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RESEARCH ARTICLE

SCREENING AND IDENTIFICATION OF POLYURETHANE (PU) AND LOW DENSITY POLYETHYLENE (LDPE) DEGRADING SOIL FUNGI ISOLATED FROM MUNICIPAL SOLID WASTE

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ABSTRACT

Plastics have become an important part of modern life and are used in different sectors of applications like packaging, building materials, consumer products and much more. Many plastics are physically, chemically robust and cause waste management problems. Bioremediation is an important approach to waste reduction that relies on biological processes to break down a variety of pollutants. This is made possible by the vast metabolic diversity of the microbial world. To explore this diversity for the breakdown of plastics, in the present study several indigenous fungi were screened from municipal solid waste to check for its ability to degrade the polyurethane (PUR) and Low Density Polyethylene (LDPE) under *in-vitro* and *in vivo* studies. Several fungi proved their ability to efficiently degrade polyurethanes and Low Density Polyethylene (LDPE) in biodegradable studies. Vigorous activity was observed in the genus *Fusarium oxysporum, Aspergillus fumigatus, Lasiodiplodia crassispora, Aspergillus niger, Penicillium* sp. and *Trichoderma harzianum* capable of degrading this polymer display a zone of clearance around the growing culture on both PUR and LDPE growth medium. These isolates were analysed on Impranil DLN medium as the sole carbon source under both aerobic and anaerobic conditions. *Aspergillus niger* was able to degrade the polymer more activity in the PUR-A solid medium and PUR-L liquid medium than other fungal isolates. *A. niger* resulted in maximum decrease in percentage of elongation and tensile strength 2.94, 4.32, and 5.11 in LDPE sheets for 30, 60 and 90 days durations and 0.78, 1.44 and 2.18 in PUR sheets for 30, 60 and 90 days durations and 25.5 Mpa with breaking load 7.5**,** Percentage of elongation of 295 in LDPE and 12.9 Mpa with breaking load 5.8**,** Percentage of elongation of 186 in PUR sheets respectively. This study can be used as valuable microbial tool in the field of bioremediation to solve the inert polythene and plastic waste management.

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INTRODUCTION

Plastic is a macromolecule, comprising of smaller repetitive units made up of either natural or synthetic substances that enabled most of the industrial and technological revolutions of the 20th century. Plastics are composed of petroleum based materials called resin which are widely used for packaging applications like Polyethylene High Density Polyethylene (HDPE), Medium Density Polyethylene (MDPE), Linear Low Density Polyethylene (LLDE), Low Density Polyethylene (LDPE), Polypropylene (PP), Polystyrene (PS), Polybutylene terephthalate (PBT), Polyurethanes (PUR) materials that are resistant to biodegradation. Due to its resistance it cannot be degraded naturally and when they are disposed in landfills they remain in their original form for long time without any degradation (Raaman *et al*., 2012). Plastics are used in a wide variety of industries, as it increases more and more the waste plastic thrown to the environment also increases. Presently industries are divided into organized and unorganized sectors. The organized sector produces high quality products, whereas unorganized sector is producing poor quality and cheap products. This poor quality of plastics generates annually around 57 million tons (Singh and Sharma 2008). However

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these low qualities do not break down in the environment. Due to their resilience, long term perseverance in the environment, plastic wastes becoming a very serious environmental problem which causes health hazards by means of contamination and epidemics (Das and Kumar, 2014). As a result accumulation of waste plastics in the environment has become a big issue and most of the studies carried out to degrade plastics involve either biotic or abiotic methods directly or indirectly. With the advances in technology and the increase in global population daily disposal of garbage including plastic wastes becoming a serious environmental problem causes health hazards by several means of contamination and epidemics. Many microbes like fungi and bacteria which are ubiquitous in nature found colonize the land fill debris preferably cellulose, lignin rich wastes including plastics (Pandey *et al*., 2015). Due to variation in temperature, moisture the organic wastes harbor the succession of microbes. Among the microbes, fungi and bacteria proved their efficacy in degrading the wastes and convert in to non toxic useful bio-fertilizer. Since, most of the plastics are being produced after blending of synthetic plastics with starch. Fungi and bacteria once colonizes the wastes as substrate and secretes extra cellular enzymes which play a major role in breaking down the polymers into simple dimmer and monomer and thus, find the carbon source for the metabolism (Howard, *et al*., 1999). Hence, microbes play an effective role in combating the environmental pollution by the degradation of garbage including plastics in the land fill sites as well in the soil and leave useful returns of organic fertilizers in to the soil. Thus, microbes serves as renewable resource of managing garbage and plastic pollutions against other methods of incineration, acidification etc. which leads to production of poisonous gases into the surrounding. In contrast microbes are eco-friendly which breakdown a majority of cellulose, lignin and plastic rich debris, eventually recycle the organic matter which will ended with the release of carbon dioxide and water (Negi *et al*., 2009). Some microorganisms even secrete several LDPE degrading enzymes in different quantities, which expressed its degradation efficiency of the microorganism. In several studies, fungi were considered favorable for the degradation of LDPE due to their higher ability to form hydrophobic enzyme proteins, which promote fungal sps. in attachment to the polymer surface (Seneviratne, *et al.,* 2006 and Bhardwaj *et al*, 2012).

