Pollution 2022, 8(2): 567-577
DOI: 10.22059/POLL.2021.331156.1195

RESEARCH PAPER

Estimation of possible Biodegradation of Polythene by Fungal Isolates Growing on Polythene Debris

Ankita Saxena[1], Sapna Jain[2], Arvind Pareek[2,*]

1. Mewar University, Gangrar,Chittoregarh,India.
2. Department of Botany وMaharshi Dayanand Saraswati University, Ajmer, India.

Received: 27.09.2021, Revised: 16.12.2021, Accepted: 18.12.2021

ABSTRACT

Consumption of polythene is unavoidable in this era and it is increasing day by day. Polythene's hazardous waste is adversely effecting environment. In fact any form of polythene is a nuisance to the environment because of strong resistance against degradation thus; they remain in nature for a very long time. Biodegradation is the only promising solution to overcome this problem. Fungi, a group of saprophytic organisms are evolved to adapt for almost every environment, specially marine and freshwater source. This property drives fungi to grown on polythene even in adverse environment. So, present study was planned to compare biological degradation of low density polythene [LDPE] and biodegradable polythene by potential fungus to find out an eco-friendly and economic solution of polythene waste. Ten fungal strains were isolated from rotting polythene debris those are *Penicillium chrysogenum, Rhizopus nigricans, Chaetomium murorum, Memnoniella echinata, Aspergillus fumigatus, Stachybotrys chartarum, Aspergillus niger, Chaetomium globosum, Aspergillus flavus* and *Fusarium oxysporum*, in which *Penicillium chrysogenum, Rhizopus nigricans, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus* showed greatest results in terms of degrading both Low density polythene and biodegradable polythene. These isolates also showed good enzymatic reaction and weight loss. SEM analysis of polythene surface was also in support of these findings.

Keyword: Biodegradable Polythene; Biodegradation; Fungi; LDPE, SEM

INTRODUCTION

Polythene is most versatile synthetic man-made material created out of the fossil-fuel (Seymour, 1989). Plastic polymers used today are made from organic raw materials and inorganic raw materials. The main polymers present in plastic are- Polyurethane (PUR), Polyethylene(PE), Polyamide(PA), Polyethylene terepthlalate (PET), Polystyrene(PS), Polyvinylchloride(PVC) and Polypropylene(PP) (Danso et al., 2019). Dilara and Briassoulis (2000)reported that polymers are the broad class of materials those are made from the repeating units of smaller molecules which is called hydrocarbon monomers, represent as $(C_2H_4)_n$. Out of all polymers polythene has unmatchable qualities like- Durability, flexibility, moulding ability, strength and most important factor i.e. low manufacturing cost (Rivard et al., 1995). Suseela and Toppo (2007) reported that polythene has become unavoidable for human life's point of view in this modern era. Truthfully, over the period polythene has become a necessity for routine life, industries and market. Polythene has unmatched

* Corresponding author Email: arvindmdsu@gmail.com

durability, because of this polythene has strong resistance against degradation thus; they remain in nature for very long time which is generating polythene pollution which is affecting adversely wildlife, marine life and our environment (Shah et al., 2008; Nanda et al., 2010; Ferreira et al., 2005). Even methods those are using for waste management are also creating pollution like- landfill is responsible for soil pollution, incineration is causing air pollution and dumping in sea damaging sea eco-system (Moharir & Kumar, 2019; Geyer et al., 2017; Orhan et al., 2004). So, there is urgent need of an eco-friendly and economic solution for this problem. There are two possible way to overcome this problem: First microbial degradation and for that a large number of scientists are trying to find out the solution by using microbes like- Bacteria, Fungi, Actinomycetes, Cyanobacteria and their combination or biofilm (Vimal Kumar et al., 2017). Second step is to evolve polymers with the quality of high degree degradability.

It might be possible, dumped polythene is getting degraded by potential microbial flora. Filamentous fungi or molds are quickly germinated and vital for the conservation of natural eco-system by breaking down organic materials into its components, which can be absorbed by decomposers or nearby plants and other microbial flora (Bandh et al., 2011). Heterotrophic nature of fungi makes it possible to grow on polythene even in adverse environment. A variety of heterotrophic fungi associated with polythene, is reported near water bodies and rooting decaying sites (Raaman et al., 2012). Polythene is consuming back by fungi has environmental friendly and this method is less expensive and natural alternative of waste management of organic pollutant (Sangale et al., 2012). To make biodegradable polythene some functional groups have to be added in the polymer chain so that microorganism can degrade it by attacking on this side. For example starch, antioxidant, colouring agent, cellulose, some substances- O, H, S, Cl, etc (Sigbritt et al., 1988). Interest in biodegradable polymer was increase in last two decades environmental concern and realization that our petroleum resources are very limited. Biodegradable polythene and biodegradation are promising solution for many problems as they are environmental friendly. Biodegradable polymers can be derived from many renewable feedstock and thereby also reducing emission of greenhouse gases into the environment. This plastic can be naturally degraded and get converted in its raw elements which can increase soil fertility and reduce waste management cost. If the practices of biodegradable polythene in society increase accordingly accumulation of bulky plastic materials in environment will also reduce and that can minimize the injuries to wild life. Keeping in mind all these facts, present piece of work was designed to find out the potential fungal strains from polythene debris around water bodies which are involve in possible degradation of polythene and to conduct a comparative study of biodegradation process of both polythene LDPE and biodegradable polythene.

