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STUDIES ON BIODEGRADATION OF SYNTHETIC POLYETHYLENE BY MICROORGANISMS ISOLATED FROM WASTE DISPOSAL SITE AT M.I.D.C AREA OF WARDHA CITY.

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ABSTRACT

In the present study biodegradation of dumped polyethylene bags by microbial activity was carried out. Microorganisms isolated from waste disposal site at M.I.D.C, Wardha city of Maharashtra was incubated with pretreated dumped polyethylene for one month. Shake-flask method was used to study biodegradation ability of isolated microorganisms. The biodegradation of Polyethylene was first detected qualitatively with the formation of clearing zone on mineral salt medium agar plates supplemented with 0.1% (w/v) pretreated polyethylene. The initial and final dry weights of plastic bags before and after incubation in the culture medium were compared and the percentage of degradation was calculated. An important test for assessing biodegradation of plastics ie. CO_2 evolution was also performed to ensure the progress of biodegradation in culture flasks. Seven isolates comprising five bacterial and two fungal species were obtained from four soil samples of waste disposal sites. Among all the treatments, isolate PD-4 was found to degrade polyethylene efficiently with 76.38% of weight loss in polyethylene and 46.87 mg of CO_2 evolution after 30^{th} day of incubation. This work reveals that the indigenous soil from waste disposal site at M.I.D.C, area of Wardha city is a good source of microbes capable of degrading plastics.

Keywords: Polyethylene degradation, biodegradation, dumped plastic, waste disposal site.

INTRODUCTION

Polyethylene is a polymer made of long chains of ethylene monomers. The use of polyethylene growing worldwide at a rate of 12% per year. Worldwide production of plastics is increasing by approximately 5% every year [1], because plastics exhibit advantages such as clarity, hardness, processability and lightness together with price competiveness. Synthetic plastic production is one of the fastest growing fields of global industry. Despite the fact that plastics have been used in daily life for 100 years, the beginning of large-scale production dates back to 1950 [2]. However plastics are mostly made from fossil resources such as petroleum, coal and natural gas [3] and lots of plastics are discarded after short service life [4]. Plastics have replaced paper and other cellulose-based products for packaging because they have better tensile strength, lightness, resistance to water and microbial attack. Commonly used plastics have been categorized as polyethylene (LDPE, MDPE, HDPE and LLDPE), polypropylene (PP), polystyrene (PS) and polyvinyl chloride (PVC) [5]. Among the synthetic plastic, polyethylene is one of the most problematic plastic. It is with high hydrophobic level and high molecular weight. Polyethylene (PE) is

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widely used for various applications like food packaging, retail industrial and agricultural uses.This aroused serious public concern so that that mankind classified plastics as one of the worst inventions ever made. With such a large amount of polyethylene gets accumulated in the environment, generating plastic waste ecological problems are needed thousands of years to efficientl degradation [6].
Biodegradation can be defined as the decomposition of substances through microbial activity.This is a complex process which involves several steps [7]. The research on degradability of plastics began in the early 1980s and numerous papers provide information on the microbial biodegradation of a variety of plastics such as polyesters, polyhydroxybutyrate (PHB), polycaprolactone (PCL), polylactic acid (PLA), polyurethane PUR, polyvinyl alcohol (PVA), nylon, and polyethylene (PE) [8]. Over 90 genera of microorganisms from bacteria and fungi, can degrade plastic, among them; *Bacillus megaterium*, *Pseudomonas* sp., *Azotobacter*, *Ralstonia eutropha*, *Halomonas* sp., etc. predominates [9]. Plastic degradation by microbes due to the activity of certain enzymes that cause cleavage of the polymer chains into monomers and oligomers. Plastic that has been enzymatically broken down further absorbed by the microbial cells to be metabolized. Aerobic metabolism produces carbon dioxide and water. Instead of anaerobic metabolism produces carbon dioxide, water, and methane as end products (6). Increasing environmental pollution and waste that can not be renewed and degrade it encourages research and studies in the field of biosynthetic and biodegradation material. One of the waste that can not be destroyed is plastic waste, which is a type of polyethylene plastic. For this reason this study aims to isolate the microorganisms from waste disposal site at MIDC, Wardha city of Maharashtra and screening of potential polyethylene degrading microorganisms and to study the extent of biodegradation of polyethylene.

