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# Environmental sustainability is achieved by Low/ high-density polyethylene degrading by biodegradables tool

**Prabha Singh*[1], Dr. Parul Sharma[2], Sanjana[3]**

*[1]Department of Botany, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan (India).
[2]Department of Biotechnology, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan (India).
[3]Department of Biochemistry, Mewar University of Rajasthan, Chittorgarh(India)
Corresponding address: sanjanagoswami9211@gmail.com, anubhutijeevanki@gmail.com

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## ABSTRACT

***The development of environmentally friendly substitutes has developed into a potent strategy for displacing petroleum-based polymers in recent years. Bioplastics, which are a more and more common biodegradable replacement for plastics made from petroleum, have the potential to significantly reduce the amount of plastic pollution in the environment. Their environment has a significant impact on how they degrade. The issue of garbage and its effects on the environment have sparked renewed interest in the study of polymers that can be broken down by bacteria and fungi. Certain enzyme activities that result in the chain cleavage of polymers into oligomers and monomers are what drive the microbial breakdown of plastics. Biodegradation is the deformation of a substance into new compounds through biochemicalreactions or the actions of microorganisms such as bacteria or fungi. It is necessary for water-soluble or water-immiscible polymers because they eventually enter streams that can neither berecycled nor incinerated. It is important to consider the microbial degradation of natural andsynthetic polymers to understand what is necessary for biodegradation and themechanisms involved. Low/high-density polyethylene is a vital cause of environmental pollution. Itoccurs by choking the sewer line through mishandling, thus posing an everlasting ecologicalthreat. Thisrequires understanding the interactions between materials and microorganisms and thebiochemical changes involved. Biodegradation of plastics has beencarried out to overcome the environmental problems associated with synthetic plasticwaste. In this study, the screening of plastic-degrading microbes was done by usingan opaque method separately for bacteria.***

*KEYWORDS: Low/high-density polyethylene, biodegradation, bacteria, environmental, sustainability*

## INTRODUCTION

Plastic is one of the major inorganic solid waste fractions in our daily municipal solid waste (MSW) production. Ever-increasing population, rapid urbanization, and industrial advancement are some of the key driving factors for creating critical man-made problems that eventually threaten the existence of living beings on this earth. Plastic has been a ubiquitous commodity for more than 50 years in every aspect of our lives, covering households, construction, packaging, health care,

automotive, etc. It is mainly a petroleum-derived non-biodegradable with a unique polymeric structure that offers low specific weight, low thermal and electrical conductivity, and high durability (plastic bags- 20 years, plastic bottles-450 years, fishing line-600 years), excellent mechanical properties, reasonable pricing, etc. **(Eagle *et al.*, 2016; Wang *et al.*, 2018; Landon-Lane 2018; Mazhandu *et al.*, 2020a, b).**

The rapid rate of urbanization and development has led to an increase in the consumption of plastic products vis-à-vis plastic waste generation. It is a fact that plastic waste constitutes a significant portion of the total municipal solid waste (MSW) generated in India. Plastics are non-biodegradable and remain on Earth for thousands of years. The burning of plastic waste under uncontrolled conditions leads to the generation of different hazardous air pollutants (HAPs), depending upon the type of polymers and additives used. However, end-of-life plastics can be recycled into a second-life application after every thermal treatment/recycling deterioration in the quality of recycled plastic products. Thus plastic waste can be recycled only 3-4 times. The visibility of huge quantities of plastic waste has been perceived as a serious problem and made plastics a target in the management of solid waste (**Mourshed *et al.*, 2017; Horodytska *et al.*, 2019)**. Different types of plastics and their uses are given in **Table:1.**

