# Screening of Plastic Degrading *Pseudomonas* spp. From Soil

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**Abstract-** The plastics of various forms such as nylon, polycarbonate, polyethylene terephthalate, polyethylene, polypropylene, polystyrene, polytetraflouro ethylene, polyurethane and polyvinyl chloride are being continuously used in our day to day life. Most of the plastic materials are generating as one of the major source of environmental pollution. Therefore, this research was conducted to find the screening of plastic degrading *Pseudomonas* spp. isolated from the different soil samples at different temperature. From the four sentinel sites Sisdol, Teku, Balkhu and Sanothimi, the soil samples were collected and processed in the Microbiology Laboratory of the Central Department of Microbiology, Tribhuvan University, Kirtipur for the isolation and identification of the *Pseudomonas* spp. The organisms were identified by the conventional microbiological methods and biochemical reactions. The *Pseudomonas* spp., potential of degradation of plastic was screened. The whole process was carried out at Central Department of Microbiology, Tribhuvan University, Kritipur form April to September 2017. It was found that, the *Pseudomonas* spp. degraded 7.6% and 8.2% of plastic at 30°C and 37°C temperature during one month. *Pseudomonas aeruginosa* degraded 7.3% and 8.5%, the *Pseudomonas fluorescence* degraded 7.8% and 7.9% of the polythene at 30°C and 37°C temperature respectively during one month. This research shows the indigenous strain of *Pseudomonas* spp. has the potency of degradation of polythene.



**Key words**: Degradation, polythene, *Pseudomonas aeruginosa*, *Pseudomonas fluorescence*

## 1. INTRODUCTION

The word plastic comes from the Greek word “plastikos” which means able in molded into varied shapes (Joel 1995). Polyethene comprises of 645 of total plastic, which is a linear hydrocarbon polymers consisting of long chain of ethylene monomers (Sangale et al 2012). Plastic is the most useful synthetic ‘manmade’ substance made up of elements extracted from the fossil fuel resources. It has made possible most of the industrial and technological revolutions of the 19$^{th}$ and 20$^{th}$ centuries. The widely used packaging plastic mainly polythene constitutes about 10% of the total municipal waste generated around the globe (Barnes et al 2009).

With continuous growth for more than 50 years, global production in 2013 has increased to 299 million tons, meaning a 3.9% increase compared to 2012.In 2012, 25.2 million tones of post consumer plastics waste ended up in the waste upstream. 62% was recovered through recycling and energy recovery processes while 38% still went to landfill (Plastics Europe 2014/15).

From total solid waste in Kathmandu Metropolitan city, the Composition of plastic waste from Household, Institutional and Commercial field is found 15.9%, 24.5% and 24.2% respectively. In aggregate, the composition of the plastic waste is 21.6% from the total solid waste in Kathmandu Metropolitan city (Banskota 2015).

Biodegradation resulting from the utilization of polyethylene as nutrient may be more efficient if the degrading microorganism forms a biofilm on the polyethylene surface. The microbial species are associated with the degrading materials were identified as bacteria (*Pseudomonas, Streptococcus, Staphylococcus, Micrococcus* and *Moraxella*) fungi (*Aspergillus niger* and *A. glaucus*), *Actinomycetes* spp. and genus *Saccharomonospora* (Swift 1997). The soil bacteria were isolated from plastic contaminated soil sample. The *P. alcaligenes* was found to be more effective than *Desulfotomaculum nigrificans* in degradation of polythene bag at 30 days. An increase in incubation period there is a dramatic increase in weight loss of polythene bag (Begum et al 2015). *P. fluorescence* was the most active of the tested microorganisms degrading approximately 18% and 16% of polythene at 9 and 12 months period respectively and 3.8% of plastics in twelve month period under field condition. Also 8% and 5.6% of polythene and plastics were respectively degraded in a month under laboratory condition. (Thomas 2015).

Unlike most polymers, biodegradable polymers when disposed favorably in the environment e.g. compost, soil and waste water are acted upon and utilized by the indigenous microorganisms as sources of carbon and energy, thus are degraded (Starnecker and Menner 1996). As new biodegradable polymers and their packaging applications are emerging, there is a need to address their environmental performance particularly the time required for their complete disintegration in nature (Kale et al 2007).

