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Biodegradation of Polythene Bag by *Aspergillus oryzae*

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The soil samples were collected from decomposed polythene bag disposal area. The collected samples were serially diluted and plate . The isolate fungal strains were identified based on their cultural morphological study. The fungal species was isolated and idendified namely *Aspergillus oryzae. A. oryzae* was subjected to polythene bag degradation based on the resistance capability. Various thickness of polythene bag were prepared. Polythene bag degradation abilities were observed by *A. oryzae* in soil and heavy metal analysis. In case if *A .oryzae* shows any loss of thickness and color, it was recorded after one month of incubation period. When size of the polythene bag was 0.5 to 5 mm it shows that fungi can be used in both natural and artificial conditions for the purpose of degradation of polymers and the microbes. Our study was mainly focus on microbes cause greatest degradation of polythene bag. Hence, the further attention is required from microbiologists for commercial degradation and eco-friendly polyethylene with *Aspergillus oryzae.* In case of *A. oryzae* loss of thickness was recorded after one month of incubation period when the size of 0.5 to 5mm. The highest degradation of polythene bag was noted in Pit-3(1.8mm). That indicate the *A.oryzae* moderately degrade the polythene .

Key words: Biodegradation, Polythene bags, *Aspergillus oryzae*.

Biodegradation is a process of chemical decomposition performed by living organisms, while microorganisms play a vital role in biological decomposition of materials, have assessed the biodegradability of some polymers by measuring changes in physical properties or enzymatic environments and by co_2 evolution (Ritmann, 2001). Polythene, commercially called as polythene, is a thermoplastic commodity mostly used for packaging goods. It is a polymer made up of long chain monomers of ethylene. Including packaging, it has got various other applications to mankind, the utility expanding at 12% per annum (shabir, 2008). About 140 million tones contribute for polyethylene manufacture(Shimao, 2001). American Society for Testing and Materials (ASTM) defines biodegradable Polythene as "a polyethylene that degrade because of the action of naturally occurring microorganisms such as bacteria, fungi and alger" and a compostable polythene as "a polythene that undergoes degradation by biological processes during composting to yield carbon dioxide, water, inorganic compounds, and biomass at a rate consistent to other known compostable materials and leaves no visually distinguishable or toxic residues" (Kale *et al.*, 2007).

Biodegradable polymer are designed to degrade upon disposal by the action of living organisms. Biodegradable polymers generally decompose in various medium in our environment.

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Table 1. Soil analysis

S. No	Soil Analysis	Types of Pits					
		Before			After		
		Pit1	Pit2	Pit3	Pit1	Pit2	Pit3
1	P^h	7	7	7	7	7	7
2	Temperature^{o}C	26^{o}C	34^{o}C	42^{o}C	26^{o}C	34^{o}C	42^{o}C
3	Nitrogen (mg)	12	13.5	16	11	12	14
4	Phosphorus (mg)	7.5	9.5	11.5	6.5	7.5	9.4
5	Potassium(mg)	5.2	6.9	7.8	4.8	5.6	6.2
6	Calcium(mg)	52	43	32	48	36	25
7	Magnesium(mg)	15.81	14.12	11.52	14.61	12.18	9.47
8	Zinc(Ppm)	3.22	3.13	3.15	3.20	3.11	3.12
9	Mercury (Ppm)	1.255	1.245	1.251	1.250	1.243	1.249
10	Lead (Ppm)	1.263	1.125	1.124	1.261	1.122	1.123
11	Copper(Ppm)	1.380	1.355	1.357	1.378	1.351	1.353
12	Cadmium(Ppm)	0.00137	0.00125	0.00012	0.00135	0.00122	0.000124
13	Iron (Ppm)	13.6	12.45	11.62	13.3	12.39	11.58

Table 2. Before and after degradation of pmolythene bag

S. No.	Size of Polythene bag	Thickness of Polythene bag			
		Before		After	
		25%	50%	25%	50%
1	0.5(mm)	0.35(mm)	0.1(mm)	0.3(mm)	0.09(mm)
2	1	0.4	0.3	6.7	0.1
3	1.5	1.1	0.5	1.3	0.3
4	2	1.9	0.7	1.7	0.4
5	2.5	2.0	1.2	1.9	0.8
6	3	2.6	1.5	2.4	1.1
7	3.5	2.9	1.5	2.4	1.1
8	4	3.0	1.7	2.7	1.3
9	4.5	3.2	2.1	2.9	1.6
10	5	3.7	2.5	3.2	1.9

The microbial species are associated with the degrading materials were identified as bacteria *(Psedomonas, Streptococcus, Staphylococcus, Micrococcus and Moraxella*) fungi (*Aspergillus oryzae Aspergillus niger*) and *Actinomycetes sp.* (Goeb and Knight 1982).

MATERIALS AND METHODS

Sample collection

Soil was collected from polythene bag disposed area at Vedharniyam, Nagoppattinam district in Tamil Nadu. The soil samples were collected at a depth of 3-5cm in a sterile container and then air dried at room temperature.