**Table 1. Different Types of Plastics & their Uses**

| S. No. | Symbol | Short Name | Scientific Name | Uses |
|---|---|---|---|---|
| 1 |  | PET | Polyethylene terephthalate | Soft drink bottles, furniture, carpet, paneling etc. |
| 2 |  | HDPE | High-density polyethylene | Bottles carry bags, milk pouches, recycling bins, agricultural pipe, base cups, playground equipment, etc. |
| 3 |  | PVC | Polyvinyl chloride | Pipe, Window profile, fencing, flooring, shower curtains, lawn chairs, non-food bottles and children's toys etc. |
| 4 |  | LDPE | Low-density polyethylene | Plastic bags, various containers, dispensing bottles, wash bottles, tubing etc. |

| | | | | |
|---|---|---|---|---|
| 5 |  | PP | Polypropylene | Auto parts, industrial fibers, food containers, dishware, etc. |
| 6 |  | PS | Polystyrene | Cafeteria trays, plastic utensils, toys, video cassettes and cases, clamshell containers, insulation board, etc. |
| 7 |  | O | Other | Thermoset Plastics, Multilayer and Laminates, Bakelite, Polycarbonate, Nylon SMC, FRP,etc. |

**DEGRADATION OF PLASTIC**

Plastic degradation involves various treatments of physical, chemical, and biological factors.

Physical factors involve heat and radiation, chemical factors include acids and alkalis and biological factors are using microbes like bacteria, fungi,and *actinomycetes***(Thamizhmarai *et al.*, 2018).** Based on the nature of the causative agents, the degradation of polymer is classified as thermal degradation, photo-oxidative degradation, mechanic-chemicaldegradation, catalytic degradation, ozone-induced degradation, and biodegradation **(Devi *et al.*, 2016)**.

The conventional techniques employed to degrade plastic in the environment are inadequate as it releases harmful by-products.The worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tons of synthetic polymers are produced worldwide each year**(CPCB, 2018-19)**. With such a huge amount of polyethylene getting accumulated in the environment, their disposal evokes a big ecological issue. It takes thousands of years for their efficient degradation. The most abundant plastic waste materialsdiscarded in landfills are plastic bags(69.13%) which are made up of low-densitypolyethylene (LDPE) **(Pramila *et al.*, 2011)**.

LDPE is amorphous innature (10-30 methyl (CH3) groups per 1000 Catoms) and composed of butene, hexene, andoctene. The branching system in LDPE chainsmakes it more accessible and susceptible to attackdue to the presence of more tertiary carbonatoms at the branch sites**(Helen *et al.*, 2017)**. In LDPE material, the physical arrangement of the polymer chains and the vinylidene content is directly related to the polymer oxidization which makes it more biodegradable**(Mohanan *et al.*, 2020)**.

**MICROBES AND THEIR ROLE IN PLASTIC BIO-DEGRADATION**

Microbes are omnipresent in the biosphere, and their presence influences the environment in which they grow. The effects of microbes on their environment can be beneficial or harmful. The most important role of the microbes on earth is their ability to decompose the organic matter and recycle the primary elements (carbon (C), oxygen (O), and nitrogen (N)) that make up all living systems**(Krapivin *et al.*, 2017)**. Plastic biodegradation is the breakdown of complex polymers into simpler monomers which depends on various factors like morphology, substrate availability, the molecular weight of the polymers, and surface characteristics **(Albertsson *et al.*, 1987; Ammala *et al.*, 2011; Harrison *et al.*, 2018)**.

The microbial role is very crucial in the degradation of plastic and different microorganisms degrade different plastic groups.Bio-degradation of plastics is brought about by the activity of microbial enzymes through the cleavage of polymer chains into monomers and oligomers, which are water soluble and get easily absorbed and metabolized by the microbial cells**(Naser *et al.*, 2021)**. Aerobic metabolism results in carbon dioxide and water, and anaerobic metabolism results in the production of carbon dioxide, water, and methane which are called end products, respectively **(Karamanlioglu *et al.*, 2017)**. The degradation leads to the breaking down of polymers into monomers creating an ease of accumulation by the microbial cells for further degradation **(Kumari *et al.*, 2013)**. The biodegradation method of polymers is classified with the use of microbes and enzymes **(Kim *et al.*, 2022)**.

