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BIOLOGICAL DEGRADATION OF LOW DENSITY POLYETHYLENE (LDPE)

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Abstract– Polythene is a serious problem which causes pollution to the environment and pollutes the land and marine ecosystem. However, the biological degradation could be a solution of polythene pollution. According to the previous studies microbes have ability to degrade polythene. Microbes use polythene as their carbon source and energy. In this present research, we found fungal strain which degrade polythene in 45 days. The isolated fungi have shown great ability to degrade and they grow rapidly and can be isolated from the soil. We isolate *Aspergillus fumigatus* and it has have ability to degrade the LDPE. Some enzymes such as Polyesterases, esterases, Lipases and hydrolases involved in degradation of polythene and produced by the *Aspergillus fumigatus.* The isolated fungi secrete Lipases enzyme showed activity on agar plate assay and halo area was measured as 4.67mm. So our study suggest that polythene can be degrade by microorganism and we can overcome from the white pollution from the natural process.

INTRODUCTION

Plastic was invented in 1942 by Whinfield and Dicson (Mary Belis, 2020). (https:// www.thoughtco.com/history-of-plastics-1992322). Plastics are generally synthetic organic product which is made from oil, coal and natural gas. (Azeko *et al.,* 2015). Due to the scarcity of resources and enhancement of technologies the plastic was introduced (Lear G. 2021). Plastic is a very versatile material because plastic can be molded in any shape and it is very durable. Plastic used in packaging 39%, construction (19%), automotive industry (9.8%), electronics and communication (6.2%), agriculture (3.4%), household leisure and sport (4.1%) and others (16.7%). Plastics have varieties but the most commonly used in packaging are polypropylene (PP), low density polyethylene (LDPE), high density polyethylene (HDPE), polyvinyl chloride (PVC), polyurethane (PU), polyethylene terephthalate (PETE) and polystyrene (PS). In all these type of plastic most of the plastic is resistant to degradation and durable for long period. Nearly 300 million tones plastic is produced worldwide most of the plastic is single used plastic and shockingly, the 300 million tons of plastic weight is equivalent to the whole human population of the world. (Xochilt *et al.,* 2019).

Plastic is one of the most threat full elements which is producing and using by the majority in the world. It is dangerous for the environment because it has low degradation ability and plastic can stay in environment for decades which is really dangerous. It is dangerous for human as well as animals because they assumed plastic on land as the food and consumed the plastics which cause death of animals (Srikanth *et al.,* 2022). There are many disadvantages from plastic it is not only dangerous for human plastic cause serious problem in the marine ecosystem also because plastic form particulate matter by UV radiation and increase the temperature. The disposal of plastic is also very problematic because when plastic disposed in to the ocean plastic accumulated toxic elements such as: polychlorinated biphenyl (PCB's), nonyphenol (NP), dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PHA) polybrominateddiphenyl esters (PBDE) and

bisphenol A (BPA). (Bryant *et al.*, 2016). Accumulation of these toxic elements in the oceans causes serious problem like indigestion, gastrointestinal blockage and reproductive problems. At least 267 species are being affected by plastic pollution in the marine ecosystem which includes 86% turtles and 44% seabirds get affected. (Coe *et al.*, 1997). As the usage of low density polyethylene (LDPE) is rising day by day and the disposal of LDPE is the main concern because in the landfill the major amount of solid waste come from households and LDPE is mainly used in packaging and carry bags. When it comes to degradation of LDPE the biological degradation is more suitable for the environmental health. In most of the previous studies the biodegradation of plastic can be carried out using microorganisms. Microbes can degrade organic wastes and increase the biological productivity directly and indirectly. There are some microbes which can degrade the organic and inorganic materials. So, there are some chances that the may be microbes have ability to degrade the plastic and polythene wastes (Kambe *et al.*, 1995). In this research article wdescribe various fungi that are involved in biological degradation of polythene. In addition, we discussed the effective role of fungi to degrade the plastic from secretion of enzymes. In biological degradation of LDPE or polythene is due to the enzymatic mechanisms of fungi. Surface degradation of polythene involves the microbial enzymes. Microbes grow on the surface of the plastic or polythene pieces and utilize the plastic as a source of nutrition and energy. Therefore, the plastic depolymerized and degradation takes part by mineralization process. (Montazer *et al.*, 2019). Fungi have ability to occupy substrate using enzymes that can detoxify the pollutants. Degradation of polythene takes place through the intercellular and extracellular enzymes. Intracellular enzyme act as an internal mechanism for detoxify and fungal adaptation (Jeon *et al.*, 2016). Whereas, the extracellular enzyme composed hydrolytic system that produce hydrolases that elaborate in polysaccharide degradation that involved in breaking down the complex structures (Sanchez, 2009). And in the recent study, scientific communities revealed that the microorganisms have capabilities to degrade the plastic polymer like polyethylene terephthalate (PET) (Wei *et al.*, 2014). The example is β-proteobacterium *Ideonellasakensis* which produces enzyme IsPETase to degrade the plastic and this enzyme had cutinase like structure (Molitor *et al*, 2019). Agar plate assay can be consider as a high throughput activity based screening from which thousands to millions of samples can be test rapidly for biological activity like pathway or molecular level (Popovic *et al*, 2015). The workload of experiment can also be reducing from this assays which can further characterized. Agar plate based activity assays are applied and the clear or colored zones are formed around the fungal plate (Molitor *et al.*, 2019).

