

Biodegradation - A Boon For Mitigating the Polyethylene Waste

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Plastic in our environment is a serious threat to the life on earth. Bioremediation is the only way out to mitigate the present problem. Actinomycetes strains from two different locations were isolated within Kerala state. Efficiency of strains for biodegradation collected from both the locations was compared using weight loss experiment. Experimental evidence on the basis of percentage degradation of LDPE strip shows that strains collected from waste dump yard are more efficient degraders of polyethylene waste. (DSR2-24%, DSR1-20%, DSR3-8%) than those collected from virgin soil. (VSR3-13.2%, VSR2-8%, VSR1-4.8%). Actinomycetes are potential group of microorganisms which can be utilized for biodegradation of plastics. Bio-augmenting the soil with potential degraders will enhance the rate of degradation even more.

Keywords: Biodegradation, Actinomycetes, Bioaugmentation.

Plastics are anthropogenic polymers of carbon, hydrogen and oxygen. Almost 140 million tons of polymers are utilized worldwide and their usage is shooting up at a rate of 12%¹. Every year an estimated 500 billion to 1 trillion plastic bags are consumed worldwide². Polythene could sometimes affect the entire marine life badly and may pave the way for their extinction³⁻⁴.

Low density polyethylene is one of the major environmental pollutants. The degradation of this pollutant is only possible if the microorganism is able to produce enzymes that cause cleavage of the polymer chains in to monomers and oligomers. These water soluble products are consumed by microorganisms as a source of carbon and broken down further to simpler products. Aerobic metabolism produces Carbon Dioxide and Water as end product⁵.

Microorganisms have a naturally occurring catabolic mechanism to degrade, transform or accumulate a huge range of compounds including hydrocarbons (PAHs), pharmaceutical substances and metals. The durability, light weight and processability of these polymers causes them to stay longer in the environment for centuries and end up in landfills and natural water resources creating a severe threat to the environment and its ecosystems .

The nature and ease of use of plastics make it very close to us and thereby its careless and excessive use leads to a great damage to the ecosystem, thereby raising severe ecological catastrophes. As microorganisms have the capacity to degrade the polymers, the only way to overcome the nightmare is by using efficient biodegraders from the soil.

Actinomycetes are gram-positive organisms that tend to grow slowly as branching elements. In addition, Actinomycetes are prokaryotes, produces spores and exhibit powdery growth. Their similarity to fungi is in the formation of branched aerial mycelium, which profusely

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sporulates, and is clearly noticeable. They are sometimes called higher bacteria, hence the name Actinobacteria. Actinomycetes are seen in a diverse and wide range of habitats as they are both mesophilic and thermophilic. Actinomycetes also produce a wide range of bioactive compounds of commercial importance⁶.

Plastic materials have been increasingly becoming popular in our day to day life as it is durable and can be moulded in to any form with much ease. Attempt has been made in this paper to study bioremediation methods to treat plastic waste accumulation and to compare the degradation capacity of Actinomycetes isolated from different locations.

MATERIALS AND METHODS

Low Density Polyethylene powder (LDPE) was received from Sigma Aldrich Chemical Company. LDPE sheets were obtained from Shaz polymers, Calicut, Kerala.

Sample collection

Soil samples were obtained from two topographical locations viz. Garbage dumping site of Kozhikode Corporation and Virgin soil of Wayanad wildlife sanctuary. Using sterile sampling bags soil adjacent to plastic waste bags in the plastic waste dumping site was taken for the study and the other sample from non-polluted virgin soil. The soil samples were dried at room temperature for 2 hrs.

Isolation of Actinomycetes

Serial dilution method was carried out for isolating microorganisms from one gram of soil sample taken from both the sites. The agar used for isolating Actinomycetes was starch casein Agar, and isolation was performed by spread plate method. 3 strains from each location were selected for further studies. Identification of bacteria was done both by Macroscopic and Microscopic tests based on Bergeys Manual⁷.

Screening of Actinomycetes for biodegradability-by Clear zone method.

