A STUDY ON DEGRADATION OF POLYTHNE BAGS USING SOIL MICROORGANISM

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ABSTRACT*- Plastics are the polymers of non-metallic mouldable compound. The most commonly used non degradable solid waste is polythene, extensively used in packaging purpose. Every year 25 million tons of synthetic plastics are being accumulated in the environment. Due to burning of solid waste releases harmful toxic material which is a major pollutant to environment so reduce that problem by the influence of degrading capacity of soil organism against non-degradable polythene. In the present study, to investigate the biodegrading ability of *Aspergilus niger*and *pseudomonas* isolated from soil and identified by using several biochemical test. The extents of biodegradability of the plastic by isolated bacterial strains were assessed in vitro in the separate liquid medium containing polythene bags. After 45 days incubation period, the biodegradation was measured in terms of weight loss.Incubation of untreated polythene with *Aspergilus niger*and *Pseudomonas* reduces its mass from 0.010 to 0.005 and0.0080respectively. As a result, the smooth surface of untreated plastic is become rough with cracking that indicates biodegradation of plastics.Finally, an attempt has been successfully made to determine the plastic degrading ability of *Aspergilus niger*and *Pseudomonas sp.

Key words: **Polythene, In vitro, Microbial biodegradation, soil.**

I. INTRODUCTION

Matter is destroyed and created all the time. But if there is anything that goes against this rule, it can create havoc to the entire universe. One such matter is PLASTIC. Plastics are characteristically inert and resistant to microbial attack and therefore they remain in the nature without any deformation for very long time (G. Gnanavel et.al, 2012).

The contamination of soil due to dispersal of industrial waste generated by human activities is of great environmental concern. (Ghosh M *et al.*, 2005)About 300 years old, cleaning the contaminated environment using plants by phytoremediation concept that is the plants have a capacity to convert the toxic compounds to non-toxic forms. One of the major environmental threats is the slow rate of degradation of the organic materials under natural condition, e.g. Plastics. (Hartman WJ, 1975) The various forms of plastics are nylon;polyethylene (LDPR, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PST), polycarbonate (PC), polytetraflouroethylene (PTFE), polyurethane (PUR) and polyvinyl chlorides (PVC) are being continuously used in our day-to-day life. The general formula of polythene is C_n2_n. (Smith, 1964)

Polythene has a wide range of applications in human's daily use. Among the synthetic plastics waste produced in environment, polythene constitutes about 64%. Huge quantity of plastic used for manufacturing of bottles, carry bags, milk jugs, packaging of food articles, textile product and laboratory instruments. (Arutchelvi *et al.*, 2008 and Lee *et al.*, 1991)This utilisation is still expanding at a high rate of 12% per annum (AribaBegam, 2015) that is annually 500 billion to 1 trillion polythene bags are being used routinely all over the world. From this report we conclude the most commonly used non-degradable solid waste is polythene which is a linear hydrocarbon polymer. With such huge amount of polythene getting accumulated in the environment and their disposal evokes a big ecological issue.(Roy *et al.*, 2008)

The widely used packaging plastic constitutes about 10% of the total municipal waste generated around the globe. (Barnes *et al.*, *2009*) Only a fraction of plastic waste is recycled whereas most of the waste enters into the landfill and takes a hundreds of years to degrade. The plastic could sometimes cause blockage in intestine of fish, birds and mammals due to consumption of plastic carry bags in the oceans. (Moore, 2008 and Yang et al., 2004) From this, an estimate 1 million birds and 10,000 marine animals die each year as a result of above said reason. Degradation of plastic is a great challenge as the materials are increasingly used. (Singh, 2005)

Recently, to employ the physico-chemical disposal methods like pH, temperature and mechanical damage used and they often cause environmental problems. Alternatively, the modern eco-friendly technologies develop microbial degradation that is usage of microorganisms that has gained notable success because inefficiency of above physicochemical methods. (Swift, 1997)

Consequently, in the present study is to investigate the biodegradability of plastic bags by *Aspergilus niger* and *Pseudomonas spp*, microorganisms isolates from plastic contaminated soil by pure culture shake flask method. The percentage of degradation was evaluated by comparing initial and final dry weight of plastic bags before and after incubated in media respectively.

