Comparative Assessment of Polythene Degradation By *Trichoderma harzianum* and *Trichoderma viride*

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Abstract

Polythene pollution is a hassle to the human beings and environment. The problems caused by polythene waste is mainly due to its persistent nature. A number of methods physical, chemical and biological are used to reduce the polythene waste. Both physical and chemical methods release toxic gases, whereas biological methods have been found to be eco-friendly and do not produce secondary pollutant. In this study two known fungal strain i.e. T.harzianum and T.viride are used to degrade the HDPE type polythene. Biodegradation experiment was performed in soil and in synthetic medium % weight loss, FTIR, SEM were used to determine the degradation of polythene. In present study biodegradation of HDPE polythene film was analyzed for 90 days in soil burial and 30 days by shake flask test in liquid synthetic medium. The surface of plastic material turned from smooth to rough and the weight of polythene strip was also less due to the fungal activity. It concludes that 2 species of Trichoderma harzianum and Trichoderma viride are able to degrade polythene 42% and 16% in soil as well as 25% and 18% in liquid synthetic medium . T.viride show high rate of degradation in soil as well as in liquid synthetic medium.

Keywords: Strains, Biodegradation, Shake flask, Synthetic medium,

1.Introduction:

Polythene are made by ethylene monomer units having high molecular weight. The uses of polythene is enormous and they are popular because of their high impact strength, good chemical resistance flexible and it is also least expensive **(Jayanthi *et al*.2016)**. Accumulation of polythene based plastic waste material in the environment is steadily increasing and has reached 25 million tons per year **(Zahra *et al*.2010).**Globally increasing polythene waste adversely affect the environment and ecosystem. So over the years rapid degradation of polythene has been a subject of interest in waste management. Among the processes which used for polythene degradation, biodegradation of polythene is promising and cost effective process. Recently scientists are focusing on the biodegradation of polythene waste to form a most effective and integrative approach **(Koutny *et al.* 2009).** Several studies have reported on biodegradation of polythene by bacteria and fungi. In **2005 Hadad** isolated a thermophilic bacterium *Brevibacillus borstelensis* which degrade the LDPE polythene and revealed the reduction in molecular weight of polythene by 30%. Studies show some bacterial strains which are capable of degrading polythene are *Streptomyces* KU5, *Streptomyces* KU1, *Streptomyces* KU6, *Streptomyces* KU8**(Usha *et al*. 2011)**. In a recent study by **Yoshida *et al*.2016**, protein was identified as ISF6-4381 secreted by the bacterial strain *Ideonella sakaiensis 201-F6* which could degrade polyethyl terephthalate rapidly.**J.D.Gu 2001 *et al*.** isolated plastic degrading fungi from soil identified as *Fusarium solani* and used in degradation of both natural and synthetic polythene as a potential carbon source. Some potential fungal species which have capability of polythene degradation are *Aspergillus niger*. *Aspergillus glaucus, Cladosporium,Fusarium, Mucor,Penicillium Phanerochaete* and *Trichoderma* **(Kathiresan 2003) (Koutny *et. al* 2006) (Yamado-Onodera *et. al* 2001)(Upreti *et. Al***

2003). This study was focused on evaluating the biodegradability of the HDPE polythene by two known fungal strains.

2. Material and Method

2.1 Collection of polythene: HDPE sheets were collected from local market of Agra as 20 micron thickness.

2.2 Micro-organism: Fungal cultures *T.herzianum and T.viride* was selected on the basis of screening for polythene degradation in the medium having no carbon source polythene powder was added to screening medium(Mineral Salt medium).

2.3 Preparation of fungal suspension: Fungi were cultured on SDA slants and incubated at 30˚C. After completion of sporulation, spore of fungi on slants were washed with 6ml physiological saline solution. The spore mixture was placed in a small beaker on stirrer and stirred for 10 minutes aseptically. Added 3ml of this spore mixer in known volume of distilled water. Fungal spore were counted by haemocytometer.