MATERIALS AND METHODS

Collection of Sample: Sample was collected from the Shobhit Deemed to be University, Modipuram, Roorkee road, Meerut. India with longitude and latitude position 29.0718" North and 77.7128" East respectively. The site was chosen based on the local dumping site mostly the polythene bags were disposed and bags were partially degraded by the microbes so the soil was collected from the site in the zip-lock bag with the help of spatula from 6 inches deep soil.

Isolation of Microbes: Soil sample was collected from sample collection site and 1gm soil diluted 100ml water in test tube and serially diluted in various dilutions (10^{-3} to 10^{-5}). And spread on the agar medium plate. On agar plate fungus growth was reported for fungus growth CDA media from himedia (Sucrose-30 g, sodium nitrate-2 g, dipotassium phosphate-1g, magnesium sulphate-0.500 g, ferrous sulphate-0.010 g, potassium chloride-0.500 g, agar-15 g) was prepared.

Identification of Microbes: The identification of isolated microbes done by the morphology and microscopic identification of microbes by the reference books. The morphological characterization and microscopic characterization such as: shape, size, color, texture, appearance, elevation were studied.

Production of Enzymes: For the production of enzymes the Sucrose broth was prepared for the degradation process through the enzymatic process. 60 ml sucrose broth was prepared and autoclaved at 121°C for 15min in 250 ml conical flask and incubate at different temperature for 15, 30 and 45 days. Fresh polythene bag pieces were cut at least 1gm in to the 1×1cm and washed and sterile from ethyl alcohol. Further added the polythene pieces in to the broth flask after that the isolated fungus was inoculated in to the flask and incubate the flask.

After 15 days remove the polythene from the flask carefully washed with ethyl alcohol air dried the polythene pieces and weighted for data collection. Repeat this procedure for rest of samples.

Determination of total weight loss: The degradation of polythene by *Aspergilljus fumigatus* determine by the calculating the percentage ofweight loss of the polythene bag. This formula used for calculation the final value of degradation.

$$\text{Percentage of weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Agar plate assay method for enzyme activity screening

The enzymatic activity of fungus was analysis by the plate essay method (Bisht *et al.,* 2019). For the plate assays, CDA media with 1% Tributyrin was autoclaved (121 °C, 15 min) (Molitor *et al.,* 2019) and pour 25 ml in to each plate after solidifying the media cut wells with the help of cork borer inoculated fungus in to wells and incubate at 37 °C for 24 h afterwards incubate at 4°C for photo documentation for 2 to 3 days.

Polyesterases: Polyesterases enzyme is lipolytic enzyme which comprises lipases and esterases. And polyesterases involved in degradation of polythene (Molitor *et al.,* 2019).

Esterases: Esterase enzymes are also involved in plastic degradation. Esterases enzyme catalyze hydrolysis enzyme of an ester group and it splits in to alcohols and acids with water. And it is produced by both bacteria and fungi (Yamamoto-Tamaro *et al.,* 2015). Polycaprolactone polyester is also degraded by lipase and esterase (Ganesh *et al.,* 2017).