II. MATERIALS AND METHODS

1. COLLECTION OF SOIL SAMPLE

Soil samples were collected from different regions. These samples were taken in sterilized polythene bags using sterilized spatula and stored at 4° C until examination. The pH of these samples was checked by using pH meter.

2. ISOLATION OF MICROORGANISMS

The soil samples were serially diluted in saline solution (0.9% NaCl). The suspension from 10-5 and 10-6 dilutions were inoculated on Nutrient agar plate for isolating bacteria and incubated at 37°C for 48 hrs.Individual colonies of bacteria which varied in shape and colour were picked up and purified by streaking on nutrient agar. The bacterial isolates were kept on nutrient agar at 4°C and re-cultured every three days.

3. IDENTIFICATION OF MICROORGANISMS

The bacterial isolates were identified on the basis of classification schemes published in (Krieg and Holt, 1984). Among this bacterial isolates, the plastic degrading microorganism were used in this study.

3.1. Grams Staining

The gram staining of bacteria was done as per the procedure published by Bergey's Manual of Systematic Bacteriology. A thin smear was prepared on a clean slide using the isolated individual colony. The smear was heat fixed and dried. The dried smear was then flooded, with the primary stain, crystal violet solution and allowed to stand for one minute. Then it was washed with water and flooded with Gram's iodine solution and allowed to stand for minute. The slide was again washed with water and decolorized with 95% ethanol for few seconds and washed with running tap water. Then the slide was flooded with a counter stain, saffranin for 1minute. After drying the stained smear was observed under microscope to identify the organisms. The colonies were stained by this method, in order to identify the morphology and gram's reaction of the bacterium.

3.2. Fungal Staining

It is used to demonstrate the fungus and its spores. Using sterile technique, a portion of fungal colony was kept on drops of lacto phenol cotton blue on slide. Using needles, it was well teased and a cover slide was kept on it without air bubbles. Excess stain was removed using blotting paper and observed under microscope.

3.3. Motility test

The motility bacteria were done as per the published by Bergey's Manual of Systematic Bacteriology.The motility was studied by employing hanging drop method. A loop full of organism was

inoculated in to a broth and incubates it for 24 hours. A drop of culture broth was placed on the centre of the cover slip. A pinch of Vaseline was applied over each corner of the cover slip. Then a cavity slide was placed in an inverted position on the cover slip and the slide was observed under the microscope.

3.4. *Biochemical Tests:*

The biochemical tests for the bacterial isolates were done as per the procedure published by Bergey's Manual of Systematic Bacteriology.Theisolated organisms were subjected to biochemical test for identification. The biochemical tests includes – starch hydrolysis, Catalase test, Oxidase test, Citrate Utilization test, Indole test, Methyl red Test, Voges - proskauer test, Triple sugar iron test, Urease test and Nitratereductase test.

4. MICROBIAL DEGRADATION OF POLYTHENE PIECES IN THE LIQUID MEDIA

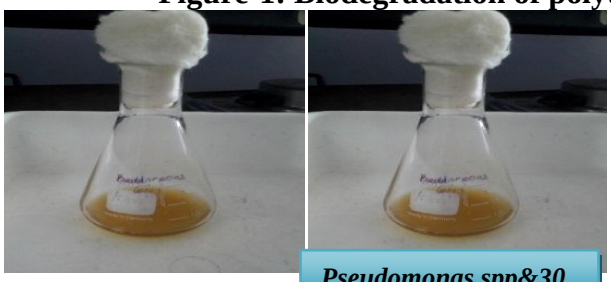
4.1. Surface Sterilization of Polythene

Polythene bags of thickness 30 micron and 60 micron were cut in to 2cm diameter. These pieces were thoroughly rinsed with tap water and then distilled water. Dry these pieces under room temperature for three hours. Then take the initial weight of the polythene pieces.

