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Saleh Agreira Ali Abu Hamrah
 Faculty of Science, Bani Walid
 University Bani Walid, Libya

A study on biodegradation of plastic by using microorganisms isolated from different soil samples

Saleh Agreira Ali Abu Hamrah

Abstract

Plastic wastes accumulating in the environment are posing an ever increasing ecological threat. Plastics that are biodegradable can be considered environment friendly, they have an increasing range of potential application and are driven by the growing use of plastics in packaging. In this study, the biodegradation of polythene bag was analyzed 1 month of incubation in liquid culture method. Low Density Polyethylene (LDPE) is one of the polymers that is up till date nearly impossible to be degraded safely. 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. This study introspected the comparative extent of plastic biodegradation by employing bacterial species and fungal species. The bacterial isolates such as *Streptococcus* and *Pseudomonas alcaligenes* were identified by morphological and biochemical characterization. The biodegradation efficacy of *Streptococcus* and *Pseudomonas alcaligenes* by using polythene bag were studied. The *Pseudomonas* was found to be more effective than *Streptococcus* in degradation of polythene bag at 30 days. An increase in incubation period there is a dramatic increase in weight loss of polythene bag.

Keywords: Biodegradation, plastics, degradation

Introduction

Plastics are defined as the polymers (solid materials) which on heating become mobile and can be cast into moulds. They are non metallic mold able compounds and the materials that are made from them can be pushed into any desired shape and sizes. Commonly plastics are used in many purposes including packaging, disposable diaper backing, agricultural films and fishing nets. Plastics and their use has become a part in all sectors of economy. Infrastructure such as agriculture, telecommunication, building and construction, consumer goods, packaging, health and medical are all high growth areas that ensures present demand for plastics. Plastic is the mother industry to hundreds of components and products that are manufactured and used in our daily life like automobiles parts, electrical goods, plastic furniture, defense materials, agriculture pipes, packages and sanitary wares, pipes and fittings, tiles and flooring, artificial leathers, bottles and jars, PVC shoes and sleepers hundreds of household items ^[1].

Plastics are used in packaging of products such as food, pharmaceuticals, cosmetics, detergents and chemicals. Approximately 30% of plastics are used worldwide for packaging applications and the most widely used plastics used for packaging are polyethylene (LDPE, MDPE, HDPE, LLDPE), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), polyurethane (PUR), polybutylene terephthalate (PBT), nylons. At present the industry is split into organized and unorganised sectors. The organized sector produce quality products whereas unorganized sector is not capable of producing quality products, it produces low quality, cheap products through excessive use of plastic scrap ^[2].

They are composed of organic condensation or addition polymers and may contain other substances to improve performance or economics. Discarded plastics, besides being highly visible are a rapidly increasing percentage of solid waste in landfills, resistant to biodegradation leading to pollution, harmful to the natural environment ^[1]. The term biodegradable plastics normally refer to an attack by microorganism on non water soluble polymer based materials ^[2]. Plastics are resistant to microbial attack, because their short time of presence in nature evolution could not design new enzyme structures capable of degrading

Correspondence

Saleh Agreira Ali Abu Hamrah
 Faculty of Science, Bani Walid
 University Bani Walid, Libya

synthetic polymers [3]. The term is often used in relation to ecology, waste management, environmental remediation and to plastic materials, due to their long life span [4]. Plastics can be classified by the chemical process that is used in their synthesis [5]. Pure plastics generally have low toxicity due to their insolubility in water and relative chemical inertness. Many microorganisms accumulate PHA as intracellular energy and storage of carbon inclusions when the carbon is in excess to the other nutrients such as nitrogen, sulphur, phosphorus and oxygen [6].

The purpose of this study was to isolate microorganism from dumped soil area and screening of the potential polyethylene degrading microorganisms and indentifying the high potential microorganism that degrade the plastics.

Materials and methods

Sample collection

The plastic contaminated soils were collected from plastic contaminated place in Acharya Nagarjuna University and polythene bags were collected from a stationary shop in Guntur, Andhra Pradesh.

Isolation of soil bacteria

The soil bacteria were isolated by spread plate technique (Kathiresan, 2003) [7]. 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 37°C 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) [8].