## METHODOLOGY

### 2.1 Sample collection site

The sample collection site was Sisdol dumping site, Teku dumping site, Balkhu dumping site and Sanothimi Bhaktapur Household garbage site. Polythene sample was purchased from local market of Kathmandu.

### 2.2 Sample collection and transportation

The soil sample was collected from the soil surrounding the plastic waste by using sterile spatula and was put in sterile container. The plastic sample i.e. polythene bags was collected and transported in the Microbiology laboratory.

### 2.3 Sample processing

**Serial dilution of soil**

One gram of the soil sample was added in 9 ml distilled water to make 1:10 dilution and 1 ml of 1:10 dilution into 9 ml distilled water to make 1:100 dilutions and so on.

**Pre-treatment of polyethylene**

The polyethylene bags were cut in small strips and were transferred into sterile beaker with distilled water and stirred for 1 hour. Further, they were aseptically placed to ethanol solution 70% v/v for 30 minutes. Then, the polyethylene strips were transferred to a sterile Petri dish (El-Shafei et al 1998). Finally, the plastic strips were air dried and were weighted in fix mass.



### 2.4 Identification of the isolates

Identification of the isolates was performed according to their morphological, staining reaction, cultural and various biochemical characteristics by following Bergey's Mannual of Systematic Bacteriology.

**Gram staining**

Gram staining was performed for the presumptive identification of the bacteria. *Pseudomonas* spp. was identified as gram negative rod.

**Biochemical Tests**

Typical colonies of bacterial isolates were sub cultured on Nutrient agar and incubated at 37°C for 24 hours. After incubation, fresh culture of test organism was inoculated into different biochemical media. *P. aeruginosa* and *P. fluorescence* were characterized and identified using a combination of colony morphology, Gram stain characteristics and different biochemical tests such as Catalase test, Oxidase test, Indole test, MR test, VP test, Citrate Utilization test, OF test, Urease test, Gelatin hydrolysis test, Xylose, Glucose fermentation test, Growth at 4°C and Growth on 7% NaCl tests. (Forbes et al 2007).

### 2.5 Inoculation of *Pseudomonas* spp. in Nutrient agar plate

The *Pseudomonas* spp. was inoculated by carpet culture method onto Nutrient agar plates containing polythene strips and incubated at 30°C and 37°C separately for one month. Negative control was maintained by adding the same quantity of plastic strips in the Nutrient agar plate without inoculation of the bacteria and incubated together with test at the same temperature.

### 2.6 Dry weight determination of recovered polyethylene

The residual polyethylene strips were recovered from the culture plates after one month. The bacterial cell mass adhering to the polyethylene surface was washed by a 2% (v/v) aqueous sodium dodecyl sulphate solution for 2 hours and finally with distilled water (Hadad et al 2005). The washed polyethylene particles were air-dried and weighed. The weight loss of the plastics was calculated by using the following formula.

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

## RESULTS

The potency of the degradation of plastic by used microorganism screened *Pseudomonas* spp. was found to be 7.9% during one month.