2.4 Experiment setup for polythene degradation-

2.4.1. Soil burial treatment: Pre-weighted polythene strips (4cm×3cm) were disinfected with 70% ethanol for 30 minutes. Later it was placed in distilled water for 10 minutes and dried for 15 minutes in laminar air flow chamber. This disinfected film was buried in pots containing sterilized soil and inoculated with fungal suspension. Set of control experiment were maintained which is devoid of fungal culture

2.4.2. Shake flask test (in liquid synthetic medium): In this biodegradation experiment 2ml of fungal suspension was added to flask containing 150ml of liquid synthetic medium. Added disinfected film (4cm×3cm) in flask and incubated at 30C in orbital shaker at 120 rpm for one month. All the experiment were run simultaneously in triplicates

2.4.3. Media Used: Synthetic medium was prepared using method given by **Esmaeili, 2014** with some modification.

Table: 1 Composition of liquid synthetic medium

Chemicals	gm/l
NH_4NO_3	1.0gm/l
$MgSO_4.7H_2O$	0.2g/l
K_2HPO_4	1.0g/l
KCl	0.15g/l
$FeCl_3.6H_2O$	5.0g/l
$ZnCl_2$	0.0084g/l
H_3BO_3	0.0001g/l
$CaCl_2.2H_2O$	0.001g/l
$MnCl_2.4H_2O$	0.0016g/l
Malt extract	1%

2.3.3Film harvest: After incubation period of nine months in soil and one month in liquid synthetic medium HDPE polythene strips (treated and untreated) were harvested and washed with running tap water. Later that 2 % (v/v) aqueous sodium dodecyl sulfate solution for 4 h (using shaker), followed by distilled water and finally with 70% ethanol was used to remove as much as cell mass from the residual film as possible. The washed polymer pieces were placed on a filter paper and dried

overnight at room temperature before weighing. The film was dried 24 hours in oven. **(Jyoti Singh *et. al 2015*)**

3. Assessment of degradation of polythene: -

3.1. Weight Loss Method- Sample weight loss was determined by an analytical balance (SHIMADZU CORPORATION TYPE AY220). The weight difference between initial and final weight indicate the extent of polythene utilization by the fungi. Percentage weight loss was determined using the formula -

$$\text{Weight Loss\%} = \frac{\textit{Initial Weight} - \textit{Final Weight}}{\textit{Initial Weight}} * 100$$

3.2. FTIR-Infrared spectra of polythene film were recorded on Cary 630 FTIR (Agilen technologies) over a range of 4000cm^{-1}-800cm^{-1}. Samples were powdered and analyzed. For monitoring, formation and disappearance of carbonyl and double bond FT-IR is necessary to elucidate the mechanism of the biodegradation process.

3.3. SEM- The surface morphology and microstructure of the polyethylene strip due to biodegradation were analyzed through scanning electron microscopy **(Gnanavel *et. al.* 2016).** The polythene film were prepared according to **Lee *et. al.* (1994)** and examined by JSM 6490 LV (JEOL JAPAN)

4. Results and Discussion The degradation of polythene was determined by calculating the percentage of weight loss of polythene. Film were weighed, with an accurate four digit balance before and after incubation in soil as well as in liquid synthetic medium. The percentage of weight loss is shown in table 2 and 3.

Table: 2 Degradation of polythene in shake flask test (synthetic medium) within one month

Fungi	Initial Weight(mg)	Final Weight(mg)	Weight of P.E.degraded(mg)	% of degraded P.E.
T.harzianum	7.0	5.1	1.9±1.06	25%
T.viride	7.0	6.0	1.0 ±0.17	18%

Table: 3 Degradation of polythene in soil within nine months

Fungi	Initial Weight(mg)	Final Weight(mg)	Weight of P.E.degraded(mg)	% of degraded P.E.
T.harzianum	7.0	5.8	1.2±0.05	16%
T.viride	7.0	4.0	3.6±0.1	42%

These 2 fungal species were separately allowed to degrade the polythene under the soil burial method and in shake flask method in liquid synthetic medium. Among the fungal species *Trichoderma viride* was found to be most active degrading 42% in soil and *T.harzianum* 25% in liquid synthetic medium.

FTIR-The FTIR spectra of untreated HDPE film incubated with *Trichoderma* species for one month in liquid synthetic medium and 9 months in soil containing polythene as sole carbon source are shown in Fig.1 and Fig.2