MATERIAL & METHODS

Samples were collected from different decaying sites near water bodies (dumping sites) of Jhansi city. Samples were inoculated and plated on PDA plates and sub cultured for pure fungal isolates (fig. 1). These pure fungal isolates were named as PE-1 to PE-10 and tested on LDPE (Low density polythene) and biodegradable polythene for bio-degradation activity. These isolates were identified microscopically.

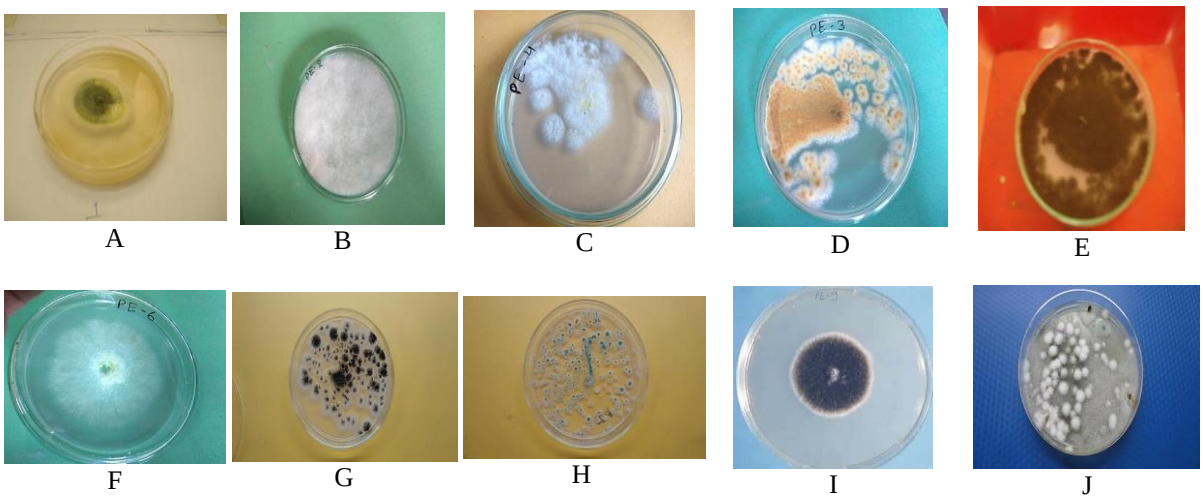

Fig. 1. Pure isolated fungi (**A)** *Penicillium chrysogenum (***B)** *Rhizopus nigrica (***C)** *Chaetomium murorum (***D)** *Memnonielle echinata (***E)** *Aspergillus fumigates (***F)** *Stachybotrys chartarum (***G)** *Aspergillus niger (***H)** *Chaetomium globosum (***I)** *Aspergillus flavus (***J)** *Fusarium oxysporum.*

Submerged fermentation (SF) method was performed to examine polythene degradation. 100 ml of modified Sabaroud's Dextrose broth was taken in 250 ml of Erlenmeyer flask and pH was set to 5.6 (Sabouraud, 1892). Each flask was inoculated accordingly with a fungal isolates and Pre-weighed 1x1 cm sterilized segments of LDPE and Biodegradable polythene, incubated for 15 days and 30 days at 27° C in a rotary shaker at 250 rpm as per the steps discussed by Gilan et al., 2004. After incubation polythene segments were removed by centrifuge and collect the biomass of fungi on pre-weighted filter paper. Polythene segments were observed and then their weight loss was calculated by subtracting pre weight. Degradation was determined in terms of percentage of weight loss. Similarly, dry biomass of fungi was determined by subtracting pre weight of filter paper. pH change was also calculated and for assessment of polythene degradation. These polythene segments were analysed by SEM for critical observation.

RESULTS AND DISCUSSION

In this study 10 fungi were isolated and identified as *Penicillium chrysogenum, Chaetomium murorum, Memnonielle echinata, Aspergillus fumigatus, Stachybotrys chartarum, Aspergillu sniger, Chaetomium globosum, Aspergillus flavus* and *Fusarium oxysporum* belongs to Ascomycota and one belongs to Zygomycota i.e. *Rhizopus nigricans*.

Initially in 15 days fungi indicate more growth in liquid medium and pH has boosted up to7.6 in *Penicillium chrysogenum* and *Aspergillus flavus i.e.* followed by PE-5, PE-7 as 7.5. Likewise, pH increased in PE-2, PE-3, PE-4, PE-6, PE-8 and PE-10 was observed as 6.4, 6.5, 7.4, 6.7, 6.9 and 6.8. It was observed that pH was increased in first 15 days in *Fusarium oxysporum* inoculated flask, and later on in next 15 days it was increased by +0.3. This change in pH might be due to enzyme production activity as shown in Table 1.

Table 1 pH observed after 15 days and 30 days incubation

Sample No.	Fungal Isolate	pH change in 15 days	pH in 30 days	Variation after 15 days
PE-1	*Penicillium chrysogenum*	7.6	7.2	-0.4
PE-2	*Rhizopus nigricans*	6.4	6.3	-0.1
PE-3	*Chaetomium murorum*	6.5	7.2	+0.5
PE-4	*Memmoniella echinata*	7.4	7.6	+0.2
PE-5	*Aspergillus fumigatus*	7.5	7.2	-0.3
PE-6	*Stachybotrys chartarum*	6.7	6.8	+0.1
PE-7	*Aspergillus niger*	7.5	6.9	-0.6
PE-8	*Chaetomium globosum*	6.9	6.6	-0.3
PE-9	*Aspergillus flavus*	7.6	7.2	-0.4
PE-10	*Fusarium oxysporum*	6.8	7.1	+0.3

Similarly, on the 30th day of incubation fungal growth was increased in every sample flask but pH was reported correspondingly decreasing in a comparable order. *Penicillium chrysogenum, Aspergillus flavus* and *Aspergillus niger* recorded the maximum pH decrement and it can consider as the more metabolic activity (Fig. 2). *Aspergillus niger* (PE-7) showed maximum fluctuation in pH, change was noted -0.6 in comparison of control and followed by PE-1 and PE-9. This huge pH fluctuation may reflect as the potential enzyme production by respective fungal isolates.