4.2. Inoculation of microorganisms

A 400ml of Nutrient liquid broth were prepared and autoclaved at 121^0 C for 15 min. 100ml nutrient liquid broth was poured into four 250ml conical flasks. The sterile pre weighed plastic pieces were aseptically transformed into respective medium. A loopful of *Pseudomonas* spp *and Aspergilus niger* was inoculated into respective medium. These flasks were incubated at 37^0C for 15, 30, and 45 days in mechanical shaking incubators for even growth of microorganisms.

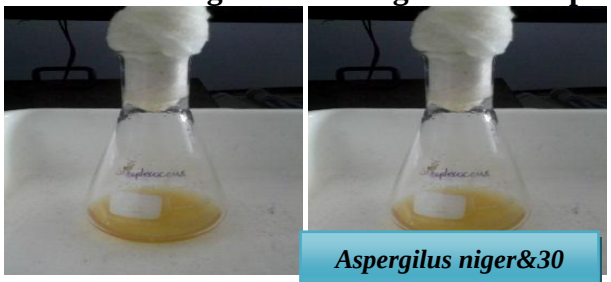
Figure-1: Biodegradation of polythene pieces in liquid media contain bacteria



Pseudomonas spp&30 micron thickness

Pseudomonas spp& 60 micron thickness

Figure-2: Biodegradation of polythene pieces in liquid media contain fungi



Aspergilus niger&30 micron thickness

Aspergilus niger& 60 micron thickness

4.3. Recovery

The polythene pieces were carefully removed from the media, after days of incubation. The collected pieces were washed with tap water and drying in oven. Now measure the final weight. Theloss in weight shows the activity of microorganisms on the polythene pieces. The same procedure was also repeated for 30 and 45 days of incubation.

4.4. Determination of Degradation of Plastics:

The percentage of degradation of plastic pieces by *Pseudomonas* spp *and Aspergilus niger* weredetermined by calculated the percentage of weight loss of plastics. The percentage of weight loss wascalculated by the following formula

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

III. RESULTS

1. ANALYSIS OF SOIL PH

The pH of the soil sample solution was checked by using the pH meter.
The pH of soil is nearly neutral. (7.1)

2. ISOLATION OF SOIL MICROORGANISM

A total of 2 different species were isolated. The bacterial isolates were purified and identified at the genus level by standard procedures described in Bergey's manual of systematic bacteriology. The fungal species isolated was *Aspergillusniger.* Among the bacterial strains *Pseudomonas*were isolated from the soil.

Table 1: Biochemical characteristics for fungal species

Organism / Tests	Aspergillusniger
Fungal staining	Hyphae and Conidio spores were observed
Starch hydrolysis	Positive

Table 2: Biochemical tests results for bacterial species

Test	Morphology	Staining	Catalase	Oxidase	Citrate	Indole
Pseudomonas	Rod	-ve	+ve	+ve	+ve	-ve

Table 3: Biochemical tests results for bacterial species

Test	Methylred	Vogesproskauer	Triple sugar iron	Nitrate reductase	Motility test
Pseudomonas	-ve	-ve	-ve	-ve	Motile

3. ROLE OF SOIL MICROORGANISMS IN DEGRADATION OF POLYTHENE PIECES

3.1. Biodegradation of 30 micron thickness plastic

The initial weight of polythene (30 micron) was 0.010gm.

The degradation of plastics by *Aspergillusniger* exposed at 15 days of incubation showed 20.0% of weight loss. Similarity degradation of 30 and 45 days of incubation showed 30.0%and 50.0% respectively.

