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Screening of polythene and plastic degrading microbes from Muthupet mangrove soil

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ABSTRACT

Polythene and plastic waste accumulating in the environment are posing an ever increasing ecological threat. Biodegradable plastics are environment friendly; they have an expanding range of potential application and are driven by the growing use of plastics in packaging. In this study, soil sample were collected from polythene and plastic dumped site waste in the area of Muthupet Mangrove. The physico-chemical parameters of the soil were studied. The isolated microbial strains were identified based on their cultural morphological and biochemical study. Degradation of polythene bag and plastic cup were analyzed 35, 45 and 55days of incubation in liquid culture method. Pot culture of Vigna radiata was performed. The morphological parameters such as Germinating ability, root length, shoot length and chlorophyll content were analyzed. Waste management was the important process to protect the environment from pollution. Polythene and plastic cup waste materials cause serious environment problems, so the waste materials removed by using the microorganism. This method was cheap and effective, so that it can be used widely for the treatment of polythene and plastic cup.

Key words: Polythene, Plastic, *S.aureus*, *P.aeruginosa*, *A.niger*, *Rhizopus sp and Streptomyces sp*

INTRODUCTION

Biodegradation is defined as decomposition or destruction of contaminant molecules by the action of the enzymatic machinery of biological system. Biodegradation is the process by which organic substances are broken down by living organisms. The term is often used in notation to ecology waste management and environmental remediation. A term related to biodegradation is biomineralisation, in which organic matter is converted into minerals. Biosurfactant, an extracellular surfactant secreted by microorganisms, enhances the biodegradation process [1].

Microorganisms have a naturally occurring in microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons (PAHs), pharmaceutical substances and metals. The durability, light weight and process ability of these polymers causes them to longer in the nature for centuries and end up in landfills and natural water resources creating a severe threat to the environment and its ecosystems [2].

Biodegradable polymers are designed to degrade upon disposal by the action of living organisms, biodegradable polymers generally decompose in various medium in our environment. The depolymerisation results due to various physical and biological forces. The physical forces such as temperature, moisture, pressure etc, deals with causing

mechanical damage to the polymer. The microbial biodegradation was widely accepted and still underway for its enhanced efficiency. Recently, several microorganisms have been reported to produce polyester degrading enzymes. The microbial species are associated with the degrading materials were identified as bacteria (*Pseudomonas, Streptococcus, Staphylococcus, Micrococcus and Moraxella),* fungi *(Aspergillus niger* and *Aspergillus glaucus), Actinomycetes sp.* and *Saccharomonospora* genus.

Hence the present study was carried out with the collection of soil sample from Muthupet mangrove. Analysis of physico-chemical properties of the soil, isolate and identify the organisms ie., *S.aureus, P.aeruginosa, A.niger, Rhizopus sp* and *Streptomycetes sp* from two different soil sample, viz., polythene and plastic degrading soil samples and study the toxicity level of the biodegraded polythene and plastic products.

EXPERIMENTAL SECTION

Collection of test sample

Polythene bag and plastic cup were collected from local market in Thiruthuraipoondi, Thiruvarur District, Tamil Nadu and India and used for degradation studies.

Soil Sample Collection

The soil sample was collected from polythene and plastic accumulated site of Muthupet Mangrove, Thiruvarur District, Tamil Nadu, South India.

Physico - chemical properties of soil

Samples were also taken from the site and analyzed the physico-chemical parameters such as pH, temperature, phosphorus, potassium, calcium, magnesium, sodium, nitrogen and trace elements like copper, iron, manganese and zinc.

Isolation and identification of microbes from soil sample

The collected 1gm of sample was taken and serially diluted using sterile distilled water upto 10^{-9} dilutions in bacteria (10^{-6}), fungi (10^{-2}) and actinomyctes (10^{-4}) specific medium. All the plates were incubated at 37°C and 28ºC for 24 to 48 hrs. The identification of bacteria was performed on the basis of microscopic examination and biochemical test according to Bergey´s manual of determinative bacteriology [3]. The fungal species were identified after staining them with lactophenol cotton blue [4]. The phenotypic and chemotaxonomic characteristic of the actinomycetes was determined by the method described by [5].