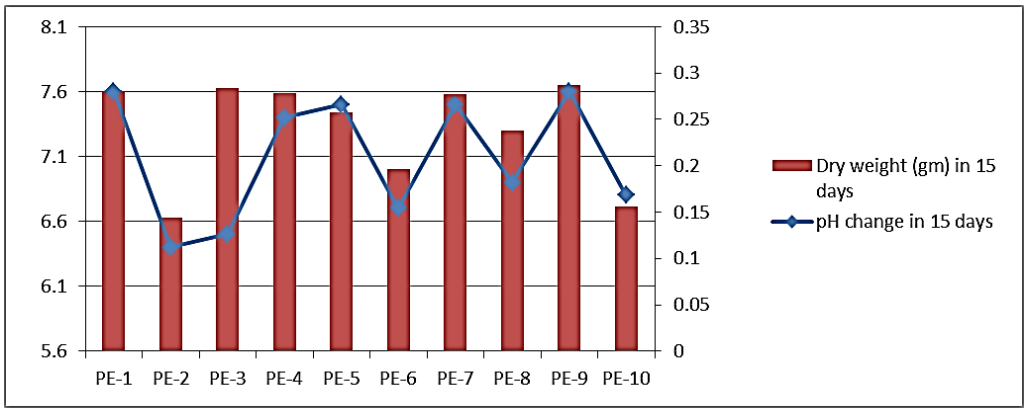

Fig. 2. Comparison of change in pH and dry weight (15 days).

Growth of fungi in every flask was also observed. *Aspergillus flavus* (PE-9) showed the maximum growth in 15 days incubation i.e. 0.287 gm. but PE-10 shows less growth in compare to PE-9 i.e. 0.156 gm. Prominently, maximum growth is noted in 15 days in PE-9 and PE-3 (*Aspergillus flavus and Chaetomium murorum*). *Memnoniella echinata* also indicated a very good growth followed by *Aspergillus niger.* Similarly, at 30th day observation, dry weight of PE-1, PE-2, PE-3, PE-4, PE-5 and PE-6 were observed as 0.307, 0.287, 0.309, 0.294, 0.359 and 0.270 gm respectively (Table 2). *Aspergillus niger* (PE-7) showed the maximum growth 0.365 gm. PE-8 and PE-9 dry weight was observed as 0.283 and 0.312 gm. PE-10 (*Fusarium oxysporum*) dry weight was calculated as minimum growth i.e. 0.206 gm. As shown in Fig. 2 and 3, small amount of fungal biomass was producing very good pH fluctuation this was an indication that fungi is still metabolically active and LDPE or biodegradable polythene or both were being utilized for growth because of carbon deficiency. After removing from medium, biodegradable polythene found lighter in color and weight loss was also observed.

Table 2 Biomass determination after incubation period

Sample no.	Fungal Isolate	Dry weight (gm) in 15 days	Dry weight (gm) in 30 days	Difference in dry weight (gm)
PE-1	*Penicillium chrysogenum*	0.280	0.307	0.027
PE-2	*Rhizopus nigricans*	0.144	0.287	0.143
PE-3	*Chaetomium murorum*	0.284	0.309	0.025
PE-4	*Memmoniella echinata*	0.278	0.294	0.016
PE-5	*Aspergillus fumigatus*	0.258	0.359	0.101
PE-6	*Stachybotrys chartarum*	0.196	0.270	0.074
PE-7	*Aspergillus niger*	0.277	0.365	0.088
PE-8	*Chaetomium globosum*	0.238	0.283	0.045
PE-9	*Aspergillus flavus*	0.287	0.312	0.025
PE-10	*Fusarium oxysporum*	0.156	0.206	0.050

Table 3 Percentage of degradation in 15 days incubation and 30 days incubation

Sample no.	Fungal Isolate	LDPE % of loss in 15 days	BIODEGRADABLE % of loss 15 days	LDPE % of loss in 30 days	BIODEGRADABLE % of loss 30 days
PE-1	*Penicillium chrysogenum*	2%	5%	8%	23%
PE-2	*Rhizopus nigricans*	0.5%	3%	6%	14%
PE-3	*Chaetomium murorum*	.01%	1%	2%	5%
PE-4	*Memmoniella echinata*	.02%	2%	3%	8%
PE-5	*Aspergillus fumigatus*	.4%	4%	7%	15%
PE-6	*Stachybotrys chartarum*	.04	2%	2%	7%
PE-7	*Aspergillus niger*	4%	10%	9%	28%
PE-8	*Chaetomium globosum*	1%	4%	5%	10%
PE-9	*Aspergillus flavus*	1%	5%	7%	18%
PE-10	*Fusarium oxysporum*	0%	1%	3%	8%

