JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Sept. 2018. Vol. **12**(3), p. 1641-1646

Bio-augmentation - Effective Method of Treating Plastic Waste - A field Study

Naveen Mohan and R. Usha*

Department of Microbiology, Karpagam Academy of Higher Education, Coimbatore 641021, Tamil Nadu, India.

http://dx.doi.org/10.22207/JPAM.12.3.71

(Received: 08 August 2018; accepted: 21 September 2018)

Polythene waste is a serious threat to our environment. There are many methods available for its degradation. The present study shows light for eco friendly approach for mitigating the ecological problem of the century. By using bio-augmentation, microorganisms having specific metabolic capabilities are introduced into the contaminated site for enhancing the degradation of plastic waste. The field trials for a period of 3 months shows clearly that the LDPE sheets are better degraded with augmented microorganism (1.6%,4%,5.2%,8%,8.8%) than non bio augmented soil (4%,1.6%,2.8%3.6%4%). The ecological importance of this study is that we will be able to tackle plastic waste by adding bio-augmented strains in to an area or dumpyard filled in with plastic waste.

Keywords: Bio augmentation, Actinomycetes, Plastic waste and Degradation.

The term "Plastic" is derived from the Greek word "plastikos" which means "able to be molded in to different shapes"[1] and is given to any synthetic or semi- synthetic organic polymers with high molecular mass that are mouldable. Plastic are man-made long chain polymeric molecules[2] mainly synthetically derived from petrochemicals. Plastic materials such as polyethylene, polypropylene, polyvinyl chloride and polyethylene tetra phthalate have wide applications in industries and human life.

Civilization, urbanization and industrialization paved the way for the mass dumping of plastic waste in to the environment . "Beat plastic pollution" is the theme for World Environment Day 2018, and is a call for everyone combat the eco-nightmare. Nearly one third of the plastic packaging we use escapes collection systems, which means it ends up as solid wastes causing irreparable damage to our environment. There are different methods of disposal of plastics such as incineration, recycling and landfills[3]. Biodegradation is the recent trend in this field giving a helping hand to the nature[4] .

The microbial species which are associated with degrading plastic materials were identified as Bacteria (*Psuedomonas, Streptococcus, Micrococcus* and *Moraxella*) Fungi (*Aspergillus*) and Actinomycetes[5].

Bio-augmentation is the addition of pre-grown microbial cultures to enhance microbial population at a site to improve contaminant clean up and reduce clean up time and cost. The success of bio-augmentation depends on both biotic and abiotic factors. The most important is a strain selection[6]. The organism for bio augmentation should be able to survive and multiply in soil as well as to compete with indigenous microorganisms for nutrients and oxygen. Moreover, after being introduced in the soil, they should not lose their degradative capacity.

Attempt has been made in this paper to find a lucrative bioremediation method to study the biodegradation of LDPE using augmented

* To whom all correspondence should be addressed.
E-mail: ushaanbu09@gmail.com

microorganisms in the field .Bio-augmentation method is preferred as the indigenous micro flora may not be adapted to consume as much as plastic waste augmented microorganisms[7].A comparative study between non augmented plastic degradation and augmented plastic degradation in field is the nerve of this work.

MATERIALS AND METHODS

1 cent land (40.46 sq.m) was selected for the field trial. The field was marked to separate it from the rest of the area. One cent land was again divided in to two equal portions of 20.23sq.m each. Both the portions were similar in nature and texture. One half of the land was marked as control land and the other half selected for bio-augmentation study.

Active Strain

An active strain of Actinomycetes (DSR2) isolated[8] from a plastic dump yard near Calicut ,Kerala was selected for bio augmentation study here. The present strain DSR2, is known for its high degradation capacity on plastic. This strain was isolated from a dump yard where the soil was seen associated with plastic wastes. Then the soil was serial diluted and spread on to a Starch casein agar. The organism DSR2 was then identified and used for weight loss experiment, pH variation study, and found to be the most active degrading strain.

Insertion of LDPE sheets in field

Low density poly ethylene sheets were collected from Shaz polymers (Calicut Kerala) The sheets were equally cut in to 5X2 cm pieces.6 small wells of 3-5 cm depth were cut in both pieces of land.LDPE sheets were placed inside the wells .

The control land is left as such without adding any microorganisms whereas to augmented land, an active strain (DSR2) of Actinomycetes, together with a suitable carrier like Rice hull[9]was inoculated equally in each of the six wells.

Prior to the inoculation, the weight of the strips was recorded .Each well was marked, both in Control and Augmented land as 15,30,45,60,75,and 90 corresponding to the day.