Polyurethanes (PUR) represent the most common class of polymers which is used as raw materials in the medical, automotive and industrial fields (Gautam *et al*., 2007). They are commonly present in furniture, coatings, fibers, synthetic skins, adhesives, elastomers, constructional materials, padding's and paints. Polyurethane is a xenobiotic substances that offer to a kind of polymers derived from the condensation of polyisocyanate and polyols having intra molecular urethane bonds (carbonmate ester bond, -NHCOO-) connected with a series of urethane linkages (Shah *et al*., 2008). Microorganisms are known to survive in environment where intractable materials like polyurethane are present, so it is possible that these microorganisms can use this substance and be useful tools in biodegradation. Therefore there are many reports on the degradation of polyester PUR and LDPE by several microorganisms especially by fungi mainly by the hydrolysis of ester bonds by esterases produced by fungi. Fungi colonize the wastes as substrate and secrete extracellular enzymes which play a major role in breaking down the polymers into simple dimer and monomers (Howard *et al*., 2001). Oda *et al.,* (1998) characterized a fungi and bacterium from soil and activated sludge which utilizes polyester polyurethane as a sole carbon and nitrogen source. Gangoiti *et al*., (2012) isolated nineteen medium chain length (mcl) poly (3-hydroxyalkanoate) PHA-degrading microorganisms were isolated and characterized for its extracellular depolymerase from natural sources. These microorganisms hydrolysed biodegradable plastics such as short chain-length (scl) PHA, poly (-caprolactene) (PCL), poly ethylene succinate (PES), and poly (L-lactide) (PLA). In the current study, indigenous fungi were isolated from municipal waste Mysore, Karnataka. A subset of these organisms was screened for their ability to degrade PUR and LDPE. Several active organisms were identified, screened for the ability to efficiently degrade and utilize PUR and LDPE using *in vitro* and *in vivo* condition. Selected microorganisms were tested for mechanical changes based on breaking load, tensile strength and elongation break to determine the effect of various transitions on biodegradation of LDPE and PUR respectively

MATERIALS AND METHODS

Indigenous fungi was isolated on colonizing soil and on plastic garbage in the land fill sites from different regions of Karnataka India, were screened on Potato dextrose media by serial dilution and spread plate techniques. Fungi isolated was maintained on Potato dextrose media and Saboraud agar plates and identified microscopically based on the habit and conidial morphology with the support of laboratory manuals provided by Ellis (1971). After incubation, individual fungal colonies were picked from the edge with a sterile needle and transferred onto potato dextrose agar (PDA) medium. Fungal species were identified based on the habit conidial and morphology by Barnett and Hunter (1972).

Screening, selection of microorganisms showing PU and Polyethylene (LDPE) activity

As a first step of the screening process, we assayed for the hydrolytic activity of the microorganisms. This is an easy, quick and cheap test to perform on solid media based on the visual inspection of plates containing the lipid substrate tributyrine for microorganisms showing a clearing zone around the colony edges. Micro-organisms capable of degrading this polymer display a zone of clearance around the growing culture. Impranil DLN and degrade PUR using the PUR halo assay for the initial screening (Russell, *et al*., 2011). Polyethylene (PE) Low density polyethylene powder (LDPE) and Polyurethane (PUR) was obtained from Sigma, Bangalore was added in mineral salt medium at a final concentration of 0.1% (w/v) respectively and the mixture was sonicated for 1hour at 120 rpm in shaker. After sonication the medium was sterilized at 121°C and pressure for 15 lbs/inch for 20 minutes. About 15 ml sterilized medium was poured before cooling in each plate. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 27-30°C for 2-4 weeks. The organisms, producing zone of clearance around

their colonies were selected for further analysis (Singh & Gupta 2014).