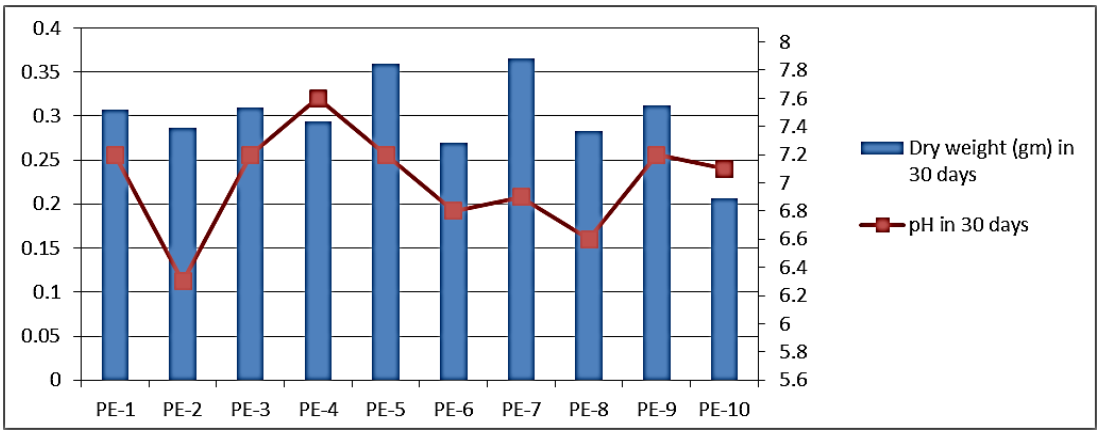

Fig. 3. Comparison of change in pH and dry weight (30 days).

Polythene segments were further examined under SEM to see how well these could stab into polythene surface and degrade it. SEM results showed that absence of carbon source can force to use polythene as carbon source and caused various changes in surface structure.

After 15 days incubation *Penicillium chrysogenum* and *Aspergillus flavus* on biodegradable polythene segments and LDPE samples were examined under SEM and showed very fine grooves, shown in Fig. 4and 5. *Rhizopus nigricans, Aspergillus fumigates* and *Chaetomium globosum* were also generating some degradation and cracks but not as good as *Penicillium chrysogenum* and *Aspergillus flavus.* Polythene segments were from *Chaetomium murorum, Memnoniella echinata, Stachybotrys chartarum* and *Fusarium oxysporum* flask were not showed as remarkable degradation results as other fungi showed. It was observed that very less channel formation in both LDPE and biodegradable polythene. *Aspergillus niger* showed the best results in both LDPE and biodegradable polythene.

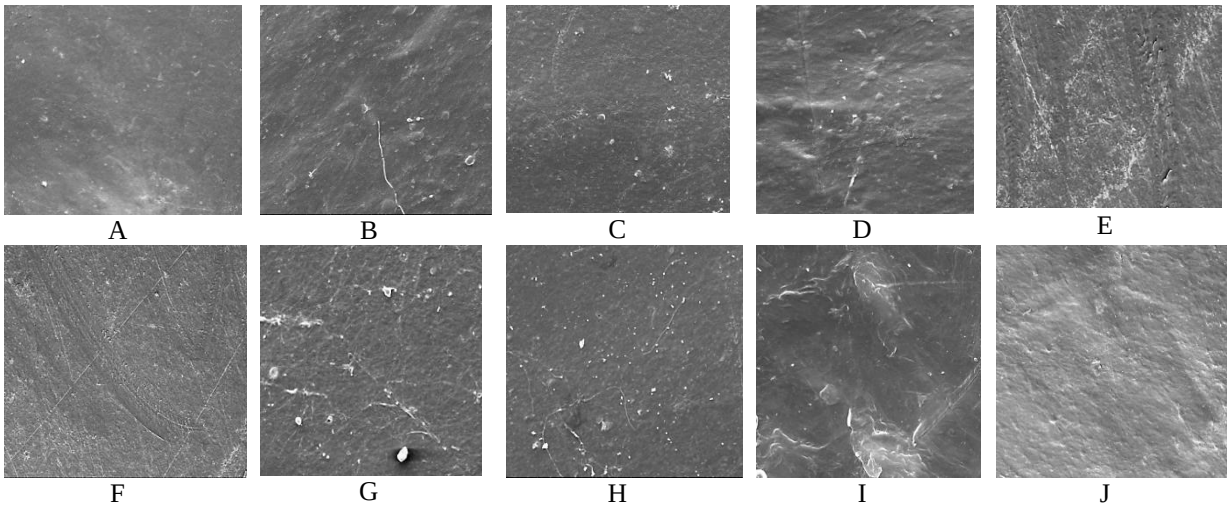

Fig. 4. SEM photographs of LDPE after 15 days incubation (**A)** *Penicillium chrysogenum (***B)** *Rhizopus nigrica (***C)** *Chaetomium murorum (***D)** *Memnonielle echinata (***E)** *Aspergillus fumigates (***F)** *Stachybotrys chartarum (***G)** *Aspergillus niger (***H)***Chaetomium globosum (***I)** *Aspergillus flavus (***J)** *Fusarium oxysporum.*

