegradation Of HDPE By Fourier Transform Infrared Spectroscopy

It was observed from the control treated groups showed a prominent were length of 1056.99 cm^{-1} indicated the C-H bond strength of the polyethylene(HDPE).A tensile strength of polymer with entity of peaks were observed. It is interacting to make that another spectral were length at peak of 3311.78 cm^{-1} and 3442.94 cm^{-1} indicated presence of intact OH-groups. The FTIR spectral wavelength of HDPE polymer amended with fungal isolates of *Aspergillus flavus* revealed prominent peaks after biodegradation leading to cleavage of bonds (aliphatic chain) by enzymatic machinery of fungus. During 60days of exposure, a peak at 1404.18cm^{-1} is a strong indication of C-H deformation, which is confirmed by shortening of peaks during experimental analysis of biodegradation of polymer. The result compared between control and 40days incubation reveal increase of 0.2% CI .A peak obtained at 1132.21cm^{-1} at control exhibited the presence of carboxyl groups where found decreased after degradation during 60days with 1124.5cm^{-1}. A band of 2333.81cm^{-1} appeared at peak no 17 was found to exhibit tensile strength of polymer, where in during 60days of incubation a peak at wavelength 2231.64cm^{-1} corresponded to formation of carboxyl peaks and occurrence of C=O stretches may be due process of biodegradation. The enzymatic machinery of fungus cleave the polymer leading to formation of ketones, aldehydes etc.,

Fig. 3. FTIR analysis of Control HDPE strips in C-Zopex Dox medium

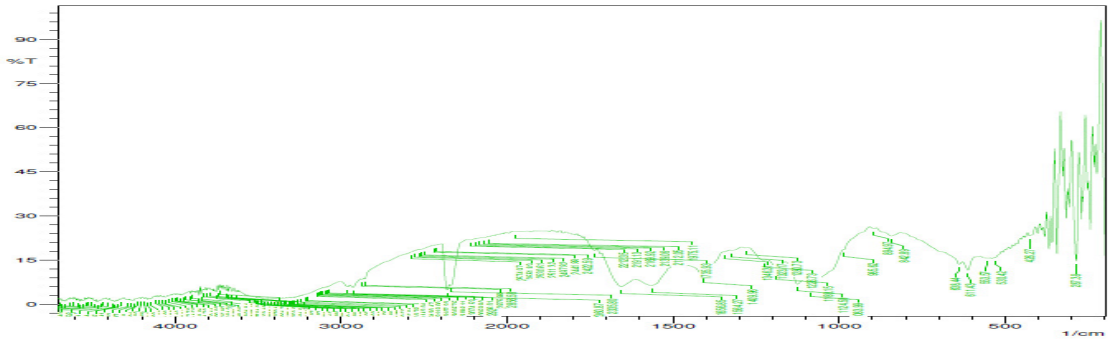


Fig.4. FTIR analysis of HDPE strips inoculated with *Aspergillus flavus* exposed to 20 days

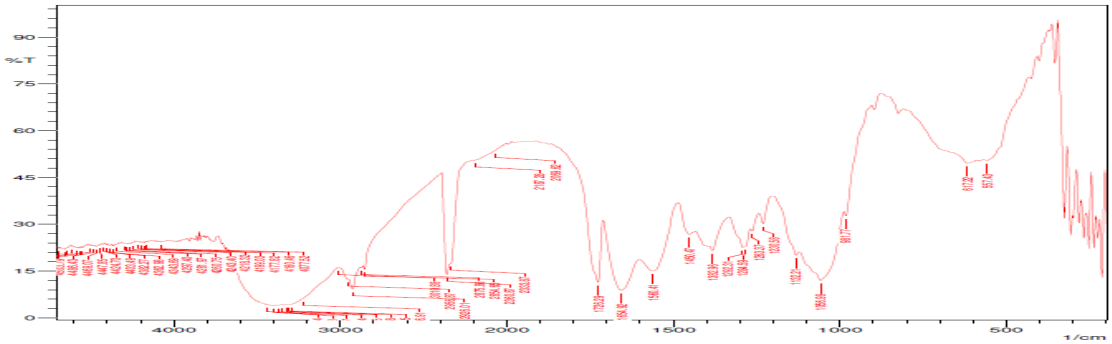


Fig. 5. FTIR analysis of HDPE strips inoculated with *Aspergillus flavus* exposed to 40 days

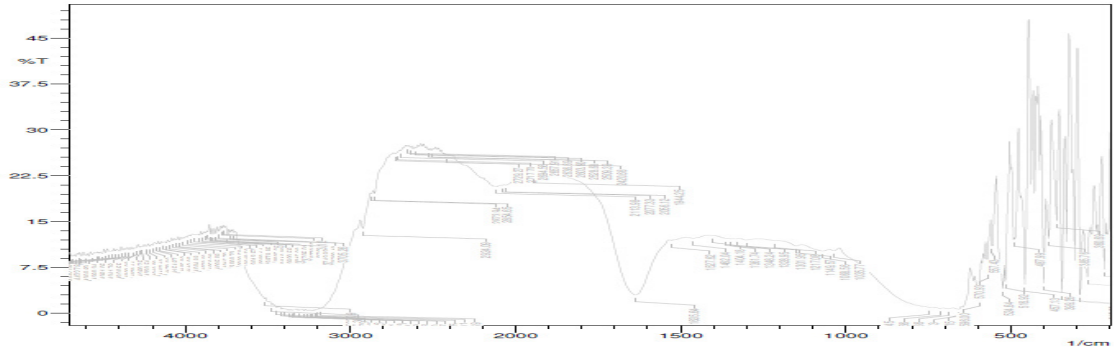
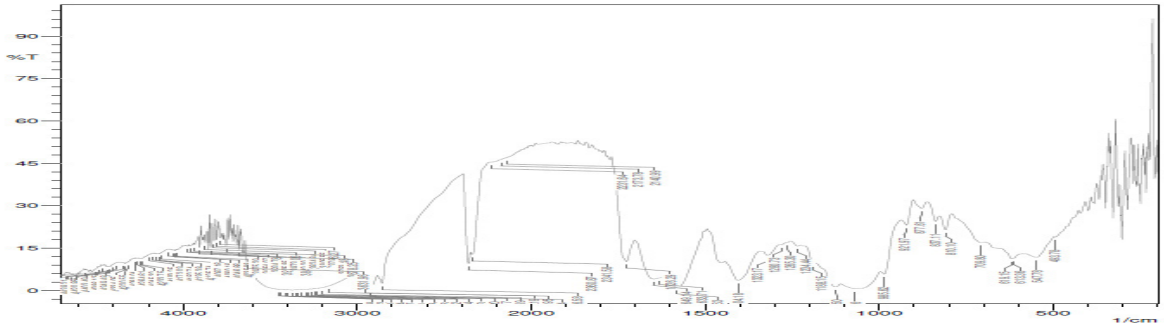


Fig.6. FTIR analysis of HDPE strips inoculated with *Aspergillus flavus* exposed to 60 days



Conclusion

The observed results presented that *A. flavus* could be used as a potential biodegradable agent for lastic materials. Studies must be conducted to improve the biodegrading ability.

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