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Microbial Degradation Of Plastic: A Short Study

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ABSTRACT

Plastic is also one of the keycauses of ecologicalcontamination. Accumulation of plastic pollutants in the environment becoming an ecological threat. The aim of this study is to isolate plastic degrading organism. Plastic is not broken down the plastic. Absence or low activity of catabolic enzymes that can attack its components. Polyesters containing a High ratio of chemically inert components, such as PET, shown resistance against microbial degradation. Buried soil and shake flask methods were used in this study. Plastic samples were buried and incubated in different soil samples for 3-month intervals. *Pseudomonas* species (15.2%), *Proteus* species (26.4%) and *Micrococcus* species (46.1%) were isolated by the Soil Buried Method. These species were tested forconfirming degradation of plastic by using the Shake Flask Method.

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Introduction

Plastic is a polymer consist of carbon along with hydrogen, nitrogen, sulfur and other organic and inorganic elements and is manufactured from fossil fuel . Plastic is a non-renewable source which is non-biodegradable, tough, sturdy, moisture resistant, and light in weight (1). The manufacture of plastic increased from 0.5 million tons in 1950 to 260 million tons now (3). Plastic is being gradually used in agriculture field also to obstruct weeds and for other purposes (4). Accumulation of plastic wastes created the thoughtful threat to the environment and wildlife (5).

Soil pollutions are mainly produced due to seven different types of human activities (6). Synthetic polymers, like plastic waste and synthetic polymers that are soluble in water are the major cause environmental pollution (7). Poly Venyle Chloride is used in common consumer commodities, containing adhesives, detergents, oiling, diluents, automotive plastics, plastic type clothing, building material and toys and soap, shampoo, deodorants, fragrances, hair spray, nail polish are Personnel care products as well as toys and building materials. Furans and dioxins deadly hazardous gases are produced due to the burning of the plastic. One of the causes of ozone depletion (8). Plastic are of two types: one is thermoplastics and the other is thermosetting polymers. Thermoplastic plastic can be hard or soft by repeating heating and cooling process and they can be shaped repeatedly. Thermosets plastics have endless molecular weight as they are highly cross linked structures (1).

Light, heat or biological processes are the part of biodegradation process (9).Biodegradation is the method in which living organisms decompose the organic substances. Biodegradation is frequently used regarding environmentalism, management of waste, bioremediation and to the plastic materials, due to their extended life cycle. Throughout aerobic biodegradation of plastic, CO_2

and H_2O are produce and throughout anaerobic biodegradation C_2O, H_2O and CH_4 are produced (10).

Microorganisms are responsible for degradation of ordinary and artificial plastic (10). Biodegradation of plastic continues under different soil circumstances because microbes that are accountable for the course of biological degradation they have their own ideal growth circumstances in the soil (1).

Initially the polymer mineralized into different monomers because the polymers of large size cannot permit through the cell membrane, so first they depolymerized to enter into the cell and then they are mineralized or biodegraded inside the cell. Many fungi that grow on polymers can also responsible of enlargement, to assist polymer permeation into the fungal cell (11).

Two sets of enzymes are involved in the biological degradation of polymers: extracellular and intracellular depolymerase (12). During degradation Exoenzymes from micro-organisms split complex polymers to yield short chains of smaller molecules, to permit the bacterial outer semi-permeable membranes, then make use of bacteria as carbon and energy sources. This whole process is called as depolymerization. The CO_2, H_2O, or CH_4 produced as an end products called mineralization (13). Degradation is made in two steps. The aerobic degradation in which oxygen is used to change complex material to produce CO_2 and H_2O and microbial biomass, as an end product.

The second type is anaerobic degradation in which microorganisms do not use oxygen for degradation. The biomass of microbial origin, CO_2, CH_4, and H_2O are products under anaerobic settings (15).

Materials and Method

Sample Collection: The soil samples were collected from Sea view soil from Karachi, Garden soil from near by area and Sludge soil from the dump site near Board office, Nazimabad, Karachi.

Isolation of Microorganisms: Multiple Soil sample was collected and serially diluted:1 gm of soil sample was serially diluted in 9.0 ml or distilled water to make 1:10, 1:100, 1:1000, 1:10000, and 1:100000 and from the last dilution 0.1 ml was taken and streak in to Nutrient agar plate and incubate the plates at 37^0 C for 24 hours. After 24 hours any visible colonies were observed and subjected to biochemical, serological identification for the conformation.