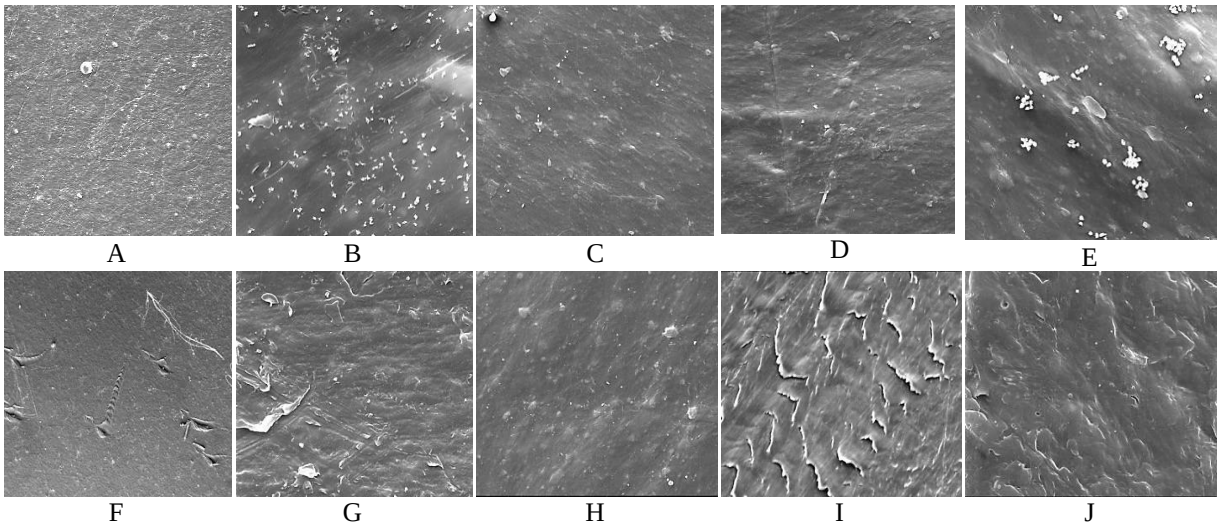

Fig. 5. SEM photographs of LDPE after 30 days incubation (**A)** *Penicillium chrysogenum (***B)** *Rhizopus nigrica (***C)** *Chaetomium murorum (***D)** *Memnonielle echinata (***E)** *Aspergillus fumigates (***F)** *Stachybotrys chartarum (***G)** *Aspergillus niger* **(H)** *Chaetomium globosum (***I)** *Aspergillus flavus (***J)** *Fusarium oxysporum.*

After completing 30 days incubation period SEM analysis of both polythenes showed marvellous results as shown in Fig. 6 and 7. SEM showed very large cracks on surface and very remarkable attachment of mycelium on polythene surface as shown in picture. *Memnoniella echinata* and *Stachybotrys chartarum* might be the first time reported from decaying sites and first time they are being studied for biodegradation of both LDPE and biodegradable polythene. All these factors are providing a very strong driving force for continuing research on biodegradation of polythene with various fungal strains or consortiums of fungi. Fungal isolates from natural decaying sites may play an important role in polythene degradation. (Gilan et al., 2004)were also worked on LDPE and starch blend polythene degradation by *C. globosum* and observed weight loss and breakdown, under SEM analysis which is similar to the finding of this study.

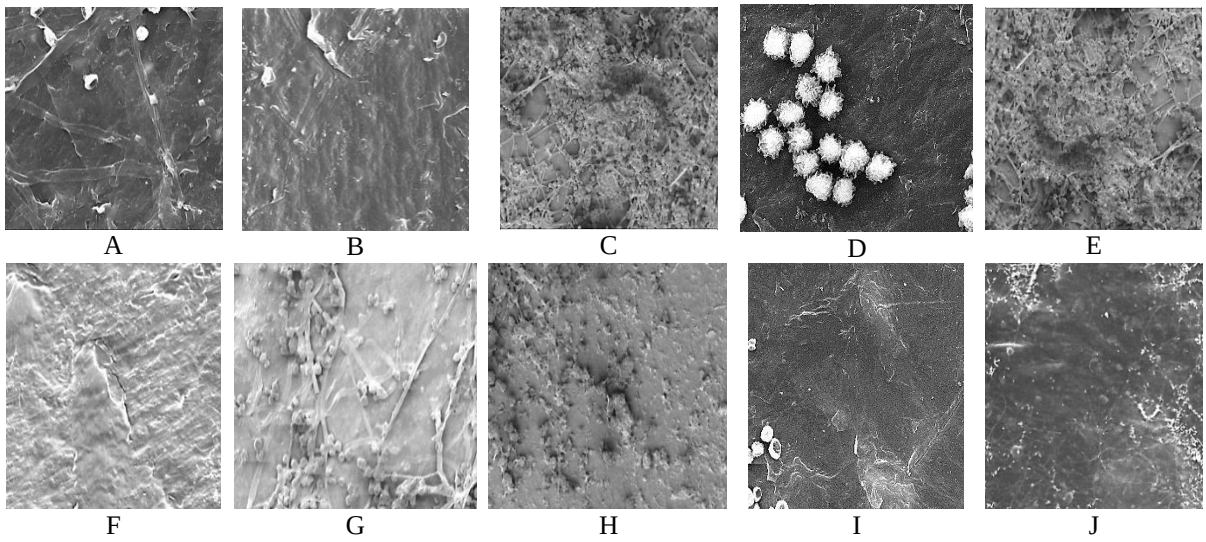

Fig. 6. SEM photographs of biodegradable polythene after 15 days incubation (**A)** *Penicillium chrysogenum (***B)** *Rhizopus nigrica (***C)** *Chaetomium murorum (***D)** *Memnonielle echinata (***E)** *Aspergillus fumigates (***F)** *Stachybotrys chartarum (***G)** *Aspergillus niger (* **H)** *Chaetomium globosum (* **I)** *Aspergillus flavus (* **J)** *Fusarium oxysporum*

