Microbial Degradation of Synthetic Polyethylene by Isolating Microorganism from Landfill Site

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***Abstract*- Polyethylene is one of the most widely used packaging material which turn into a waste material after its use which is the major cause of environmental pollution. In this paper the degradation ability of polyethylene is investigated by isolating microorganism from soil sample collected from landfill site Attakulangara, Trivandrum district. Synthetic plastic High Density Polyethylene (HDPE) was purchased from the local market and this plastic was used to study the biodegradation of polyethylene. By using the isolated strain I6, is capable of degrading fine particles of high density polyethylene by 1.81% (without any pretreatment) which increases to 3.44% (UV treatment), 5.08% (mineral oil addition), 3.57% (UV and thermal treatment) and 6.77% (UV, thermal and mineral oil addition) with 30 days of incubation.**

***Index Terms*- Polyethylene, Biodegradation, Weight Reduction, Scanning Electron Microscopy.**

I. INTRODUCTION

In India, plastic waste generation in metro-cities like Delhi, Chennai, Mumbai and Kolkata were 689,429, 408 and 425 tons/ day respectively according to the status of plastic waste management in the year 2015 [1]. According to the annual report of State Pollution Control Board (SPCB) or Pollution Control Committee (PCC) submitted for the year 2011-2012 for the implementation of Plastic Waste Management (PWM) Rules 2011, the plastic waste generation in Kerala is about 109500 tons/Annum [2]. As per the statement on waste generation and handling at Thiruvananthapuram Municipal Corporation total plastic waste collected is about 122.5 ton/day during the year 2016. Based on the field study conducted by Central Institute of Plastic Engineering and Technology (CIPET) and Central Pollution Control Board (CPCB), the assessment and quantification of plastics waste was conducted at Vilappilsala dump sites, Thiruvananthapuram having the total MSW of about 250 MT/Day, and the plastics were assessed as with an average of 60.22 Kg/MT. As per the survey data about 71% of plastics were HDPE or LDPE which comprising of carry bags, milk pouches and packing films. The data obtained with a minimum Plastic Waste of 59.15 Kg/MT and a maximum of 61.28 Kg/MT [1]. Plastic is a long-chain synthetic man-made hazardous polymer. Due to their excellent moisture barrier properties, lightweight, bio-inertness and low cost make them excellent packing materials [3]. From the Greek word “plastios” the word plastic is derived which means “able to be molded into different shapes and sizes” [4]. The most widely used synthetic plastics are polyethylene (LDPE, MDPE, HDPE, LLDPE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), poly carbonate (PC), poly urethane (PU). The widespread applications of plastics are not only due to their promising thermal and mechanical properties but also due to their stability and durability [5]. Microorganisms involved in the degradation of both natural and synthetic plastics are bacteria and fungi [6]. During degradation process the polymer is first converted to into its monomers and these monomers are further mineralized. First large polymer must be depolymerized to small monomers to pass through cellular membranes before they can be absorbed and biodegraded within microbial cells [7]. A variety of physical and biological forces result in the initial breakdown of a polymer [8]. A bench scale study is conducted by isolating microorganism capable of degrading polyethylene from landfill soil and the potentiality of degrading High density

polyethylene (HDPE) were analyzed with various techniques to enhance the rate of biodegradation process.

II. MATERIALS AND METHODS

A. Collection of polyethylene sample

The polyethylene film HDPE was purchased from local market which is then cut into size approx. 1.5 × 1.5 cm.

B. Collection of Soil Sample

The soil sample was collected from landfill site Attakulangara, Trivandrum district and brought to the laboratory and preserved at 4°C.

C. Isolation of Microorganism

1 g of soil was transferred into a test tube containing 10 ml of sterile water to make 10^{-1} dilution and adding 1ml of the 10^{-1} to 9 ml of sterile water to make 10^{-2} dilution and so on up to 10^{-9}. Then 0.1 ml of various dilutions (10^{-4} to 10^{-9}) was spread on the plate containing nutrient agar medium and plastic strips by using L-rod and incubated at 37°C for 24 hours. After incubation isolated colonies were selected for screening test. Pure slants were prepared and preserved at 4˚C

D. Screening of Polyethylene Degrading Isolated Microorganism

Minimal media having a composition of 0.45 g of NH_4Cl, 0.75g of K_2HPO_4, 0.5g of NaCl, 0.03 g of $MgSO_4.7H_2O$, 2.25 g of Agar in 150 ml of distilled water was prepared and 2 ml of polyethylene glycol (PEG) was added to it. The isolated colonies were grown in this medium by keeping it in a 37°C incubator for 24 hrs. The colonies grown in this medium was selected for zone of clearance test.