Screening of polythene and plastic degrading microorganisms by clear zone method [6]

Polythene and plastic powder were added in mineral salt medium at a final concentration of 0.1% (w/v) respectively and the mixtures were sonicated for 1 hour at 120 rpm in shaker. After sonication, the medium were sterilized at 121° and pressure for 15 Lbs/inch^2 for 20 minutes. About 15 ml of sterilized medium was poured before cooling in each plate. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 22-30°C for 2-4 weeks. The organisms producing zones of clearance around the colonies were selected for further analysis.

Microbial degradation of polythene and plastics

The degradation of polythene and plastics by microorganisms were studied by following the method [7]. The collected polythene bag and plastic cups were cut into small pieces. The pieces were cleaned with tap water (to remove dust particles) and sterilized with ethanol and again washed with distilled water. They were weighed about 1cm each, used for both test and control. The plastic pieces were carefully removed from the culture (by using forceps) after different days of incubation. The collected pieces were washed thoroughly with tap water, ethanol and then with distilled water. The pieces were shade dried and weighed for final weight. The data were recorded. The same procedure was also repeated for 35, 45 and 55 days of treated samples.

Effect of the biodegraded polythene and plastic products

Biodegraded polythene and plastic products toxicity effect was studied by the planting method [8].

Percentage of germination [9]

The percentage of germinating ability was calculated for each treatment by the following formula

$$\text{Percentage of germinating ability} = \frac{\text{Total no. of seed germinate}}{\text{Total no. of seed shown}} \times 100$$

Morphological parameter
Following morphological parameters were studied. The height of the plant (in cm), number of leaves (per plant), shoot length (in cm), root length (in cm)

Phytochemical analysis
Chlorophyll Estimation [10]
❖The chlorophyll contents were calculated by using for formula.

$$\text{mg of total chlorophyll/g tissue} = 20.2\ (A_{645}) + 8.02\ (A_{663}) \times \frac{V}{1000 \times W}$$

Where,
A = absorbance at specific wave lengths.
V = final volume of chlorophyll extract in 80% acetone
W = fresh weight of tissue extracted

Statistical analysis
Random sampling was used for the entire test. The data of all values were statistically analyzed and expressed as Mean ± Standard Deviation by using the formula given by[11].

$$\text{Mean} = \bar{X} = \frac{\Sigma X}{N}$$

Where,
Σ X = Sum of all values of variable
N = Number of observation.

$$\text{Standard deviation} = \sqrt{\frac{\sum (X - \bar{X})^2}{N}}$$

Where,
$\Sigma(X-\bar{X})^2$ = The sum of the square of the deviations of each value from the mean.
N=Number of observation.

RESULTS AND DISCUSSION

Sample collection
The present study deals with polythene bag and plastic cup degradation by using *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Rhizopus sp* and *Streptomyces sp* were isolated from the Muthupet mangrove soil, Thiruvarur (Dt), Tamil Nadu, South India.

Physico - chemical characteristics
The physico- chemical characteristics of polythene and plastic degrading soil sample such as pH, temperature, calcium, magnesium, nitrogen, phosphorus, phosphate and trace elements like copper, manganese, zinc and iron were analyzed in standard methods [12] in table-1.

Isolation and identification of organism
Serial dilution and plating method used for isolation and characterization of the organisms. Hence the isolated organisms were confirmed as *S. aureus*, *P. aeruginosa*, *A. niger*, *Rhizopus sp* and *Streptomyces sp* . The bacterial and actinomycetes colonies were compared with Bergey's Manual of systematic Bacteriology. The fungal colony were confirmed according to manual of soil fungi [13].

Screening of polythene and plastic degrading microorganisms by clear zone method

The polythene and plastic containing mineral salt agar plates were inoculated with the isolated bacteria, fungi and actinomycetes. All the isolated bacteria, fungi and actinomycetes were screened for their degradation assay. On this screening of polythene and plastic degrading microorganisms such as *S. aureus, P. aeruginosa,A. niger, Rhizopus sp* and *Streptomyces sp* were noted in 2nd week, 3rd week and 4th week of incubation (Table -2). [14] Reported that the extracellular hydrolyzing enzymes secreted by the target organism hydrolyse the suspended polyesters in the turbid agar medium into water soluble products thereby producing zones of clearance around the colony.