The purpose of this study was to isolate microorganisms from dumped soil areas and screenthe potential plastic-degrading microorganisms and identify the high-potentialmicroorganism that degrades the plastics.

## MATERIAL AND METHODS:

### Sample collection

The soil sample (Municipal solid waste, where plastic bags were buried) was obtained from a compost plant, atdifferent dumping yard sitesin Jaipur Municipal Corporation (JMC) Rajasthan, India**(Figure:1)**. The compost inoculum was free from larger inert materials (glass, stones, metals, etc.) as much as possible. These items are removed manually as much as possible to produce a homogenous compost inoculum. The soil sample had the following basic properties: total solids (%TS) 81%; volatile solids at 550°C (%VS) 18%, pH 7.2, C/N ratio 15.3. It was used for the isolation of polymer-degrading microorganisms.

(a) (b)

**Figure.1: Collection of soil samples from (A-B) different dumping yard sites Jaipur Municipal Corporation (JMC) Rajasthan, India.**

**Plastic Material:** Commonly available plastic bags were collected from Solid waste plantsof different dumping yard sites in Jaipur Municipal Corporation (JMC) Rajasthan, India.

### Media for cultivation and degradation experiments

### Preparation of Nutrient agar (NA) media and Nutrient broth (NB) media

- **Nutrient agar media**

The composition of nutrient agar was beef extract (1gm/l), peptone (5 gm/l), NaCl (5 gm/l), yeast extract (2 gm/l), and agar (2%). The pH was maintained at 6.8±2. The media was sterilized and prepared NA plates.

- **Nutrient broth**

The composition of the nutrient broth was beef extract (1gm/l), peptone (5 gm/l), NaCl (5 gm/l), and yeast extract (2 gm/l). The pH was maintained at 6.8±2. The broth was sterilized and prepared NB flasks.

### Isolation of polymer-degrading microorganisms:

The plastic-degrading microorganisms were isolated from the soil with the help of serial dilution.

### Bacterial isolation and identification:

The bacterial strains were isolated with the ability to degrade and performed based on macroscopic and microscopic examination and biochemical tests. The bacterial isolates were identified macroscopically by examining colony, morphology, surface pigment, size shape, margin, and surface on media plates and microscopic examination including, grams staining to study the staining behavior, shape, and cell arrangement, and granulation, spore staining. The motility test was also performed as a biochemical test. The isolates were identified by using the selective medium.

### Microbial Degradation of Plastics in Laboratory Condition:

### Pre-treatment of polyethylene

The polyethylene was cut into small strips and transferred into the sterile beaker with distilled water and stirred for 1 hour. Further, they were aseptically placed inan ethanol solution of 70% v/v for 30 minutes. Then, the polyethylene strips were transferred to a sterile petri plate. Finally, the plastic strips were air-dried and weighed in fixed mass.

**Inoculation of Identified bacteria from soil samples in Nutrient agar plate**

Identified bacteria from the soil sample were inoculated onto Nutrient agar plates and in Nutrient broth flasks containing polythene strips and incubated at 37°C separately for one month. Negative control was maintained by adding the same quantity of plastic strips in the Nutrient agar plate and Nutrient broth flasks without inoculation of the bacteria and incubated together with a test at the same temperature.

**Dry weight determination of recovered polyethylene**

The residual polyethylene strips were recovered from the culture plates and flasks after one month. The bacterial cell mass adhering to the polyethylene surface was washed with a 2% (v/v) aqueous sodium dodecyl sulfate (SDS) solution for 2 hours and finally with distilled water. The washed polyethylene particles were air-dried and weighed. The weight loss of the plastics was calculated by using the following formula:

$$\text{Percentage of weight loss} = \frac{\text{Initial weight - Final weight} \times 100}{\text{Initial weight}}$$

**RESULT & DISCUSSION:**

The present study aimed to degrade the plastic strips using microbes isolated from soil samples. Many different bacterial isolates were obtained from the soil samples. But only predominant bacterial colonies were chosen by screening and they were identified based on their Morphological and biochemical characteristics.

