

Research Article

In Vitro Degradation of Plastics (Plastic Cup) Using *Micrococcus Luteus* and *Masoniella Sp*

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Abstract: Plastic is a broad name given to different polymers with high molecular weight, which can be degraded by various processes. However, considering their abundance in the environment and their specificity in attacking plastics, biodegradation of plastics by microorganisms and enzymes seems to be the most effective process. When plastics are used as substrates for microorganisms, evaluation of their biodegradability should not only be based on their chemical structure, but also on their physical properties (melting point, glass transition temperature, crystallinity, storage modulus etc.). Present paper investigates the possibility of plastic degradation by microbes isolated from forest soil. The invitro degradation was studied by litter bag experiment by taking 1 g of each plastic and buried under forest soil at a depth of 15 cm from the surface during the month of September to February, 2010. An in-vitro experiment was started after collecting the plastic samples from the litter bag experiment and the microbes were isolated from the surface of the plastic. Then the isolated microbes inoculated in the nutrient agar. Result showed that no variety of plastic comfortable degraded under burial condition during 45 days. In the present study, plastics are highly resistant to degradation of plastics by using microorganisms is a great challenge. Hence an attempt has been made to determine the plastics degrading ability of *Micrococcus luteus* and *Masoniella sp*.

Keywords: Poly ethylene, in vitro, biodegradation, enzymatic degradation, bio-based plastics, microbial degradation

INTRODUCTION

Plastics are defined as the polymer (Solid materials) which became mobile on heating and thus can be cast into moulds. Plastics are non-metallic moldable compounds and the materials made from them, can be pushed into almost any desired shape and retain that shape [1]. They are durable and degrade very slowly These are highly to biodegradation, leading to pollution and harmful to the natural environment.

Plastic materials have been increasingly used in Food clothing shelter, transportation, Construction, medical and recreation industries. Plastics are advantageous as they are strong, light weighted and durable. However, they are disadvantageous as they are resistant to biodegradation leading to pollution and harmful to the natural environment. In the past 10 years, several biodegradable plastics have been introduced in the market. However, none of them is efficiently biodegradable in landfills. For this reason, none of the products has gained widespread use [2].

Biodegradable plastics opened the way for new consideration of waste management strategies since these materials are designed to degrade under

environmental conditions or in municipal and industrial biological waste treatment facilities [3]. A number of biodegradable polyesters, namely Polyhydroxyalkanoates (PHA), polylactides, aliphatic polyester, polysaccharides and copolymer or blends of the have been developed Successfully to meet Specific demands in various fields and industries [4].

MATERIALS AND METHODS

Collection of sample

Polystyrene Collection

Plastics cup were collected from waste disposal in and around Thanjavur.

Soil Collection:

The samples are collected from the site dumped with large amount of plastic waste disposal in the area of Thanjavur.

Methodology

Inoculum Preparation

Nutrient Broth

Beef extract	- 3 gm
Peptone	- 5 gm
Sodium chloride	- 5 gm
Distilled water	- 10000 ml

Potato Dextrose Broth

Potato (peeled) - 200 gm
Dextrose - 20 gm
Distilled water - 1000 ml

Processing of Soil Sample:

The soil samples were serially diluted as per the method of Aneja [5]. The suspension from 10^{-5} and 10^{-6} dilutions were inoculated on Nutrient agar plate for the isolation of bacteria. The suspension from 10^{-2} and 10^{-3} dilutions were inoculated on potato dextrose agar medium for the isolation of fungi. Both the plates were incubated for 24 hrs and respectively.

Identification of Bacterial Isolates

Gram Staining

The gram staining of bacteria was done as per the procedure given by Aneja [5]. The colonies were stained by staining method, in order to identify the morphology and gram's reaction of the bacterium. 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. Then it was washed with water and flooded with Gram's Iodine solution and allowed to stand for 1 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, safranin for 1 minute. After drying the stained smear was observed under microscope to identify the organisms.

