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Evaluation of HDPE Biodegradation by Bacteria isolated from Soil Sample Solapur, India

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ABSTRACT

High density polyethylene a synthetic polymer constitutes an important part of our life since decades. All the plastic waste generated by human activities is entering into ecosystem and causing environmental pollution. Natural degradation of high-density polyethylene is difficult and time consuming. Chemical methods used to overcome this problem have resulted in further pollution by releasing toxic chemicals during degradation process. Plastic pollution may be managed with the fewest negative effects by microbial degradation, a promising environmentally friendly technique. Six bacterial isolates in total were obtained from plastic waste dump site in Solapur, Maharashtra.

Keywords: High Density Polyethylene, Synthetic Polymer, Polyvinyl Chloride, Polyethylene Terephthalate

I. INTRODUCTION

Plastic is a synthetic material made from polymers, which are long chains of molecules. It is produced through a process called polymerization, where small monomers are chemically bonded together to form larger chains. Plastics have become ubiquitous in modern society due to their versatility, durability, and affordability. Global plastic production has doubled in the last two decades, reaching 460 million tonnes in 2019, an increase of nearly 230-fold since the 1950[1]. They are used in a wide range of applications, including packaging, construction, automotive, electronics, textiles, and healthcare. Common types of plastics include polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), polystyrene (PS), and many others. While plastics have provided numerous benefits and convenience, they also pose environmental challenges [10]. Plastics are known for their resistance to natural degradation processes, leading to their accumulation in the environment. Improper disposal and littering of plastic waste have resulted in pollution of land, water bodies, and ecosystems.

Efforts are being made globally to address the plastic waste issue. Strategies include recycling and reusing plastics, implementing waste management systems,

reducing plastic consumption, and promoting the development of alternative materials. Additionally, the development of biodegradable and compostable plastics aims to offer more environmentally friendly options.

HDPE (High-Density Polyethylene) is a thermoplastic polymer made from ethylene monomer units. HDPE is known for its high strength, durability, and resistance to chemicals and UV radiation. It is commonly used in various applications, including packaging (such as bottles and containers), pipes, geomembranes, electrical insulation etc. HDPE is produced through a polymerization process that involves the catalytic reaction of ethylene monomers [14].

The resulting polymer has a high molecular weight, leading to its high density and strong intermolecular forces [13]. This gives HDPE its characteristic properties, such as toughness and rigidity. Pre-treating microplastics with nitric acid and UV radiation has shown promising results in enhancing their biodegradation [2]. Nitric acid treatment can modify the surface of microplastics, making them more accessible to microorganisms for degradation. UV radiation, on the other hand, can break down microplastics into smaller fragments, increasing their surface area for microbial attack. Blending polymers with natural biodegradable polymers, such as polylactic acid (PLA), can also enhance their biodegradability. PLA is derived from renewable resources and is known to be biodegradable.

Mixing non-biodegradable polymers with PLA can help initiate the degradation process and reduce the overall persistence of the material in the environment. Additionally, mixing plastics with pro-oxidants can also improve their biodegradability. Pro-oxidants are additives that can initiate oxidation and degrade plastics more rapidly. They can break down the polymer chains, making the plastics more susceptible to microbial degradation [2].

Microorganism's naturally present in the environment, such as in fecal matter, have the potential to contribute to the degradation of HDPE waste. The evaluation of the biodegradation efficiency of strain *Bacillus cereus* present in cow fecal matter provides important insights into its degradation capabilities [3].

Pseudomonas sp. and *Arthrobacter* sp. have been acknowledged for their capacity to break down high-density polyethylene (HDPE). Both types of bacteria have demonstrated promising results in the degradation of HDPE, although *Pseudomona*s sp. has been observed to be more effective [4]. In this aspect *Pseudomonas* sp. is renowned for its versatility in metabolism and has been extensively studied for its ability to degrade various pollutants, including plastics. It produces enzymes like lipases and esterases that can cleave the long chains of HDPE into smaller fragments, which can then be utilized as a carbon source by the microorganism.

Arthrobacter sp. has also exhibited the capability to degrade HDPE, although its efficiency may be lower compared to *Pseudomonas* sp. *Arthrobacter* sp. is a common bacterium found in soil and has been found to possess enzymes that can break down HDPE, including esterases and oxidoreductases [4].

Public awareness about plastic pollution and its negative impacts on the planet and aquatic ecosystem should be increased through the media. Many people are not aware of the harm caused by synthetic plastics, and educating them through various media platforms can be an effective way to raise awareness [5].

In addition to raising awareness, it's important to provide proper waste disposal systems to both urban and rural areas. Lack of access to waste management facilities often leads to improper disposal of plastic waste, which ultimately ends up in our oceans and waterways [1]. By ensuring that everyone has access to such systems and making them convenient and easy to

use, we can encourage responsible waste disposal and reduce the amount of plastic pollution entering our environment [5].

