Research Journal of Agricultural Sciences
An International Journal

*Res. Jr. of Agril. Sci.* **12**(1): 190–192

**P**- ISSN: 0976-1675 | **E**- ISSN: 2229-4538

www.rjas.org

*Short Communication*

# *Klebsiella Sp*. and *Micrococcus Sp*. Induced Degradation of Polystyrene

Meenashi Subramani*[1] and Umamaheswari Sepperumal[2]

Received: 08 Oct 2020 | Revised accepted: 05 Jan 2021 | Published online 07 Jan 2021


***Key words*:** Polystyrene, Biodegradation, *Klebsiella sp., Micrococcus sp*., CFU, Weight loss

Synthetic polymers such as polyethylene terephthalate, polystyrene, polypropylene, polyurethane etc., are composed of elements extracted from fossil fuel resources. It has been widely used in food, clothing, shelter, transportation, construction, medical and packaging industries because of their light weight and unbreakable nature. The disposal of these plastic wastes is of environmental concern. Hence, eco-friendly approaches should be adopted for the management of disposal of these solid wastes. The accumulation these wastes could have negative impact on the biota. Polystyrene is a polymer of styrene and the polystyrene transformation produce styrene oxide which is toxic to humans [1]. Several microbial species *Pseudomonas sp., Klebseilla sp*. [2] *Aspergillus niger, luteus, Rhodococcus fa Figuero* [3] have been reported to degrade these synthetic polymers. The biotic components of the ecosystem elicit a vital role in the degradation of these synthetic polymers. With this view, the present was designed to determine the ability of soil bacteria to grow using polystyrene as a sole source of carbon and the weight loss parameter was adopted to evaluate the ability of soil bacteria to degrade polystyrene.

*Isolation of bacteria from polystyrene waste dumped soil*

Soil samples were collected from polystyrene waste dumped sites. 1 g of soil was dissolved in 99 ml sterile distilled water and serially diluted. The diluted samples were inoculated on nutrient agar plates and bacterial colonies were counted by colony counter and also the bacterial isolates were identified using Bergeys manual of Determinative Bacteriology [4]. *Klebsiella sp.*, and *Micrococcus sp.*, were the dominant bacteria.

*Preparation of polystyrene samples*

The polystyrene foam was cut into beads and flakes of equal sizes and used for degradation studies (Plate 1).

*Degradation of polystyrene by bacteria in MSM*

*Klebsiella sp.*, and *Micrococcus sp.*, were used for Polystyrene degradation studies. 10µl of the broth culture of bacteria were inoculated each in 100 ml sterile minimal salt medium (MSM) containing polystyrene beads and flakes separately and kept in the shaker at 37°C and 120 rpm for a period of one month.


Plate 1 Sample PS beads and flakes

*Determination of weight loss*

Equal sized polystyrene beads and flakes were weighed and placed aseptically in conical flasks containing 100 ml of sterile MSM which were inoculated with *Klebsilla. sp* and *Micrococcus. sp* separately and kept in orbital shaker at 37°C and 120 rpm for a period of one month. MSM containing polystyrene beads and flakes separately was simultaneously maintained as control. After one month, these polystyrene beads and flakes were removed and washed with sterilized distilled water and dried completely. They were weighed accurately and percentage loss in their weight was calculated by the formula:

$$\text{Total weight loss \%} \quad \frac{W_i - W_f}{W_f} \times 100$$

Where,
$W_i$ = Initial Weight (Weight of polystyrene before incubation)
$W_f$ = Final weight (Weight polystyrene after incubation)

From (Table 1), it is evident that both *Klebsiella sp.*, and *Micrococcus sp.*, grew well in MSM inoculated with polystyrene for a period of one month when compared to the control. In the control, *Klebsiella sp*., growth was evinced till 20 days and *Micrococcus* sp., till 23 days after which no bacterial growth was evinced (Plate 2-5). Thus, indicating that *Klebsiella* sp., and *Micrcoccus sp*., utilized polystyrene as a sole source of carbon for their growth. Comparatively, *Micrococcus sp*., exhibited higher growth in MSM inoculated with polystyrene flakes (71.4 $\times 10^9$ CFU/ml) than *Klebsiella*