Motility Test: (Hanging Drop method).

The motility bacteria were done as per the procedure described by Aneja [5]. The motility was studied by employing hanging drop method. A drop of culture both 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.

Biochemical Tests:

The biochemical tests for the bacterial isolates were done as per the procedure described by Aneja[5]. The isolated organisms were subjected to biochemical test for identification. The biochemical tests includes - Indole test, Methyl red Test, Voges - proskauer test, Citrate Utilization test, Catalase test, Oxidase test and Triple sugar iron test .

Identification of Fungal isolates:

The fungal isolates were identified as per the procedure described by Aneja [5]. The fungal isolate that were obtained in potato dextrose agar plates were identified by lactophenol cotton blue staining.

Lactophenol cotton blue is a stain commonly used for making semi permanent microscope preparation of fungi. It stains the fungal cytoplasm and provides a light blue back ground against which the walls of hyphae can readily be seen. It contains four constituent's namely phenol- which serves cotton blue) and a cover glass was placed over the preparation which was then ready for microscopic examination.

Requirements:

A Young culture of *Masoniella sp*, Lactophenol cotton blue in a dropper bottle mounted needles, Glass slides, Cover slips, Bunsen burner, 2% alcohol, Nail polish, and Microscope.

Procedure:

- A drop of lactophenol cotton blue was placed on a clean glass slide.
- A small test of fungus, probably with spores and spore bearing structures was transferred into the drop, using a flamed, cooled needle.
- Then the material was gently teased using the two mounted needles.
- Then the stain was gently mixed with the mold structure.
- A cover slip was placed over the preparation by avoiding air bubbles.
- Finally, the slide was observed under the microscope.

Microbial Degradation of Plastics:

Surface Sterilization of Polystyrene

The degradation of plastics by microorganisms were studied by following the method of Kathirasan [6]. The collected plastics cups were cut into small pieces. The pieces were cleaned with tap water (to remove dust particles), Surface sterilized with ethanol and again washed with distilled water. They were weighed about 1g one and used for both test and control.

Media Preparation:

1000ml of Nutrient broth Potato dextrose were prepared and autoclaved at 121°C for 15 min. 250ml of cooled. Nutrient broth and potato dextrose broth was poured into four 500ml conical flasks. The sterile pre weighed plastic pieces were aseptically transformed into respective medium. A loopful of *Micrococcus Luteus* a *Masoniella* was inoculated into respective medium. A 250 ml of flask containing only the plastics were maintained as control. These flasks were incubated at 37°C for 35, 45, and 55 days in shaking incubators.

Recovers:

The Plastic pieces were carefully removed from the culture (by using forceps) after days of incubation. The collected pieces were washed thorough with tap water, ethanol and then with distilled water. The pieces were shade diced and weighed for final weight. The data

were recorded. The same procedure was also repeated for 45 and 55 days of incubation.

Determination of Degradation of Plastics:

The percentage of degradation of plastic pieces by *Micrococcus Luteus* and *Masoniella SP* were determined by calculated the percentage of weight loss of plastics. The percentage of weight loss was calculated by the following formula.

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

RESULTS

Isolation and identification of *Micrococcus Luteus* and *Masoniella sp*

The Microorganisms used for the present study includes *M.Luteus* and *Masoniella sp*. The *Micrococcus luteus* and *Masoniella sp* were isolated from the soil sample and were identified by using biochemical test. The results were presented of biochemical tests of bacteria in Table 1. The results of *Masoniella sp* identified by lactophenol cotton blue staining were presented in Table2.

Degradation of plastics by *Micrococcus Luteus*:

The results of degradation of plastics by *Micrococcus Luteus* were presented in Table .3. The results clearly showed that *Micrococcus luteus* were able to degrade plastics.

Degradation of plastics by *Masoniella sp*:

The results of degradation of plastic by *Masoniella sp* were presented in Table 4. The results clearly showed that *Masoniella sp* were able to degrade plastics.