Isolation and Identification of polythene Degrading Microorganisms

One gram of soil sample was transport into a conical flask containing 99ml of sterile distilled water. This content was shaken and serially diluted. To isolate microorganisms associated with material (Polythene bag) by pour

plate method was adapted using the Rose Bengal agar for fungi. For each dilution, three replicates were made. The plates were then incubated at 30°c for 2-7 days. The developed colonies were isolated and sub cultured repeatedly to get pure colonies and then preserved in slant at 4°c. The fungus was identified after staining them with cotton blue by following the keys Raper and Fennel.

Microbial Degradation of Polythene bag under the pit formation

The polythene bag was prepared in various thickness in 0.5 to 5cm were aseptically transferred into the conical flask containing 100ml distilled water and then inoculated with identified polythene degrading microorganism *(Aspergillus oryzae*). Control was maintained with polythene bag in the microbes free pit. The pits was incubated at one mouth period. After the period of polythene bag were collected, washed through by using distilled water, shade dried and then weighted to check the final weight. Finally the weight loss of the polythene bags were calculated and compared with control.

Preparation of polythene bag

The polythene bag was prepared in various thickness from 0.5 to 5cm. *A.oryzae* plays a vital role in the degradation of polythene bag. Three pits were prepared in 1 fact dimension for the degradation of polythene bags. One pit acts as control which has no fungi. (Booth *et al.,* 2006).

Analysis of degradation study

The degradation study was analyzed in polythene bag at various thickness and the fungi was applied at various concentration in pit. Before and after 30 days incubation the following parameter's were analyzed. Determination of P^H was done by (Hansa Rostocki ,1954), Determination of Nitrogen was done by (Sakuma, 2006) , Estimation of Phosphorus was done by (Jorgensen ,1973) ,Determination of Potassium was done by (pengx *et al.,*2005), Calcium estimation using EDTA method was done by (Ferrer-Roca *et al.,1*997), Determination of iron using AOAC method (Waldbaum, Jane Bronze 1978), Determination of zinc using AOAC method was done by (Peter and Winch,1805), Estimation of mercury was done by(John Keller 1896), Estimation of lead and cadmium, Determination of copper.

RESULTS AND DISCUSSION

Aspergillus oryzae are the potential microbes to degrade polythene bag in the environment and keep clear. The present study deals with polythene bag degradation by using *A.oryzae.* Which was isolated from soil.

Before degradation of the pit 3 have highest level of (nitrogen 16mg, phosphorus 11.5mg, potassium 7.8mg). The pit 1 have highest level of (Calcium 52mg and Magnesium 15.8 mg). After degradation the nitrogen level was noted in pit1, pit2 and pit 3 are (11mg, 12mg and 14 mg) respectively. The phosphorus level was noted in pit1, pit2 and pit3 are (6.5mg, 7.5mg, 9.4 mg) respectively. The potassium level was noted in pit1, pit2 and pit3 are (4.7mg, 5.6mg and 6.2mg) respectively. The calcium level was noted in pit1, pit2, and pit3 are (48mg, 36mg and 25mg) respectively. The magnesium level was noted in pit-1, pit2, and pit-3 are (14.61mg, 12.48mg and 9.47mg) respectively. Among this study pit3 have highest level (Nitrogen 14mg, Phosphorus 9.4mg, potassium 6.2mg). Before degradation of pit 1 have highest level in (zinc 3.22ppm, mercury 1.255ppm, lead 1.263ppm, copper 1.380ppm, cadmium 0.00137 ppm and 13.6ppm). After degradation the Zinc level was noted in pit-1, pit2 and pit-3 was (3.20Ppm, 3.11Ppm and 3.12 Ppm) respectively. Among this study pit-1 have highest level in pit-1 (iron - 13.3Ppm and Zinc 3.20Ppm).(Table-1)

Determination of weight loss

Before degradation size of the polythene bag ranging from 0.5 to 5 and the thickness of polythene bag ranging from 3.7mm– 2.5% mm (Fig. 3. Table 3 and plate-III). After degradation size of polythene bag ranging from 0.5 to 5mm. And the thickness of polythene bag ranging from 3.2mm to 1.9mm(Fig. 4. Table 4 and plate-III). *Aspergillus oryzae* was more active degrading in 1.8mm level of polythene bag with in a month. This may be attributed to the thickness of the polythene bag is low (Table 2).

Our work was supported by Kathiresan. 2003 and Ji-Chul Jang *et al.,* 2002. In soil these are known to be ubiquitous and are generally found in soil including garbage as well as mangrove soil. In case of *Aspergillus oryzae* loss of thickness was recorded after one month of incubation periodwhen the size of 0.5 to 5mm. The highest degradation of

polythene bag in Pit- 3 (1.8mm). That indicate the *A. oryzae* moderately degrade the polythene bag. Same result reported in degradation of polyurethane by (Goeb and Knight 1982).Some previous reports showed the degradation of petroleum–degrading microorganism (bacteria, yeast, and fungi) by direct plating and enrichment culture, (Springer 1974). Degraded solid polyester PUR, with diethyleneglycol and adipic acid released as the degradation products. The optimum PH for this enzyme 6.5, and optimum temperature was 45°C.

CONCLUSION

Plastic pollution has emerged as one of the most challenging problems of the mankind, and can be satisfactorily addressed through biodegradation. From the study, it was concluded that *A.oryzae* degrade the polythene materials effectively. Thus the *Aspergillum species* have efficient to degrade the polythene bag materials. This method was cheap and effective, so that it can be used widely for the treatment of polythene bag.

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