**Isolation of bacteria from soil samples**

The plastic degrading (PD) bacteria were isolated and assayed for biochemical tests for identification from the soil samples collected from different dumping yard sites in Jaipur.

**4.4.1 Gram's staining**

During the Gram's staining, it was observed that PD 1,3, and 5 were Gram-positive, and PD 2 and 4 were Gram-negative as shown in **Figure:2**.

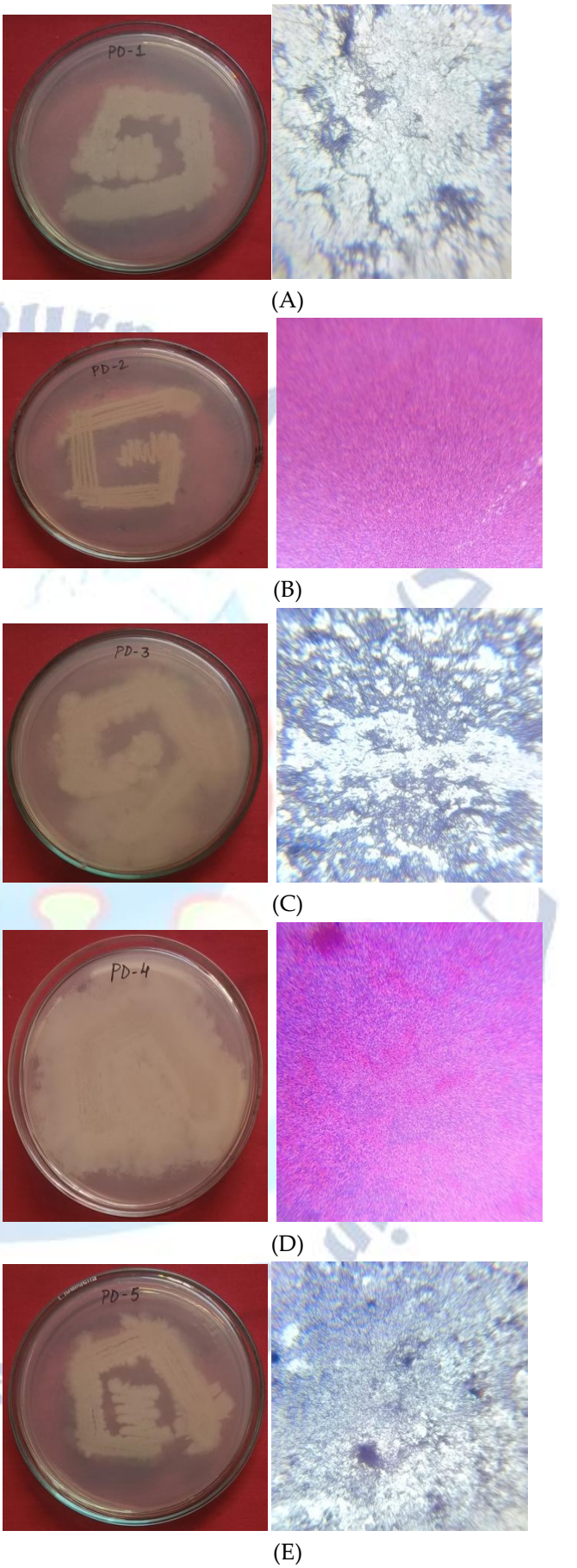



**Figure. 2: Isolated bacteria (A-E) from collected soil samples and their Gram staining**

#### 4.4.2 Catalase test

As shown in **Figure:3**, all the isolated bacteria were able to produce catalase enzyme and gave a positive result to the catalase test.


**Figure.3: Catalase test of isolated bacteria**

#### 4.4.3 KOH test

KOH test was performed for all the bacterial isolates and gave a negative response to the test hence all bacteria were KOH negative as shown in **Figure:4**.

