



(RESEARCH ARTICLE)



## Biodegradation of plastic by *Pseudomonas aeruginosa*

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### Abstract

The threat of plastic pollution has become a global concern for the environment, resulting to the exploration of sustainable measures that reduce its adverse effects. Bioremediation is one such method that uses microorganisms to degrade soil and water contaminants, it is cost effective, eco-friendly and renewable. *Pseudomonas* is a ubiquitous genus of bacteria that can be used in biodegradation of several kinds of plastic. The current studies focus on the utilization of *Pseudomonas aeruginosa* for the biodegradation of polythene. *Pseudomonas* strain was isolated from soil contaminated with plastic waste. Based on morphological, physiological, biochemical, and pigmentation shifts the isolated strain was identified as *Pseudomonas aeruginosa*. This strain was capable of degradation of polythene material in 16 days of incubation with the highest percentage being an 11.5% reduction.

**Keywords:** *Pseudomonas*; Contaminated site; Plastic; Biodegradation

### 1. Introduction

Bioremediation is a process by which microbes degrade target pollutants at the site of contamination. This can be done by altering the environmental conditions and providing them with the required nutrients. Bioremediation is a new treatment technology based on fundamental processes of microorganisms to utilize synthetic organic molecules as a sole source of energy. Bioremediation usually involves redox reactions where either an electron acceptor is added to stimulate the oxidation of a reduced pollutant or an electron donor is added to reduce an oxidized pollutant. Bioremediation exploits the catabolic diversity of microorganisms to transform contaminants into eco-friendly products. Many species of bacteria and fungi have evolved the metabolic capacities to degrade plastics [1]. However, the event of biotechnology for the removal of plastics from commercial effluents remains to be adequately addressed even today. Various approaches are being developed to treat the plastic. The constraints are the availability of suitable microorganisms that can overcome their culturing limitations from their natural habits to the effluent conditions. *Pseudomonas* is a genus of aerobic, non-sporulating, motile Gram-negative Bacilli. This genus is found to have considerable heterogeneity [2]. This genus of bacteria is well known for its metabolic versatility allowing it to inhabit a range of environments and utilize an unusually wide range of polymers [3].

Earlier studies have shown *Pseudomonas aeruginosa* to be capable of degrading diesel, crude oil, n-alkanes, and polycyclic aromatic hydrocarbons (PAHs) in petroleum [4]. *Pseudomonas* is manifested with the capacity to degrade several aliphatic, aromatic, polyaromatic hydrocarbons and various derivatives, among a vast variety of miscellaneous organic compounds [5]. *Pseudomonas aeruginosa* is a predominant microbial species for phenol degradation [6,7]. *Pseudomonas aeruginosa* KBM13 exhibited maximum degradation of phenol at a concentration of 500 mg/L [8].

Plastic usage has transformed our lives in various ways. The production and utilization of plastics are always increasing due to the rising demand. They are inexpensive, strong, lightweight, corrosion-resistant, have duration and

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electrical insulation properties, and have high thermal [9]. They play an important part in every sector of the economy worldwide due to their extensive use in agriculture, building and construction, health, and consumer goods. They are the backbone of many industries because they are used in the manufacturing of different products including defense materials, sanitary wares, tiles, plastic bottles, artificial leather, and other household items. Plastics are also used in the packaging of food items, pharmaceuticals, detergents, and cosmetics. Excessive use of plastics poses a serious threat to the ecosystem and human life on the planet hundred billion to one trillion/annum PE (polythene) covers have been under regular use worldwide. The useful breakdown of plastic bags takes more than a thousand years. Plastic causes global warming and pollution not only as a major issue of waste disposal but then also releases dioxides and CO<sub>2</sub> while burning [10]. Plastic accumulation on land and sea has aroused interest in degrading these polymers. There is a need to use adequate biodegradable methods to reduce the plastic burden on the environment. Methods like biodegradation, plastic degradation, and bioremediation potential make these microorganisms propitious for green chemistry to eliminate harmful plastics from the ecosystem. [11,12]

In our previous study, an aerobic microorganism with the ability to utilize phenol as a carbon and energy source was isolated from a site contaminated with plastic waste. The isolate was identified as a *Pseudomonas sp.* based on morphological, physiological, and biochemical tests. The isolated strain showed optimal growth at 25 °C and pH of 7. The phenol utilization studies with the *Pseudomonas sp.* showed that the complete assimilation occurred in 24 hours. The microorganism metabolized phenol up to 53mM concentrations. The bacterial strain was immobilized in alginate beads and its phenol degradation efficiency was observed to increase many folds. [13]

The present study reports the biodegradation of plastics such as polythene by the *Pseudomonas* strain. This is a new agent for the biodegradation of plastic and is capable of mineralizing plastic as the sole source of carbon and energy. The study shows its applicability in the bioremediation of plastics.