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

Microbial Degradation of Plastics in Laboratory Condition

Prewashed discs of 2x2 cm prepared from polythene bags were aseptically transferred to the conical flask containing 50 ml of culture broth medium, inoculated with different bacterial species and fungal isolates. Control was maintained with plastic discs in the microbe free medium. Different flasks were maintained for each treatment and left in a shaker. After one month of shaking, the plastic discs

were collected, washed thoroughly using distilled water and then with acetone, shade dried and then weighed for final weight.

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.

$$\text{Percentage of weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 1000$$

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) [9].

Plastic degrading microorganisms were isolated from two different soil samples and degradation of plastic strips by the isolated micro organisms was determined using weight loss method. The isolate which shows high opacity was selected and further used. The isolates obtained was subjected to standard biochemical test results showed the presence of *Streptococcus sp.*, *Pseudomonas sp.*, and *Bacillus sp.* for bacteria and *Aspergillus sp.* and *Fusarium sp.* were observed in fungi. The organisms identified was further inoculated into different culture media and their biodegradative ability was determined by loss of weight after a period of 30 days and observed that bacterial *sp.* degrades up to 23% and fungal *sp.* up to 44%. Thus fungi degrade more plastic than bacteria. Thus it may takes 120 days complete degradation of plastics by using bacteria and its takes approximately 75 days while using fungi for complete degradation of plastic. Further biochemical test can be done to identify the exact bacterial and fungal species. Further characterization of the obtained microbes can be done to increase the level of biodegradation of plastic.

Conclusion

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

Table 1: Morphological and Biochemical Characterization of Isolated bacteria

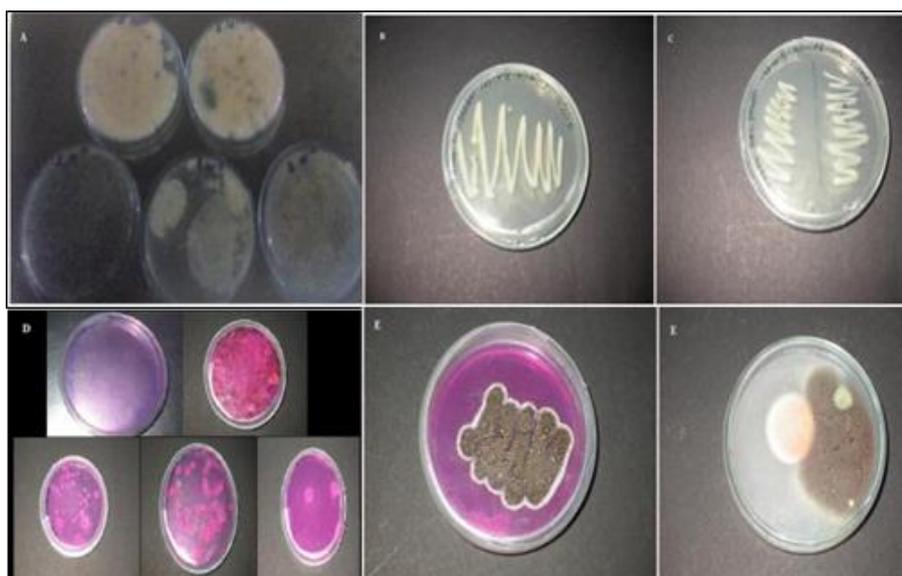
S. No.	Gram Staining	Motility	M R	V P	Indole	Nitrate	Glucose	Citrate	Oxidase	Catalase	Urease	TSI	Bacterial isolates
1.	+ve Rod (chain)	Motile	-	+	+	ND	+	+	ND	+	-	H ₂ S	<i>Streptococcus</i>
2.	-ve Rod	Non motile	+	+	-	+	+	+	+	+	+	-	<i>Pseudomonas alcaligenes</i>

Table 2: Biodegradation of Polythene Bag by *Streptococcus*

S. No.	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1.	10	1.000	0.8750	10.70
2.	20	1.000	0.8620	12.00
3.	30	1.000	0.8300	16.00

Table 3: Biodegradation of Polythene Bag by *Pseudomonas*

S. No.	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1.	10	1.000	0.8885	10.5
2.	20	1.000	0.8520	15.00
3.	30	1.000	0.8400	21.00

**Fig 1:** Sample of Polythene bag**Fig 2:** Isolation of Microorganisms.

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