Lipases: Lipase enzyme is a subclass of esterases enzyme. *Aspergillus spp.* Produce lipase enzyme and involved in the degradation of polythene (Eberl *et al*. 2009).

Hydrolases: Hydrolases enzyme breakdown the large molecules in to small molecules. Hydrolases enzyme was produced by the *Aspergillus fumigatus* and involved in the degradation of polythene (Molitor *et al.,* 2019).

RESULTS AND DISCUSSION

Microbial diversity varies on the environmental conditions such as soil, water and compost. So it is necessary to investigate the microbial population of polythene degrading microbes in various ecosystems (Tokiwa *et al.,* 2009).

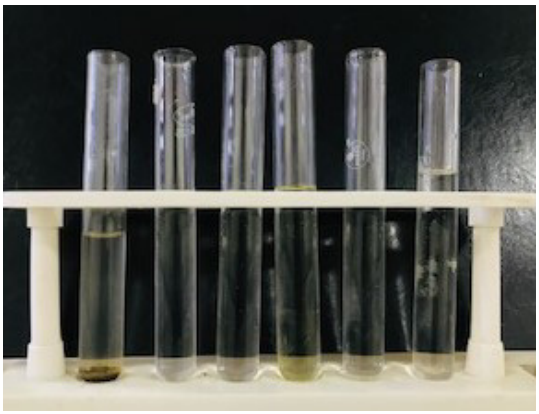

Fig. 1. Serial dilution of plastic contaminated soil from dump site

Soil dilution method has done in Fig. 1. Soil microbes enumerated, so the sample was serially diluted (10^{-3}- 10^{-5}) and then dispersed on to the growth medium to isolate the polythene degrading microbes

In this research article, *Aspergillus fumigatus* was isolated from the contaminated soil. The fungus identified by the morphology. In previous researches Shrikant *et al.,* 2022 and Raman *et al.,* 2012 reported that fungus has ability to degrade the polythene.

CONCLUSION

Polythene bag is a serious threat to the environment because disposal of plastics in open environment is causing serious problems. Non-degradable plastic stays in environment for decades and low grade plastics are more harmful for environment, animals,

Table 1. Total Weight loss of polythene degradation by *Aspergillus fumigatus* (Each figures of 3- independent replicates)

S. No.	Enzymes weight of polythene bag (1g)	Initial	Percentage of weight loss		
			15 Days	30 Days	45 Days
1.	Lipase	1	18.9±0.02	38.45±0.04	47.25±0.04
2.	Hydrolase	1	4.65±0.04	9.85±0.04	11.95±0.08
3.	Esterase	1	2.82±0.04	7.95±0.04	11.15±.0.6
4.	Polyesterase	1	4.95±0.07	10.95±0.04	22.65±0.07

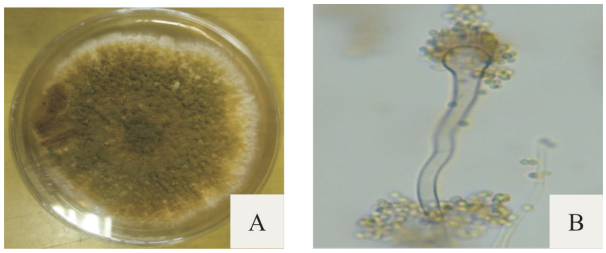

Fig. 2. (A) Isolated fungus on CDA plate, (B) Microscopic view of *Aspergillus fumigatus*