Initial Polyurethane (PUR) Clearance Screen

Indigenous fungi were first assayed for their ability to degrade PUR by growing them in the presence of Impranil DLF an anionic aliphatic aqueous PUR dispersion with 3% *N*-methyl pyrrolidone. Among the fungi screened in initial assay on PU and PUR clearance test, 06 organisms grown on solid PUR medium. The solid medium screening assay followed the general method with minor modification of Crabbe *et al*., (1994). The PUR solid medium was added to sterile culture tubes in 10-ml aliquots. Fungus grown on PDA was added to each test tube using aseptic technique and allowed to grow undisturbed at 26± 2^0 C. Distilled water is used as control for polyurethane degradation (Darby *et al*., 1968). PUR degradation was confirmed by a change in medium appearance from opaque to translucent. After 2 weeks of incubation, the depth of polyurethane clearance was measured from the top of the medium to the lowest point of visible clearance (Filip, 1979). PUR-L liquid medium assay was followed by the minor modification described by (Gautam *et al*. 2007). PUR-L liquid medium was prepared using the same recipe as for the solid PUR-A medium without agar. PUR-L medium was added to sterile culture tubes in 10-ml aliquots and inoculated with a fungus grown on PDA. At the end of 2 weeks, the liquid cultures were homogenized by vigorous shaking. A 1.5-ml portion of each culture was transferred to an Eppendorf tube and centrifuged for 30 seconds at 6000 x *g* in a centrifuge to selectively pellet the fungal matter. Supernatant was measured on a UV-visible spectrophotometer at a wavelength of 600 nm, with sterile water as a blank. Dilutions of PUR-L medium with sterile water were measured to construct a standard curve for converting absorbance to percent clearance. Samples were measured every day up to 2 weeks for optical absorbance to determine a relative rate of clearance.

Sole carbon source assay

After screening of microorganisms for the PUR-degrading activity were tested for their ability to use PUR as the sole carbon source. Impranil DLN, as a source of substrate, which contains PUR suspended in distilled water (without *N*-methyl pyrrolidone). Only 06 fungal isolates was used for initial PUR-degrading activity which were grown on Impranil DLN without any other carbon sources (PUR-C). The fungal samples were washed with distilled water 3 times to remove all residual medium and prior to inoculation make sure that the polymer is the only source of carbon for fungal metabolism and growth (Russell, *et al*., 2011). PUR-C was prepared without adding sodium citrate, thiamine and Casamino acids but similar to that for the PUR-L medium. Fungal cultures were grown for 1 week in potato dextrose broth (PDB). Stock cultures were homogenized by vigorous shaking, and 1 ml of each culture was centrifuged at 10,000x g for 3 min. The supernatant was removed, and the fungal pellet was re-suspended in 1 ml of the PUR-C liquid medium. Samples were centrifuged and re-suspended a second time to ensure removal of all residual PDB. 1-ml sample of washed fungal material was added to sterile culture tubes to a final volume of 10 ml PUR-C. The cultures were monitored for visual clearance of the opaque medium. Samples were measured every 2 days for 2 weeks for optical absorbance at 600 nm to determine an approximate rate of clearance. An increase in fungal mass correlating to PUR degradation was measured by lyophilizing mycelial mass from triplicate cultures containing minimal medium with and without PUR. For these studies we used Impranil DLF, (an anionic aliphatic PUR dispersion in water) used for the previous studies but does not contain *N*-methyl pyrrolidone. If an organism grows in Impranil DLN, it can use PUR as the sole carbon source for metabolism and growth.

Biodegradation studies

Soil burial assay

Polyurethane (PU) films and Polyethylene film (LDPE) inoculated with 06 indigenous fungal isolates was buried in compost rich in nitrogen, phosphorus, trace elements and co-substrates 3 months at room temperature (26± 2^0 C) in large plastic trays amended with distilled water aseptically to maintain the availability of mineral salts and moisture (Konduri *et al*., 2011). Uninoculated control samples will be washed in sterilized distilled water and subjected for the analysis of presence of microbial community involved in the degradation of polyurethanes was observed.

Treatment of polyethylene samples

Polyurethane (PU) films and Polyethylene film (LDPE) used in the study was subjected to continuous exposure to UV irradiation (312 nm) for 50 h. Prior to pre-treatment of polyethylene films were cut into pieces (about 10 x 10 cm^2 each), weighed, disinfected in 70% ethanol and air dried for 15 min in a laminar-flow head.

Film harvest

After exposing of fungal isolates for three months of Polyurethane (PU) films and Polyethylene film (LDPE) pieces were harvested, washed in 70% ethanol to remove as much biomass as possible, dried at 45^0 C and equilibrated and weights were determined. Each of the films with and without treatment

Determination of weight loss

Recovered PU and LDPE films were analyzed for degradation by weight loss before and after microbial treatment using electronic balance. The percentage weight loss of the inoculated samples is given by the formula

% weight loss = (Final weight –Initial weight)/Initial X 100

Mechanical tests

Mechanical tests

The mechanical properties of chemically pretreated and inoculated LDPE films were examined using Universal Testing Machine, Shimadzu, KN model. Thin film grips were used to avoid damage to the test samples at the contact surface

between the grips and polymer. All tests were performed at 25^0 C using a crosshead speed of 10 mm/min with gauge length 5 cm (Konduri *et al*., 2011). Three replicates were tested for each sample, and average values of the breaking load, tensile strength and % of elongation were determined.

growing culture. During the first round of selection, 23 indigenous fungal isolates isolated from municipal solid waste biomass were screened for initial PUR and LDPE halo assay to check for their ability to display a zone of clearance around the culture.