Mineral salt medium constituted by adding polyethylene powder at a concentration of 1%(w/v) was used. It was then sonicated for 1 hour at 120RPM. Agar was then added to the sonicated medium and sterilized by autoclaving at 120°C and 15lbs pressure for 15 mins. The sterilized medium was then allowed to cool to 45°C and then poured

in to sterile petriplates. After solidification, the isolated colonies were inoculated and plates were kept for incubation at 30-35°C for 2-4 weeks. The organisms producing zone of clearance were selected for further study⁸.

Biodegradation of LDPE by weight loss experiment

LDPE sheets were cut into 5cmx2cm equal pieces and then surface sterilized with ethanol and air dried. The strips were then introduced into conical flasks containing 100ml Mineral salt medium and later inoculated with polythene degrading microbes under study. It was mandatory to keep a control flask without inoculation of microorganism to confirm biodegradation.

The flasks were then kept in orbital shaker incubator at 30°C, 120RPM for 3 months. After every month, the strips were taken out, washed with 70% ethanol to remove biofilms, then air dried and checked for weight. Weight loss was reported and compared⁵.

Colonization of Actinomycetes on the LDPE sheet.

Mineral salt agar medium containing no carbon source was prepared and autoclaved at 120°C, and 15lbs pressure for 15mins. After sterilization the medium was poured into petriplates and Actinomycetes were spread evenly over the medium. On to the seeded plate LDPE sheet (2cmx2cm) was placed at the centre. Plates were then incubated at 30-35°C for 3 months and morphological changes noted twice a month for a period of 3 months (Plates 1, 2, 3)

Determination of variation in pH value of the medium

The pH value variation in Mineral salt medium containing LDPE sheet were observed, recorded and compared with its initial pH 6.8 value after an incubation period of 90 Days.

Evaluation of cell surface hydrophobicity

Bacterial Cell Surface Hydrophobicity is determined by [BATH] test⁹. With more and more bacteria becoming hydrophobic, their affinity towards hydrocarbon also increased resulting in change of turbidity of the culture due to the migration of cells from aqueous phase to organic phase. For BATH assay, Actinomycetes were cultured in Starch casein broth until logarithmic phase and then centrifuged and washed twice with PUM buffer (per litre) 17gK₂HPO₄·7.26g

KH_2PO_4 , 1.8g UREA and 0.2 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. The washed cells were resuspended in PUM buffer until they reached an OD value of 1.0 to 1.2 at 400nm. 1.2ml of suspension was poured to a set of test tubes to which hexadecane is added in progressive volume (range 0 to .2ml). The test tubes were shaken for 10mins and allowed for Phase separation of 2mins. The OD_{400} value of the aqueous suspension was measured. Cell free buffer served as the blank.

RESULTS

Six strains of Actinomycetes were considered for the present study. According to the location, strains were named as from dump yard DSR1, DSR2, DSR3 and other three from virgin soil VSR1, VSR2, and VSR3.

Clear zone formation

Growth began within 3-4 days of inoculation. Initially an opaque zone was seen around the colony, but gradually a clear and transparent zone was visualized with in 15-20- days at 30-35°C in polythene incorporated mineral salt medium.

Morphological changes on sheet

Microbial growth was visible over the sheet after 4-5 days of incubation, and the growth extended around the sheet in 10-15 days. Within 30-35 days a clear zone halo appeared around the sheet and the colonization was very evident on the sheet when compared to the uninoculated sample.

Biodegradation of LDPE by Weight loss method

After one week of incubation, the conical flasks were checked for visible growth. Thin bio film was observed over the surface of LDPE sheet and every month weight of the sheet was taken for a period of 3 months.

Based on results given on (Table 1&2) it confirms that Actinomycetes utilized polyethylene as a sole source of carbon. The strains isolated from Dumpyard, where they were already associated and exposed to polyethylene wastes, showed a good biodegradation capacity (DSR2-24%, DSR1-20%, DSR3-8 % (Fig. 1). When compared to the Actinomycetes isolated (VSR3-13.3%, VSR2-8%, VSR1-4.8%) from virgin soil (Fig. 2).