MATERIALS AND METHODS

Sample collection: Soil samples adhered to polyethylene (dumped with polythene bag) were collected randomly from four selected points of waste disposable sites at MIDC, Wardha city Maharashtra. Four samples of soil along with dumped, deteriorated polythene was collected in a ziploc bags and transported to the laboratory for further study. Temperature of the sampling site and pH of the samples was also measured and recorded.

Pre-treatment of polyethylene: The dumped polyethylene was cut into small pieces (1 cm) and washed with ethanol and then with distilled water 2-3 times and dried. It is cited in most literature that the pretreated polymers degrade more easily than the untreated polymers. Therefore the polyethylene pieces were crushed with NaCl till they get mixed and form a fine thread. It is then mixed with distilled water in conical flask and mixed well in a shaker for 1 hour. The content was filtered through Whatman No.41 filter paper and air dried.

Isolation of polyethylene degrading microorganisms: One gram of soil sample was added into 99 ml sterile distilled water in a conical flask. The soil suspension is then serially diluted. The respective dilution was plated onto Nutrient agar (NA), Sabouraud Dextrose Agar (SDA) to isolate bacteria and fungi respectively. Culture flasks for fungal incubation were augmented with 200mg/ml ampicillin to inhibit bacterial growth. The nutrient agar plates were then incubated at 37^{o}C and that of SDA at 28^{o}C for 2-7 days. The developed colonies were sub-cultured to get pure culture and then preserved on nutrient agar slant at 4^{o}C for further analysis.

Screening of polyethylene degrading microorganism:

Screening of potential polyethylene degrading microorganism was carried out by clear zone method (10).

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Mineral salt medium is incorporated with 0.1% (w/v) pretreated polyethylene sterilized powder and solidified with agar at pH 7. The mixture was sonicated for 1 hr at 120 rpm in shaker. The medium was inoculated with pure culture of isolated organisms and incubated in incubator shaker at 150 rpm for up to 4 weeks at 25-30^{o}C. The organisms producing zone of clearance around their colonies were selected for further analysis. Culture media with 0.1% (w/v) pretreated polyethylene sterilized powder without inoculum was used as the negative control.

Identification of polyethylene degrading microorganisms:

The identification of bacteria was performed on the basis of macroscopic, microscopic and biochemical test according to Bergey's manual of determinative bacteriology (9th edition). The fungus was identified after staining with lactophenol cotton blue.

CO_2 Evolution Test:

The biodegradability of plastics can be determined by exposure to microorganisms or controlled composting environment under laboratory conditions and calculating the percentage of conversion of carbon into carbon dioxide in the test material as well as their rate of conversion. CO_2 evolution test was performed after every third day to check whether the biodegradation in culture medium is in progress or not. CO_2 was measured by indirect test as cumulative carbon dioxide evolved as dissolved inorganic carbon (DIC) after absorption in sodium hydroxide solution by titration method. It was performed as per the method described by ASTM D-5338 (CIPET, Chennai) with some modifications.

Steps: The CO_2 produced in each flask reacted with Ba (OH) $_2$ which was then precipitated as Barium carbonate ($BaCO_3$). The amount of carbon dioxide produced was determined by titrating the remaining Barium hydroxide with 0.05 N hydrochloric acid to a phenolphthalein as end point. Unreacted Ba(OH) $_2$ was calculated by using formula $N_1V_1 = N_2V_2$, from this 1gm of $Ba(OH)_2$ unreacted in each vessel was found by, Weight = N x equivalent wt. x volume /100

Dry weight determination of polyethylene:

To prove the polythene degradation ability of isolates calculation of the percentage of degradation was carried out by mehod given by Hadad et al., 2005 [11]. To facilitate accurate measurement of the weight of the polyethylene, the biofilm was washed off with 2% (v/v) aqueous sodium dodecyl sulphate solution for 3 hr and then with distilled water. The washed film was placed on a filter paper and air dried overnight before weighing. The dry weights of recovered polyethylene from the culture media were taken after 3th, 7th and 30th day of incubation for accounting the rate of biodegradation.The initial and final dry weights of polyethylene before and after incubation in the culture medium were recorded and % weight loss was calculated by following formula –

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

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Fourier Transform-Infrared Spectroscopy (FTIR) analysis:

Fourier Transform Infrared Spectroscopic analysis was carried out for detecting the formation of new functional groups or changes in the existing functional groups **(12).**