FTIR spectra of the polythene strip sample exhibit the formation of new peak and break down of some bond. In the present study FTIR spectra showed the native band at 1463 cm^{-1} was decreased to 1461-1459 cm^{-1} showing methylene C-H bend asymmetric/symmetric on HDPE film **(V Mahalaxmi *et. al.*2012).** FTIR spectra of polythene film inoculated with fungi *Trichoderma harzianum* , *Trichoderma viride* indicate that peak at 3756-3758 correspond to carboxylated compound. Peak at 2050 cm^{-1} and 1949 cm^{-1} aromatic combination bands. Peak at 1209 corresponds to aromatic C-H bend in plane. Peak at 1756 are alkyl carbonate group frequency. Peak at 2348 possibly due to

transition metal carbonyl group frequency. In addition the bands at 913cm^{-1} and 911cm-^{1} also appeared by the action of *Trichoderma viride* in both the degrading medium (soil and liquid synthetic medium) due to C-H bend (vinyl C-H out of plane bend The native band 2913cm-^{1} is decreased to 2910cm-^{1} in soil medium by *Trichoderma harzianum,, Trichoderma viride* in liquid synthetic medium due to strong stretch in methyne C-H group and also show broadening of band in these ranges **(K. Ambika devi *et. al*.2014).**

FTIR results exhibit changes or either new peak formation or disappearance of a peak or else change in the peak range as accounted as monitoring parameter and regarded as the change occurred on the surface of polythene due to the action of fungi **(kavitha *et.al*.2014).** The change in the peak values of almost all functional groups supported the conformational change on polythene surface.

Figure1. Graphs of FTIR showing degradation of polythene through breakdown and stretching of bond between hydrogen and carbon and in liquid synthetic medium