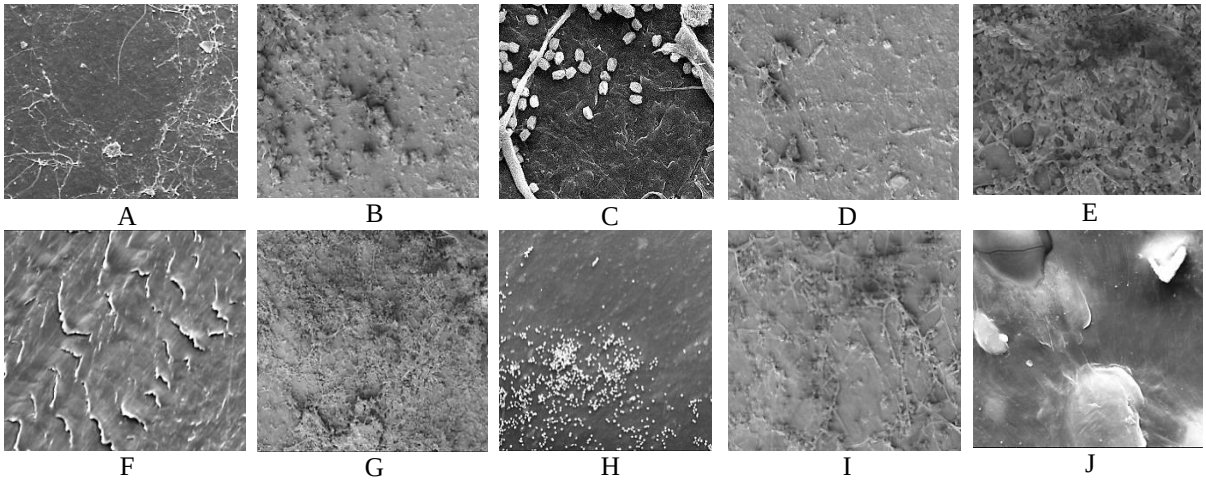

Fig. 7. SEM photographs of biodegradable polythene after 30 days incubation (**A)** *Penicillium chrysogenum (***B)** *Rhizopus nigrica (***C)** *Chaetomium murorum (***D)** *Memnonielle echinata (***E)** *Aspergillus fumigates (***F)** *Stachybotrys chartarum (***G)** *Aspergillus niger (* **H)** *Chaetomium globosum (* **I)** *Aspergillus flavus (* **J)** *Fusarium oxysporum.*

The fact cannot be denied that synthetic polythene is easily replacing natural products, but final disposable waste causing pollution. Polythene can remain in the same environment for more than ten decades because of resistant to biodegradation (Raaman et al., 2012). Fungi are widely used in biodegradation studies because of its robust nature and for their great source of enzymes production like laccases, peroxidase and oxidases enzyme (Ruiz-dueñas & Martínez, 2009).

Several polythene samples were found slimy, soft textured, with heavy fungal growth and brittle, fragile and tearing easily, these samples were further studied for microbial flora. A variety of species were isolated and identified from polythene as shown in Fig. 1. Maximum number of *Aspergillus* species were isolated from decaying waste and followed by *Chaetomium* species. Mostly fungal isolates were saprophytic fungi. Konduri et al., (2010) was also worked on many *Aspergillus* species and reported that *Aspergillus* species can grow on polythene and able to degrade it.

(Shah et al., 2008) worked on *Fusarium* species isolated from sewage sludge.(Raaman et al., 2012) conducted a similar research near Chennai and reported *Aspergillus niger, A. japonicas, A. tereus, A. flavus* and *Mucor* species and observed 8% degradation in one month period by *Aspergillus niger*.

pH change in 15 days can be considered as the enzyme production. Enzyme production usually shows alkaline tendency. But later on pH of many flasks got decreased in similar pattern, it may be considered as degradation of polythene because after degradation secondary metabolites may be acidic in nature or shows acidic tendency. Reduction in pH (Fig. 4) is not only affirms that consumption of polythene film can utilized as carbon source but also confirm the potential of fungal strains to degrade polythene (Arutchelvi et al., 2008; Duddu and Guntuku, 2015; Das and Kumar, 2015).

In present finding, *Rhizopus nigricans* showed almost similar results to previous research; 6% deduction was noted in weight loss in LDPE and 14% deduction noted in biodegradable polythene in control environment within 30 days. Comparable study was also carried out by (Ibrahim et al., 2013) on PS PUR and found significant weight loss in PS PUR blocks by shaken culture method and observed up to 100% degradation by *Fusarium solani*. Finding of present study were different to them. *Fusarium oxysporum* (PE-10) was isolated from polythene dumping site and was not appeared as efficient as *F. solani.* Similarly, (Awasthi et al., 2017) also worked on LDPE with *Rhizopus spp.* and observed around 8.4% weight loss and 60% reduction noticed in tensile strength of polymer.

(Mendez et al., 2007) isolated *Aspergillus flavus* from sanitary landfill and testified on polyethylene, found to be capable to degrade it. Similar study was also reported by Yamada-Onodera et al., (2001) was identified a fungus named *Penicillium simplicissimum,* which could efficient enough to degrade the untreated HDPE (High Density Polyethylene). Łabuzek et al., (2004) stated 100% degradation of modified polythene by *Penicillium funiculosum.* was not co-relate with the results of *Penicillium chrysogenum* (PE-1) showed 2% degradation of LDPE and 5% degradation of biodegradable polythene over the 15 days incubation but later on when fungus started utilizing polythene and degrade it up to 8% LDPE and 23% biodegradable polythene as given in Table 3. This decrease in weight can be associated with the finding of other studies those were carried out on degradation of Low-density polythene (LDPE) using *Aspergillus fumigatus* and *Penicillium spp.* (Salleh et al., 1993; Gilan et al., 2004; Manzur et al., 2004 and Awasthi et al., 2017). According to their findings, *A. fumigatus* was able to degrade 4.65 percentage of polyethylene and *Penicillium spp.* degraded 6.58% polythene. After incubation period both LDPE and biodegradable polythene segments were measured for weight loss and maximum weight loss was found in biodegradable polythene

i.e. 28% by *Aspergillus niger*, followed by LDPE 9% in SDB medium. Present experiment gives slightly different results to them.