Table 1. Indigenous fungal isolates collected from municipal solid waste and screened for initial screening for PUR and LDPE for zone of clearance test

S. No.	Isolates from Municipal waste disposal sites		PUR	LDPE
1	001	*Colletotrichum gloeosporioides*	+	-
2	002	*Fusarium solani*	-	+
3	003	*Fusarium solani*	-	+
4	004	*Fusarium solani*	+	-
5	005	*Fusarium moniliformae*	-	+
6	006	*Fusarium oxysporum*	+	+
7	007	*Aspergillus fumigatus*	+	+
8	008	*Lasiodiplodia theobromae*	-	+
9	009	*Fusarium* sp.	+	-
10	010	*Alternaria alternata*	+	-
11	011	*Lasiodiplodia crassispora*	+	+
12	012	*Phlyctis argena*	-	+
13	013	*Periconia* sp.	+	-
14	014	*Corynespora cassiicola*	+	-
15	015	*Phialimonium sp.*	-	+
16	016	*Sordariomycetes sp.*	-	+
17	017	*Aspergillus niger*	+	+
18	018	*Penicillium sp.*	+	+
19	019	*Botrydiplodia theobromae*	+	-
20	020	*Alternaria* sp.	-	+
21	021	*Rhizopus* sp.	-	+
22	022	*Trichoderma harzianum*	+	+
23	023	*Colletotrichum* sp.	-	+

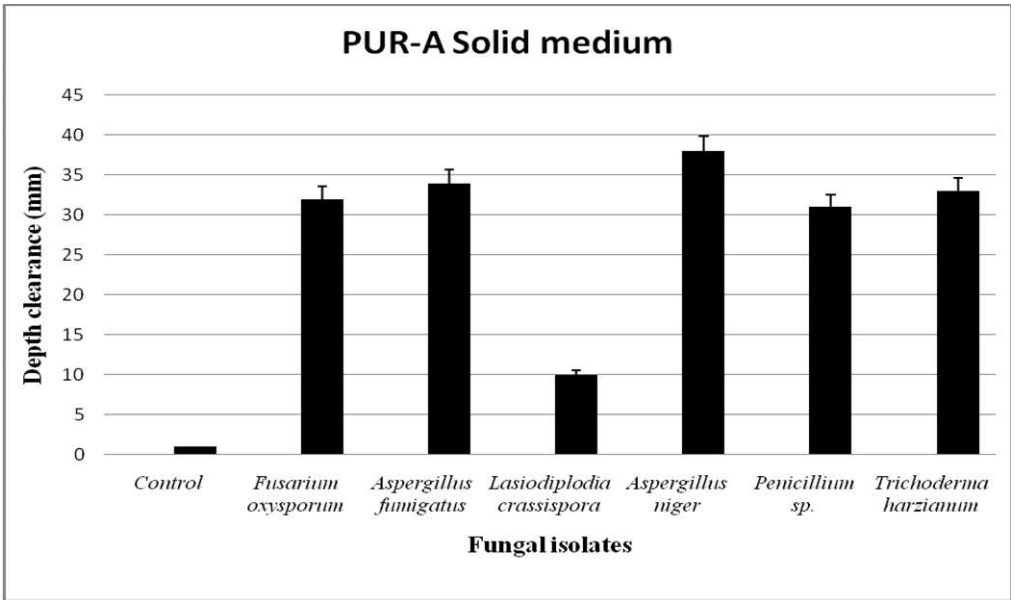

Figure 1. *A. niger* exhibited high activity, whereas *F. oxysporum*, *A. fumigatus*, *Penicillium* sp. and *T. harzianum* exhibited moderate activity and remaining *L. crassispora* exhibited very less depth clearance after 2 weeks of growth. Error bars represent the standard deviation for each data set

RESULTS

Initial Polyurethane PU and Low density polyethylene (LDPE) Clearance Screen

Impranil DLN (polymer), Polyester Polyurethane (PUR), and LDPE are an opaque milky suspension that becomes transparent upon degradation. Micro-organisms capable of degrading this polymer display a zone of clearance around the

Among the fungi screened, only 06 organisms viz., *Fusarium oxysporum, Aspergillus fumigates, Lasiodiplodia crassispora, Aspergillus niger*, *Penicillium* sp. and *Trichoderma harzianum* capable of degrading this polymer display a zone of clearance around the growing culture on both PUR and LDPE growth medium as shown in (Table 1). Remaining fungal isolates either showed zone of clearance on PUR medium or in the LDPE growth medium.

Based upon the initial polyurethane PU and low density polyethylene (LDPE) clearance test six isolates were screened for PUR –A solid medium assay. Among them *A. niger* showed the highest clearance of 38 mm in PUR solid medium, whereas *A. fumigatus* showed 34 mm depth clearance, *T. harzianum* showed 33 mm, *F. oxysporum* showed 32, *Penicillium* sp. showed 30 and *Lasiodiplodia crassispora* showed only 10 mm depth clearance when compared to control which showed negligible clearance of 1 mm as shown in Figure 1.

