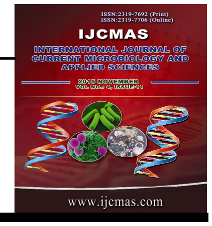

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Original Research Article

Biodegradation of Polythene Bag using Bacteria Isolated from Soil

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A B S T R A C T

Keywords

Soil Bacteria, Biochemical, Biodegradation, Polythene Bag

In the present investigation the soil bacteria were isolated from plastic contaminated soil sample. The bacterial isolates such as *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* were identified by morphological and biochemical characterization. The biodegradation efficacy of *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* by using polythene bag were studied. The *Pseudomonas alcaligenes* was found to be more effective than *Desulfotomaculum nigrificans* in degradation of polythene bag at 30 days. An increase in incubation period there is a dramatic increase in weight loss of polythene bag.

Introduction

One of the major environmental threats is the least rate of degradation or non biodegradability of the organic materials under natural condition, e.g. plastics. The plastics of various forms such as nylon, polycarbonate, polyethylene-terephthalate, polyethylene, polypropylene, polystyrene, polytetraflouro ethylene, polyurethane and polyvinyl chloride (Smith, 1964) are being continuously used in our day-to-day life.

Polythene finds a wide range of applications in human's daily use because of its easy processing for various products used for carrying food articles, for packaging textiles, for manufacturing laboratory instruments and automotive components (Arutchelvi *et al.*, 2008). Polythene constitutes 64% of the total synthetic plastic as it is being used in huge quantity for the manufacture of bottles, carry bags, disposable articles, garbage containers, margarine tubs, milk jugs, and water pipes (Lee *et al.*, 1991). Polyethene bags are made of polyethylene. The synthetic polymers are high hydrophobic level and high molecular weight. Annually 500 billion to 1 trillion polythene bags are being used routinely all over the world.

The worldwide utility of polyethylene is expanding at a rate of 12% annum and approximately 140 million tones of synthetic polymers are produced worldwide each year (Roy *et al.*, 2008; Vatseldutt and Anbuselvi, 2014). With such huge amount of polyethylene getting accumulated in the environment and their disposal evokes a big ecological issue.

To the marine life polythene waste is recognized as a major threat. Sometimes, it could cause intestinal blockage in the fishes, birds and marine mammals (Spear *et al.*, 1995; Secchi and Zarzur, 1999; Denuncio *et al.*, 2011). Due to plastic pollution in the marine environment minimum 267 species are being affected which includes all mammals, sea turtles (86%) and seabirds (44%) (Coe and Rogers, 1997). The death of terrestrial animals such as cow was reported due to consumption of polythene carry bags (Singh, 2005).

The widely used packaging plastic (mainly polythene) constitutes about 10% of the total municipal waste generated around the globe (Barnes *et al.,* 2009). Only a fraction of this polythene waste is recycled whereas most of the wastes enter into the landfills and take hundreds of years to degrade (Lederberg, 2000; Moore, 2008). The use of polythene is increasing every day and its degradation is becoming a great challenge.

The polythene is the most commonly found non-degradable solid waste that has been recently recognized as a major threat to life. The polythene could sometimes cause blockage in intestine of fish, birds and mammals. Degradation of polythene is a great challenge as the materials are increasingly used. An estimated one million birds and ten thousand marine animals die each year as a result of ingestion of or trapping by plastics in the oceans.

Recently, the biodegradation of plastic waste and the use of microorganisms to degrade the polymers have gained notable importance because of the inefficiency of the chemical and physical disposal methods used for these pollutants, and the environmental problems they cause. Consequently, in the present investigation we designed to evaluate the biodegradation efficacy of bacterial isolates from plastic contaminated soil,

Materials and Methods

Sample collection

The plastic contaminated soils were collected from plastic contaminated place in Thanjavur and polythene bags were collected from a stationary shop in Thozhkoppier square, Thanjavur (Dt.), Tamil Nadu, India (Fig. 1).

Isolation of soil bacteria

The soil bacteria were isolated by spread plate technique (Kathiresan, 2003). 1g of plastic contaminated soil sample was taken and mixed in 100 ml of distilled water in a conical flask and serially diluted. 0.1 ml aliquot of various dilutions (10^{-3} to 10^{-5}) was spread on nutrient agar medium (Himedia, Mumbai) by using L-rod and incubated at 37ºC for 24 hrs.