RESULTS AND DISCUSSION

The microorganisms associated with the soil adhered to dumped polyethylene were isolated from waste disposal site M.I.D.C, Wardha and identified. Total 7 isolates (5 bacterial and 2 fungal spp.) were screened for the potentiality to degrade polyethylene in laboratory conditions. Inoculation of the microbes isolated from dumped soil in a medium containing polyethylene as a carbon source showed that the microbes were alive in that medium. After 30 days of incubation surface deformation was observed on the surface of the sample. The value for CO_2 evolution from the degradation of polyethylene sample by bacterial isolates showed that isolate PD-4, PD-6, PD-2 and PD-3, PD-5 degraded polyethylene with a higher efficiency. The change in weight of polyethylene during and at the end of experiment was reduced significantly. Highest percent weight loss was shown by isolate PD-4 ie. 76.38 % after 4 weeks. PD-6 also showed percent weight loss up to 54.88 % indicating that the microbiological process leading to the degradation of the polyethylene. Detailed results of dry weight determination are depicted in table 1. The CO_2 evolution test gave a valid data about the degradation rate by the bacterial isolates. CO_2 evolved by the PD-4, PD-5, and PD-6 was measured to be 46.87, 46.25, and 46.24 % respectively. Hadad *et.al*, 2005 [11] recorded similar kind of results. The degradation of the polyethylene under laboratory conditions with different methods showed that microorganisms utilized polythene as a sole source of carbon resulting in partial degradation of polyethylene. Comparative analysis of polyethylene and weight loss with different microbial species in shaker flask cultures under laboratory conditions showed the biodegradation leads to decrease in molecular weight and formation of new functional groups such as carbonyl, hydroxyl etc. In the present study, all the isolates showed a similar trend in degrading polyethylene, with slight increase in initial days as they might have had a long lag phase in adapting to the changing environment but as the incubation period increases a boost in biodegradation rate was found which would have been possible for the enhanced biofilm formation and enzymatic activities on polyethylene particles. These results are in support with S. Nanda and S.S. Sahu [12], 2010 who stated that biofilm helped the bacteria to act collectively and produced sufficient metabolites to degrade the polymer and utilize it as the carbon source. FTIR spectra obtained by the films of 7 different samples showed that some news peaks arose after the biodegradation. They show that specific peaks such as carbonyl (1720 cm^{-1}), CH3 deformation (1463 cm^{-1}) shows that introduction of ketocarbonyl functional group (1718 cm^{-1}). It may correlate with the results obtained by Sudesh *et.al*, 2007 (14). Decrease in carbonyl region in FTIR was reported. The appearance of some new peaks-C=C and increase in already existing peaks at a region of 1400-1600 indicated the formation of new intermediate products.

Table 1: Dry weight Determination of polyethylene

Sr.No	Isolates	Incubation period				
		Zero time	3rd day	7th day	30th day	% weight loss
		Dry weight (gm)				
1	PD-1	0.6	0.3471	0.3110	0.2734	54.43
2	PD-2	0.6	0.2973	0.2845	0.2748	54.20
3	PD-3	0.6	0.5457	0.4630	0.3205	46.58
4	PD-4	0.6	0.2973	0.2960	0.1417	76.38
5	PD-5	0.6	0.3205	0.3140	0.2860	52.33

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6	PD-6	0.6	0.3229	0.3080	0.2707	54.88
7	PD-7	0.6	0.5478	0.5457	0.3471	42.15