### Objectives

- To study the growth of isolated bacteria *Pseudomonas*, in the presence of plastic
- To study the degradation of plastic by the isolated strain of bacteria

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## 2. Material and methods

### 2.1. Chemicals

All chemicals used were of analytical grade and purchased from commercial suppliers.

### 2.2. Isolation of microorganisms from source

Soil samples were collected from plastic-contaminated sites around the Uttarahalli domestic area and were used to isolate microorganisms adopting selective enrichment techniques. The bacterial strain was grown on a mineral salt medium supplemented with varied concentrations of phenol as the sole source of carbon and energy.

### 2.3. Culturing of bacteria

The organism was maintained and propagated on nutrient agar and substrate-mineral salt media. For purification of the bacterial strain, the microorganism was grown on a nutrient agar medium. For metabolic studies, the bacterial strains were grown on mineral-salt medium (MSM) containing (g/lit: K<sub>2</sub>HPO<sub>4</sub>, 1.6; K<sub>2</sub>HPO<sub>4</sub>, 0.2; (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1.0; MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.2; NaCl, 0.1; CaCl<sub>2</sub>.2H<sub>2</sub>O, 0.02; FeSO<sub>4</sub>.H<sub>2</sub>O, 0.01; Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O, 0.5; MnSO<sub>4</sub>.H<sub>2</sub>O, 0.5; Na<sub>2</sub>WO<sub>4</sub>.2H<sub>2</sub>O, 0.5. The growth substrate was supplemented to the sterilized mineral salt medium aseptically. The flasks were then inoculated with 5% inoculum aseptically and were incubated at 25°C (± 2°C) on a rotary shaker for 24 hours. Uninoculated flasks were incubated in parallel as controls [13,14].

### 2.4. Identification and characterization of bacteria

According to previous studies, a series of different tests were performed to identify and differentiate bacteria. The bacteria were isolated and cultured in a nutrient agar medium as they are the basic media and enhance the growth of non-fastidious bacteria due to the presence of nutrients in abundance. The identification and characterization of bacteria is an important as well as a systematic procedure that has to be performed. The biochemical tests and gram staining categorize bacteria using various properties like composition of cell wall (Gram staining), production of hydrolytic enzymes (Indole test, Urease test), ability to ferment glucose (Methyl red test and Voges Proskauer test), ability to use citrate as the sole carbon source (Citrate test), and the ability to reduce compounds like nitrate and

sulphate (Nitrate reduction and H<sub>2</sub>S production test) respectively. The motility of the bacteria was determined by the hanging drop technique. All the results together have indicated the isolated organism to belong to the strain of *Pseudomonas*. The results are tabulated in Tables 1 and 2.

### 2.5. Degradation of polythene by isolated bacteria

Polythene material, collected from the soil dumps at Uttarahalli, Bengaluru served as plastic sample for investigation. Three distinct weights of the plastic sample 0.1, 0.2, and 0.3 grams were sliced into thin strips. These were then rinsed with water, dried, and exposed to the bacterial culture after being immersed in ethanol for five minutes. The entire process of inoculation and incubation was done under aseptic circumstances. We took three conical flasks and filled each with 1000µl of *Pseudomonas aeruginosa* inoculum and 100ml of MSM medium. 0.1g of polythene was added to the 1<sup>st</sup> flask, 0.2 g to the 2<sup>nd</sup> flask, and 0.3g to the 3<sup>rd</sup> flask. The flasks were incubated and kept on a rotatory shaker at 120 rpm, 37°C. A control was maintained with uninoculated MSM media. During the full three weeks of incubation, the plastic strips were removed, and plastic weights were tested for weight decrease on days four, eight, twelve, and sixteen. Later, the percentage reduction in the weights of polythene strips was calculated.