Table 1. Determination of Weight Loss (Sample 1- Calicut)

S. No	Polyethylene Treated with Isolates	Initial wt	Weight of Polyethylene (mg)			Percentage of Polyethylene Degraded(%)
			Aug 2016	Sep 2016	Oct 2016	
1	Control	25	25	25	24.8	.66
2	<i>DSR 1</i>	25	23	21	20	20
3	<i>DSR2</i>	25	22	20	19	24
4	<i>DSR 3</i>	25	24	23.8	23	8

Table 2. Determination of Weight Loss (Sample 2- Wayand)

S. No	Polyethylene Treated with Isolates	Initial wt	Weight of Polyethylene (mg)			Percentage of Polyethylene Degraded(%)
			Aug 2016	Sep 2016	Oct 2016	
1	Control	25	25	25	24.9	0.4
2	<i>VSR 1</i>	25	24	24	23.8	4.8
3	<i>VSR2</i>	25	24.5	24	23	8
4	<i>VSR 3</i>	25	24	22.8	21.7	13.2

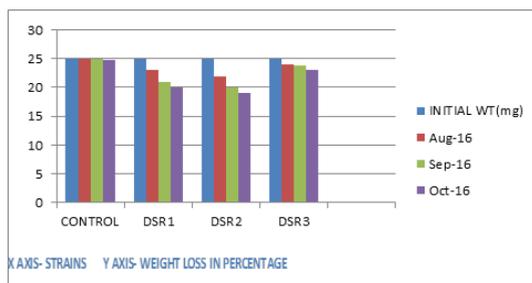


Fig. 1. Determination Of Weight Loss (Sample 1)

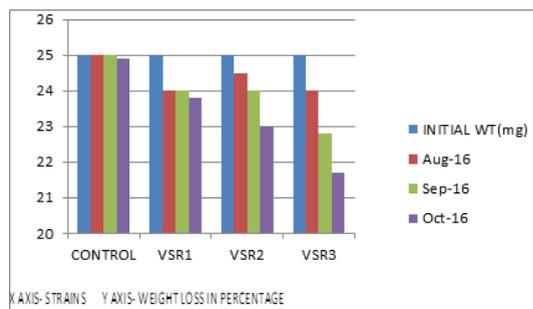


Fig. 2. Determination Of Weight Loss (Sample 2)

Variation of pH of the medium

The initial pH of the medium was 6.8 and it was seen reduced to acidic side over a period of 90 days of incubation. The reduction in pH proved that the culture was still metabolically active and it was utilizing LDPE for its growth. (Table 3&4).

Table 3. Variation in pH (Sample 1)

S. No	Isolates	pH (initial)	pH (30days)	pH (60days)	pH (90days)
1	Control	6.8	6.6	6.4	6.3
2	DSR1	6.8	6.5	6.1	5.9
3	DSR2	6.8	6.3	6.0	5.5
4	DSR3	6.8	6.6	6.3	6.1

Cell surface hydrophobicity

DSR 2 strain exhibited 11% OD where other strains from the same location showed much higher percentage of optical density, DSR1-39%, DSR3-32% and the samples taken from Virgin soil exhibited VSR3-30%, VSR2-33%,

Table 4. Variation in pH (Sample 1)

S. No	Isolates	pH (initial)	pH (30days)	pH (60days)	pH (90days)
1	Control	6.8	6.8	6.8	6.7
2	VSR1	6.8	6.6	6.6	6.5
3	VSR2	6.8	6.6	6.3	6.0
4	VSR3	6.8	6.5	6.2	5.9

Table 5. Cell Surface Hydrophobicity (Sample 1)

S. No.	Vol of Culture (ml)	Vol of Hexadecane (ml)	DSR1 Od ₄₀₀	DSR1 % OF Initial Od	DSR2 od ₄₀₀	DSR2 % OF od ₄₀₀	DSR3OD ₄₀₀ Initial Od	DSR3 % of Initialod
1	1.2	0	1.26	100	1.12	100	1.24	100
2	1.2	0.04	.96	88	0.50	41	0.89	71
3	1.2	0.08	0.89	55	.29	22	0.85	67
4	1.2	0.16	.71	53	0.21	16	0.72	60
5	1.2	0.20	0.88	39	0.13	11	0.76	32

Table 6. Cell Surface Hydrophobicity (Sample 2)