In a liquid culture assay relative rate of Polyurethane clearance, optical density was measured at 600nm. Due to the scattering of the suspended polymer, it was considered as an indication of the extent of clearance by the fungal isolate. The relative order of the liquid clearance assay was similar to that observed for the solid clearance assay.

Aspergillus niger was the most active organism in the liquid medium clearance screen showed 85% along with *A. fumigatus* 78% *Penicillium* sp. 75% *F. oxysporum* 74% and *T. harzianum*72% followed by *L. crassispora* 40% when compared with control as shown in Figure 2.

Sole carbon source assay

All six fungal isolates were tested for their ability to degrade PUR in liquid culture using PUR as the sole carbon source (PUR-C). All fungal isolates were washed with minimal medium to remove residual carbon and enzymes from the stock culture. Experiments performed using Impranil DLF as the substrate showed that the presence of *N*-methyl pyrrolidone did not affect the growth rate for the fungal isolates tested. *A. niger* demonstrated the highest rate of PUR clearance.

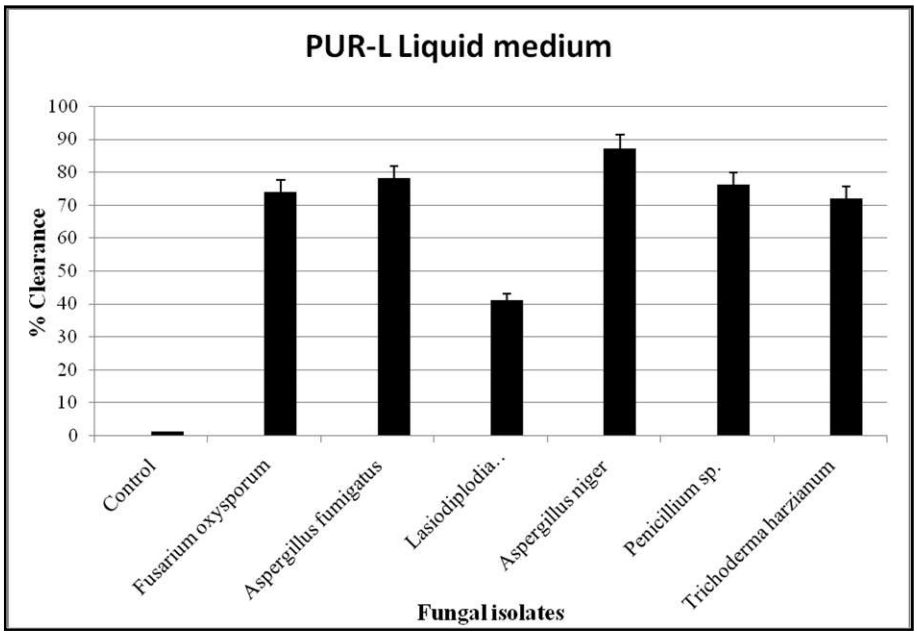

Figure 2. *A. niger* was the most active organism in the liquid medium clearance screen, along with *F. oxysporum*, *A. fumigatus*, *Penicillium* sp. and *T. harzianum* exhibited moderate activity and remaining *L. crassispora* exhibited very least percentage of depth clearance. Error bars represent the standard deviation for each data set

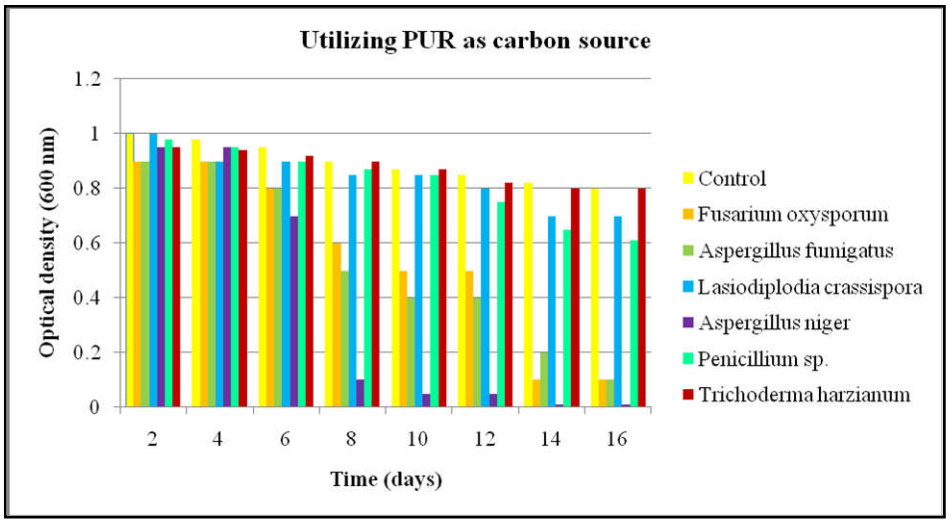

Figure 3. Degradation of PUR as a sole carbon source by endophytes was monitored over a 16-day time course. Cultures containing PUR-C medium with Impranil DLN as the sole carbon source were inoculated with washed fungal inoculums. Error bars represent the standard deviation for each data set

The cultures were visually transparent by the end of the 16-days, results in the degradation of the Impranil DLN in *A. niger* was cleared at 16th day which shows the transparent in PUR liquid culture, whereas *F. oxysporum, A. fumigatus*, *Penicillium* sp. and *T. harzianum* showed moderate clearance of turbidity of PUR in liquid media at the 16th day when compare to *L. crassispora* and control as shown in Figure 3.