Raaman et al., (2012)also reported 8% degradation of LDPE by *Aspergillus niger* and 12% degradation by *Aspergillus japonicas*. Finding of present study were better than previous research, 9% weight loss was observed in LDPE and 28% weight loss in biodegradable polythene by *Aspergillus niger*. Whereas in control there was no weight loss observed in flask.

The mechanism of degradation is not exactly known till date but it have been examined that surface of polythene has turned from smooth to rougher and weight reduction was also reported. The biodegradation was found under different growth condition, according to microbial properties and growth condition (Lucas et al., 2008). Filamentous fungi generally have the ability to produce various and vast amount of enzymes in a constant manner (Christensen et al., 1988). First and foremost was the visual observation and then polythene was examined under SEM after 15 days. *Memnoniella echinata* and *Stachybotrys chartarum* might be the first time reported from decaying sites and first time they are being studied for biodegradation of both LDPE and biodegradable polythene. All these factors are providing a very strong driving force for continuing research on biodegradation of polythene with various fungal strains or consortiums of fungi. Fungal isolates from natural decaying sites may play an important role in polythene degradation. Gilan et al., (2004) were also worked on LDPE and starch blend polythene degradation by *C. globosum* and observed weight loss and breakdown, under SEM analysis which is similar to the finding of this study.

CONCLUSION

This study was a step towards finding a solution of this global issue. In this study, ten fungal strains were isolated- *Penicillium chrysogenum, Rhizopus nigricans, Chaetomium murorum, Memnoniella echinata, Aspergillus fumigatus, Stachybotrys chartarum, Aspergillus niger, Chaetomium globosum, Aspergillus flavus* and *Fusarium oxysporum*, in which *Penicillium chrysogenum, Rhizopus nigricans, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus* showed greatest results in terms of degrading both Low density polythene and biodegradable polythene. These isolates also showed good enzymatic reaction and weight loss. They are beneficial in many ways such as- *Penicillium* as medicine, *Rhizopus* as bio-fertilizer and *Aspergillus* as fermenting agent. Out of these, four isolates (*Penicillium chrysogenum, Rhizopus nigricans, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus*), *Aspergillus niger* showed maximum degradation in both low density and biodegradable polythene.

This was an attempt to find out an eco-friendly biodegradation method which can convert hazardous waste into beneficial product. Waste that thrown in environment can be the source of raw materials for some productive things. *Penicillium chrysogenum, Rhizopus nigricans, Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus* converting polythene into its component and absorbing back as nutrition and final biomass of fungi can use as antibiotic, fertilizer or as fermenting agent. In this study the fungi identified were able to achieve all the features mentioned above. The outcome of the study can be used as a method for production of fungi by using biodegradable polythene. Effects of various fungal species were seen on biodegradable polythene in comparison to low density polythene.

GRANT SUPPORT DETAILS

The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

REFERENCES

Arutchelvi, J., Sudhakar, M., Arkatkar, A., Doble, M., Bhaduri, S. and Uppara, P. V. (2008). Biodegradation of polyethylene and polypropylene. Ind. J. Biotech., 7(1); 9–22.

Awasthi, S., Srivastava, N., Singh, T., Tiwary, D. and Mishra, P. K. (2017). Biodegradation of thermally treated low density polyethylene by fungus Rhizopus oryzae NS 5. 3 Biotech., 7(1);1–8. https://doi.org/10.1007/s13205-017-0699-4.

Bandh, S. A., Kamili, A. N. and Ganai, B. A. (2011). Identification of some Penicillium species by traditional approach of morphological observation and culture. Af. J. Micro.Res., 5(21); 3493–3496. https://doi.org/10.5897/AJMR11.677.

Christensen, T., Woeldike, H., Boel, E., Mortensen, S. B., Hjortshoej, K., Thim, L. and Hansen, M. T. (1988). High level expression of recombihant genes in aspcrgiuus oryzae. Bio/Technology, 6(12); 1419–1422. https://doi.org/10.1038/nbt1288-1419.

Danso, D., Chow, J. and Streita, W. R. (2019). Plastics: Environmental and biotechnological perspectives on microbial degradation. App. and Environ. Micro., 85(19). https://doi.org/10.1128/AEM.01095-19.

Das, M. P. and Kumar, S. (2015). An approach to low-density polyethylene biodegradation by Bacillus amyloliquefaciens. 3 Biotech., 5(1); 81–86. https://doi.org/10.1007/s13205-014-0205-1.

Dilara, P. A. and Briassoulis, D. (2000). Degradation and stabilization of low-density polyethylene films used as greenhouse covering materials. J. Ag. and Eng. Res., 76(4); 309–321. https://doi.org/10.1006/jaer.1999.0513

Duddu, M. K. and Guntuku, G. (2015). Isolation and Screening of Actinomycetes for Biodegradation of Low Density Polyethylene from Mangrove Sediment. Int. J. Pharm. Res. & Rev., 4(11); 14–22.

Ferreira, L. M., Falcão, A. N. and Gil, M. H. (2005). Modification of LDPE molecular structure by gamma irradiation for bioapplications. Nucl Instrum Methods in Phys Res.B., 236(1–4); 513–520. https://doi.org/10.1016/j.nimb.2005.04.030

Geyer, R., Jambeck, J. R. and Law, K. L. (2017). Production, use, and fate of all plastics ever made - Supplementary Information. Science Advances, 3(7); 19–24.