Focusing on areas near the aquatic environment is crucial because these are often the most affected by plastic pollution. Coastal regions and rivers are particularly vulnerable, with plastic waste often finding its way into these water bodies and causing harm to marine life [10]. Implementing effective waste management systems, such as waste collection and recycling programs, can help prevent plastic waste from reaching water bodies and protect the health of aquatic ecosystems.

II. METHODS AND MATERIAL

A. Sample Collection

The samples were collected with utmost care to avoid any contamination. Special attention was given to collecting the samples from different depths to ensure a representative sample of the soil at the dump site.

The plastic dump site at Hipparga, Solapur region, Maharashtra in India was selected as it is known for its high accumulation of plastic waste. It has been used as a dumping ground for a long time, making it an ideal location to study plastic degradation. The presence of plastic degrading organisms was expected due to the continuous exposure to plastic waste.

To collect the soil samples, aseptic techniques were employed to prevent any contamination. The samples were collected from three different depths, namely 1cm, 2cm, and 3cm, to capture any variation in the distribution of plastic degrading organisms within the soil layers. Sterile polythene bags and a sterile spatula were used to collect the samples. Each depth was sampled separately, and the collected soil was carefully transferred into the sterile bags using the spatula. This ensured that the samples were not contaminated by any external sources and that the integrity of the samples was maintained.

The soil samples were then properly labelled and transported to the laboratory for further analysis.

B. HDPE Preparation

The first step in the process was to cut the HDPE sheets into small pieces. These small pieces were then immersed in xylene, a solvent commonly used for dissolving and cleaning various materials. The xylene and HDPE sheet mixture was then boiled for 15 minutes. After boiling, to remove the xylene from the solution, the HDPE solution was treated with ethyl alcohol. This step facilitated the removal of the xylene from the HDPE solution. The xylene-ethyl alcohol mixture was then evaporated, allowing for the separation of the HDPE powder. Next, the obtained HDPE powder was washed with ethanol. This step was essential to remove any remaining residue of xylene present in the HDPE powder. Again, the mixture of HDPE powder and ethanol was allowed to evaporate.

Finally, the HDPE powder was dried in a hot air oven at a temperature of 40-50°C for overnight. This process ensured the complete removal of any residual solvents and moisture from the HDPE powder, resulting in a dry and pure HDPE powder ready for further analysis.

C. Isolation of HDPE degrading Bacteria

1g of each collected soil samples were suspended in 10 ml of sterile water and vortexed for thorough mixing and then left undisturbed for 15 min. 5 ml of each soil suspensions were transferred into 250 ml conical flasks containing 100 ml of the sterile minimal salt media and 1gm of polymer beads (HDPE). All conical flasks were incubated on rotary shaker at 120 rpm and at 37°C. After one week of incubation, growth was observed as Optical density at 600nm in UV-Visible spectrophotometer. 5 ml of the enrichment culture was transferred into 100 ml of freshly prepared minimal media with 1gm of polymer beads as the sole source of carbon and energy. The second and third transfers were performed successively under identical conditions. 0.1 ml of the third enrichment culture of

overnight grown were transferred on agar plates containing 0.1% polymer powder for isolation of bacteria. After 14 days of incubation at 37°C, individual colonies were picked and streaked on the Nutrient Agar plates for isolation and purification. The organisms, producing zone of clearance around their colonies were selected for further analysis.

D. Identification of Isolated Strain

Once the pure cultures of the isolated bacterial colonies are obtained, they can be further characterized and identified using Gram's staining, colony morphology and biochemical test [19].

E. Determination of degradation rate of HDPE

The percentage of degradation of polyethylene plastic by bacteria can be calculated using the formula:
%Degradation = (initial weight - final weight / initial weight) x 100%
Where:
- final weight is the weight of the polyethylene plastic after incubation with isolated strain and drying.
- initial weight is the weight of the polyethylene plastic before incubation.

F. SEM analysis

Changes in the topography of HDPE film before and after bacterial degradation were examined by scanning electron Microscopy.

III.RESULTS AND DISCUSSION

A. Isolation of HDPE degrading bacteria

The six isolates that showed HDPE degrading activity on the minimal salt medium by enrichment culture technique were further examined for their potential for HDPE degradation. The zone of clearance around the colonies indicated that these bacteria have the ability to degrade HDPE.

By measuring the Optical density at 600 nm, the growth of the bacteria can be determined. This allows for the identification and quantification of the bacteria responsible for the HDPE degradation. The Optical density measurements can provide information about the concentration and activity of the bacteria in the culture.

Figure 1: Minimal medium with HDPE powder as carbon source

Figure 2 : Turbidity in the medium were observed after 14 days of incubation.