Biodegradation studies

Microbial degradation of LDPE and PUR sheets was determined under different durations as 30, 60 and 90 days by using *Fusarium oxysporum, Aspergillus fumigates, Lasiodiplodia crassispora, Aspergillus niger*, *Penicillium sp.* and *Trichoderma harzianum*. Physical modifications and degradation rate of LDPE sheets after microbial degradation were determined by mean weight loss. The result obtained from microbial degradation of LDPE sheets by *A. niger*, *A. fumigatus*. *Penicillium sp.* and *T. harzianum* followed by *F. oxysporum* and *L. crassispora,* are as shown in Table 2 and 3.

A. niger showed 2.94, 4.32, and 5.11 bio-degradation efficiency in LDPE sheets for 30, 60 and 90 days durations respectively. *Fusarium oxysporum* showed 2.79, 3.75 and 4.09 bio-degradation efficiency in LDPE sheets for 30, 60 and 90 days of durations respectively. *Penicillium sp.* showed 2.02, 3.11 and 3.87, *Trichoderma harzianum* showed 1.79, 2.26 and 3.42, *Aspergillus fumigates* showed 1.77, 2.49 and 3.86 and *Lasiodiplodia crassispora* showed 1.05, 1.37 and 1.58 degradation efficiency in LDPE sheets for 30, 60 and 90 days as shown in Table 2. All results obtained are clearly showing considerable level of microbial activities for the destruction and breakdown of LDPE sheets. *A. niger* showed 0.78, 1.44 and 2.18 bio-degradation efficiency in PUR sheets for 30, 60 and 90 days durations respectively. *Fusarium oxysporum* showed 0.73, 1.24 and 1.54 bio-degradation efficiency in PUR sheets for 30, 60 and 90 days of durations respectively. *Penicillium* sp. showed 0.52, 1.36 and 1.46, *Trichoderma harzianum* showed 0.43, 0.94 and 1.29, *Aspergillus fumigates* showed 0.27, 1.26 and 2.15 and *Lasiodiplodia crassispora* showed 0.25, 0.50 and 0.63 degradation efficiency in PU sheets for 30, 60 and 90 days as shown in Table 3.

Table 2. Biodegradation of LDPE sheets was determined under different durations

Fungi	Incubation time	Initial weight in (mg)	Final weight in (mg)	Weight loss in mg	Biodegradation efficiency in %
	30	0.0532	0.0532	0.0000	0
Control	60	0.0532	0.0532	0.0000	0
	90	0.0532	0.0532	0.0000	0
Fusarium oxysporum	30	0.0537	0.0522	0.0015	2.79
	60	0.0532	0.0512	0.0020	3.75
	90	0.0537	0.0515	0.0022	4.09
Aspergillus fumigatus	30	0.0621	0.0610	0.0011	1.77
	60	0.0562	0.0548	0.0014	2.49
	90	0.0621	0.0597	0.0024	3.86
Lasiodiplodia crassispora	30	0.0568	0.0562	0.0006	1.05
	60	0.0581	0.0573	0.0008	1.37
	90	0.0568	0.0559	0.0009	1.58
Aspergillus niger	30	0.0645	0.0626	0.0019	2.94
	60	0.0578	0.0566	0.0025	4.32
	90	0.0645	0.0612	0.0033	5.11
***Penicillium* sp**	30	0.0542	0.0533	0.0011	2.02
	60	0.0578	0.0560	0.0018	3.11
	90	0.0542	0.0521	0.0021	3.87
Trichoderma harzianum	30	0.0613	0.0602	0.0011	1.79
	60	0.0752	0.0735	0.0017	2.26
	90	0.0613	0.0592	0.0021	3.42

Table 3. Biodegradation of PUR sheets was determined under different durations

Fungi	Incubation time (days)	Initial weight in (mg)	Final weight in (mg)	Weight loss in (mg)	Biodegradation efficiency in %
	30	0.0932	0.0932	0.0000	0
Control	60	0.0852	0.0852	0.0000	0
	90	0.0992	0.0992	0.0000	0
Fusarium	30	0.1229	0.1220	0.0009	0.73
oxysporum	60	0.1532	0.1513	0.0019	1.24
	90	0.1818	0.1790	0.0028	1.54
Aspergillus	30	0.1845	0.1840	0.0005	0.27
fumigates	60	0.1658	0.1637	0.0021	1.26
	90	0.1347	0.1318	0.0029	2.15
Lasiodiplodia	30	0.1562	0.1558	0.0004	0.25
crassispora	60	0.1581	0.1573	0.0008	0.50
	90	0.1568	0.1558	0.0010	0.63
	30	0.1278	0.1268	0.0010	0.78
Aspergillus	60	0.1311	0.1292	0.0019	1.44
niger	90	0.1466	0.1434	0.0032	2.18
	30	0.1523	0.1518	0.0008	0.52
***Penicillium* sp.**	60	0.1322	0.1304	0.0018	1.36
	90	0.1745	0.1725	0.0020	1.46
Trichoderma	30	0.1623	0.1616	0.0007	0.43
harzianum	60	0.1689	0.1673	0.0016	0.94
	90	0.1545	0.1525	0.0020	1.29