Biodegradation of LDPE - Weight loss Method

After every 15 days, up to 90 days , the strips from corresponding wells, both Control and Augmented were taken out and then washed in 70%ethanol,air dried and weighed. The weight loss was then calculated and compared.[10]

Weight loss %= [(Initial weight-Final weight)/ Initial weight] x100

Table 1. Control Field-weight Loss)

Initial WT(mg)	15th Day	30th Day	45thDay	60th Day	75thDay	90th Day
25	25	24.9	24.6	24.3	24.1	24

Tables 2. Augmented Field-weight Loss)

Initial WT(mg)	15th Day	30th Day	45thDay	60th Day	75thDay	90th Day
25	25	24.6	24	23.7	23	22.8

Table 3. Variation In Ph(control)

Initial pH	15th Day	30th Day	45thDay	60th Day	75thDay	90th Day
6.8	6.8	6.7	6.7	6.6	6.6	6.5

Determination of Variation in pH value of the medium

The variations in the pH value of the soil containing LDPE sheet were determined by soil pH meter and compared with its respective initial pH value at every 15 days of field trial. Study of pH change was adopted to verify the metabolic efficiency of the organism in degrading plastics.[11]

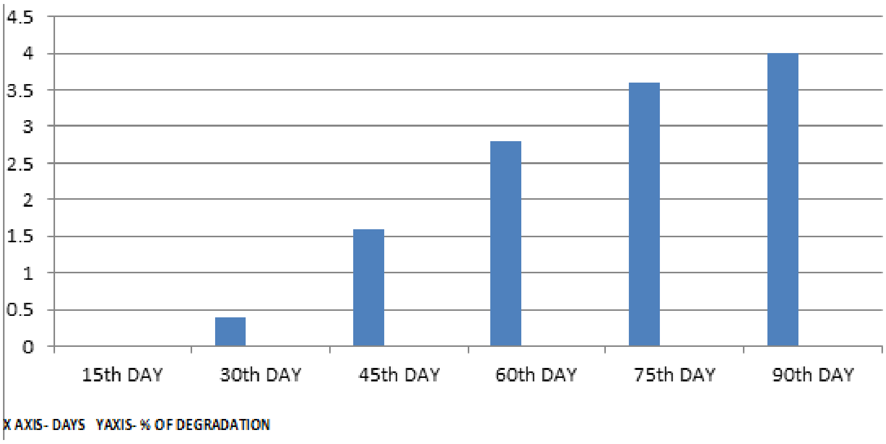


Fig. 1. Percentage of Degradation-control Land

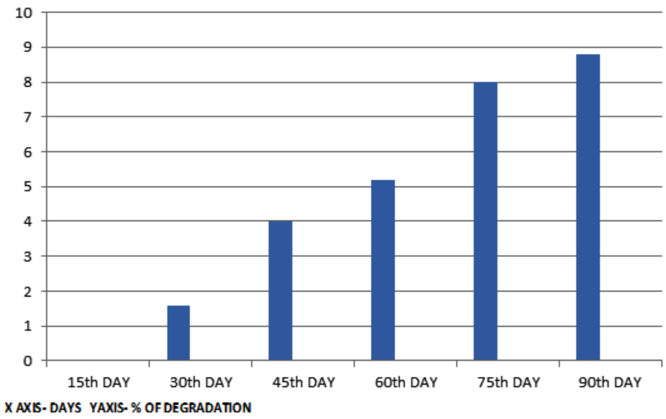


Fig. 2. Percentage of Degradation-augemented Land

Table 4. Variation In pH (augmented)

Initial pH	15th Day	30th Day	45thDay	60th Day	75thDay	90th Day
6.8	6.8	6.7	6.5	6.5	6.2	5.7

Table 5. Percentage of Degradation

	15thDay	30thDay	45th Day	60thDay	75thDay	90thDay
Control	0	0.4	1.6	2.8	3.6	4
Augmented	0	1.6	4	5.2	8	8.8

RESULTS

The initial weight of the strips of LDPE and pH of the soil near well were recorded before introducing the sheet into the soil. Soil augmentation was carried out in the next stage.[12]

The strips of LDPE were taken out from both Control and Augmented land at an equal interval of 15 days up to 90 days (3 months). pH of the soil near wells was also tested and recorded[4].

The LDPE sheets taken out of the well were tested for weight loss and the results were recorded at each interval. As shown in table 1 (control) no considerable weight loss was seen over a period of 90 days as the well contained only indigenous micro organisms (0.4%,1.6%,2.8%,3.6%,4%). The maximum percentage of degradation was found to be 4%.

The considerable weight loss (1.6%,4%,5.2%,8%,8.8%) (Table 2) was experienced due to the degradative work carried out by augmented strain. Imam et al[13] observed that significant biodegradation occurred only after colonization of the plastic. So, increase in bacterial load had a correlation with the degradation of polymer. The maximum degradation was found to be 8.8% within a period of 3 months in the field.

Table 3 (pH variation-control) No significant variation in pH of the soil was seen in the study of control land (pH6.8 -6.5) but in Table 4 **(**pH variation-augmented) was seen dropping drastically towards acidic side (pH 6.8-5.7) The reduction in pH not only confirmed the usage of the polyethylene sheets as their sole source of carbon but also paved the way for the idea regarding production of monomers.