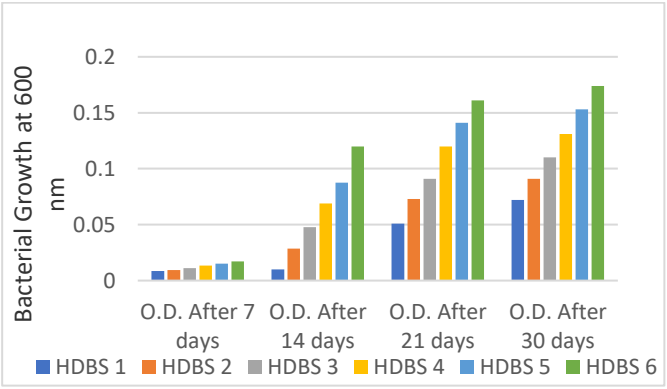

Figure 3: The growth of HDPE degrading bacteria in mineral salt medium.

B. Identification of Isolated Strain

After isolating the bacteria from plastic dumpsite soil sample Bergey's Manuals systematic approach were followed for identification. Gram staining revealed that prominent isolates are gram positive cocci shaped

organisms, while subsequent biochemical tests like citrate, mannitol, oxidase, glucose fermentation, and coagulase provided key clues to identify the bacterial species. By comparing the observations of biochemical test, it is confirmed that isolate HDBS 1, HDBS 2 and HDBS 4 belongs to *Bacillus* species whereas HDBS 3, HDBS 5 and HDBS 6 belongs to *pseudomonas*.

TABLE 1 BIOCHEMICAL TEST OBSERVATIONS OF ISOLATES

Characteristic	HDBS 1	HDBS 2	HDBS 4
Colony Characteristics			
Size	Small	Small	Large
Shape	Round	Round	Round
Colour	White	White	White
Margin	Entire	Entire	Undulate
Gram Satin	+	+	+
Motility	+	+	+
Glucose Fermentation	+	+	+
Catalase	+	+	+
Indole Test	-	-	-
MR Test	-	-	-
VP Test	+	+	+
Citrate	+	+	+

Characteristic	HDBS 3	HDBS 5	HDBS 6
Colony Characteristics			
Size	Small	Small	Large
Shape	Round	Round	Round
Colour	Green	Green	Green
Margin	Entire	Entire	Undulate
Gram Satin	+	+	+
Motility	+	+	+
Glucose Fermentation	+	+	+
Catalase	+	+	+
Indole Test	-	-	-
MR Test	-	-	-
VP Test	+	+	+
Citrate	+	+	+

C. Determination of degradation rate of HDPE

A simple method to determine biodegradation of polymer is weight loss method. The surface area of the polymer material plays a crucial role in the initiation and progress of biodegradation. Microorganisms that feed on the polymer typically colonize and grow on the surface, gradually breaking down the polymer structure. As the biodegradation process continues, the polymer may become brittle or lose integrity, leading to physical weight loss. The larger the surface area of the polymer, the greater the potential for microbial colonization and subsequent biodegradation, ultimately resulting in more weight loss.

In the present study, the polymer degrading efficiency of bacterial isolates was measured by determining the weight loss of the HDPE film after 30 days of incubation.

The maximum HDPE film weight reduction 0f 6.67% was observed by HDBS 5 strain. The percentage weight loss of HDPE film by HDBS 1, HDBS 2, HDBS 3, HDBS 4 and HDBS 6 was 2.04%, 2.27%, 4.10%, 1.29% and 6.01% respectively. In control experiment without the bacterial isolates showed weight loss of 0.11%. These findings indicated that isolated bacterial strain have potential to degrade high density polyethylene.

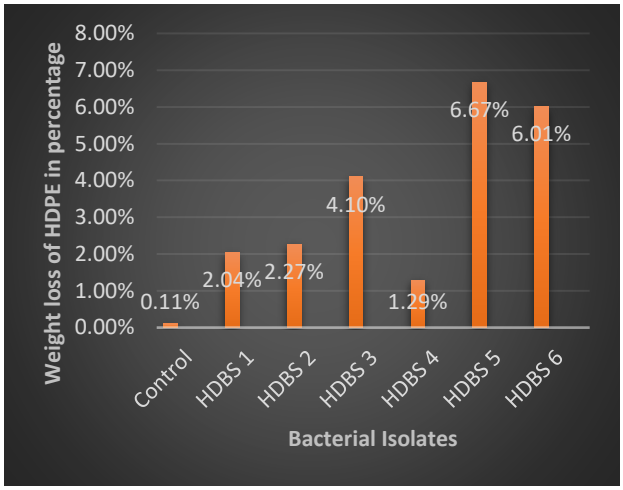

Figure 4: Weight reduction of HDPE by after 30 days of incubation with isolated bacterial strains.