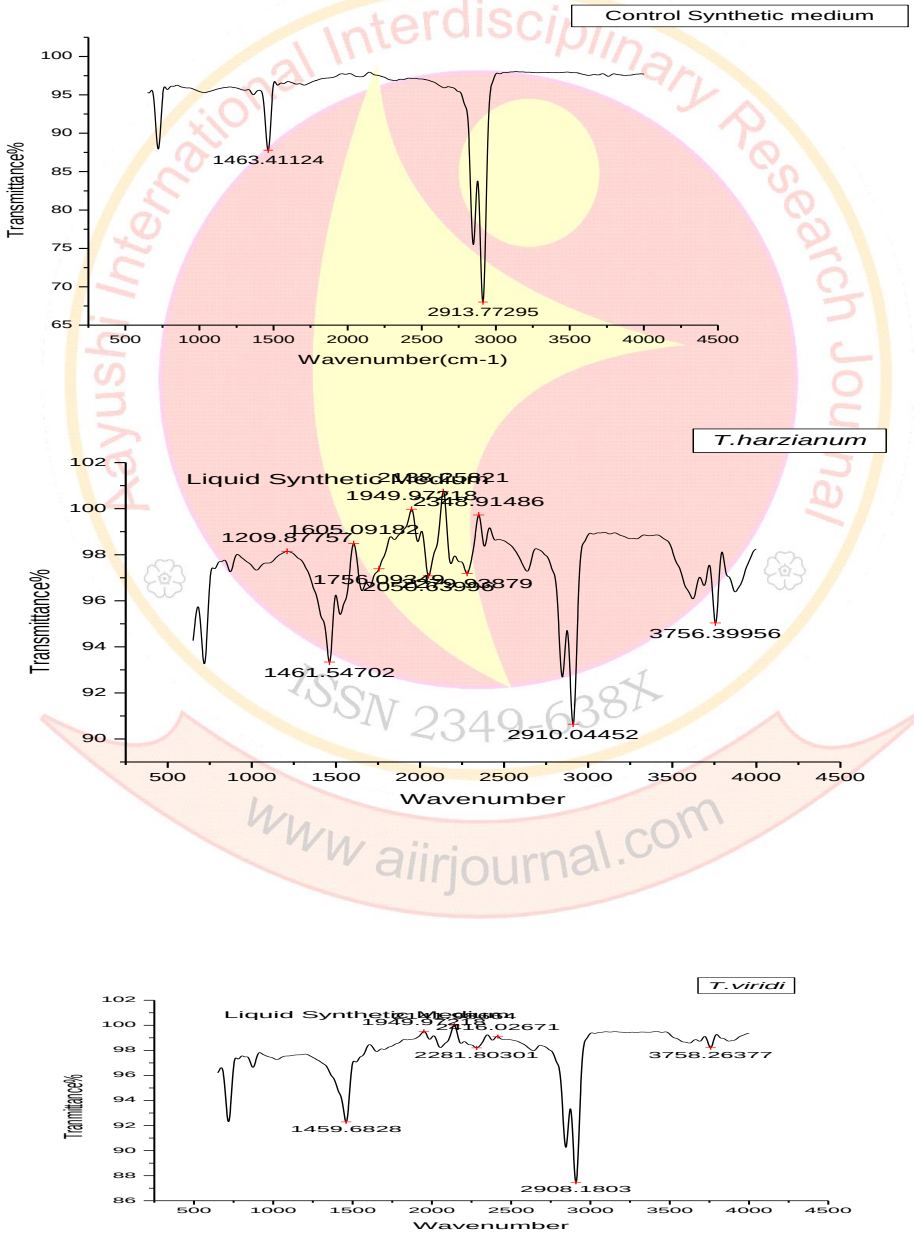
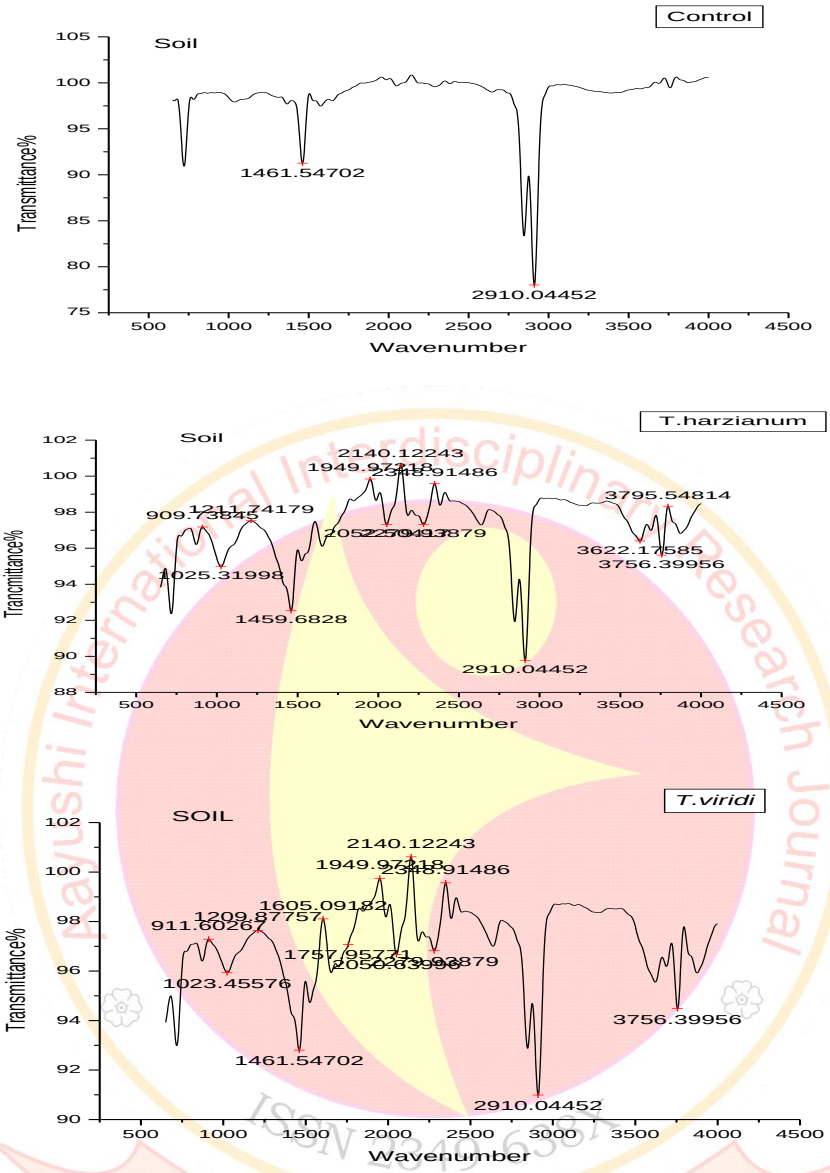
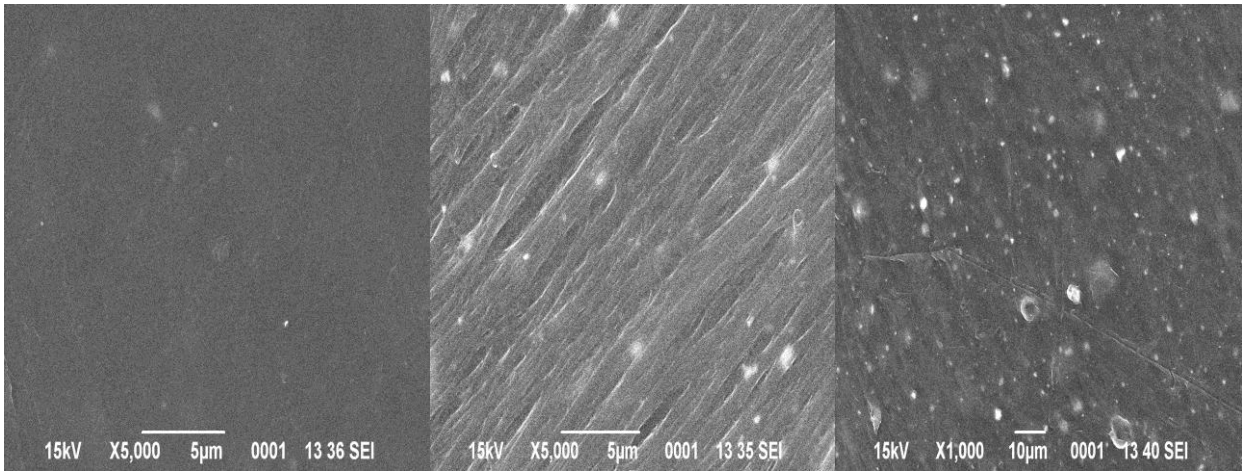