Biodegradation:

The degradation of plastics by *M.Luteus* exposed at 35 days of incubation. Showed 19.6% of weight loss. Similarly degradation of 45 and 55 days of incubation showed 27.6% and 32 % respectively.

The degradation of plastics by *Masoniella sp* exposed at 35 days of incubation showed 88% of weight loss. Similarly degradation of 45 and 55 days of incubation showed 17.8% and 27.4% respectively.

Table-1: Morphological characteristics of bacteria

Colony Observation						
shape	Margin	Elevation	Size	texture	Appearance	pigment
circular	Entire	Flat	Small	Smooth	Glistening	Non Pigmented

Table- 2: Identification of Bacteria

Tests	<i>Micrococcus Luteus</i>
Gram Staining	(+)
Molility Test	Nonmotile
Indole Test	(-)
Methy 1 Red Test	(-)
Voges – Proskauer Test	(+)
Citrate Utilization Test	(-)
Catalase Test	(+)
Onidase Test	(-)
Triple Sugar Iron Test	Alkaline Slant

Table-3: Identification of *Masoniella sp*

Colony morphology	Microscopic
Colonies growing on laboratory media, dark or white	Vegetative hyphae, dematiaceous, phialides and irregular, conodia dry, born in long chains, pyriform in shape

Table-4: Biodegradation of plastics by *Micrococcus luteus*.

S.No	Bacteria Used	Duration exposed	Initial weight of plastic	Final Weight	% of Weight
1.	<i>M.Luteus</i>	35 days	1.000 gm	0.804	19.6%
2.	<i>M.Liteus</i>	45 days	1.000 gm	0.724	27.6%
3.	<i>M.Luteus</i>	55 days	1.000 gm	0.680	32%

Table- 5: Biodegradation of plastics by using *Masoniella sp*

S.No	Fungi used	Duration exposed	Initial weight of plastic	Final Weight	% of Weight loss
1.	<i>Masoniella</i>	35 days	1.000 gm	0.912	8.8%
2.	<i>Masoniella</i>	45 days	1.000 gm	0.822	17.8%
3.	<i>Masoniella</i>	55 days	1.000 gm	0.726	27.4%

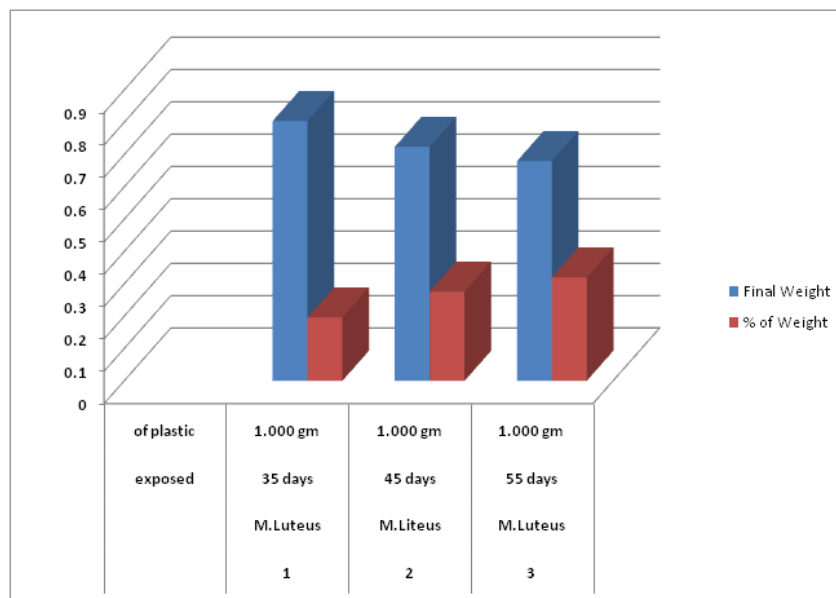


Figure-1: Biodegradation of plastics by *Micrococcus luteus*.

