TITLE- Isolation of plastic degrading bacteria from contaminated soil.

INTRODUCTION

A solution to an environmental issue between physical and chemical degradation techniques or other plastic structures is biodegradation. Plastic biodegradation has been widely investigated for the past 30 years. Enzymatic degradation is a prominent approach for treating environmental plastic waste. This process involves biodegradation by bacteria that generate Enzymes capable of degrading plastics without harming the surrounding environment. Several factors determine the rate at which bacteria degrade polymers. These parameters include humidity, microbes, temperature, pH, polymer type, and thickness. To optimize biodegradation, modify pH, temperature, nutrients, minerals, oxygen, and humidity according on the microorganisms utilized (Muhammad Diki Juliandi et al 2020). In this research, isolation of fungi from Plastic contaminated soil collected from the NMC's garbage dumping station Nashik in Maharashtra and screening of polyethylene plastic-degrading fungi will be carried out.

MATERIAL AND METHODS

- **Materials -**

The material used in this study was **Solution A**: Distilled Water 50 ml. KH_2PO_4 12.5gm NH_4Cl 2 gm $MgSO_4$ 0.5 gm $CaCl_2$ 0.25 gm**Solutions B**: Distilled water 100 ml Citric acid 2.5 gm$ZnSO_4$ 2.5 gms$CuSO_4$ 0.01 gm$MnSO_4$0.001gm $Na_2MOO_4$0.001 gm

- **Preparation of LDPE Film**

Readymade LDPE film (2cm X 2cm) is used for the action of fungal degradation on its surface areas.

- **Isolation and Purification of Polypropylene Plastic fungi**

A LDPE film (2 cm × 2 cm) was placed into 50 mL of mineral salt medium broth in a 1000 mL Erlenmeyer flask prior to the inoculation of five agar plugs (5 mm diameter, 7 days old) of each strain. All solution flasks were stored at pH 7.0 ± 0.2. Fungal cultures were incubated at room temperature (27.0 ± 2.0 °C) for 30 days under static conditions (Khruengsai et al 2021). The fungus *A. niger* was used as the control to confirm the degradation of LDPE film. After 30 days, fungal mycelium was filtered and vacuum dried for 24 h. Mycelium dry weight obtained from each fungus was measured by digital weighing balance.

SrNo.	Organisms	Label
1	Bacteria	B1
2	Bacteria	B2
3	Fungi	F1
4	Fungi	F2
5	Fungi	F3
6	Fungi	F4
7	Fungi	F5

Table:1-Fungal and bacterial Isolates

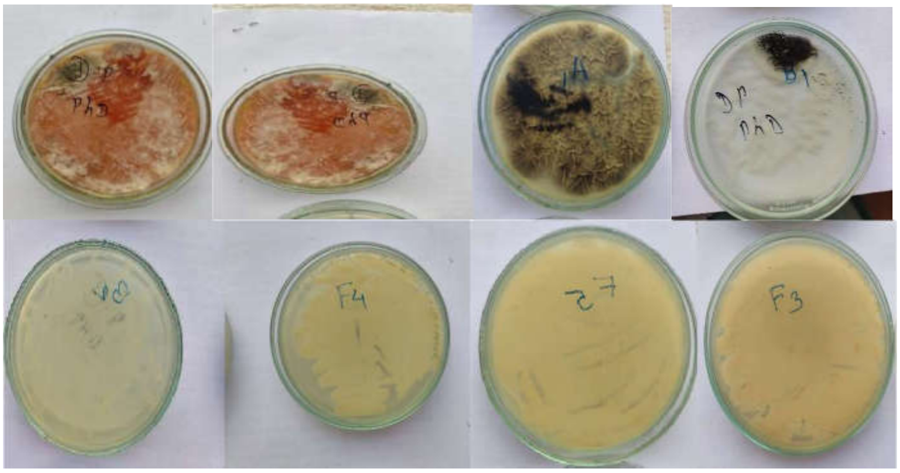


Figure1: Identification of isolated bacterial and fungi species

- **fungal Isolates Stock Preparation of**

Pure fungall cultures were obtained, inoculated on PDA slants, and kept at 4°C for collection.

- **To study the polyethylene degrading microorganisms.**

For this experiment soil sample, media (vogel), plastic (PE film) has been used. Approximate amount of mix cultured sample placed into 50ml of vogel media along with measured P.E.film.andInoculation of sample and PE film were kept on observation for 15 to 30 days.Mix culture sample and P.E film was observed after 15 days that the earlier weight of plastic film was 0.0212mgm and after 15 days the weight of P.E film was 0.0208mgm. It observeredthat the weight was reduced upto 0.0004 mgm(Ojha et al 2017).