Degradation of polythene bag and plastic cup by *S. aureus* and *P. aeruginosa*

The degradation of Plastics by bacteria was assessed based on the percent weight loss of plastics during treatment. The results of polythene and plastic cup degradation by *S. aureus* and *P. aeruginora* were shown in [Table-3]. The weight loss of polythene in 35 days incubation of *S. aureus and P. aeruginosa* were found to be 6.61 ±0.042 and 9.3±0.02. And the weight loss of plastic in *S. aureus* and *P. aeruginosa* noted that 8.16±0.65 and 8.3±0.01. In 45 days of incubation, the weight loss of polythene in *S. aureus and P. aeruginosa* found to 8.21 ±0.61 and 1.2 ±0.09. And the weight loss of plastic in *S. aureus* and *P. aeruginosa* showed to 10.1 ±0.91 and 12.1 ±0.25. After 55 days of incubation, the weight loss of polythene in *S. aureus* and *P. aeruginosa* were recorded as 16.3 ±0.13 and 17.3 ±0.04. And plastic weight losses in both bacteria were noted in 15.2 ±0.01 and 19.1 ±0.35. From this data, it was very clear that polythene and plastic cup degradation was maximum in sample subjected to long time of incubation.

Degradation of Polythene bag and plastic cup by *A. niger and Rhizopus sp*

The results of degradation of polythene and plastic by *A. niger* and *Rhizopus sp* were presented in the table-3. The weight loss of polythene in 35 days incubation in *A. niger* and *Rhizopus sp* were found us 6.26±0.27 and 7.07±0.27. And the weight loss of plastic in *A. niger* and *Rhizopus sp* were noted that 4.31±0.01 and 4.01±0.02. In 45 days of incubation, above mentioned fungi in weight loss of polythene were noted that 8.21 ±0.42 and 7.0 ±0.29 and the weight loss of plastic in both fungal species were found to be 6.01 ±0.26 and 5.03 ±0.26. Similarly the weight loss of polythene in *A. niger* and *Rhizopus sp* subjected to 55 days of incubation was found to be 11.01 ±0.5 and 7.09 ±0.31. And is the plastic weight loss treated in *A. niger* and *Rhizopus sp* were recorded that 10.1 ±0.86 and 7.04 ±0.86.

Degradation of Polythene bag and Plastic cup by *Streptomyces sp*

The results of degradation of polythene and plastics by *Streptomyces sp* were presented in [Table-3]. The weight loss of polythene and plastics in 35 days treated samples was found to be 7.03 ± 0.31 and 7.07 ± 0.02. The weight loss of polythene and plastic by *Streptomyces sp* subjected to 45 and 55 days was found to be 8.06 ± 0.35, 9.06 ± 0.31 and 11.01 ± 0.41, 12.04 ± 0.33. The data from the studies showed that maximum degradation had occurred on samples incubated for longer time.

In case of *S. aureus, P. aeruginosa, A. niger, Rhizopus* and *Streptomyces sp* loss of weight were recorded after 2, 4 and 6 month of incubation period when the size of 1 cm. The highest degradation of polythene and plastic cup in 6 months. The results were similar to polythene and plastic – degrading microbes from polythene and plastic – degrading microbes from the Muthupet mangrove soil [15]. Once the organisms get attached to the surface, it starts growing by using the polymer as the carbon source. In primary degradation, the main chain cleaves leading to the formation of low-molecular weight fragments (oligomers), dimmers or monomers[16].

Effect of the biodegraded polythene and plastic products

The toxicity level of the biodegraded polythene and plastic products used for the planting method. Here the effect of biodegradable polythene and plastic product were analyzed in *Vigna radiata* for their growth. **[**17**]** Pointed out that apart from nutrient availability, addition of organic manure also increased porosity and moisture content of soil that enhances root growth and water intake of the plant. However, addition of polythene granules reduced soil pores size, which may have negative effect on the nutrient intake by the root of plant.

Morphological parameter

Morphological parameters such as height of the plant, number of leaves, shoot length, chlorophyll analysis were also determined (Table 4 and 5).

