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BIODEGRADATION OF LOW DENSITY POLYETHYLENE BY SELECTED FUNGUS SPECIES FROM SOIL SAMPLES OF SILTARA INDUSTRIAL AREA, CHHATTISGARH

Biological Science

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ABSTRACT

Siltara is the industrial area located some about 15 km away from the Raipur city on Raipur-Bilaspur Road located at about 21022 N and 81039 E. There are two industrial sites in Siltata, one on the left and another on the right side of the road, named as: Siltara I and Siltara II. Almost all types of industries, from small to relatively large are located within the area. Plastics have been widely used as a packing material in the form of low density polyethylene (LDPE). Continuous accumulation of plastic in the environment can cause threat to humanity and environment. In order to stop the accumulation of plastic and to make the surroundings free from plastic, fugues are isolated from Bhilai, Chhattisgarh area soil. These fugues are screened by clear zone technique using polythene powder to confirm the degradation activity. Biodegradation of polymer granules by the isolated organisms and it makes physical and structural changes over a period of time after fungus adhesion to the granules. To check the efficiency of biodegradation, weight method was performed under laboratory conditions for 2, 4 and 6 months. Experimental data revealed that *Fusarium oxysporum* sps have highest plastic degradation capacity and it degrades up to 48.9%, this degradation was followed by the Aspergillus niger (28.27%), and Rhizopus stolonifer (23.10%) for the period of 6 months. This work revealed that *Fusarium oxysporum* plays a vital role in degrading polythene powder and polymer granules.

KEYWORDS

Biodegradation - polyethylene -LDPE - Fungus- soil

1. INTRODUCTION

Environmental pollution is any discharge of material or energy into water, land, or air that causes or may cause acute (shortterm) or chronic (long-term) detriment to the Earth's ecological balance or that lowers the quality of life. Pollution and development are almost always correlated, positively. Industrialization has its beginning since the beginning of the eighteenth century and since then, till now, industries are the major sources of pollution. To restrict pollution in limited areas, it has now become customary to identify areas for locating industries. Siltara is a similar area located near Raipur city, the capital city of Chhattisgarh state, where a large number of industries have been located. Siltara is the industrial area located some about 15 km away from the Raipur city on Raipur-Bilaspur Road located at about 210 22 N and 810 39 E. There are two industrial sites in Siltata, one on the left and another on the right side of the road, named as: Siltara I and Siltara II. Almost all types of industries, from small to relatively large are located within the area. However, most of them are sponge iron factories. Most prominent of the area is the air pollution. There are as many types of gaseous effluents as there are types of industries. More apparent is the dust pollution, depositing on every structure in and beyond the industrial area. With the human inhabitants of the area plants are other sufferers of the dust pollution. Present studies were made therefore to analyze the herbaceous vegetation within the Siltara industrial area.

Plastics are the synthetic polymers of carbon, hydrogen and oxygen which are derived from petrochemicals. About 140 million tons of synthetic polymers are produced worldwide annually with their utility escalating at a rate of 12% per annum (Shimao, 2001). Each year, an estimated 500 billion to 1 trillion plastic bags are consumed worldwide (Roy et al., 2008). After their usage these packing materials are dumped in landfills leading to pollution since they are non-biodegradable under natural environmental conditions (Burd, 2008). LDPE is a thermoplastic polymer with significant short branches, commonly made by copolymerization of ethylene with longer-chain olefins. There are different methods of disposal of plastics such as incineration, recycling and landfills (Sharma & Sharma, 2004). However, biodegradation plays a key role in reducing the molecular weight of the polymer by naturally occurring microbes like bacteria, fungi and actinomycetes isolated from different environments (Gu, 2003; Sivan et al 2006).

Although literature exists on degradation of many types of plastics, all of them are starch blended. Reports on microbes able to degrade pure form of polyethylene are not much highlighted, especially on polymer granules. The ability to degrade polymers depends on the enzymes produced by the microbes to convert the polymers to oligomers and then to monomers. These water soluble enzymatically cleaved products are further absorbed by the microbial cells as carbon source where they are metabolized (Vasile, 1993). Hence the present manuscript aims to isolates and investigate on the different fungus species that are capable to degrade polymer granules and LDPE bags produced from polymer granules.

2. MATERIALS AND METHODS

2.1 Materials Low density polyethylene powder (LDPE) with 53-75µm particle size was obtained from Sigma Aldrich Chemical Co (Product of USA) with density 0.94g/ml at 250C. Low density polyethylene granules from local market of Bhilai, Chhattisgarh.

2.2 Samples Collection

Soil samples were collected from two different areas viz three different area of sample was collected from Siltara Industrial Area, Chhattisgarh. The samples were collected in sterile Nasco sampling bags. The soil samples associated with polythene waste bags were collected at a depth of 3-5cm and dried at room temperature for 2hrs.