D. Scanning Electron Microscopic analysis of HDPE treated with bacterial isolates

The surface morphology of High-density polyethylene (HDPE) films recovered from Minimal medium inoculated with bacterial isolates HDBS 1, HDBS 2, HDBS 3, HDBS 4, HDBS 5 and HDBS 6 for a period of 30 days was observed using scanning electron microscopy (SEM).

The SEM micrographs of the untreated HDPE samples (control) revealed a smooth surface morphology. This smooth surface suggests that the HDPE films did not undergo significant degradation under the given experimental conditions.

In contrast, the SEM images of HDPE films recovered from the Minimal medium inoculated with bacterial isolates showed noticeable changes in surface morphology. These changes included the presence of pits, cracks, and irregularities on the surface of the films.

The occurrence of these surface irregularities indicates that the bacterial isolates had the potential to degrade and modify the HDPE films. The presence of pits and cracks suggests that the bacterial activity resulted in the physical breakdown of the polymer structure. This could be due to the production of enzymes or other extracellular metabolites by the bacteria, which facilitated the degradation process.

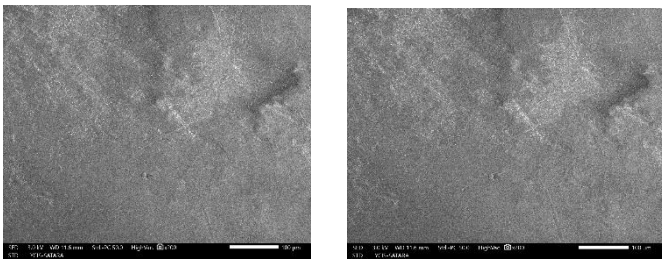

Figure *5:* Scanning electron micrograph of untreated HDPE film

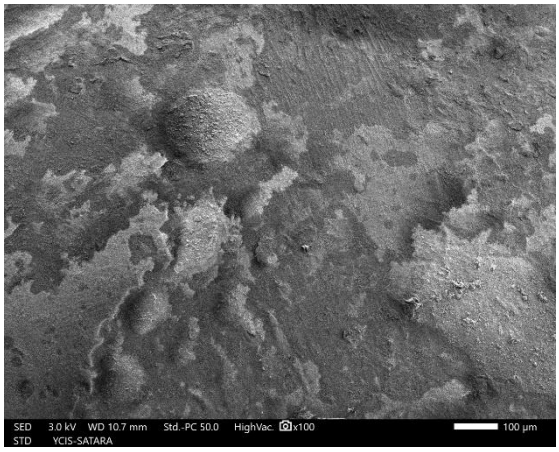

Figure 6*:* Scanning electron micrograph of HDPE film treated with bacterial isolate HDBS 5.

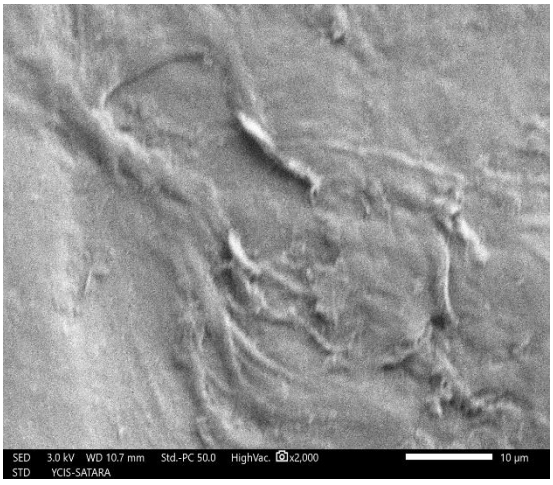

Figure 7 : Scanning electron micrograph of HDPE film treated with bacterial isolate HDBS 1.

IV. CONCLUSION

Bacterial strain capable of utilizing high density polyethylene as carbon source were isolated from plastic dump site Solapur, Maharashtra. HDBS 5 bacterial isolate was found more efficient high density polyethylene degrading bacteria than others. Bacterial isolates HDBS 1, HDBS 2 and HDBS 4 were confirmed as *Bacillus* species whereas HDBS 3, HDBS 5 and HDBS 6 confirmed as *pseudomonas*. This finding is significant because plastic waste, including HDPE, is a major environmental pollutant that takes hundreds of years to degrade naturally. By harnessing the potential of these microorganisms, it may be possible to accelerate the degradation process and reduce the accumulation of plastic waste in the environment.

Further research is needed to understand the specific mechanisms by which these microorganisms degrade HDPE and to optimize their activity. Additionally, it is important to assess the potential impact of using these microorganisms for bioremediation on other organisms and ecosystems.

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