Identification of Microorganism: Identification of the isolates were performed according to their morphological, cultural and biochemical characteristics. All the isolates were subjected to Gram stain for the conformation of morphology and subjected to specific biochemical tests and spot test such as Coagulase test, Oxidase Test, IMVIC and Motility test were performed.

Results

Plastic is also one of the key causes of ecological contamination. Accumulation of plastic pollutants in the environment is becoming an ecological threat. The aim of this study is to isolate plastic degrading organism. Three bacterial strains were used in this study are checked for their morphological, biochemical, and cultural characteristics. The organism isolated and inoculated with the sea view soil sample was *Proteus* mirabilis are gram negative rods and scattered in arrangement and gives gray swarming colonies with a uniform film ad fishy odor. Catalase positive, Coagulase negative, Oxidase negative, highly motile, Indole negative. On TSI butt acidic and slant alkaline, with H_2S and gas production. From Buried Soil Method percentage of plastic degraded by *Proteus* mirabilis was 7.1% in comparison from a Shake Flask Method percentage of plastic degraded by *Proteus* mirabilis was 26.4%.The microbe isolated and inoculated with garden soil sample was *Pseudomonas* being short gram negative rods which are scattered in arrangement and gives large, opaque, flat colonies with irregular margins with green pigmentation and fruity odor. Catalase positive, Coagulase negative, Oxidase positive, Motility positive, Indole negative.At TSI both butt and slant are acidic with no H_2S and no gas production. From Soil Buried Method percentage of plastic degraded by *Pseudomonas* was 8.1% and in comparison Shake Flask Method percentage of plastic degraded by *Pseudomonas* was 15.2%.The microbe isolated and inoculated with the sludge soil sample was *Micrococcus* lutes are gram positive cocci arranged in pairs or in tetrads and gives Circular, pinhead, bright yellow colonies with entire margins. Catalase positive, coagulase negative, Oxidase negative, Motility negative, Indole negative. At TSI both butt and slant are acidic with no H_2S and no gas production. From Soil Buried Method percentage of plastic degraded by *Micrococcus luteus* was 34% and from Shake Flask Method percentage of plastic degraded by *Micrococcus* luteus was 46.1%.

Discussion

This study was conducted to overcome the pollution caused by plastic and its polymers. This study deals about the isolation, identification and the plastic degradative ability of microorganism isolated from different soil samples. Microorganisms play a vital role in plastic degradation. In this depolymerization process 2 types of enzymes are actively involved, i.e.extracellular and intracellular depolymerases (10). During degradation Exo- enzymes from microorganisms breaks complex polymers into smaller molecules of short chain, e.g., oligomers, dimmers, and monomers, and are smaller that can pass the semipermeable outer membranes of the microbes, and then utilized as carbon and energy sources (14). Depolymerization results caused by physical strength or biological that produced aerobic metabolism those are carbon dioxide and water, whereas anaerobic metabolism produced carbon dioxide, water and methane as a final product. 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 (17).

Each microbe has different characters, so degradation ability possessed will be varied between one microbe to another microbe. Different characteristic includes type of enzyme produced in degradation process that helped in polymer degradation.

From the sea view soil sample isolated the microbe was *Proteus* mirabilis which can degrade plastic about 7.1%. Thomas (2011) reported that *Staphylococcus aureus* had the lowest degradation power. Our result agreed with Thomas who also found from the garden soil sample isolated the microbe was *Pseudomonas* sp. which can degrade plastic about 8.1% (17). Kathiresan K. 2003 reported that the *Pseudomonas* sp. was able to degrade plastic by 8.16%. We agree with Katherisan results. Reported in 2003 (2).

From the sludge, soil sample the isolated microbe was *Micrococcus* luteus which can degrade plastic about 34%. Sivasankari et.al (2014) reported that the *Micrococcus luteus* were found most active in degrade 46.1% of plastic. Our results are closely related to that value so we agree with Sivasankari's statement. In the test flask of sludge, soil the degradation rate of plastic by *Micrococcus luteus* was about 46.1%. Sivasankari et. al (2014) reported that the *Micrococcus luteus* were found most active in degrade 38% of plastic. Our results are higher than Sivasankari's results (16).

Table I: Colonial morphology after the collection of samples were serially diluted and plated for the observable colonies.