2.3 Isolation and identification of microorganisms

Microorganisms were isolated from one gram of soil sample by serial dilution method. The Czapek Dox Agar for fungi was prepared and isolation was performed by spread plate technique. For each sample, three replicas were maintained and kept for incubation at room temperature (3-5days). The isolated colonies developed were sub cultured in agar slants and preserved under refrigeration temperature. The identification of bacteria was performed on the basis of macroscopic and microscopic examination as per Bergey's Manual of Determinative Bacteriology Holt et al. (1994). The fungus was identified after staining with cotton blue by following the keys of Raper & Fennell (1987). The phenotypic and chemotaxonomic characteristics of the actinomycetes were determined by the method described by Shirling & Gottlieb (1966).

2.4 Screening of isolated microorganisms by clear zone method

Polyethylene powder was added to Mineral Salt medium at a concentration of 0.1% (w/v) and sonicated for 1hr at 120rpm. After sonication, agar was added and autoclaved at 1200 C, 15lbs pressure for 15 min. Sterilized media was cooled to 450C and poured into sterile petriplates. Once solidified, the isolated colonies were inoculated and then incubated at 30 -350C for 2-4 weeks. The organisms producing zone of clearance were selected for further analysis (Usha et al., 2011) with minor modification in incubation temperature.

2.5 Microbial degradation of polythene and polymer granules under laboratory conditions

2.5.1 Biodegradation of polymer granules

Mineral Salt media prepared and autoclaved at 1200C, 15lbs pressure for 15 min. After sterilization the media was poured into petriplates. Biodegradation studies were performed by using polymergranules, a new approach not mentioned in earlier studies. Polymer granules were kept at three locations in the plate prior to solidification. The polymer

granules become stacked to Agar media after solidification and the isolated organisms were inoculated and incubated at 30-350C and for a period of 4-8 weeks to check the physical and structural changes of polymer granules.

2.5.2 Biodegradation of LDPE by weight method

Low density polyethylene bag was cut into equal pieces 5cm X 2cm (1x b) and disinfected with ethanol and air dried. The disinfected strips were added into conical flask containing 100ml of Mineral salt medium and then inoculated with polythene degrading organism.

Control has been maintained for further reference and to confirm the reduction of molecular weight of the polyethylene. The flasks were left in orbital shaker at 300C, 120rpm for 6 months. After a period of time, the strips were washed in 70% ethanol, air dried and weighed to check the final weight. Finally the weight loss was calculated and compared based on the below formula.

$$\text{Weight loss (\%)} = \frac{\text{(Initial weight - Final weight) X 100}}{\text{Initial weight}}$$

3 RESULTS

3.1 Soil Microbial count

The fungi isolates were enumerated separately based on their incubation period. The microbial population increased tremendously from the beginning to the end of incubation period. The total counts of heterotrophic microbes isolated from soil sample are calculated in colony forming units multiplied by dilution factor (Table 1). As results represented in table 1, total three funguses isolates viz Fusarium oxysporum sps, Aspergillus niger and Rhizopus stolonifer sps were identified based on the mycelial structure and cotton blue staining following the keys of Raper & Fennell (1987).

Table 1 Soil microbial count in cfu /g.

S.No	Description	Bacteria	Fungi	Actinomycetes
1	Sample-1	Absent	2x10-4	Absent
2	Sample-2	Absent	1x10-4	Absent
3	Sample-3	Absent	4x10-4	Absent

Where: Sample 1: Site-1-Siltara Industrial Area Sample 2: Site-2 Siltara Industrial Area and Sample 3 Site-3 Siltara Industrial Area.

3.2 Clear Zone formation

Growth was started within 4- 5 days at respective incubation temperatures. Initially an opaque zone was observed around the colony, but slowly a clear transparent zone formed within 10-20 days at 30 -35^{0}C.The diameter of the zone is ranging from 0.25 to 0.75cm.Where as in fungal plates (3f), initially growth has been initiated all over the plate but instead of zone formation clear transparency has been started from one end and slowly spreading to other end.

3.3 Morphological changes in polymer granule

Fugues growth has been initiated over a period of 4-5 days, attaching to the surface of polymer granule, extended around the granule in 10- 15 days. Within 40-45 days, center halo liquefaction was clearly observed compared to un inoculated control sample Thus structural or morphological change in the polymer granules associated with the microbes indicating degradation of polymer was observed visually in the inoculated plates when compared to control.

3.4 Biodegradation of LDPE by weight method

In screening by weight method, it has been observed that fugues isolate from soil Fusarium oxysporum sps, Aspergillus niger and Rhizopus stolonifer showed positive result for polymer degradation. Hence the selected funguses were further tested by weight method.