Table 4. Effect on different fungal species on LDPE showing different properties on Tensile strength, Breaking load and Percentage of elongation

UV treated with different fungal species	Tensile Strength (Mpa)	Breaking load (N)	Percentage of elongation
Control	32.2	12.2	335
Fusarium oxysporum	30.2	10	328
Aspergillus fumigatus	30.0	9.5	308
Lasiodiplodia crassispora	33.0	9.0	332
Aspergillus niger	25.5	7.5	298
***Penicillium* sp.**	30.5	8.4	304
Trichoderma harzianum	30	8.5	308

Table 5. Effect on different fungal species on PU showing different properties on Tensile strength, Breaking load and Percentage of elongation

UV treated with different fungal species	Tensile Strength (Mpa)	Breaking load (N)	Percentage of elongation
Control	27.2	10.2	435
Fusarium oxysporum	22.2	8.0	347
Aspergillus fumigatus	14.9	6.5	248
Lasiodiplodia crassispora	26.8	9.9	422
Aspergillus niger	12.9	5.8	186
***Penicillium* sp.**	16.1	6.6	289
Trichoderma harzianum	18.5	7.5	291

All results obtained are clearly showing considerable level of microbial activities for the destruction and breakdown of PU sheets. The results obtained are also revealed that the degradation rate of PU sheets depend on duration of microbial activities.

Mechanical test

The mechanical properties of chemically pretreated and inoculated LDPE films were examined for tensile strength, breaking load and percentage of elongation. Among all the fungal species *Aspergillus niger* showed highest tensile strength of 25.5 Mpa with breaking load 7.5**,** Percentage of elongation of 295 to determine the effect of various transitions on biodegradation of LDPE, whereas *F. oxysporum* showed 30.2 tensile strength, 12.2 breaking load and 328 of percentage of elongation, *A. fumigatus* showed 30 tensile strength, 10 breaking load and 335 of percentage of elongation, *Penicillium* sp. showed 30.5 tensile strength, 8.5 breaking load and 305 of percentage of elongation, *T. harzianum* showed 30 tensile strength, 8.4 breaking load and 308 of percentage of elongation, whereas *L. crassispora* showed very least of 33 tensile strength, 12, breaking load and 332 of percentage of elongation, when compare to other fungal isolates as shown in Table 4. The mechanical properties of chemically pretreated and inoculated PUR films were examined for tensile strength, breaking load and percentage of elongation. Among all the fungal species *Aspergillus niger* showed highest tensile strength of 12.9 Mpa with breaking load 5.8**,** Percentage of elongation of 186 to determine the effect of various transitions on biodegradation of PU, whereas *F. oxysporum* showed 22.2 tensile strength Mpa, 8.0 breaking load and 347 of percentage of elongation, *A. fumigatus* showed 14.9 tensile strength, 6.5 breaking load and 248 of percentage of elongation, *Penicillium* sp. showed 16.1 tensile strength, 6.6 breaking load and 289 of percentage of elongation, *T. harzianum* showed 18.5 tensile strength, 7.5 breaking load and 291 of percentage of elongation, whereas *L. crassispora* showed very least of 26.8 tensile strength, 9.9, breaking load and 422 of percentage of elongation, when compare to other fungal isolates and control as shown in Table 5.