Identification of soil bacteria

The selected bacterial isolates were identified by morphological and biochemical characterization. In morphological characterization, macroscopic characteristics like shape, size, structure, texture, appearance, elevation and colors were studied. Phenotypic characteristics such as microscopic characterization of gram reaction, motility and biochemical test including catalase, oxidase, indole, methyl red, Voges-Proskauer, triple sugar iron, citrate utilization, urease, nitrate reduction and carbohydrate fermentation test were performed the standard protocols (Holt *et al.*, 1994).

Biodegradation of polythene bag

The degradation of by bacteria was studied by following the method of Kathiresan (2003).

Surface sterilization of polythene bag

The collected plastics bags were cut into small pieces and cleaned with tap water and surface sterilized with ethanol. Then washed with distilled water, 0.1% mercuric chloride and again washed with distilled water.

Degradation of polythene bag

Nutrient broth was prepared and autoclaved at 121°C for 15 minutes. 200 ml of cooled, nutrient broth was poured into eight 250 ml sterile conical flasks. The sterile pre weighed polythene bag pieces were aseptically transferred into nutrient broth. A loopful of bacterial cultures such as *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* was inoculated into nutrient broth. One 250 ml of flask containing the polythene bag pieces without bacterial cultures was maintained as control. These flasks were incubated at 37°C for 10, 20 and 30 days.

The polythene bag pieces were carefully removed from the culture (by using forceps) after different days of incubation. The collected pieces were washed thoroughly with tap water, ethanol and then distilled water. The pieces were shade dried and weighed for final weight. The data's were recorded. The same procedure was also repeated for all the treated samples.

Determination of degradation of polythene bag

The percentage of degradation of polythene bag pieces by *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* was determined by calculating the percentage of weight loss of plastics. The percentage of weight loss was calculating by the following formula.

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

Results and Discussion

Biodiversity and occurrence of polymer-degrading microorganisms vary depending on the environment, such as soil, sea, compost, activated sludge, etc. It is necessary to investigate the distribution and population of polymer-degrading microorganisms in various ecosystems. Generally, the adherence of microorganisms on the surface of plastics followed by the colonization of the exposed surface is the major mechanisms involved in the microbial degradation of plastics (Tokiwa *et al.*, 2009).

In the present investigation two soil bacteria were isolated from plastic contaminated soil and identified its morphological and biochemical characterization. The results were summarized in table 1. The bacterial isolates were *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes*. The results were comparable with the earlier findings of Kathiresan (2003), Vijaya and Reddy (2008), Usha *et al.* (2011) and Deepika and Jaya Madhuri (2015) who reported that the plastic associated soils are rich in bacterial species.

Bacterial species importantly involved in the biodegradation process include, *Bacillus* (capable of producing thick-walled endospores that are resistant to heat, radiation and chemical disinfection), *Pseudomonas*, *Klebsiella*, *Rhodococcus*, *Flavobacterium*, *Comamonas*, *Escherichia*, *Azotobacter* and *Alcaligenes* (Sangale *et al.*, 2012).

The biodegradation efficacy of two isolates such as *Desulfotomaculum nigrificans* and *Pseudomonas alcaligenes* were investigated by using polythene bag. The degrading ability of bacterial cultures showed variability and the results of degrading ability were presented in table 2 & 3. Plate 1 & 2 showed the degradation process of two bacterial isolates.

Table.1 Morphological and biochemical characterization of isolated bacteria

S. No.	Gram Staining	Motility	MR	VP	Indole	Nitrate	Glucose	Citrate	Oxidase	Catalase	Urease	TSI	Bacterial isolates
1.	+ve Rod (chain)	Motile	-	+	+	ND	_	+	ND	-	-	H_2S	*Desulfotomaculum nigrificans*
2.	-ve Rod	Non motile	-	-	-	+	-	+	+	+	+	-	*Pseudomonas alcaligenes*

MR: Methyl Red, VP: Voges Proskauer, TSI: Triple Sugar Iron, +: Positive, - : Negative, ND: Not Defined