Table – 1.The physico - chemical analysis of mangrove soil

S. No	Soil Analysis	Polythene degrading soil	Plastic degrading soil
1	pH	7	7
2	Temperature °C	27°C	29°C
3	Calcium (mg)	12	14
4	Magnesium (mg)	36	35
5	Nitrogen (mg)	6.5	7.5
6	Phosphorus (mg)	14.61	12.18
7	Phosphate (mg)	4.8	5.6
8	Copper (ppm)	1.351	1.353
9	Manganese (ppm)	1.243	1.240
10	Zinc (ppm)	3.11	3.42
11	Iron (ppm)	12.39	11.58

Table – 2. Comparative analysis in screening of polythene and plastic degrading microorganisms

S. No	Organisms	Name of Microbes	Microbial degradation of weight loss					
			2nd week		3rd week		4th week	
			Polythene (mm)	Plastic (mm)	Polythene (mm)	Plastic (mm)	Polythene (mm)	Plastic (mm)
1	Bacteria	*S. aureus*	3.61±0.01	2.21±6.12	6.31±0.08	4.083±0.08	17.89±0.06	16.12±0.11
		P. aeruginosa	9.67±.0.14	6.45±0.16	25.80±0.02	20.00±0.10	37.09±0.01	28.42±0.11
2	Fungi	*A. niger*	4.21±10	3.22±6.02	6.45±0.27	8.42±0.06	12.63±0.05	11.29±0.05
		Rhizopus sp	8.06±0.14	7.36±0.26	16.12±0.05	11.57±0.19	20.96±0.15	16.84±0.12
3	Actinomycetes	*Streptomyces sp*	22.58±0.03	10.52±0.31	35.48±0.01	21.05±0.23	46.16±0.01	35.78±0.25

Values are triplicate mean ± standard deviation

Table – 3.Degradation of polythene and plastics incubated with two bacterial species in shaker cultures under laboratory condition

S. No	Organisms	Days of Treatment	Microbial degradation (weight loss (µg))	
			Polythene	Plastics
1	*S. aureus*	35 days	6.61±0.042	8.16± 0.65
		45 days	8.21±0.61	10.1±0.91
		55 days	16.39± 0.13	15.2± 0.01
2	*P. aeruginosa*	35 days	9.3± 0.02	8.3± 0.03
		45 days	11.2± 0.09	12.1±0.25
		55 days	17.3± 0.14	19.1±0.35
3	*A. niger*	35 days	6.26±0.27	4.31± 0.01
		45 days	8.21±0.42	6.01±0.26
		55 days	11.01± 0.51	10.1± 0.86
4	*Rhizopus sp*	35 days	7.07± 0.27	4.01± 0.02
		45 days	7.0± 0.29	5.03±0.26
		55 days	7.09± 0.31	7.04±0.86
5	*Streptomyces sp*	35 days	7.03±0.31	7.07± 0.02
		45 days	8.06±0.35	9.06±0.31
		55 days	11.01± 0.41	12.04± 0.33

Values are triplicate mean ± standard deviation

Table – 4.Effect of the biodegraded polythene on *Vigna radiata* growth

S. No	Morphometric parameters	Treatment					
		I	II	III	IV	V	VI
1	Germinating ability (%)	78	38	54	72	40	68
2	Height of plant(cm)	10.2±0.67	11.1±0.37	10.3±0.27	12.6±2.57	11.6±0.57	9.01±0.11
3	No. of leaves/ plant	11.2±0.26	12.1±0.01	15.2±0.01	14.5±0.02	13.1±0.01	11.11±0.1
4	Shoot length (cm)	9.06±0.21	10.3±0.02	11.01±0.03	13.01±0.01	12.01±01	14.01±.02
5	Root length (cm)	9.02±0.11	10.9±0.01	11.1±0.03	13.1±0.3	12.0±0.1	14.01±0.03
6	Chlorophyll (mg/g)	0.121	0.016	0.018	0.158	0.293	0.190

Values are triplicate mean ± standard deviation

I. Treatment – I (sterile soil + seeds)
Treatment – II (sterile soil + seeds + *S. aureus* degraded polythene)
Treatment – III (sterile soil + seeds + *P. aeruginosa* degraded polythene)
Treatment – IV (sterile soil + seeds + *A. niger* degraded polythene)

Treatment – V (sterile soil + seeds + *Rhizopus* degraded polythene)
Treatment – VI (sterile soil + seeds + S*treptomyces sp* degraded polythene)