E. Zone of Clearance Test

After 24hr of incubation at 37°C the plates were flooded with Coomassie blue R-250 (0.02g) with 40% ethanol and 10% glacial acetic acid for 25 minutes and the solution was poured off and the plates were flooded with 40% ethanol and 10% glacial acetic acid for 25 minutes and the solution was poured off [9]. The organism showing clear zone were selected for the further studies. The strain I6 shows positive result. The isolate were further characterized by Gram's staining and biochemical tests [10].

F. Preparation of Inoculum

Nutrient broth (0.5 g peptone, 0.3 g beef extract, 0.5 g NaCl in 100ml distilled water) were prepared in a conical flask and it is inoculated with the isolated strain I6 and is kept for incubation at 37°C for 24 hrs.

G. Preparation of Mineral Salt Media

Mineral salt medium were prepared g/L 1.g K_2HPO_4, 0.2 g KH_2PO_4, 1g $(NH_4)_2$ SO_4, 0.5g $MgSO_4.7H_2O$, 0.01g $FeSO_4.7H_2O$, 0.01g $CuSO_4.5H_2O$, 0.01g $ZnSO_4.7H_2O$, 0.1g yeast, 1 g NaCl.

H. Biodegradation Studies of Polyethylene without and with Pretreatment

100 ml of medium was poured into each conical flask to it 3 ml of inoculum (24 hour old culture) and 5 strips (1.5×1.5 cm) of plastic with and without pretreatment were added separately and kept for incubation at 37°C for 30 days and intermittent shaking was given. The plastic were subjected to UV radiation for 96 hrs at a distance of 30cm by placing the plastic inside the box. The mineral oil was purchased and 0.05 ml was added along with the plastic and microorganisms for enhancing the rate of biodegradation [11]. The UV treated plastics were then subjected to thermal treatment by placing the plastic in hot air oven at a temperature of 60°C for 24 hrs. The UV, thermally treated polyethylene added to a conical flask containing 100 ml of mineral salt medium, 3ml of bacterial species and 0.05ml of mineral oil added was added to it and kept for incubation at 37°C.

I. Determination of pH Change in the Medium

This observation was adopted to ensure the metabolic activity of bacterial species in the medium. The metabolism shown by the bacterial species may have great support to the evidence of degradation. The pH change was measured at an interval of 10 days. The turbidity is measured to ensure the bacterial growth in the medium. It can also be considered as the evidence of biodegradation

J. Determination of Turbidity Change in the Medium

The turbidity is measured to ensure the bacterial growth in the medium. It can also be considered as

the evidence of biodegradation. The turbidity is measured after 30 days of incubation

K. Gravimetric Analysis

Before biodegradation studies the initial weight of both PE films added to individual conical flask were taken and after the biodegradation studies the polyethylene films were recovered from the conical flask and it was washed with distilled water followed by 70% ethanol and 2% sodium dodecyl sulphate this was done to remove the attachment of biofilm. The final weight was taken. The weight difference between initial and final weight indicate the extent of polyethylene utilizing by microorganisms. The weight loss percentage was analyzed by the equation 1.

$$\text{Weight loss (\%)} = \frac{Initial\ weight - final\ weight}{Initial\ weight} \times 100 \quad (1)$$

L. Scanning Electron Microscopy

The untreated and treated samples after 30 days were subjected to scanning electron microscopy (SEM) analysis to examine the degradation of polymer films. It was analyzed by using a High Resolution Scanning Electron Microscope CLARK ZEISS EVO 18 iS50. The recovered polyethylene without washing was subjected to SEM analysis to ensure the microbial attachment. The polyethylene after washing it with distilled water followed by washing it with 2% sodium dodecyl sulfate (SDS) solution and finally with 70% ethanol. This washing ensures maximum possible removal of cells.

III. RESULTS

A. Gram's staining and Biochemical test

Gram staining identifies the morphology of the isolated strain. The isolated strain I6 belongs to Gram-negative (-ve), rod shaped bacterium. The biochemical test result of isolated strain (I6) shows a negative (-ve) result for indole test and citrate test, it shows a positive (+ ve) result for methyl red test, Voges Proskauer test, lactose fermentation and catalase test the strain I6 was identified as *Enterobacter* and is shown in Table 1.

Table 1: Biochemical characterization and gram staining

Test carried	I6
Gram staining	Gram-ve bacilli
Indole	Negative (-ve)
Methyl red	Positive (+ve)
Vp test	Positive (+ve)
Citrate test	Negative (-ve)
Catalase test	Positive (+ve)
Lactose fermentation	Positive (+ve)
Microorganism identified as	*Enterobacter*

B. Determination of pH Change in the Medium Containing HDPE Plastic Strips

The pH change in medium was due to the presence of the microorganism in the medium and is shown in Table 2. The initial pH of the mineral salt medium were measured as 7 and as the medium were kept for incubation along with the plastic and organisms, observed a change in pH the medium. The pH changes indicate the metabolic activity and survival of microorganisms.