Table.2 Biodegradation of polythene bag by *Desulfotomaculum nigrificans*

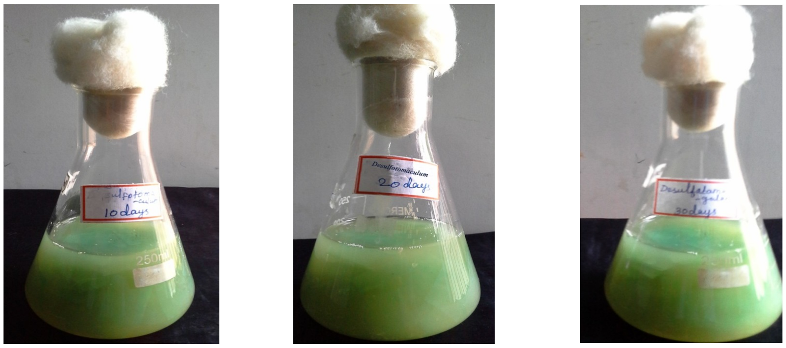
S. No.	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1.	10	1.000	0.8980	10.2
2.	20	1.000	0.8679	13.2
3.	30	1.000	0.8382	16.2

Table.3 Biodegradation of polythene bag by *Pseudomonas alcaligenes*

S. No.	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1.	10	1.000	0.8950	10.5
2.	20	1.000	0.8531	14.7
3.	30	1.000	0.7986	20.1

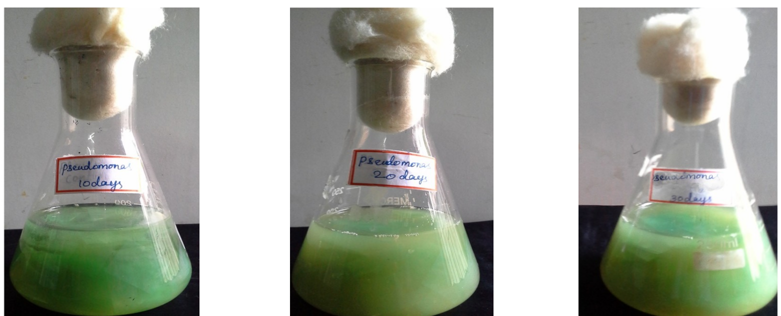
Fig.1 Sample - polythene bag and pieces

Plate.1 Biodegradation of polythene bag by *Desulfotomaculum nigrificans*



10th day 20th day 30th day

Plate.2 Biodegradation of polythene bag by *Pseudomonas alcaligenes*



10th day 20th day 30th day

In the present study, *Desulfotomaculum nigrificans* degrade 10.2, 13.2 and 16.2 % of polythene bag at 10, 20 and 30 days incubation respectively. At the same time *Pseudomonas alcaligenes* degrade 10.5, 14.7 and 16.2 % of polythene bag at 10, 20 and 30 days incubation respectively. An increase in incubation period there is a dramatic increase in weight loss of polythene bag. Among the two isolates tested, *Pseudomonas alcaligenes* was found to be more effective in degradation of polythene bag at 30 days. Previously, Tadros *et al.* (1999) and Norman *et al.* (2002) have reported on the biodegradability potential of *Pseudomonas fluorescens and P. aeruginosa* on synthetic plastics. Hadad *et al.* (2005) also isolated a thermophilic bacterial strain, identified as *Brevibacillus borstelensis*, which utilized standard and photo-oxidised polyethylene.

Evidently, Sivan *et al.* (2006) isolated a biofilm producing strain of *Rhodococcus ruber* (C208) that degraded polyethylene at a rate of 0.86% per week. The ability of *Bacillus* species to utilize polyethylene, with and without pro-oxidant additives, was also evaluated (Abrusci *et al.*, 2011).

Recently, Deepika and Jaya Madhuri (2015) reported that *Pseudomonas* sp. have significant plastic degradation capacity and it degrades up to 24.22% for the period of 6 months. Similarly, Kyaw and co workers (2012) studied that biodegradation of low density polythene (LDPE) by *Pseudomonas* species. They reported that after 120 days of incubation period, the percentage of weight reduction was 20% in *Pseudomonas aeruginosa* (PAO1), 11% in *Pseudomonas aeruginosa* (ATCC) strain, 9% in *Pseudomonas putida* and 11.3% in *Pseudomonas syringae* strain.

The overall investigation can be concluded that *Pseudomonas alcaligenes* exhibited significant polythene degradation ability and in the near future, *Pseudomonas alcaligenes* can be used to reduce the quantity of plastic waste, which is rapidly accumulating in the natural environment. Various polythene degradation methods are available in the literature but the cheapest, eco-friendly and adequate method is degradation using microbes.

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