## 3. Results and discussion

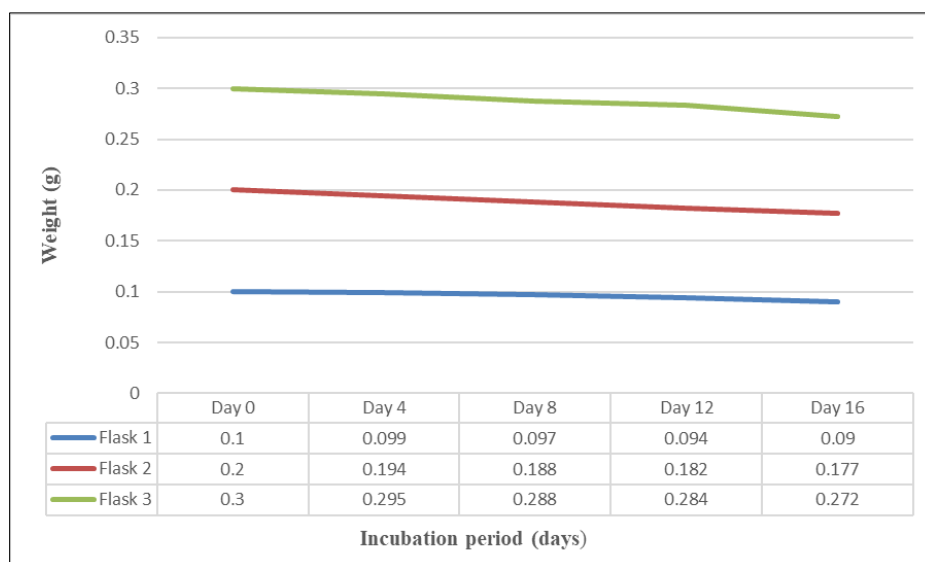
The bacteria was isolated from the source and characterized. It was then subjected to polythene biodegradation study.

**Table 1** Biochemical tests for identification of bacteria

Biochemical Tests	Results obtained
Shape	Short rod-shaped
Gram staining	Gram-negative
Indole Production	Negative
Methyl Red	Negative
Citrate Utilisation	Positive
Voges Proskauer	Negative
Cetrimide agar	Positive
Catalase	Positive
Oxidase	Positive

**Table 2** Carbohydrate Fermentation Test

Carbohydrate Fermentation Test	Acid	Gas
Glucose	Negative	Negative
Lactose	Negative	Negative
Sucrose	Negative	Negative
Mannitol	Positive	Positive
Maltose	Positive	Negative



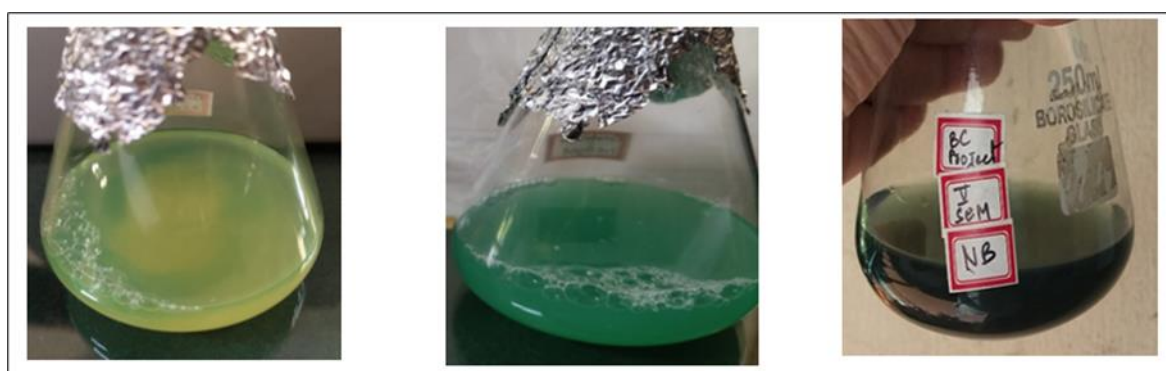
**Figure 1** Biodegradation of Plastic by *Pseudomonas aeruginosa*

**Table 3** Percentage reduction in weight of plastic after 16 days of incubation

Flask number	Initial plastic weight (g)	Final plastic weight (g)	Percentage reduction
1	0.1	0.09	10%
2	0.2	0.177	11.5%
3	0.3	0.272	9.33%

In three weeks of incubation and with only a modest inoculation, *Pseudomonas aeruginosa* can metabolize and break down plastic up to 11.5%, as shown by Graph 1 and Table 3. Also, the plastic weight in Flask 1 drops from 0.1 g to 0.09 g, in Flask 2 from 0.2 g to 0.177 g, and in Flask 3, from 0.3 g to 0.272 g.