Graph 1: Dry weight Determination of polyethylene

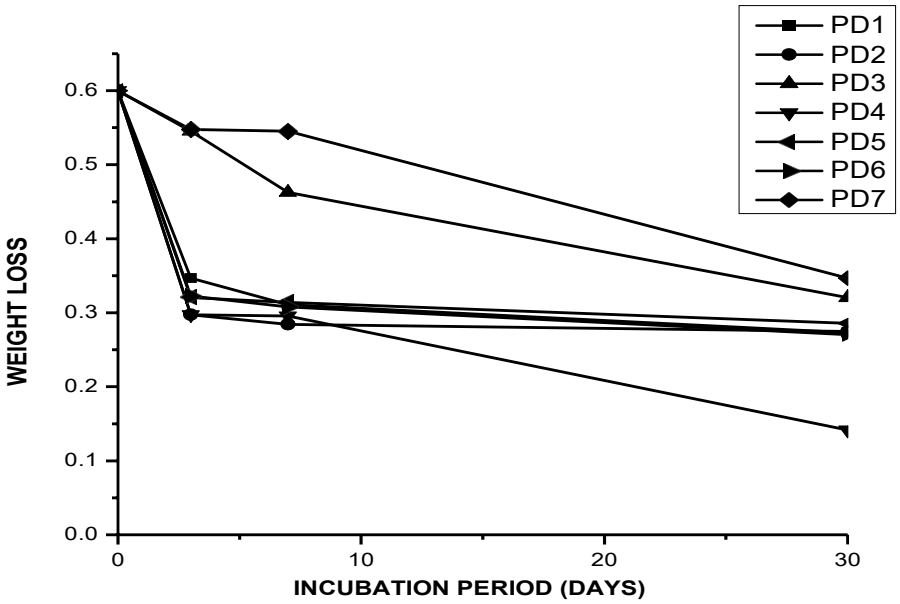

Table 2: CO_2 evolution during biodegradation of polyethylene.

Sr.No	Isolates	Incubation period			
		Zero time	3rd day	7th day	30thday
		CO_2 evolved (mg)			
1	PD-1	0.16	1.150	1.42	43.59
2	PD-2	0.16	1.167	1.83	43.78
3	PD-3	0.16	1.159	1.54	27.75
4	PD-4	0.15	1.169	1.52	46.87
5	PD-5	0.15	1.168	1.75	46.24
6	PD-6	0.16	1.163	1.69	46.25
7	PD-7	0.16	1.151	1.62	43.60

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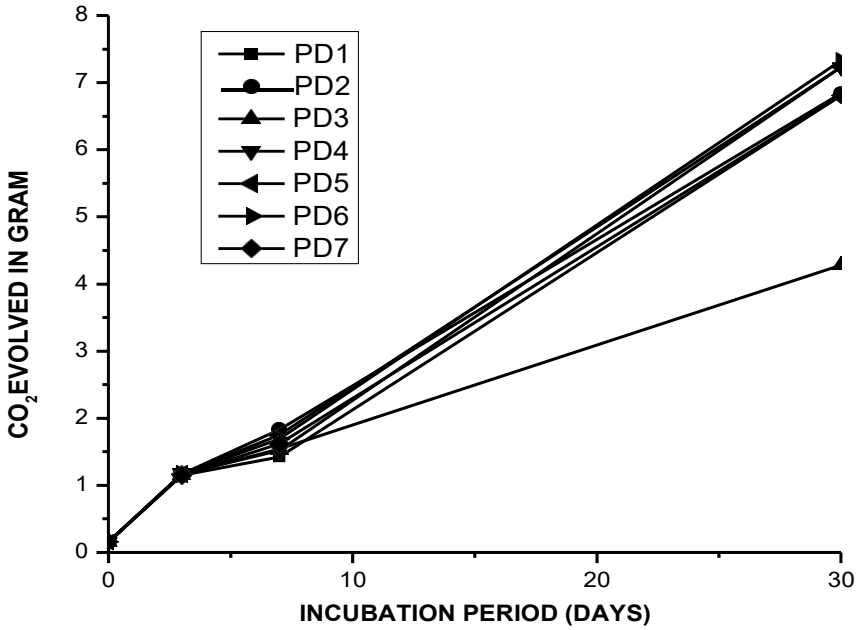
Graph 2: CO_2 evolution during biodegradation of polyethylene.



Conclusion:

Among the seven isolates obtained from waste dump soil, isolate PD-4 was found to be the most efficient degrader of polyethylene. Isolates were able to degrade the CH2 backbone of polymer. Biodegradation leads to decrease in molecular weight and formation of new functional groups such as carbonyl, hydroxyl etc. The mechanism of degradation is not known. The surface of plastic materials has turned from smooth to rough with cracking. This may be due to the compounds secreted extracellularly by the microbes that may break the complex molecular structure of plastics. Hence, further study on microbial enzymes or organic acids in degradation of the polythene and plastics will open the window for finding technology for degrading the plastic materials, which are otherwise hazardous to environment. This work reveals that the indigenous soil from waste disposal site at M.I.D.C, area of wardha city of is a good source of microbes capable of degrading polythene and plastics.

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