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Table 2: Comparative analysis of weight loss of Polyethylene film in cultures under laboratory conditions

Sl	Description	Weight loss of Polyethylene (%)		
		2nd Month%	4th Month%	6th Month%
01	Control	00	00	00
02	Fusarium oxysporum	13.20%	29.10%	48.9%
03	Aspergillus niger	4.55%	16.78%	28.27%
04	Rhizopus stolonifer	06.12%	14.60%	23.10%

DISCUSSION AND SUMMARY

Biodegradation of polymer granules by the isolated organisms and it makes physical and structural changes over a period of time after fungus adhesion to the granules. To check the efficiency of biodegradation, weight method was performed under laboratory conditions for 2, 4 and 6 months. Experimental data revealed that *Fusarium oxysporum* sps have highest plastic degradation capacity and it degrades up to 48.9%, this degradation was followed by the Aspergillus niger (28.27%), and Rhizopus stolonifer (23.10%) for the period of 6 months. This work revealed that *Fusarium oxysporum* plays a vital role in degrading polythene powder and polymer granules. The results were compared with earlier research studies done by Vijaya & Reddy (2008) in which they reported the average number of heterotrophic bacteria and fungi found in association with polythene film and plastic cups were 37.08 X 104 and 38.04 x 104, 26.94 x 102 and 35.13 x 102 respectively. Kathiresan (2003) reported that the plastic materials in mangrove soil are rich total heterotrophic bacterial counts (79.67 x 104) and fungal counts (55.33 x 102) and the plastic materials have been colonized commonly by five species of bacteria (Pseudomanas sps, Staphylococcus sps, Moraxella sps, Micrococcus sps and Streptococcus sps) and two species of fungi (A. glaucus and A.niger). In this manner results of the study are in conformity with these previous findings and similar microbes were reported during the study.

et al. (2000) reported that the abundance of polymer degrading microorganisms were in seabed solid waste disposal site. Similarly, Imam et al. (1999) observed that significant biodegradation of plastic can be occurred only after colonization by resident microbial populations and he concludes that an increase in the bacterial load has correlation with degradation of the polymer. The mechanism of biodegradation of polymer granules happened in three steps, in first step microorganism attached to the polymer granule, in second steps they grow around the granule and in last step theses microorganisms degradation the polymer and utilized it as carbon source. Augusta et al. (1993), reported that the zone of clearance around the colony is due to extracellular hydrolyzing enzymes secreted by the target organism into suspended polyesters agar medium. All the microbes involved in forming a clear zone were selected for further studied weight method. All minerals were supplied along with polyethylene as carbon source for the growth of the organism. The Bacteria, Fungi and Actinomycetes were separately inoculated to check the degradation activity under aerobic condition in an orbital shaker. After a week to cross check the ability of inoculated microbe to degrade the LDPE strips were taken out, a slimy growth on the surface of polyethylene was reported; it is a type of biofilm formation. Study of biofilm formed by Penicillium frequentans and Bacillus mycoides showed that Penicillium frequentans had polyethylene (DPE-chemical or photo initiator added polyethylene) degradation capacity and because of this it can colonized on polyethylene Arutchelvi et al (2008). Microorganisms utilize polythene films as a sole source of carbon resulting in partial degradation of polythene and plastics. They colonize the surface of the polyethylene films or plastics forming biofilm (Vijaya & Reddy, 2008). Though initially there is increase in the weight (0.02%) of the polyethylene due to biofilm formation but later on a drastic reduction in the weight of the of LDPE strip confirming the usage of polyethylene as carbon source. When weight was measured for every two months to six months bacterial isolate Pseudomonas sps caused biodegradation from 4.34% to 24.22%, A.niger caused 10.78% to 26.17%, A. flavus caused 5.69% to 16.45% and Streptomyces showed high degradability i.e12.42% to 46.7% as compared to other isolated species. Kathiresan & Bingham (2001) reported that biodegradation of polythene by bacteria was 2.19 to 20.54% for polythene and 0.56 to 8.16% for plastics. Among all the species, Aspergillus glaucus was more active than A. niger in degrading 28.8% of polythene and 7.26% of plastics within a month. Singh et al (2012) reported that Penicillium sp. was more active in reducing LDPE i.e up to 6.58% compared to A. fumigatus as it reduced the weight upto 4.65%.

Once the organism attached to the surface of the polymer it starts growing by using polymer as carbon source. In the primary degradation, the main chain cleaves to low molecular weight fragments (oligomers), dimers or monomers Vasile (1993). The degradation is due to extracellular enzymes release by the organisms. The breakdown products of polymer should be completely utilized by microorganisms as carbon source to control environmental pollution

Narayan (2006). Furthermore it is clearly reported by Oskay et al. (2004) Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil.

The present work concludes that naturally growing soil microbes specially fungi shows great efficacy in degrading synthetic polymer granules and LDPE polyethylene bags produced from polymer granules. Among all the isolates, *Fusarium oxysporum* sps exhibit high rate of degradation compared to , Aspergillus niger and Rhizopus stolonifer.

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