***Meenashi Subramani**
meenashi73@gmail.com

[1]P.G. and Research Department of Zoology, Government Arts College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Karur- 639 007, Tamil Nadu, India

[2]P.G. and Research Department of Zoology, Periyar EVR College, Tiruchirappalli, Tamil Nadu India

*sp.*, ($46.7\times10^9$ CFU/ml). On the other hand, with respect to polystyrene beads *Klebsiella* sp., exhibited higher growth ($23\times10^9$ CFU/ml) when compare to *Micrococcus sp*. ($17.2 \times 10^9$ CFU/ml). Percentage of weight loss was high in polystyrene flakes inoculated with *Micrococcus sp*., (68.09%) when compared to *Klebsiella sp*., (24.03%). Irrespective of the bacterial species, there was no much percentage loss of weight in polystyrene beads inoculated in MSM (Table 2). Further, it is observed that higher the bacterial colonization greater the weight loss of polystyrene.

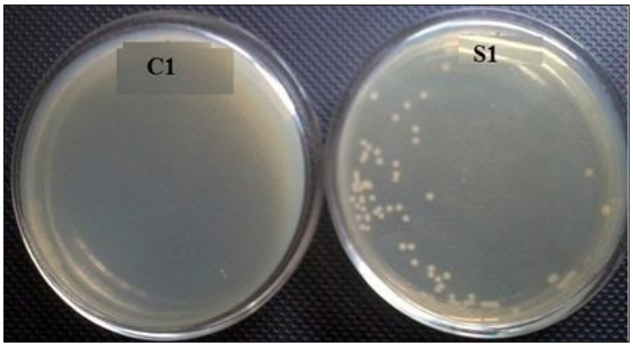


Plate 2 CFU of *Klebsilla Sp* after one-month degradation of PS beads

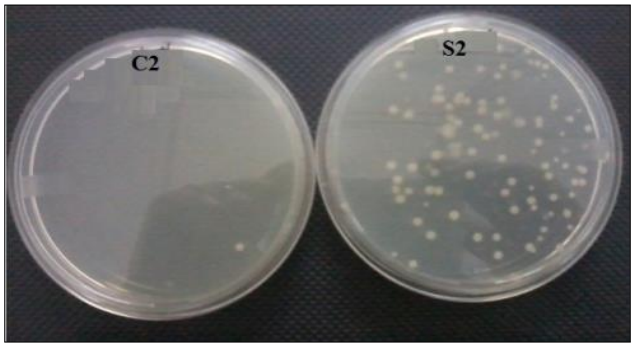


Plate 3 CFU of *Micrococcus Sp* after one-month degradation of PS beads

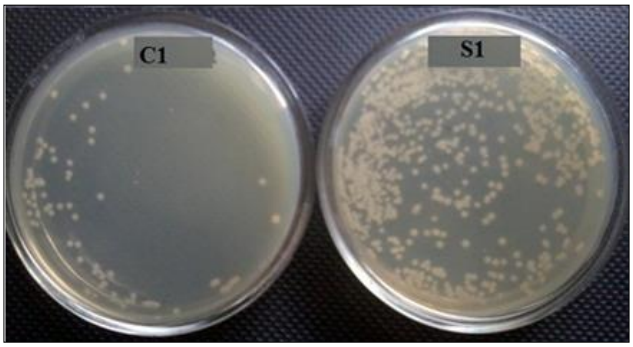


Plate 4 CFU of *Klebsilla Sp* after one-month degradation of PS flakes

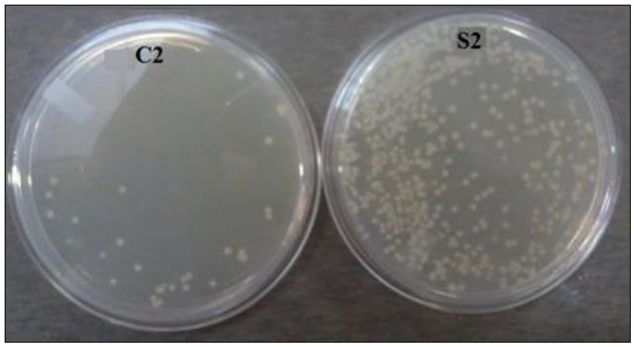


Plate 5 CFU of *Micrococcus Sp* after one-month degradation of PS flakes

Table 1 Variation in *Klebsiella sp., and Micrococcus sp.,* population inoculated in MSM with polystyrene flakes and beads for a period of one month