Table – 5 .Effect of the biodegraded plastic on *Vigna radiata* growth

S. No	Morphometric parameters	Treatment					
		I	II	III	IV	V	VI
1	Germinating ability (%)	68	48	64	82	40	68
2	Height of plant(cm)	11.1±0.02	13±0.2	12±0.01	14.1±.0.3	8.9±0.1	15.2±0.03
3	No. of levels plant	12.1±0.1	16.2±0.01	13.1±0.02	11.2±0.01	15.1±0.03	14.1±0.02
4	Shoot length (cm)	11±0.3	12±0.08	15±0.06	14±0.07	13±0.06	16.1±0.02
5	Root length (cm)	11±0.4	12±0.09	15±0.03	14±0.04	13±0.06	16.1±0.06
6	Chlorophyll (mg/g)	0.012	0.016	0.167	0.189	0.019	0.099

Values are triplicate mean ± standard deviation

II. Treatment – I (sterile soil + seeds)
Treatment – II (sterile soil + seeds + *S. aureus* degraded plastic)
Treatment – III (sterile soil + seeds + *P. aeruginosa* degraded plastic)
Treatment – IV (sterile soil + seeds + *A. niger* degraded plastic)
Treatment – V (sterile soil + seeds + *Rhizopus* degraded plastic)
Treatment – VI (sterile soil + seeds + S*treptomyces sp* degraded plastic

CONCLUSION

The results from this study, it was concluded that *S. aureus, P. aeruginosa, A. niger, Rhizopus sp* and *Streptomyces sp,* degrade the polythene and plastic materials effectively. Thus the *P. aeruginosa* have efficient to degrade the polythene and plastic material. The seedling of *Vigna radiata* were transplanted in 12 pots of equal size, which were noted as Treatment I – VI (Polythene) and I – VI (Plastic). The morphological parameters such as germinating ability, root length, shoot length and chlorophyll content were analyzed. Polythene and plastic cup waste materials cause serious environment problems, so the waste materials removed by using the microorganism. This method was cheap and effective, so that it can be used widely for the treatment of polythene and plastic cup.

REFERENCES

[1] AM Ali; T Nakajima-Kemble; N Nomura and T Nakahara T, *Appl. Environ. Microbiol*. **1998. 64(1)**: 32-36
[2] KL Hoffmann; J Renickova; K Kozakova; P Ruzicka; D Alexy Bakos and L;Precnerova, *Polym. Degrad. Stab*. **2003,76**: 511-519
[3] JG Holt; NR Krieg; PHA Sneath; JT Staley and ST Willims,Gram positive cocci.In:Bergey´s Manual of Determinative Microbiology,Hensyl,W.R.(Ed).9th Edn.,Williams and Wilkins, Baltimore, USA., **1994**.527-558.
[4] KB Raper and DI Fennell, The genus *Aspergillus* Robert E. Krieger (ed) Co., Huntington,New York.**1987.2**:686.
[5] EB Shirling and D Gottlieb, *Int.J.Syst.Bcteriol*, **1966. 16:** 313-340.
[6] J Augusta; RJ Muller and K Widdecke, *Appl. Microbiol. Biotechnol*. **1993**, **39**: 673 – 678.
[7] K Kathiresan, *International Journal of Tropical Biology* and *Conservation*. **2003**, **51 (3)**:21-29.
[8] P Aswale, *Bioinfolect.* **2010, 5:** 239.
[9] DL Korade and MH Fulekar , *Journal of Hazardous Material*. **2009**, **172**:1344 – 1350.
[10] DI Arnon, *Plant physiol*.**1949, 24**: 1-15.
[11] SP Gupta, Measures of Dispersion. In statistical methods.**1977:** 833 – 834.
[12] APHA, Standard methods for the examination of water and waste water. American Public Health Association. Water environment Federation Green berg. AEClescerils, Eaton AD (eds) 18th edition.**1985**, 1100-1115.
[13]JC Gilman, A manual of soil fungi revised and 2nd edition oxford publishing company (Indian reprint calcutta) Bombay, New Delhi**.1957**. (**5):** 450-455.
[14] J Augusta; RJ Muller and K Widdecke, *Appl. Microbiol. Biotechnol*.**1993**, **39**: 673 – 678.
[15] K Kathiresan, *Rev. Biol. Trop*.**2003**. **51** : 629 – 634.
[16] C Vasile, Degradation and decomposition,in Handbook of polylefines synthesis and properties,edited by C Vasile and RB Seymour (Marcel Dekker Inc, New York).,**1993** pp:479-509.
[17] TM Agbede;SO Oeniyi and AJ Adeyemo, *Journal of Sustainable Agriculture*,**2008, 2(1):**72-77.