DISCUSSION

From the previous study of Orhan Y *et al*[14] and Tokiwa Y *et al* [15]. It has become evident that plastic undergoes degradation and microorganisms utilize plastic as a source of food. Vijaya and Reddy[16]have also suggested that microorganism have the capability to degrade polythene.They colonize the surface of polythene forming biofilms reducing the weight of LDPE strips, confirming the usage of plastic sheet as a source of carbon. Dey U *et al*[17] have suggested that the reduction in pH not only confirms the usage of polythene sheet as their sole source of carbon but also paves the way for the idea regarding production of monomers. The present work is a comparative study between the capacity of Indigenous microorganisms and bio-

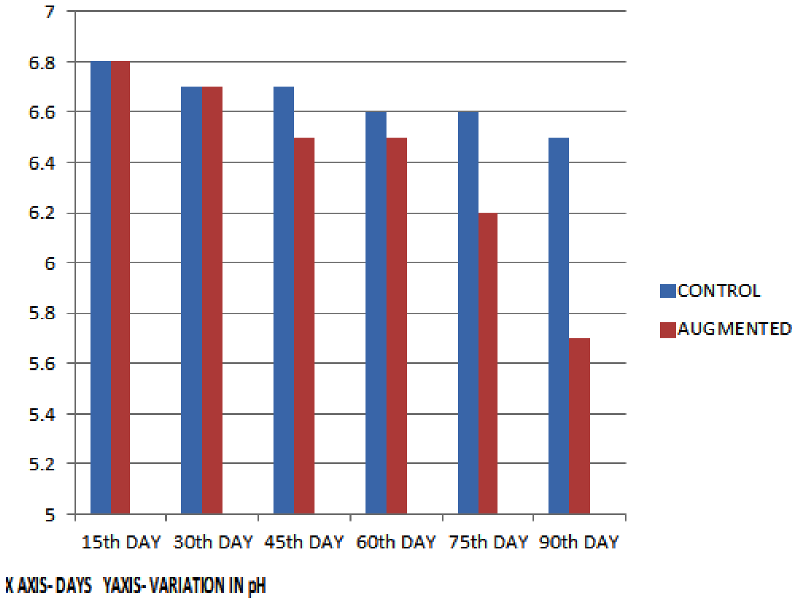
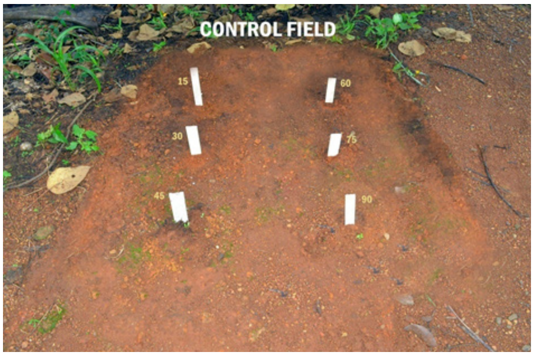
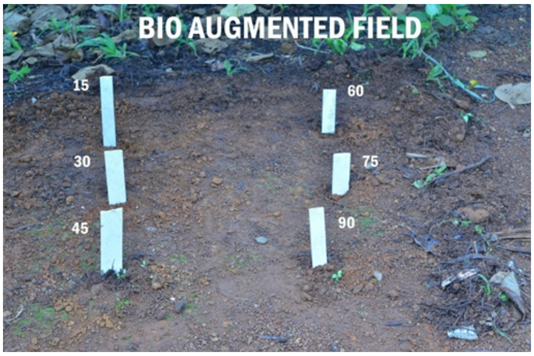


Fig. 3. Variation in pH - Control and Augmented Soil





augmented microorganisms revealing the high need for bio-augmentation for the effective treatment of plastic waste. A previous study carried out by Deepika *et al*[18] reported that Actinomycetes shows great capability in degrading synthetic polymer granules and polythene bags when compared to fungi under laboratory conditions. The present study shows that indigenous microorganisms are less efficient in degrading polythene waste in field study (0.4%,1.6%,2.8%,3.6%,4%) over a period of 90 days. The maximum percentage of degradation shown by indigenous microorganism is 4%.

In bio-augmentation we are introducing microorganism capable of degrading plastic waste to soil to aid indigenous microorganism or to degrade the waste to enhance the rate of biodegradation. In the present study actinomycete strain (DSR2) is used for bio-augmenting the soil. The percentage of its degradation (1.6%,4%,5.2%,8%,8.8%) was far better than those of the indigenous microorganism. The maximum percentage of degradation obtained was 8.8% over a period of 90 days.

Conclusion

In the present study the process of biodegradation is accelerated by bio- augmenting with an active strain of microorganism, which helps the biodegradation to happen much faster in field conditions, as the indigenous strain is unable to degrade the LDPE strip faster when compared to the augmented strain. The ecological importance of this study is that we will be able to tackle plastic waste by adding bio-augmented strains in to an area or dumpyard filled in with plastic waste and leave the degradation occur naturally thereby avoiding an ecological nightmare. The economic value of this work is that we will be able to combat the plastic waste problem by introducing the solution based on nature.

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