The rates of decline could rise dramatically if given more time, which would result in a greater reduction.



**Figures 2, 3, and 4** Depict the pigmentation changes in *Pseudomonas* inoculated nutrient broth after 24 hrs, 30 hrs, and 34 hrs. *Pseudomonas aeruginosa* may display color variations upon incubation, frequently due to the synthesis of different pigmented secondary metabolites. These alterations may function as markers of environmental adaptation and metabolic activity

*Pseudomonas aeruginosa* frequently produces the following pigments

- Pyocyanin: *Pseudomonas aeruginosa* produces a blue-green pigment called pyocyanin, which gives the bacteria its distinctive greenish hue. Pyocyanin has antibacterial qualities and is linked to virulence.
- Pyoverdine: A yellow-green fluorescent pigment, involved in iron chelation and uptake. The bacteria can produce fluorescence and, be observed under UV light.
- Pyorubin and pyomelanin: These are red and brown pigments, respectively, which can also contribute to the overall coloration of *Pseudomonas aeruginosa* cultures.

Following incubation, variations in the presence or strength of these pigments reveal details about the physiological status of the bacteria, including their growth phase, metabolic activity, and reaction to external stimuli. These alterations in pigmentation are frequently employed in microbiological tests to identify and characterize *Pseudomonas aeruginosa*. Hence from Figures 1,2 and, 3, it is evident that the isolated strain is *Pseudomonas aeruginosa*.

This study demonstrates that the *Pseudomonas* strain isolated from a contaminated site can metabolize plastic. Acquisition of degradative abilities by selective enrichment has been seen in laboratory ecosystems for many organic compounds [15]. Consistent with this observation a bacterial strain degrading plastic has been isolated from the contaminated site adopting the selective enrichment technique. The strain of *Pseudomonas* used in this study proves to be a novel strain with the ability to degrade not only polythene but also capable of degrading other plastics. The advantage of applying bacterial systems for effluent remediation is that they pose a higher rate of biodegradation than fungi.

The study suggests that the bacteria already present at a polluted site can often adapt to degrade the plastic contaminants if sufficient time is given. The use of biological systems for bioremediation is more cost-effective than traditional cleaning techniques.

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#### 4. Conclusion

The use of biological systems bioremediation is more cost-effective than traditional treatment techniques. The microorganisms isolated from the contaminated site used plastic as the sole source of energy. This was identified and characterized as the *Pseudomonas aeruginosa* strain. The organism had abilities to degrade polythene. The study also reveals that the *Pseudomonas* strain can efficiently degrade plastics even at various concentrations. Since the bacterium is capable of degrading various plastics there exists a possibility for its use in the development of microbial technology for the decontamination of plastic-contaminated sites.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

The authors Mahesh Arvind, Jayanth D.R, Eesha Prasad, Saleem Ahmed, and Sumukh Srinath show no conflict of interest.



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## Authors short Biography

	<p><b>Dr. Mahesh Arvind</b>, Professor and Head of, the Department of Chemistry and Biochemistry of Vijaya College, Bengaluru City University has visited various countries abroad and has presented research papers in the field of Bioremediation. He is a recipient of Bharath Jyothi", Senior Scientist" and "Dr. APJ Abdul Kalam Lifetime Achievement" National awards with gold medals.</p>
	<p><b>Jayanth D R</b> received a BSc degree from Bengaluru City University in 2023. He has completed a few in-house projects and achieved a national-level internship (IIT Roorkee) and he has also presented research papers in national and international conferences.</p>

	<p><b>Eesha Prasad</b> received a BSc degree from Bengaluru City University in 2023. She has completed several in-house projects and publications. She has presented research papers at various National and International Conferences to her credit and received a couple of awards.</p>
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