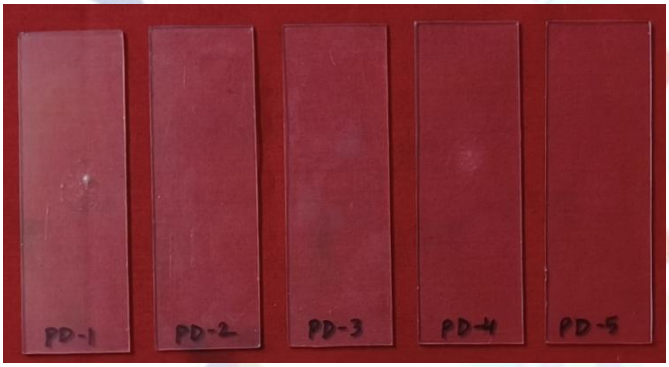



**Figure.4: KOH test of the isolated bacteria**

#### 4.4.4. Oxidase test

As shown in **Figure:5**, PD 2 and 3 were oxidase positive while PD 1, 4, and 5 gave a negative result for the oxidase test.

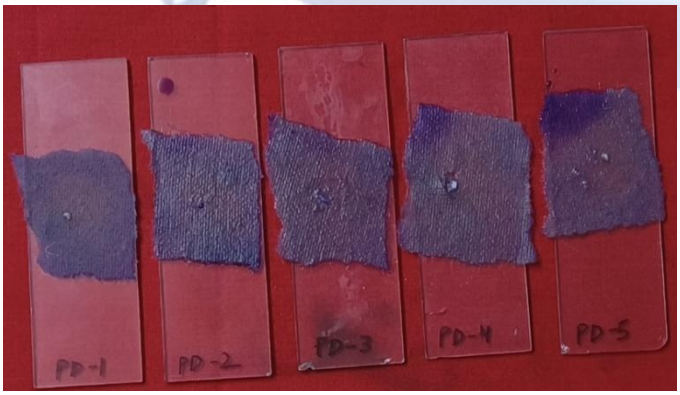



**Figure:5: Oxidase test of the isolated bacteria**

#### 4.4.5 Indole production test

**Figure:6** shows the results for the indole production by the isolated bacteria in which all the bacteria were negative for indole production.

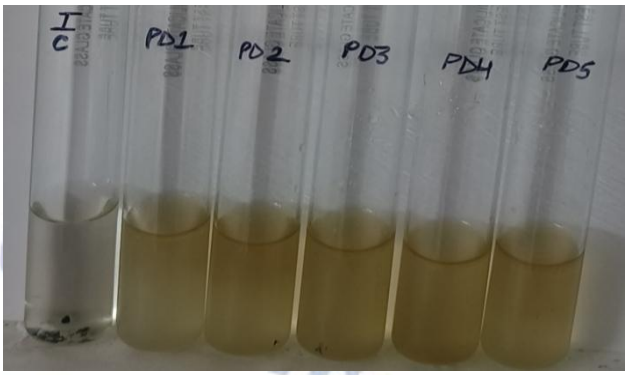



**Figure.6: Indole production test of the isolated bacteria**

#### 4.4.6 Citrate utilization test

All the isolated bacteria utilized citrate from the prepared nutrient medium and changed the color from pale yellow to bluish color as shown in **Figure:7** which gave a positive result for the citrate utilization test.

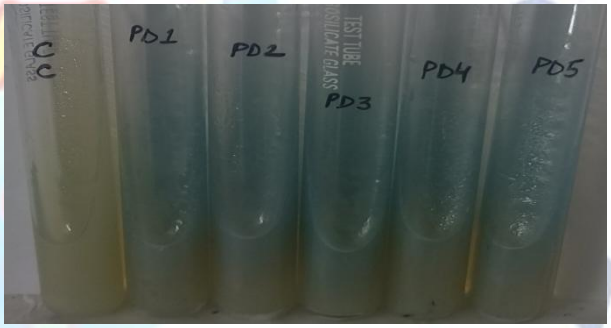



**Figure.7: Citrate utilization test of isolated bacteria**

#### 4.4.7 Methyl Red test

As shown in **Figure:8** all isolated bacteria gave positive responses in the methyl red test except the PD 2 isolated bacteria.