| Date | *Klebsiella sp.,* | | | | *Micrococcus sp.,* | | | |
|---|---|---|---|---|---|---|---|---|
| | PS Flakes | | PS Beads | | PS Flakes | | PS Beads | |
| | Control CFU $\times10^9$ /ml | Test CFU $\times10^9$ /ml | Control CFU $\times10^9$ /ml | Test CFU $\times10^9$ /ml | Control CFU $\times10^9$ /ml | Test CFU $\times10^9$ /ml | Control CFU $\times10^9$ /ml | Test CFU $\times10^9$ /ml |
| 26.12.15 | 6.4 | 6 | 3 | 3 | 3.3 | 3.6 | 3.3 | 3.3 |
| 29.01.16 | 4.3 | 16 | 2.3 | 13 | 2.9 | 17.3 | 2.6 | 8.3 |
| 03.01.16 | 3.5 | 38.6 | 1.2 | 16 | 2.1 | 33.1 | 2.0 | 15.2 |
| 06.01.16 | 2 | 53.6 | 1.1 | 22.6 | 1.9 | 45.5 | 1.8 | 25.2 |
| 11.01.16 | 1.4 | 64.6 | 1 | 113 | 1.2 | 47.3 | 1.3 | 29.9 |
| 14.01.16 | 1 | 41.6 | 0.9 | 20.8 | 0.8 | 64.3 | 1 | 31.6 |
| 17.01.16 | - | 39 | 0.4 | 25.3 | 0.3 | 73.9 | 0.5 | 46.3 |
| 20.01.16 | - | 46.4 | - | 8 | - | 79.2 | - | 30.4 |
| 23.01.16 | - | 35 | - | 16.3 | - | 63.2 | - | 28.1 |
| 26.01.16 | - | 46.7 | - | 23 | - | 71.4 | - | 17.2 |

Table 2 Weight loss of polystyrene flakes and beads incubated in MSM after a period of one month by bacteria

| Bacteria | Polystyrene | Weight of polystyrene before incubation (g) | Weight of polystyrene after incubation (g) | Total weight loss (g) | Percentage of weight loss (%) |
|---|---|---|---|---|---|
| *Klebsiella sp.,* | Flakes | 0.320 | 0.258 | 0.2403 | 24.03 |
| | Beads | 0.310 | 0.277 | 0.1191 | 11.91 |
| *Micrococcus sp.,* | Flakes | 0.432 | 0.257 | 0.6809 | 68.09 |
| | Beads | 0.431 | 0.387 | 0.1162 | 11.62 |

The higher number of bacterial growth in polystyrene flakes observed in this study could be attributed to more surface area of polystyrene that enables the bacteria to colonize and utilize it as a carbon source for its growth. The prevalence of bacteria in polystyrene waste dumped soil coincides with the higher bacterial count in mangrove soil which could play a vital role in the microbial decomposition process in the soil [5]. Moreover, [6] have correlated the

increase in bacterial population with the disintegration of mechanical properties of natural polymer films reflecting the role of bacteria in the degradation process. Our findings are also in parallel to the findings of [7] has opined that biodegradation of plastics is dependent on the resident bacterial population. The beads are shredded form of polystyrene structurally having a pentane phenyl ring which gives them protection by preventing the polymer chains from packing into close and also restrict rotation of the chains around the carbon-carbon bonds, lending the polymer more rigid. The flakes are polystyrene films cut from polystyrene foams which are coated by a protective epoxy. The epoxy material used in foam production is the combination of polyurethane and polyuria. Some species of bacteria were capable of utilizing the polyurethane as a sole carbon and energy source [8].

## SUMMARY

The CFU and weight loss studies of *Klebsiella sp.,* and *Micrococcus sp.,* in MSM of polystyrene flakes and beads indicates that due to more surface area of the polystyrene flakes may provide a comfortable substratum for microbes to adhere and utilize polystyrene as a sole source of carbon and increase their population.

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