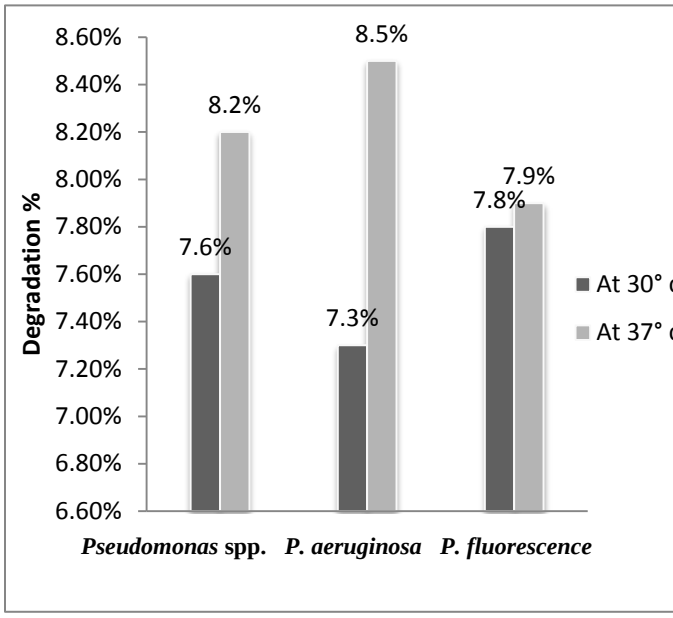
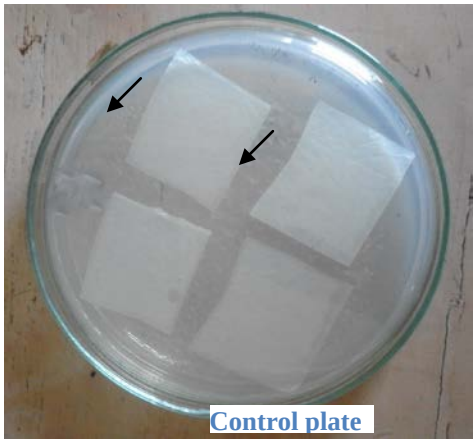



**Figure 1: Polythene degradation by *Pseudomonas* spp.**

### 3.1 Degradation based on surface change in plastic sample

The surface change of the polythene sample was observed after incubation with soil isolates. The surface of the plastic samples has turned from smooth to rough with cracking after one month represents the degradation of the polythene strips by *Pseudomonas* spp.




**Photograph 1: Nutrient media containing polythene strips as control**


**Photograph 2: Surface change i.e. degraded strips of polythene after one month**

| Place → Microorganism↓ | Sisdol | Sanothimi | Balkhu | Teku |
|---|---|---|---|---|
| *Pseudomonas* Spp. | 7.8% | 8.9% | 6.8% | 6.3% |
| *P. aeruginosa* | 6.1% | 8.8% | 8.0% | 6.2% |
| *P. fluorescence* | 9.7% | 8.9% | 5.7% | 6.6% |

### 3.2 Polythene degradation based on temperature

**Degradation at 30**°C

The polythene degraded by the *Pseudomonas* spp. isolated from the Sisdol dumping site soil was found to be 7.8%. From the same site, the *P. aeruginosa* and *P. fluorescence* were degraded 6.1% and 9.7% of polythene respectively at 30°C temperature during one month. The polythene degraded by the *Pseudomonas* spp. isolated from the Sanothimi household garbage site soil was found to be 8.9%. The *P. aeruginosa* and *P. fluorescence* isolated from same site soil degraded the 8.8% and 8.9% of polythene respectively.

At the same temperature and time duration, the *Pseudomonas* spp. isolated from the Balkhu dumping site soil was found to be degraded 6.8% of polythene. The *P. aeruginosa* and *P. fluorescence* were able to degrade 8.0% and 5.7% of polythene from same site. The *Pseudomonas* spp. isolated from the Teku dumping site soil was found to be degraded 6.3% of polythene. From the same site, the *P. aeruginosa* and *P. fluorescence* were able to degrade 6.2% and 6.6% of polythene.

**Table 1: Degradation of polythene by *Pseudomonas* spp. at 30°C**

**Degradation at 37°C**

The polythene degraded by the *Pseudomonas* spp. isolated from the Sisdol dumping site soil, was found to be 7.5%. From the same site, The *P. aeruginosa* and *P. fluorescence* were degraded the 8.1% and 6.9% of polythene respectively at 37°C temperature during one month. The polythene degraded by the *Pseudomonas* spp. isolated from the Sanothimi household garbage site soil was found to be 9.0%. The *P. aeruginosa* and *P. fluorescence* isolated from same site soil degraded the 9.2% and 8.8% of polythene respectively after one month of incubation.

At the same temperature and time duration, the *Peudomonas* spp. isolated from the Balkhu dumping site soil was found to be degraded 8.2% of polythene. The *P. aeruginosa* and *P. fluorescence* were able to degrade 8.9% and 7.6% of polythene from same site. The *Pseudomonas* spp. isolated from the Teku dumping site soil was found to be degraded 8.1% of polythene. From the same site, the *P. aeruginosa* and *P. fluorescence* were able to degrade 8.0% and 8.1% of polythene.