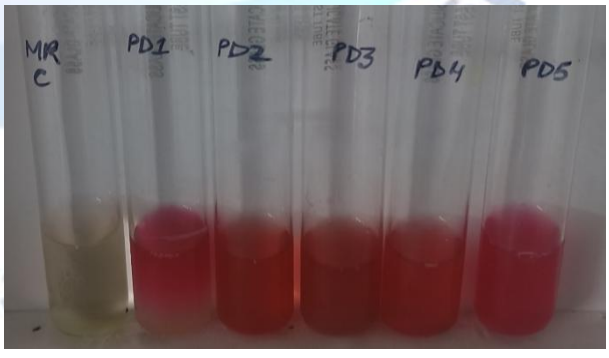



**Figure:8: Methyl Red test of isolated bacteria**

#### 4.4.8 Triple Sugar Iron (TSI) test

As shown in **Figure:9** all the isolated bacteria gave negative results to the triple sugar iron (TSI) test as they did not produce any $H_2$ gas.

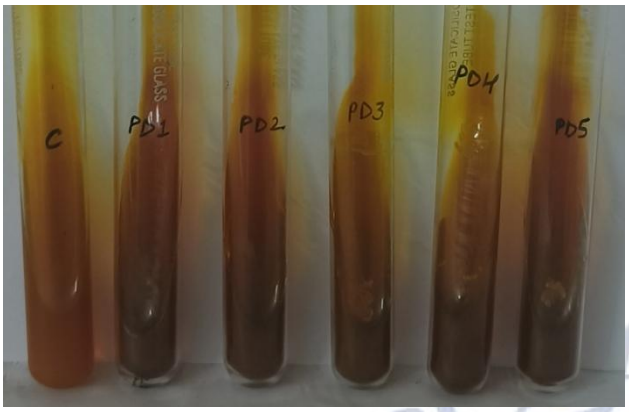



**Figure.9: TSI test of isolated bacteria**

#### 4.4.9 Nitrate Reduction test

As shown in **Figure:10**, bacterial isolates PD 1,2, and 5 showed positive results for nitrate reduction whereas PD 3 and 4 gave negative results for it.

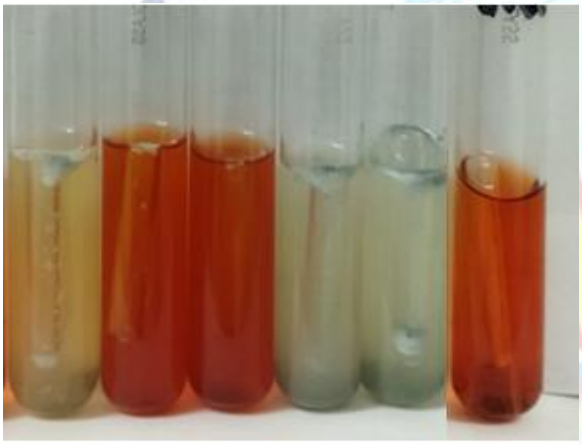


**Figure.10: Nitrate Reduction of isolated bacteria**

#### 4.4.10 Carbohydrate Fermentation Test

As shown in **Figure:11**, Also in the carbohydrate fermentation test, all the bacterial isolates gave positive results for the carbohydrate fermentation except PD 2 which was negative for it.