S. No.	Vol of Culture (ml)	Vol of Hexadecane (ml)	DSR1 Od ₄₀₀	DSR1 % OF Initial Od	DSR2 od ₄₀₀	DSR2 % OF od ₄₀₀	DSR3OD ₄₀₀ Initial Od	DSR3 % of Initialod
1	1.2	0	1.30	100	1.23	100	1.20	100
2	1.2	0.04	0.93	86	0.89	71	0.48	49
3	1.2	0.08	0.87	73	0.84	67	0.50	37
4	1.2	0.16	0.71	53	0.72	60	0.49	36
5	1.2	0.20	0.81	42	0.75	33	0.23	30

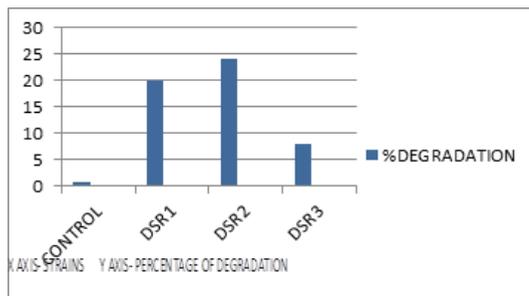


Fig. 3. Percentage of Degradation Capacity of Strains

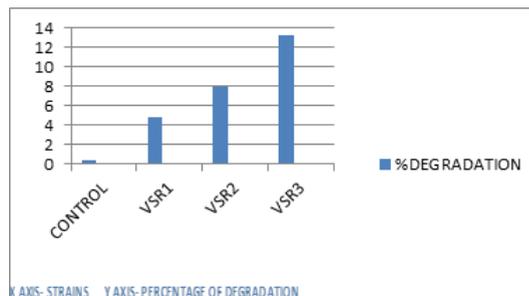


Fig. 4. Percentage of Degradation Capacity of Strains (Sample 2)

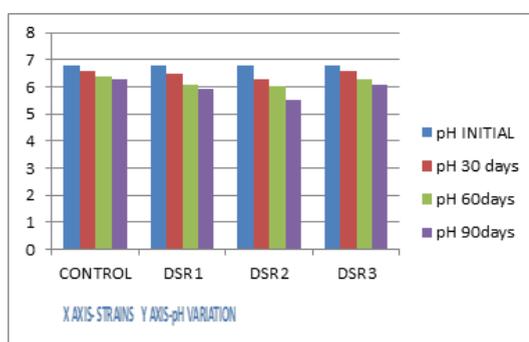


Fig. 5. Variation in pH (sample 1)

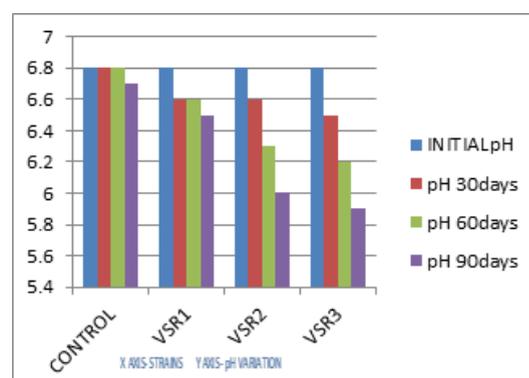


Fig. 6. Variation in pH (sample 2)

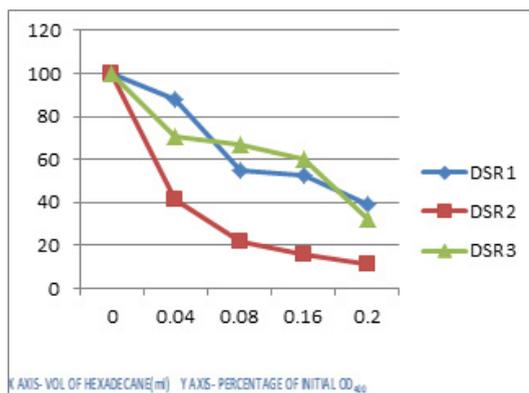


Fig. 7. Cell Surface Hydrophobicity (sample 1)