DISCUSSION

The indigenous fungi screened and identified from polluted sites which can utilize both PUR and LDPE as carbon source were noticed in *Fusarium oxysporum, Aspergillus fumigates, Lasiodiplodia crassispora, Aspergillus niger*, *Penicillium* sp. and *Trichoderma harzianum* which are capable of degrading this polymer display a zone of clearance around the growing culture on both PUR and LDPE growth medium. Russell *et al*., 2011 also reported zone of clearance for degrading PUR from *Aspergillus* sp. Isolation and identification of low density polyethylene degrading microorganisms from contaminated soil was reported by Hussein *et al*., 2015. Experiments performed using Impranil DLF as the substrate showed that the presence of *N*-methyl pyrrolidone did not affect the growth rate for the fungal isolates tested. This established that PUR alone is sufficient for the growth of fungi. As a result, Impranil DLN was used for subsequent studies to characterize indigenous microorganisms growth and the nature of the degradation reaction when compare to control. It was also reported that *Pestalotiopsis microspora* isolates were uniquely able to grow on PUR as the sole carbon source under both aerobic and anaerobic conditions (Filip, 1979). Soil fungi comprise the majority of organisms screened for PUR degradation activity. Some strains which are capable of degrading the polyethylene are *Brevibacillus* spp. and *Bacillus* spp., isolated from soil produces proteases that are responsible for biodegradation and enzymatic degradation of PUR by fungi and bacteria was reported by several researchers Crabbe *et al*., 1994, Cosgrove, *et al*., 2007 and Howard, *et al*., 1999. *Alternaria*, *Aspergillus*, *Phoma*, *Penicillium*, *Plectosphaerella*, *Geomyces*, *Nectria* and *Neonectria* were isolated with access to mixed nutrient sources from buried PUR samples was reported by Fusako and Hu, (2009). Four species of fungi *Cuvularia senegalensis*, *Fusarium solani*, *Aureobasidium pollulans* and *Cladosporium* sp. were isolated based on their

ability to utilize a colloidal polyester PU (Impranil DLN) as sole carbon and energy source was reported by Kim and Rhee (2003). Degradation efficiency in LDPE sheets for 30, 60 and 90 days of durations respectively. In the present study the screening and identification of LDPE degrading fungi from polythene polluted sites were focused. All results obtained are clearly showing considerable level of microbial activities for the destruction and breakdown of LDPE and PUR sheets. The results obtained are also revealed that the degradation rate of LDPE and PUR sheets depend on duration of microbial activities. The microbial degradation results are clearly revealed about higher degradation rates of LDPE sheets by using *A. niger* which showed 2.94, 4.32, and 5.11 bio-degradation efficiency in LDPE sheets and in PUR sheets showed 0.78, 1.44 and 2.18 for 30, 60 and 90 days durations respectively bio-degradation efficiency when compared with other fungal species. Das and Kumar (2014) studied the LDPE degradation ability of four *Aspergillus* and one *Fusarium* sps after the exposure for 60 days. They observed that the degradation potential of *Fusarium* sp. was more when compared to the *Aspergillus* sps. Vasile, (1993) concluded that the organisms get attached to the surface of polythene film, it starts growing by using its PUR as the carbon source. In the primary degradation, and by the cleavage of the main chain, led to the formation of low-molecular weight fragments (oligomers), dimers or monomers. The degradation was due to the extra cellular enzyme secreted by the organism (Narayan, 2006). He also observed that the low molecular weight compounds were further utilized by the microbes as carbon and energy sources. These fungal isolates were responsible for decreasing the weight of LDPE films by adhering on its inert surface. Loss in tensile properties is the most relevant practical criterion to determine its degradation. Microorganisms exposed to were tested for mechanical changes based on breaking load, tensile strength and elongation break to determine the effect of various transitions on biodegradation of LDPE and PUR. *A. niger* showed greater reduction in tensile strength, breaking load and percentage of elongation was compared after 3 months resulted in weight loss by 0.0458 (g) compared to control and other fungal species. In most applications envisaged for films and fibers in contact with microorganisms, loss in tensile properties is the most relevant practical criterion to determine its degradation.

Microorganisms exposed to UV irradiation were tested for mechanical changes based on breaking load, tensile strength and elongation break to determine the effect of various transitions on biodegradation of LDPE. *A. oryzae* showed greater reduction in tensile strength, breaking load and percentage of elongation compared with other microorganisms. Orhan *et al*. (2004) it was shown that polyethylene exposed to UV light for 60 h resulted in only 39% degradation. PUR incubated in compost bags under controlled soil conditions for 1 month showed only 5.33% tensile strength loss. Sudhakar *et al*. (2008) also reported, tensile strength of starch blend LDPE which were exposed to *B. sphericus* showed only 29 and 30.5% tensile strength loss. Singh and Gupta (2014) reported *Aspergillus* sps. and other 3 fungal sps. were screened based on their ability to utilize LDPE as the sole carbon source as a primary carbon source. Tensile strength loss was also noticed on LDPE and PUR sheets showed 23 % loss after two months. The results obtained in our experiment revealed that the degradation rate of LDPE and PUR sheets depend on duration of microbial activities. Mechanical test also revealed about higher degradation rates of LDPE and PUR sheets observed higher rates in *A. niger* when compared with other species. This study can be used as valuable microbial tool in the field of bioremediation to solve the inert polythene and plastic waste management.

Conclusion

Low quality plastics are very serious environmental problem which causes health hazards. Plastics made from polymers like polyethylene, polypropylene, polyurethane and polystyrene do not break down in the environment and these should be disposed eco-friendly. In view of these problems, several tasks should be addressed in order to get safe waste disposal. One of the most successful method to handle these problems is enzymatic biodegradation of plastic that will enhance the biodegradation rate. There are many reports on the degradation of PUR and LDPE by several microorganisms, especially by fungi. More and more research should be concentrated to degrade completely from the environment.

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