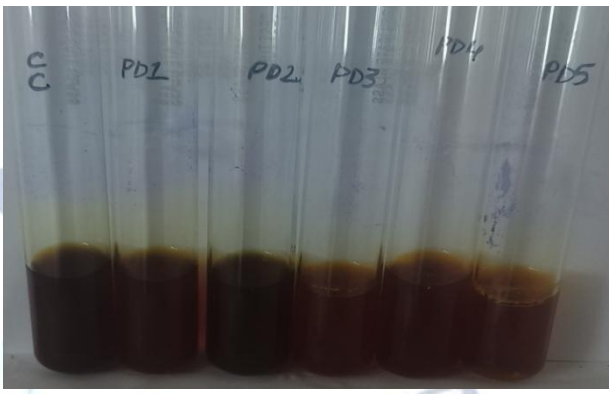



**Figure.11: Carbohydrate fermentation test of isolated bacteria**

A total of five isolates were isolated from dumped soil ofdifferent dumping yard sites in Jaipur Municipal Corporation (JMC) Rajasthan, India. These five isolates were purified to tilt to the next test and screened for plastic degradation by incubation for 1 month in an incubator shaker at 130 rpm agitation in 37$^0$C temperature conditions. The bacteria which were identified from the above biochemical tests are *Staphylococcus epidermis,Pseudomonas aeruginosa, Bacillus subtilis,Enterobactersp.*, and *Staphylococcus aureus*, by the software PIBWIN (Probabilistic identification of bacteria) as shown in **Table:2**.

**Table.2: Biochemical identification of bacterial isolates from soil samples.**

| Biochemical Test | PD 1 | PD 2 | PD 3 | PD 4 | PD 5 |
|---|---|---|---|---|---|
| Gram's staining | + | - | + | + | + |
| Catalase test | + | + | + | + | + |
| Oxidase test | - | + | + | - | - |
| KOH test | + | - | + | + | - |
| Citrate test | + | + | + | + | + |
| Indole test | - | - | - | - | - |
| Methyl red test | + | - | + | + | + |
| TSI test | - | - | - | - | - |
| Nitrate test | + | + | - | - | + |
| Carbohydrate | + | - | + | + | + |

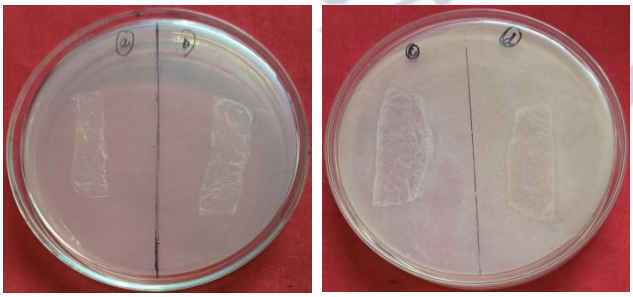
| test | | | | | |
|---|---|---|---|---|---|
| **Identification** | *Staphylococcus epidermis* | *Pseudomonas aeruginosa* | *Bacillus subtilis* | *Enterobacter* sp. | *Staphylococcus aureus* |

*PD- plastic degrading bacteria

From the data collected,the weight loss of the polythene bag was calculated. The species tested were *Staphylococcus epidermis,Bacillus subtilis,Enterobactersp.*, and *Staphylococcus aureus*. Among the bacteria, *Pseudomonas aeruginosa* was found most active in degrading53-79%of polythene in one month period.

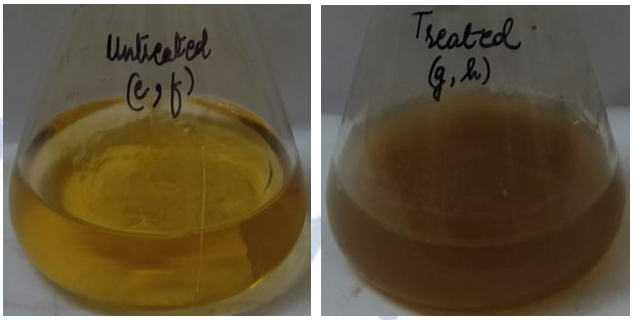
***Pseudomonas aeruginosa*degradation of plastics calculated by dry weight:**