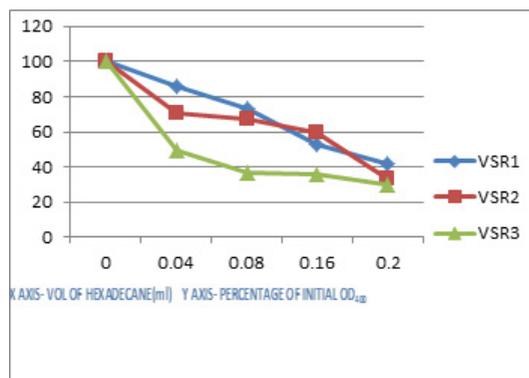


Fig. 8. Cell Surface Hydrophobicity (Sample 2)

and VSR1-42%. The results clearly indicated that the strain DSR2-11% had more adhesion capacity to hydrocarbon than all the other strains. The migration of hydrophobic cells from aqueous phase to the organic phase (hexadecane) is shown as a decrease in turbidity of bacterial suspension. (Table 5&6)

DISCUSSION

Six strains of Actinomycetes were isolated by using Starch Casein Agar. Three strains from dump yard soil (DSR1,2 and 3) and three from virgin soil (VSR1,2,and3) were considered for the entire study.

The total average count of identified actinomycetes was 12×10^5 in Dumpyard samples and 7×10^5 in virgin soil ones. The results were at par with previous studies done by Vijaya & Reddy¹⁰ in which the average number of organisms associated with polythene film was 37.08×10^4 .

All the Six strains (DSR1,2,3 and VSR1,2,3) were considered for weight loss studies in which it was shown that the Actinomycetes colonized the surface of polythene forming films (Plates 1,2, and 3). The loss in weight of LDPE strip suggested the usage of polythene strip as an alternative source of carbon in the absence of readily available carbon. In a previous study conducted by Imam¹¹ observed that biodegradation was triggered only after the colonization of microbes on the plastic sheet. The more the microbial load on the plastic, the higher is the chance for its biodegradation.

The weight loss study conducted clearly showed that, DSR2 strain obtained from dumpyard was the most efficient strain, degrading the polymer by 24% over a period of 3 months when compared with other strains under study.

Table 3 and 4 shows the variation in pH of the medium during biodegradation studies. The actinomycetes isolates in the present study produced some enzymes or metabolites which made pH to drop supporting the viability or growth of actinomycetes on LDPE substrate. DSR2 from dump yard could reduce the pH from 6.8 to 5.5 over a period of 90 days. In a similar study carried out by Dey U *et al*¹², he showed that microorganisms secreted a wide range of enzymes which were utilized for breaking down the complex polymers into monomers that were small enough to permeate through the cell wall of organisms to be utilized as carbon and energy source .

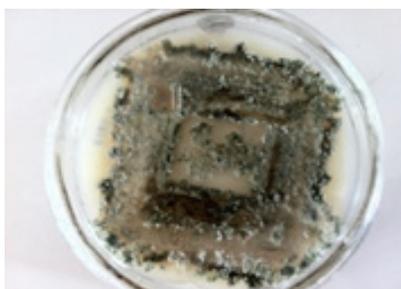


Plate 1. Attachment of Dsr2 Strain On Ldpe Sheet (15days)



Plate 2. Attachment of Dsr2 Strain On Ldpe Sheet (30days)



Plate 3. Attachment of Dsr2 Strain On Ldpe Sheet (after 45 Days)

For degradation of polythene, the microbes should adhere to the polythene sheet. The hydrophobic nature of the cell surface of Actinomycetes was responsible for their affinity towards hydrocarbon. When the microbes become more hydrophobic, their affinity for hydrocarbons also increased. DSR2 strain (11%) was the most hydrophobic Actinomycete and could be regarded as the most potential plastic degrading Actinomycete among all in the present study.

The growth, viability, weight loss and pH change clearly proved that the isolates taken from dump yard soil, where they were already associated with plastics, were more efficient degraders compared with the isolates from virgin soil, where they were not at all exposed to plastics.

CONCLUSION

By closely examining these results, we can conclude that Actinomycetes are a potential group of microorganisms which can be utilized for biodegradation of plastics, if isolated from a place where they are seen associated with plastic waste in soil rather than isolating them from virgin soil. Bio augmenting the soil with potential degraders will enhance the rate of degradation even more.

Conflict of Interest: There is no conflict of interest among the authors.

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