- **To study the process of degradation of PE by mixed culture of various strains of fungi(Without using sucrose)**

Materials: Mixed culture of various fungal strains, Polyethylene (P.E.)All samples (A1, B1, B2, F2, F3, F4, F5), Media (50 ml). For this experiment soil sample, media (vogel), plastic (PE film) has been used. Approximate amount of mix cultured sample placed into 50ml of vogel media along with measured P.E.film.and Inoculation of sample and PE film were kept on observation for 15 to 30 days. Mix culture sample and P.E film was observed after 15 days that the earlier weight of plastic film was 0.0210mgm and after 15 days the weight of P.E film was 0.0208mgm. It observeredthat the weight was reduced upto 0.0002mgm. As well as the growth of sample cultured also observed. Growth of fungus (Sample cultured) increased which is turned in to black brown colour.

- **To study the process of degradation of PE by separate sample culture of various strains of fungi(With sucrose)**

Materials: Media with sucrose + separate Samples(A1, B1, B2, F2, F3, F4, F5) + PE film + conical flask.

* Sucrose has been added into the media in order to enhance the growth of fungus, which may faster the rate of degradation of PE. Sucrose also acts as a substrate for the growth of microorganisms.

Procedure:

For this experiment 50 ml media(with sucrose) has been taken in labeled separate seven conical flasks,inoculated with separate samples A1, B1, B2, F2, F3, F4, F5, plastic (PE film) has been introduced into each conical flask and Inoculation of sample and PE film were kept on observation for 15 to 30 days. Each culture sample and P.E film was observed after 15 days.It is observered that the weight was reduced. As well as the growth of sample cultured also observed. .(Merina Paul Das et al 2018) Growth of fungus (Sample cultured) increased and turned in to black brown colour. The observation table of |Reduced weight of PE and Growth of cultured given below in table 1.

- **Determination of Plastic Polymers Dry Weight which has been Degraded by Fungal Isolates-**

  To determine the dry weight of fungal-degraded polyethylene plastic films, they were washed with 70% alcohol and rinsed, then dried at 80oC until a consistent weight was attained.(Merina Paul Das et al 2018) The percentage of plastic weight reduction achieved was estimated using the following formula:

$$\% \text{ Plastic Weight Reduction} = \frac{R1-R2}{R1} X\ 100\%$$

Description: R1 = Initial Weight of Plastic Film (g)

R2 = Final Weight of Plastic Film (g)

RESULTS

In this study, isolation of potent fungi from Plastic contaminated soil collected from the NMC's garbage dumping station Nashik in Maharashtra and screening of polyethylene plastic-degrading fungi is carried out and from this study we get polyethylene degrading fungal strains.

1. **Growth of Microorganism** :

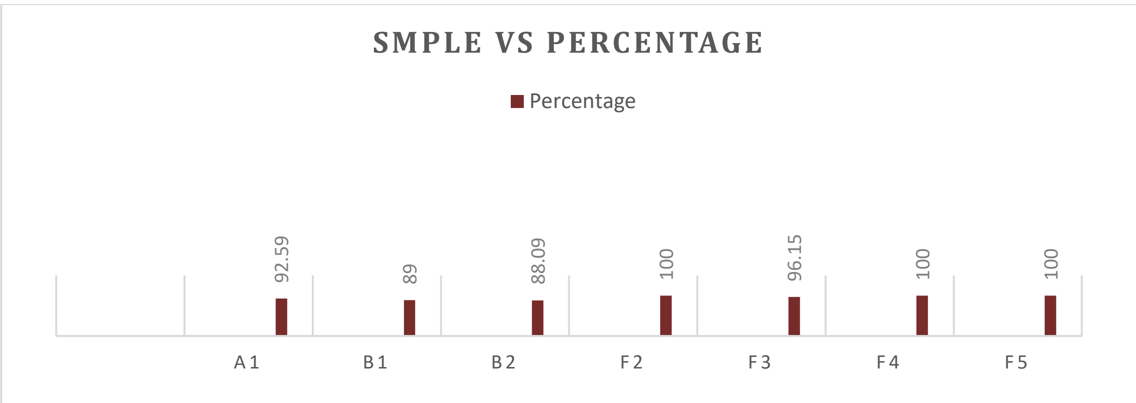
Samples	**with sucrose**	**without sucrose**	**Colour of the growth**
A1	+++	+	Black
B1	+++	+	Gray
B2	++	+	Yellow
F2	+	+	Yellow
F3	++	++	Yellow
F4	+++	+++	White
F5	+++	+++	Black