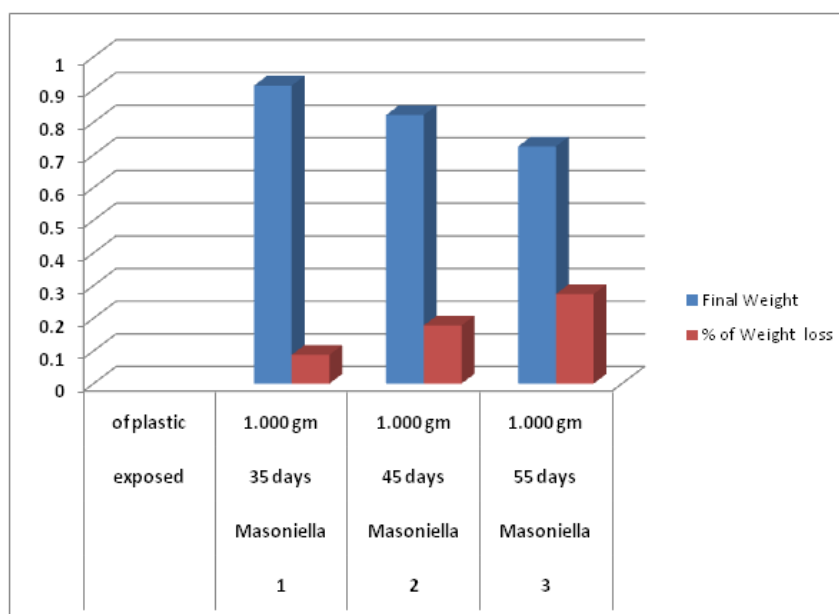


Figure- 2: Biodegradation of plastics by using *Masoniella sp*

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 [1]. 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.

In the present study an attempt have been made to evaluate the biodegradation of plastic cup by using bacteria (*Micrococcus luteus*) and fungi (*Masoniella sp*) were identified from the soil collected from plastic buried land.

The bacteria caused the biodegradation ranging from 19.6% + 32% for plastics among the bacteria *Micrococcus luteus* were found most active in degrade 38% of plastics.

The fungi caused the biodegradation ranging from 8.8% to 27.4% for plastics. Among the fungi *Masoniella sp* were found most active in degrading 27.4% of plastics.

In the present study the *Micrococcus luteus* was found most active in degrading of plastic cup, when compared with *Masoniella sp*.

Degradation of plastics was determined by the weight loss of sample, tensile strength, carbon dioxide production. Chemical changes measured infrared spectrum and bacteria activity in soil. The results can be ranked in order of decrease in susceptibility.

The present study reveals that longer time to be needed for the biodegradation of polythene than plastics, the results show that *M. Luteus* degrades 32% plastic cup in 55 days, similarly *Masoniella sp* degrades 27.4% of plastic cup in 55 days only.

CONCLUSION:

In the present study an attempt has been made to assess the biodegradation of plastics in laboratory condition using the bacteria, fungi viz, *Micrococcus Luteus* and *masoniella sp*, for which, soil sample, plastic cup were collected from plastic buried land in and around Thanjavur.

Serial dilution plating technique was made to isolate the bacteria and fungi Gram's staining and Biochemical tests have also been done. Identification of the bacteria was done by using Bergy's manual of determinative bacteriology. Identification of fungi was

done by lactophenol cotton blue staining method. Plastics to be degraded were surface sterilized with 0.1% mercuric chloride and washed twice with distilled water.

For bacterial degradation 24 hrs culture of *Micrococcus luteus* and *Masoniella sp* were inoculated into the flask containing 250ml of sterile nutrient broth, 1.000gm of plastic cup were transferred into separate flask control was maintained without bacterial culture. Inoculated flask were placed in shaking incubator for 35, 45 and 55 days.

After the incubation, plastic pieces were collected and surface sterilized with ethanol. The percentages of weight loss in plastic pieces were measured.

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