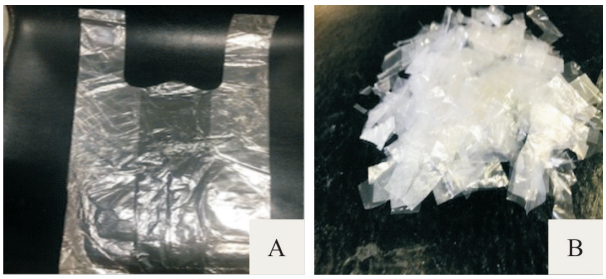

Fig. 3 (A) Polythene bag from local market (B) Polythene pieces 1×1 cm

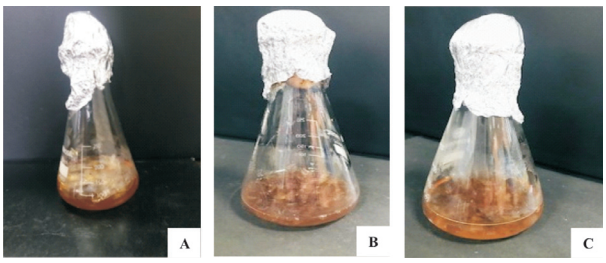

Fig. 4. Production of extracellular secretory enzyme for degradation of polythene by *Aspergillus fumigatus*at different days of incubation (where= A- 15 days, B-30 days and C- 45 days)

human and ecosystem. In the present research, we have isolate a fungi from the soil which was collected from the dump site (Fig. 1) than isolate the fungus from the soil and degrades the polythene with the help of isolated strain (Fig. 2). The *Aspergillus fumigatus* have ability to degrade the polythene bag. The fungus was identified by the morphology characteristics from (Barnet, 1972).

Polythene was degraded in the presence of moisture, temperature, heat etc. the surface of polythene was become smooth to rough with cracking and reduction in weight is due to microorganisms (Weiland *et al.,* 1995). In the process of degradation of polythene two categories of enzymes are involved: extracellular and intracellular enzyme (Gu *et al.,* 2000). In Fig. 5 different types of enzymes such as polyesterases, esterases, lipases, and hydrolases enzymes was involved in degradation of polythene. And after every 15, 30 and 45 days polythene pieces were separated aseptically and washed with ethanol and weighed them and the degradation percentage rate was 18.9±0.02, 38.45±0.04, and 47.25±0.04 for 15, 30 and 45 days of incubation respectively at temperature 37 °C and this degradation was take place when the *Aspergillus fumigates* secrete lipases enzyme in previous stidies it has shown that lipases enzyme has ability of degradation different type of polymers (Bettache *et al*., 2012, Ko *et al.,* 2005; Maciel *et al*., 2018). The isolated fungi *Aspergillus fumigatus* showed different type of enzymatic activity in which halo area was measured which is shown in Table 2. In the recent studies Lear *et al.,* 2022 and Ren Wei *et al.,* 2021 reported that the microorganism have ability to degrade polythene bags. And Srikanth *et al.,* 2022 reported that the enzymatic mechanism involved in plastic degradation.

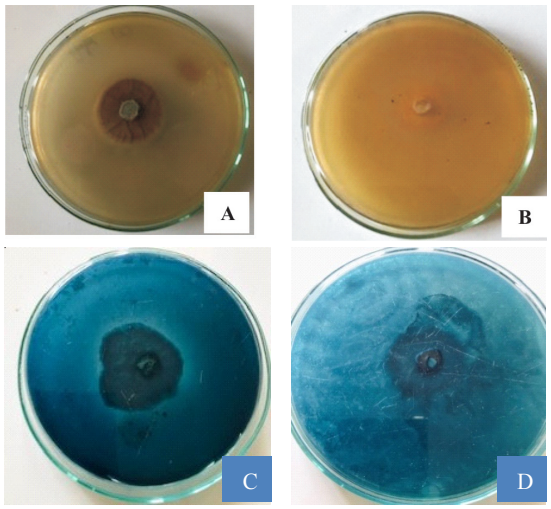

Fig. 5. Enzymatic activity by Plate assay method (where A- Lipase activity, B- Polyesterases, C-Esterases activity and D- Hydrolases activity)

Table 2. Zone of activity on the agar plate assay method by *Aspergillus fumigatus* (Each figures of 3-independent replicates)

S.No	Enzymes	*Aspergillus fumigatus*
1.	Polyesterases	4.21±0.05
2.	Esterases	2.65±0.08
3.	Lipases	4.67±0.04
4.	Hydrolases	3.46±0.06

The overall investigation of this research suggest that the *Aspergillus fumigatus* has ability to degrade

polythene in 45 days from surface and by secreting different types of enzymes and lipases enzyme has ability to degrade. There are several studies available about the degradation of plastic but the microbial degradation of polythene is cheapest, ecofriendly, and easy method.

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