Table:2.1- Growth of Microorganism

2. P.E Growth without sucrose:

Sr No.	**Sample**	**Weight**			**Percentage**
		Before	**After**	**Difference**	
1	A1	0.0027	0.0025	0.0002	92.59
2	B1	0.0037	0.0033	0.0004	89
3	B2	0.0042	0.0037	0.0005	88.09
4	F2	0.0041	0.0041	0	100
5	F3	0.0026	0.0025	0	96.15
6	F4	0.0023	0.0023	0	100
7	F5	0.0027	0.0027	0	100

Table:3- The observation table of Reduced weight of PE and Growth of cultured fungal strains



Graph-1.1-The above graph is sample Vs percent reduction of weight loss of PE film. Threr is no reduction of PE film in F2, F4 and f5 samples. The percentage of reduction in F3 is very less. But the reduction in A1, B1, and B2 is good.

3. **P.E Growth without sucrose**:

Sr No.	Sample	Weight			Percentage
		Before	after	Difference	
1	A1	0.0048	0.0038	0.001	79
2	B1	0.0037	0.0033	0.0004	89
3	B2	0.0042	0.0042	0	100
4	F2	0.0032	0.0031	0.0001	97
5	F3	0.0041	0.004	0.0001	97.56
6	F4	0.0042	0.004	0.0002	95
7	F5	0.0035	0.0039	-0.0004	110

Table:4- P.E Growth without sucrose

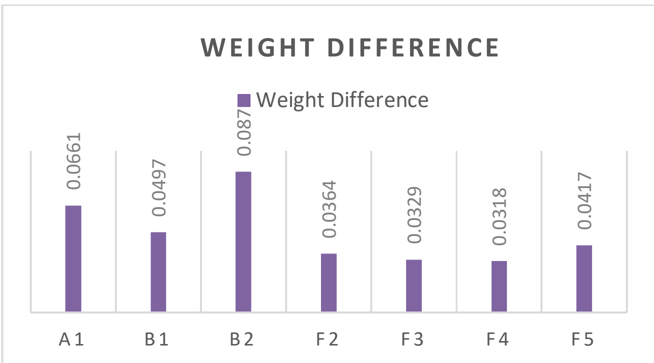


Chart:1.2- Weight Difference

4} (PE + separate sample/ strain A1, B1**)**

To study the Polythene degrading microorganisms with separate samples.

Sr No.	**Samples**	**Weight of P.E.**		
		before	**after**	**Difference**
1	B1	0.0037	0.0036	0.0001
2	B1	0.0037	0.0036	0.0001
3	A1	0.0035	0.0034	0.0001
4	A1	0.0036	0.0035	0.0001
5	Control	0.0034	0.0034	0

Table:5- Polythene degrading microorganisms with separate samples

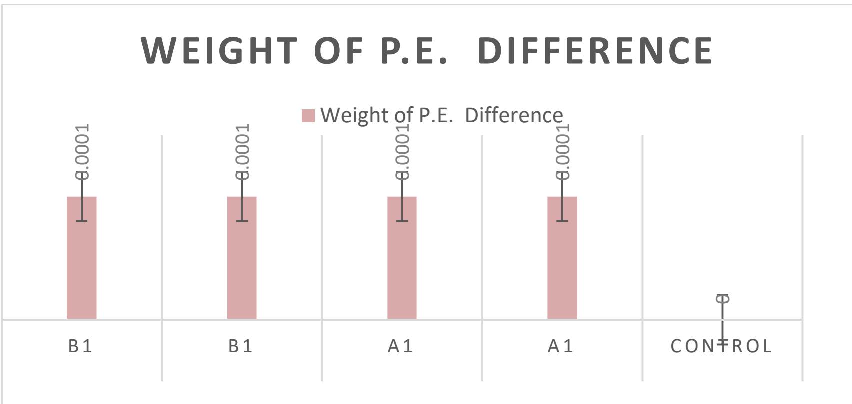


Chart:1.3-P.E microorganisms with separate samples

4.1 (P.E.)-Media + Fungal Samples A1 and B1 + P.E. (Polyethylene)