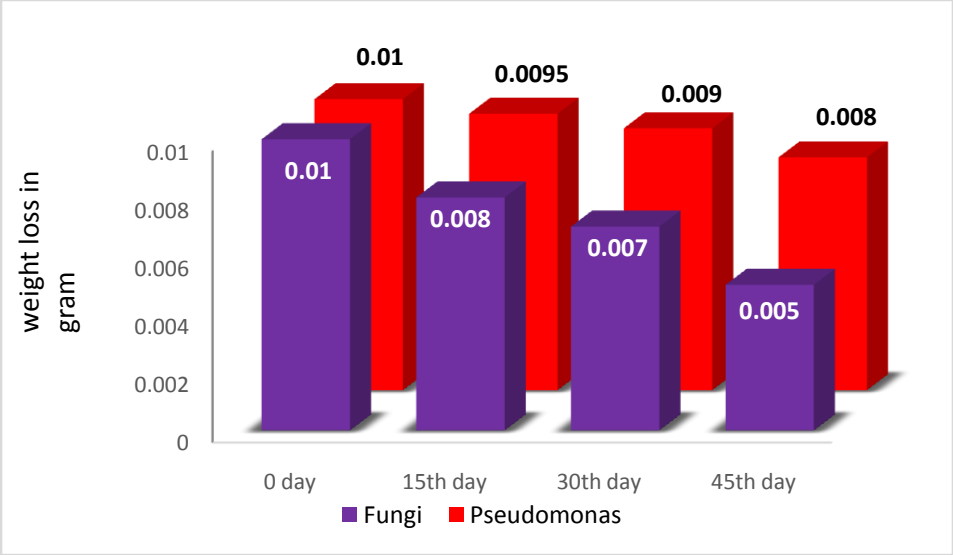
The degradation of plastics by *Pseudomonassp* exposed at 15 days of incubation showed 5.0% ofweight loss. Similarly degradation of 30 and 45 days ofincubation showed 10.0% and 20.0% respectively.

Table 4: Biodegradation of polythene bags (30 micron)

S.NO	Time interval / Organisms	0th day	15th day	30th day	45th day
1.	*Aspergillusniger*	0.01 (0.0%)	0.008 (20.0%)	0.007 (30.0%)	0.005 (50.0%)
2.	*Pseudomonas*	0.01 (0.0%)	0.0095 (5.0%)	0.009 (10.0%)	0.0080 (20.0%)

The results showed that degradation of polythene is relatively faster in fungi than that of other microorganisms.

Chart 1: Biodegradation of polythene pieces (30 micron)



3.2. Biodegradation of 60 micron thickness plastic

The initial weight of polythene pieces (60 micron) were 0.018gm.

The degradation of plastics by *Aspergillusniger* exposed at 15 days of incubation showed 11.1% of weight loss. Similarity degradation of 30 and 45 days of incubation showed 22.2%and 33.3% respectively.

The degradation of plastics by *Pseudomonas sp* exposed at 15 days of incubation showed 1.60% ofweight loss. Similarly degradation of 30 and 45 days ofincubation showed 3.3% and 5.5% respectively.

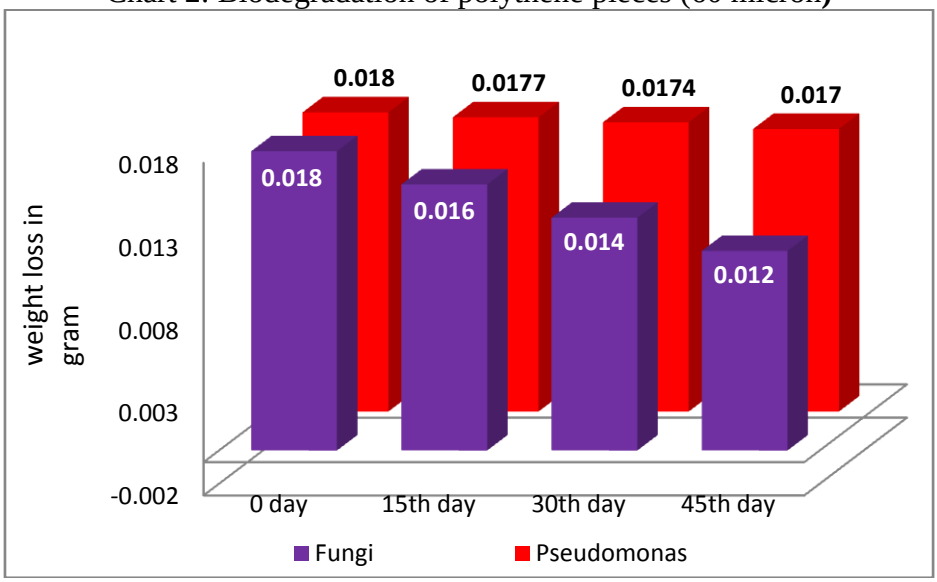
Table 5: Biodegradation of polythene pieces (60 micron)