Table 2: pH change in the medium

Isolated strains	After 10 days	After 20 days	After 30 days
I6 + HDPE (without any pretreatment	7	6.9	6.7
I6 + HDPE (after UV pretreatment	7	6.8	6.8
I6 + HDPE (with the addition of mineral oil	6.9	6.7	6.6
I6 + HDPE (after UV and thermal pretreatment	6.9	6.9	6.9
I6 + HDPE (after UV, thermal and mineral oil addition)	6.9	6.9	6.7

C. Determination of turbidity Change in the Medium containing HDPE Plastic Strips

Turbidity measurement of medium containing each isolated strains and polyethylene was monitored after 30 days incubation and is shown in table 3. The turbidity change in the medium is due to the increase or decrease of the microorganism in the medium. The turbidity measured for the mineral salt medium was 0.042 (control).

Table 3: Turbidity Change in the Medium

Isolated strains	Absorbance
I6 + HDPE (without pretreatment)	0.154
I6 + HDPE(after UV pretreatment	0.174
I6 + HDPE(with addition of mineral oil)	0.215
I6 + HDPE(after UV and Thermal pretreatment	0.187
I6 + HDPE(after UV, thermal pretreatment and mineral oil addition)	0.308

D. Gravimetric analysis of PE films

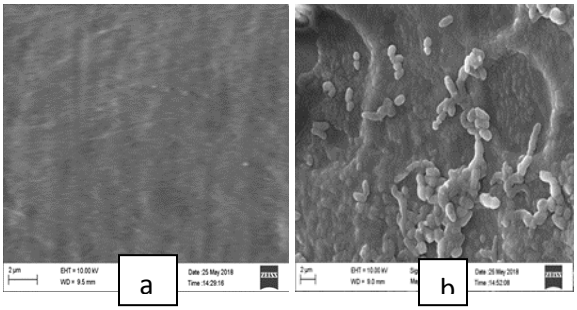
The substrate utilization by the organisms was observed by the weight reduction in the polyethylene films and is shown in table 4.

Table 4: Gravimetric analysis of polyethylene

Isolated strains	HDPE initial	HDPE final	% Reduction HDPE
I6 + HDPE(without any pretreatment)	0.055	0.054	1.81%
I6 + HDPE(After UV pretreatment	0.058	0.056	3.44%
I6 + HDPE(with addition of mineral oil	0.059	0.056	5.08%
I6 + HDPE(After UV and Thermal pretreatment)	0.056	0.054	3.57 %
I6 + HDPE (After UV, thermal and mineral oil addition)	0.059	0.055	6.77%

E. Morphological changes

The surface change of polyethylene was investigated by Scanning Electron Microscopy (SEM) after 30 days of incubation and is shown in Fig 1. The control images have an appearance of smooth surfaces having no pits. In case of treated polyethylene with isolate I6 observed several pits on the surface after the incubation period. It was observed that formation of bio-films on the surface of the polyethylene. The microbes noticed on the surface of the polyethylene indicate its strong adhering capabilities and polyethylene utilizing capabilities.



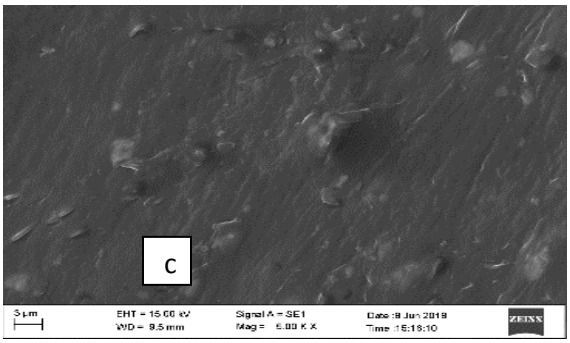


Figure 1: SEM micrograph before treatment a) HDPE control b) HDPE (biofilm layer) c) HDPE (after washing the biofilm)

VII. CONCLUSION

Microorganism capable of degrading polyethylene was isolated from the landfill site. By using the isolated strain I6 the study of pH change was analyzed to ensure the microbial activity of isolated strain in the medium. The turbidity was measured to determine the increase or decrease of microorganisms in the medium. Here medium containing pretreated and mineral oil addition shows increase in the turbidity. The weight reduction indicates the utilization of polyethylene by the microorganisms. The SEM image of polyethylene showed a clear evidence of polyethylene utilizing by the microorganisms. By using the isolated strain I6 shows degradation of fine particles of HDPE by 1.81% (without any pretreatment) which increases to 3.44% (UV treatment), 5.08% (mineral oil addition), 3.57% (UV and thermal treatment) and 6.77% (UV, thermal and mineral oil addition) with 30 days of incubation.

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