Sample	Dilution No.	Colonial Morphology
Seaview Soil	10^{-5}	Gray swarming colonies with a uniform film ad fishy odor later identified as *Proteus mirabilis*
Garden Soil	10^{-5}	Large, opaque, flat colonies with irregular margins with green pigmentation and fruity odor later identified as *Pseudomonas*
Sludge Soil	10^{-5}	Circular, pinhead, bright yellow colonies with entire margins later identified as *Micrococcus lutues*

Table II: Results of Gram staining of different sample process

Sample	Shape And Arrangement	Gram Reaction	Identification
sea view soil	Rods and scattered in the arrangement	Negative	*Proteus mirabilis*
garden soil	Short rods and scattered in the arrangement	Negative	*Pseudomonas*
sludge soil	Cocci in tetrads and pair	Positive	*Micrococcus luteus*

Table III: Results Of Biochemical And Other Confirmation Tests.

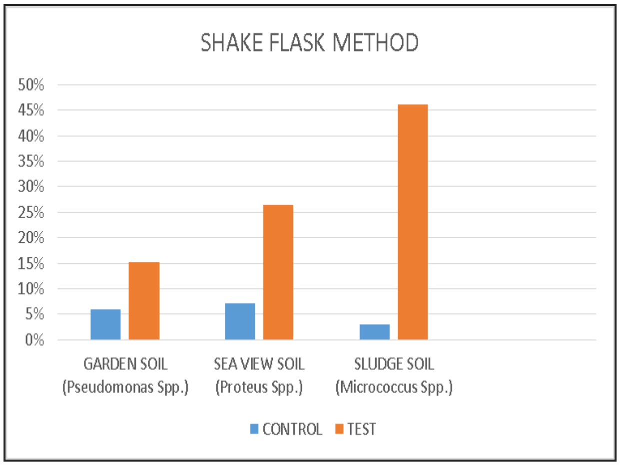
Sample	Catalase	Coagulase	Oxidase	TSI	Motility Test	Indole Test
Sea View Soil	+ve	-ve	-ve	Butt acidic and slant alkaline, with H_2S and gas production	Motile	-ve
Garden Soil	+ve	-ve	+ve	-ve	Motile	-ve
Sludge Soil	+ve	-ve	-ve	-ve	Non Motile	-ve

Table IV: Result of degradation of plastic sample by the Soil Buried Method after 3 months.

Sample	Initial Wt. (Gm.)	Final Wt. (Gm.)	Difference	Weight Loss/Month In %
Sea View Soil	11.02	10.23	0.79	7.1%
Garden Soil	12.3	11.3	1	8.1%
Sludge Soil	7.5	4.95	2.55	34%

Table V: Results of degradation of plastic samples by Shake Flask Method.

Sample	Initial Wt. (Gm.)	Final Wt. (Gm.)	Difference	Weight Loss/Month In %
Sea View Soil	1.4	1.03	0.31	26.4%
Garden Soil	2.5	2.12	0.38	15.2%
Sludge Soil	1.3	0.7	0.6	46.1%



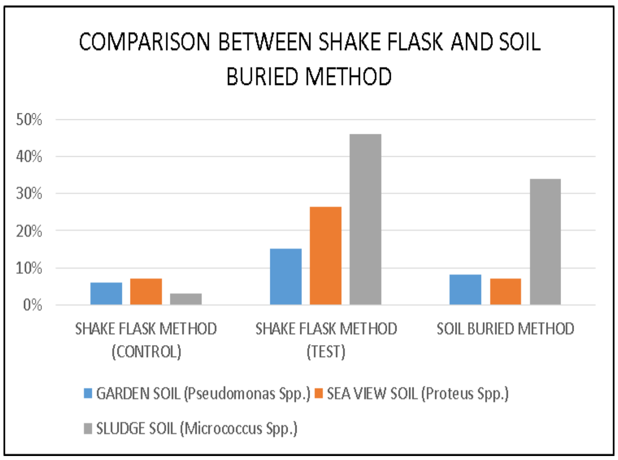


Figure 1. Graphical representation of results of Shake flask method b) Graphical representation of results of buried soil method.

Conclusion

Microbes isolated are: *Pseudomonas* spp., *Micrococcus luteus* and *Proteus mirabilis*, *Micrococcus luteus* has ability to degrade plastic too much that of other bacteria. Although other *Proteus mirabilis* and *Pseudomonas* spp has not as much of an ability to degrade plastic as compared *Micrococcus luteus*. *Micrococcus luteus* show much better degradation of plastic as compare to other org like pseudo, *Proteus* in other study.

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