Sr No.	**Samples**	**Weight of P.E.**		
		before	**after**	**Difference**
1	B1	0.0037	0.0036	0.0001
2	B1	0.0037	0.0036	0.0001
3	A1	0.0035	0.0034	0.0001
4	A1	0.0036	0.0035	0.0001
5	Control	0.0034	0.0034	0

Table:6-(P.E.)-Media + Fungal Samples A1 and B1 + P.E. (Polyethylene)

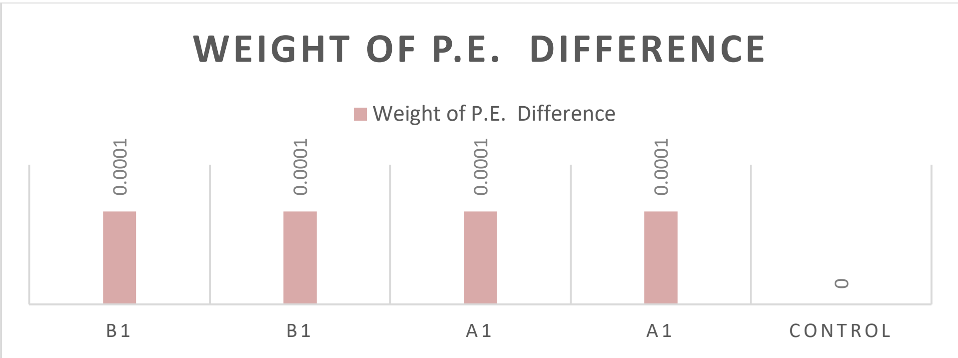


Chart:1.4- P.E.)-Media + Fungal Samples A1 and B1 + P.E. (Polyethylene)

4.2Growth of Hyphe in culture(PE)

Growth of Hyphe in a culture is removed by the process of filtration by muslin cloth and the amount of growth is measured.

Sr No.	Growth Sample	Weight of growth		
		before	after	Difference
1	B1	0.7443	0.7443	0.000
2	B1	0.5586	0.6538	0.0952
3	A1	0.6530	0.8106	0.1576
4	A1	0.6671	0.9073	0.2402
5	Control	0.6458	0.6644	0.0186

Table:7-**Growth of Hyphe in culture**

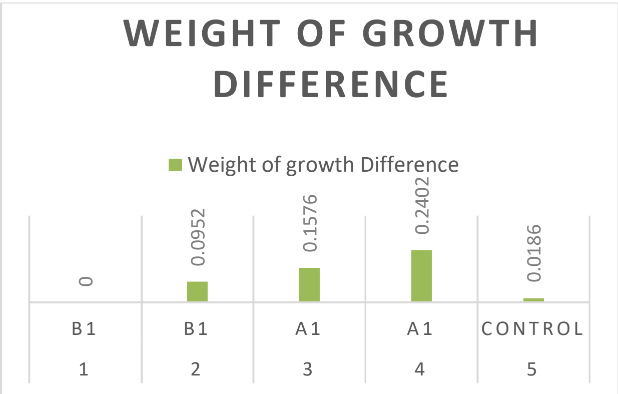


Chart-1.5- Growth of Hyphe in culture

DISCUSSION

The results of this study highlight the potential of fungal isolates in polyethylene (PE) plastic biodegradation. The research successfully isolated and identified several fungi from plastic-contaminated soil collected from NMC's garbage dumping station in Nashik, Maharashtra. These fungi were screened for their ability to degrade polyethylene, both in the presence and absence of sucrose, and their effectiveness was measured through weight reduction analysis.

The results demonstrated that certain fungal strains were more effective in degrading PE than others. In the absence of sucrose, the samples A1, B1, and B2 exhibited significant reductions in plastic weight, indicating their strong degradative abilities. However, samples F2, F4, and F5 showed no or minimal weight reduction, suggesting that these strains may not have the necessary enzymatic capabilities for PE degradation under these conditions. In contrast, when sucrose was introduced to the media, weight reduction was observed in most samples, with A1, B1, and F4 showing the highest degradation rates. This suggests that sucrose acted as a substrate to enhance fungal growth, thereby facilitating increased enzymatic degradation of PE films.

CONCLUSION

The present study successfully isolated and screened fungal strains capable of degrading polyethylene (PE) plastic from plastic-contaminated soil collected at the NMC's garbage dumping station in Nashik, Maharashtra. Through systematic experimentation, the degradation efficiency of different fungal strains was assessed under various conditions, including the presence and absence of sucrose.

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