S.NO	Time intervals / Organisms	0th day	15th day	30thday	45th day
1.	*Aspergillusniger*	0.018 (0.0%)	0.016 (11.1%)	0.014 (22.2%)	0.012 (33.3%)
2.	*Pseudomonas*	0.018	0.0177	0.0174	0.017

		(0.0%)	(1.60%)	(3.3%)	(5.5%)

The results showed that degradation of polythene is relatively faster in fungi than that of other microorganisms.

Chart 2: Biodegradation of polythene pieces (60 micron**)**



IV. DISCUSSION

Plastics are non-metallic moldable compounds and the materials made from them, can be pushed into almost any desired shapes and then retain that shape. (Seymour, *et al.*, 1989&Suman*et al.*,2014)Plastics being xenophobic compounds resistant to degradation constitute about 5-8 percent of dry weight of municipal solid waste the instrumental effects of these polymers on the environment, range from ozone depletion to the environmental toxicology of agriculture and aquatic ecosystem. (Derraik, 2002 &Sivasankari, *et al.*, 2014)

In the present study an attempt have been made to evaluate the biodegradation of plastic bags by bacteria (*Pseudomonas sp) and* fungi *(Aspergillus niger)* were identified from the soil collectedfrom different area land.The bacteria caused the biodegradation of plastic ranging from 19.6% to 32%. Among the bacteria *Pseudomonas sp* was found most active in degrade 20% and 5.0 % of 30 micron and 60 micron thickness plastics pieces respectively. The fungi caused the biodegradation of plastic ranging from8.8% to 27.4%. Among the fungi *Aspergillus niger*were found most active in degrade 50% and 33.3% of 30 micron and 60 micron thickness plastics pieces respectively.

In the present study the *Aspergillus niger* was formal most active in degrading of plastic bags, when compare with *Pseudomonas sp*.Degradation of plastics was determined by the weight loss of sampleand bacteria activity in media.

The present study reveals that longer time tobe needed for the biodegradation of polythene than plastics; the results shows that *Aspergillus niger*degrade 50%plastic bags in 45 days, similarly *Pseudomonas sp* degrade 20% of plastic bags in 45 days only.

Finally this reveals a general technical idea for biodegradation of plastics by microorganisms and reduces the plastics waste in the environment.

V. SUMMARY AND CONCLUSION

In this present study, the microorganism involved in the biodegradation of polythene pieces of 30 micron and 60 micron were identified as *Aspergillus niger* and *Pseudomonas sp* by various biochemical tests. These microorganisms were separately allowed to degrade the polythene pieces in laboratory condition. The microorganisms were grown in nutrient broth along with polythene pieces and that was kept in mechanical shaker for 45 days.

Among these microbes the highest biodegradation of polythene pieces ranging from 50% for polythene of 30 micron thickness and from 33.3% for polythene of 60 micron thickness during the duration of 45 days.The polythene of 30 micron thickness shows more degradation than the polythene of 60 micron thickness.

Among the microorganism, fungi show the highest degradation than other microorganism, followed by *Pseudomonas sp.*This study concluded that the bacterial biodegradation of polythene pieces of 30 micron is faster than the polythene pieces of 60 micron thickness. Further Fungi shows the highest biodegradation of polythene pieces of both 30 and 60 micron.

Plastics are one of the major threats to the environment. Studies are made all over the world are to degrade the polymer or bring out a biodegradable plastic. There are some plastics which resist degradation and some degrade to certain extent. (Kavitha, *et al.*, 2014) Some of them remain as persistent organic pollutant (POP).

The studies are about the degradation of different kinds of plastic in biological means and other means. The plastics which were studied are polyethylene.Bacterial and fungal species are used widely for degradation. The organisms which degrade the hydrocarbon in the plastics and use them as carbon source can be employed. Through these studies degradation of plastic can be made effective.

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