Gilan, I., Hadar, Y. and Sivan, A. (2004). Colonization, biofilm formation and biodegradation of polyethylene by a strain of Rhodococcus ruber. Appl. Microbiol. Biotechnol., 65(1); 97–104. https://doi.org/10.1007/s00253-004-1584-8

Ibrahim, D., Bankole, O. C., Ma'aji, S. A., Ohize, E. J. and Abdul, B. K. (2013). Assessment Of The Strength Properties Of Polystyrene Material Used In Building Construction In Mbora District Of Abuja,Nigeria. Int. j. eng. res. dev., 6(12); 80–84.

Konduri, M. K. R., Anupam, K. S., Vivek, J. S. and Narasu, L. M. (2010). Synergistic effect of photo- and chemical treatment on the rate of biodegradation of low density polyethylene by fusarium sp. AF4. Int. j. biotechnol. biochem., 6(2); 157+. https://doi.org/10.1002/app.26328

Łabuzek, S., Nowak, B. and Pajak, J. (2004). The susceptibility of polyethylene modified with bionolle to biodegradation by filamentous fungi. Pol. J. Environ. Stud., 13(1); 59–68.

Lucas, N., Bienaime, C., Belloy, C., Queneudec, M., Silvestre, F. and Nava-Saucedo, J. E. (2008). Polymer biodegradation: Mechanisms and estimation techniques - A review. Chemosphere, 73(4); 429–442. https://doi.org/10.1016/j.chemosphere.2008.06.064.

Manzur, A., Limón-González, M. and Favela-Torres, E. (2004). Biodegradation of Physicochemically Treated LDPE by a Consortium of Filamentous Fungi. J. Appl. Polym. Sci., 92(1); 265–271. https://doi.org/10.1002/app.13644.

Mendez, C. R., Vergaray, G., Bejar, V. R. and Cardenas, K. J. (2007). Isolation and characterization of polyethylene-biodegrading mycromycetes. Rev. peru. biol., 13(3); 203-205.

Moharir, R. V. and Kumar, S. (2019). Challenges associated with plastic waste disposal and allied microbial routes for its effective degradation: A comprehensive review. J. Clean. Prod., 208; 65–76. https://doi.org/10.1016/j.jclepro.2018.10.059.

Nanda, S., Sahu, S. and Abraham, J. (2010). Studies on the biodegradation of natural and synthetic polyethylene by Pseudomonas spp. J. Appl. Sci. Environ. Manag., 14(2); 57-60. https://doi.org/10.4314/jasem.v14i2.57839

Orhan, Y., Hrenović, J. and Büyükgüngör, H. (2004). Biodegradation of plastic compost bags under controlled soil conditions. Acta Chimica Slovenica, 51(3); 579–588.

Raaman, N., Rajitha, N., Jayshree, A. and Jegadeesh, R. (2012). Biodegradation of plastic by Aspergillus spp . isolated from polythene polluted sites around Chennai. J. Acad. ind. res., 1(6), 313–316. http://jairjp.com/NOVEMBER 2012/08 RAAMAN.pdf

Rivard, C., Moens, L., Roberts, K., Brigham, J. and Kelley, S. (1995). Starch esters as biodegradable plastics: Effects of ester group chain length and degree of substitution on anaerobic biodegradation. Enzyme Microb. Technol., 17(9); 848–852. https://doi.org/10.1016/0141-0229(94)00120-G

Ruiz-dueñas, F. J. and Martínez, Á. T. (2009). Review Microbial degradation of lignin : how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. Microb. Biotechnol., 2(2); 164–177. https://doi.org/10.1111/j.1751-7915.2008.00078.

Sabouraud R., 1892, Ann. Dermatol. Syphil. 3 : 1061.

Salleh, A. B., Musani, R., Basri, M., Ampon, K., Yunus, W. M. Z. and Razak, C. N. A. (1993). Extra- and intra-cellular lipases from a thermophilic Rhizopus oryzae and factors affecting their production. Can. J. Microbiol., 39(10); 978–981. https://doi.org/10.1139/m93-147.

Sangale, M. K., Shahnawaz, M. and Ade, A. B. (2012). A Review on Biodegradation of Polythene: The Microbial Approach. J. bioremediat. biodegrad., 03(10). https://doi.org/10.4172/2155-6199.1000164

Seymour, R. B. (1989). Polymer science before and after 1899: Notable developments during the lifetime of maurits dekker. J. Macromol. Science.A . , 26(8), 1023–1032. https://doi.org/10.1080/00222338908052032.

Shah, A. A., Hasan, F., Hameed, A. and Ahmed, S. (2008). Biological degradation of plastics: A comprehensive review. Biotechnol. Adv., 26(3); 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005

Sigbritt, K., Olle, L. and Ann-Christine, A. (1988). Biodegradation of Polyethylene and the Influence of Surfactants. Polym. Degrad. Stab., 21; 237–250.

Suseela, M. R., and Toppo, K. (2007). Algal biofilms on polythene and its possible degradation. Curr. Sci., 92(3), 285–287.

Vimal Kumar, R., Kanna, G. R. and Elumalai, S. (2017). Biodegradation of Polyethylene by Green Photosynthetic Microalgae. J. bioremediat. biodegrad., 08(01). https://doi.org/10.4172/2155-6199.1000381

Yamada-Onodera, K., Mukumoto, H., Katsuyama, Y., Saiganji, A. and Tani, Y. (2001). Degradation of polyethylene by a fungus, *Penicillium simplicissimum* YK. Polym. Degrad. Stab., 72; 323–327.