The potency of the degradation of plastic was screened by using *Pseudomonas aeruginosa* for one month. From all samples, we show two samples of polythene were isolated from the Malviya Nagar dumping yard site and the Mansarover dumping yard site of Jaipur Municipal Corporation (JMC) Rajasthan, India. The degradation efficiency was checked on two types of growth mediums; one was Nutrient Agar and the second was Nutrient Broth as shown in **Figure:12**and **Figure:13**. The weight loss of the plastics was calculated and plotted in a graph **(Figure:14)**. The average weight of control (untreated plastic) was 0.065 gm that was reduced in the range of 0.025-0.011 gm with the tested samples in both type growth media. The sample obtained from Malviya Nagar has a weight loss of **78%** in Nutrient Agar and **53.33%** in Nutrient Broth in comparison to the control whereas the sample obtained from Mansarover has a weight loss of **78.33%** in Nutrient Agar and **68.75%** in Nutrient Broth as compared to control. From the result obtained, it can be said that *Pseudomonas* aeruginosa is an effective and efficient candidate for the degradation of plastic.



(a) (b)

**Figure.12: Nutrient media containing polythene strips as (A) Control (B) Treated**




(a) (b)

**Figure.13: Nutrient broth containing polythene strips as (A) Control (B) Treated**

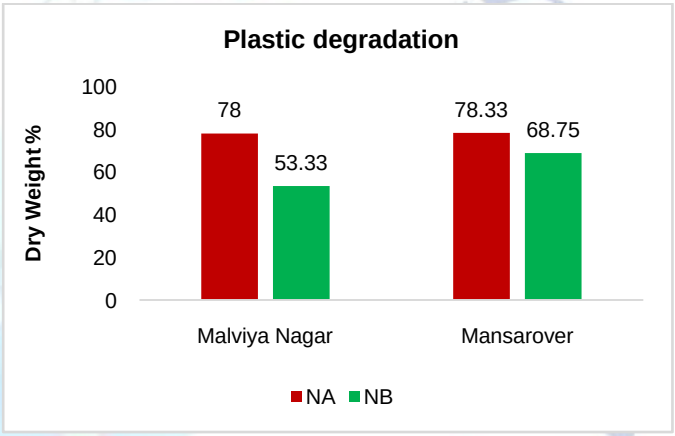



**Figure.14: Plastic degradation by *Pseudomonas aeruginosa***

First of all, microorganisms were attracted to the plastic material as a source of carbon.

Microorganisms consumed plastic in the polymer matrix and caused a fracture in the LDPEchain.The isolated microbes were native to the site ofpolyethylene disposal and might show some degradability in natural conditions, yet they alsoexhibited biodegradation in laboratory conditions on synthetic media. This gives somesuggestions that these microbes can be used in both natural and artificial conditions for the degradation of polymers. Our knowledge, microbes cause the greatest degradation ofpolythene and plastics.Among the bacteria, viz *Pseudomonas aeruginosa* followed by*Staphylococcus epidermis,Bacillus subtilis,Enterobactersp.*, and *Staphylococcus aureus*, having greaterdegradation ability. It is concluded that isolated strains are solely

dependent on plastic for theircarbon source. Hence, further attention is required from microbiologists for commercial degradation and eco-friendly polyethylene.

## CONCLUSION

A total of five bacterial strains capable of degrading plastic have been isolated from dumped soil. The isolates obtained were subjected to standard biochemical test results showed the presence of.*Pseudomonas aeruginosa* for bacteria. The organisms identified were further inoculated into different culture media and their bio-degradative ability was determined by loss of weight after a period of 30 days and observed that bacterial *sp*. degrades up to 79%. Thus it may take 30 days to complete the degradation of plastics by using bacteria for the complete degradation of plastic. Further biochemical tests can be done to identify the exact bacteria. Further characterization of the obtained microbes can be done to increase the level of biodegradation of plastic.The polluted environment containing plasticwastes can be cleaned easily with the inclusionof microbes without causing any detrimental effect on the environment. Plastic biodegradationis an eco-friendly, beneficial, and also cost-effective approach to plastic wastemanagement. Biodegradation of plastics by bacteriacan be made most efficient by altering the factors thatgovern the process. It promises a reduction in plasticpollution in the future.

### Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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