Figure2. Graphs of FTIR showing degradation of polythene through breakdown and stretching of bond between hydrogen and carbon and in soil



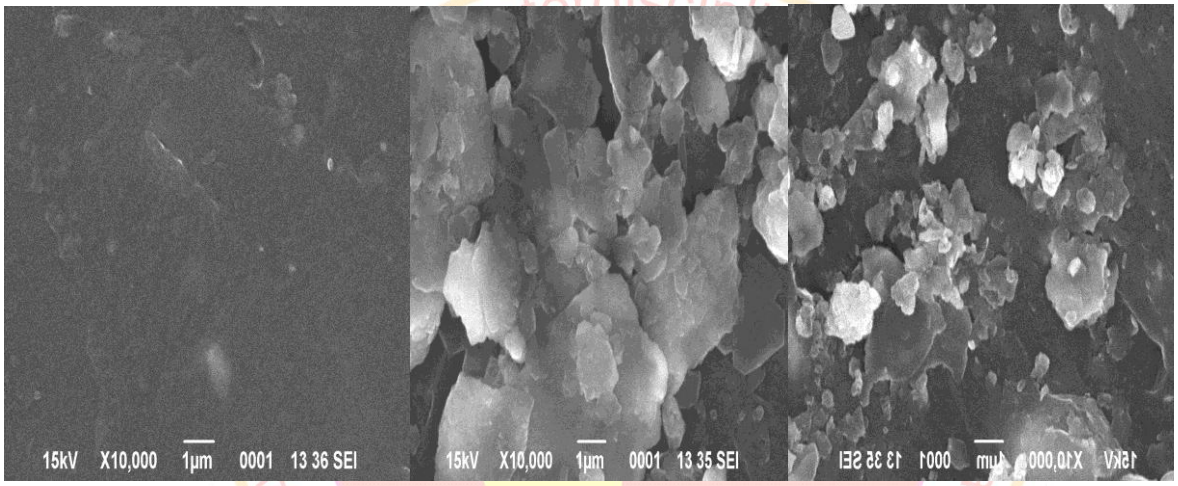
Sem Results: Figure of SEM show different kinds of surface alterations. Controls are used to establish the baseline degradation for comparison. The polythene film buried in soil and in synthetic medium showed the holes and cracks on its surface. It was also observed that the control show no such changes. **(Mona . Gouda *et al*. 2012)**

Sem Result Of Polythene Degradation In Synthetic Medium



Control ***T.harzianum*** ***T.viride***

Sem Result Of Polythene Degradation In Soil



Control ***T.harzianum*** ***T.viride***

SEM results exhibit the surface of the polythene film becomes pitted and eroded. SEM pictures of polythene film revealed that the film treated with *T.viride* show more cracks and holes than polythene film treated with *T.harzianum* in soil degradation. In shake flask test *T.harzianum* show more holes and cracks on polythene.

Conclusion:

Polythene is probably the polymer used frequently in the world, because it is easily processed, stable and low cost product. In order to get rid of polythene waste with an ecofriendly way there is only one solution to exploit microorganism to degrade polythene. In present study biodegradation of HDPE polythene film analyzed for 90 days in soil burial and 30 days by shake flask test in liquid synthetic medium. The surface of plastic material has turned from smooth to rough and the weight of polythene strip is also less due to the fungal activity. The present work concludes that these 2 species of *Trichoderma* fungi are able to degrade polythene in natural environment as well as lab conditions. Among both the fungi *T.viride* show high rate of degradation in soil as well as by shake flask test in liquid synthetic medium.

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