**Table 2: Degradation of polythene by *Pseudomonas* spp. at 37° c**

| Place→ Microorganism↓ | Sisdol | Sanothimi | Balkhu | Teku |
|---|---|---|---|---|
| *Pseudomonas* spp. | 7.5% | 9.0% | 8.2% | 8.1% |
| *P. aeruginosa* | 8.1% | 9.2% | 8.9% | 8.0% |
| *P. fluorescence* | 6.9% | 8.8% | 7.6% | 8.1% |

## DISCUSSION

Plastic waste is the major component of solid waste and source of environmental pollution of the world. The solid waste management is necessary for the developing countries for the reduction of risk due to solid waste. In our research, the *Pseudomonas* spp. was isolated and identified and used for the screening of polythene degradation.

In our research, The *Pseudomonas* spp. isolated from the different dumping sites of Kathmandu Valley and Sisdol dumping site was degraded 7.6%and 8.2% of the polyethene at 30°C and 37°C temperature during one month. The *P. aeruginosa* able to degrade 7.3% and 8.5%, the *P. fluorescence* able to degrade 7.8% and 7.9% of the polythene at 30°C and 37°C temperature respectively during one month. In our study, comparison among these isolates of all four sentinel sites, the highest amount of polythene was degraded by *P. fluorescence* at 30°C isolated from Sisdol landfill site soil and by *P. aeruginosa* at 37°C temperature isolated from Sanothimi household garbage site soil.

This is due to the accumulation of microbial cell and production of enzymes by microorganism during the degradation process acted on the surface of polythene. The enzyme produced by the microorganism breakdowns the carbon, hydrogen bond of the polymer into monomer is easily utilizes the microbial cells (El-Shafei et al 1998).

Hadad et al (2005) reported the *Desulfotomaculum nigrificans* degrade 10.2%, 13.2% and 16.2 % of polythene bag at 10, 20 and 30 days incubation respectively. At the same time *P. alcaligenes* degraded 10.5%, 14.7% and 16.2 % of polythene bag at 10, 20 and 30 days incubation respectively. An increase in incubation period there is a dramatic increase in weight loss of polythene bag. Among the two isolates tested, *P. alcaligenes* was found to be more effective in degradation of polythene bag at 30 days. Previously, (Norman et al 2002; Tadros et al 1999) have reported on the biodegradability potential of *P. fluorescens* and *P. aeruginosa* on synthetic plastics.

After pretreatment with nitric acid, *P. aeruginosa* was able to degrade 0.25 gram of LDPE by 50.5% in 2 months (Rajandas et al 2012). However, no chemical pretreatment was needed for *Pseudomonas* spp. AKS2 to degrade LDPE films, albeit only 5% of the total mass of 300 mg was degraded within 45 days (Tribedi and Sil 2013c). Also without any pretreatment, an uncharacterized *Pseudomonas* spp. was found to degrade 28.6% of low MW PE (MW 1700 Da) in a sterilized compost condition after 40 days (Yoon et al 2012).

## CONCLUSION

The *P. fluorescence* and *P. aeruginosa* isolated from Sisdol landfill site and Sanothimi household garbage site soil isolates were found to higher amount degradation of polythene at 30°C and 37°C temperature respectively than other isolates of them from same site and from different sites soil isolates during one month. Therefore, the degradation of polythene by *Pseudomonas* spp. screened was found to be significantly different potential during one month at different temperature.



## RECOMMENDATIONS

*Pseudomonas* spp. was found to be potential of degradation of polythene of13 micron size plastic can be useful for general purpose.

Continuous degradation of the polythene by *Pseudomonas* spp. should be done in order to reduce, manage and control the solid waste and environmental pollution.

## ACKNOWLEDGEMENT

First and foremost, I would like to acknowledge the Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu. I would like to acknowledge to the University Grant commission, Sanothimi Bhaktapur, Nepal and my supervisor, teacher, colleges and laboratory staff who helped me contributed to the completion of my dissertation.

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