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Evaluation of Biodegradation of Plastics and Polythene Bags from Various Soils

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Plastic pollution has emerged as one of the most challenging problems of the mankind, and can be satisfactorily addressed through Biodegradation. The present study is an attempt to explore the bio degradation of plastic cups and polythene bags (carry bags) using mangrove soils, petroleum soil, and molasses soil. Plastic cups and polythene bags were incubated for a period of 2,4,6,9, months in various soils using microbes like *Pseudomonas species, Staphylococcus, Aspergillus niger* and *Aspergillus glaucus.* Degradation of plastics was determined by the weight loss of the sample and bacterial activity in soil. The microbial counts were recorded as 45.68×10^3/g for total bacteria and 33.33×10^2 /g for fungi in mangrove soil, 26.70×10^3 /g for total bacteria and 20.22×10^2 /g for fungi in petroleum soil, and 23.49×10^3/g for total bacteria and 22.33×10^2 /g for fungi in molasses soil, The present work reveals that the above soils have the potential to degrade plastic cups and polythene bags among which Mangrove soil has the high degradability rate.

Key words: Microbes, bio degradation, mangrove soil, plastic pollution, Microbial Count, Molasses, Polythene, Bacterial activity, incubation, fungi.

Potentially huge environmental accumulations and very low biodegradability of commercial polymers, particularly commodity plastics used in industry and agriculture, drawn public attention on pollution problems that could persist for centuries(Albertsson, 1987) and currently increased emphasis is paid to research on development of biodegradable plastics and biodegradation of plastics.

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Biodegradation is a process of chemical decomposition performed by living organisms, while microorganisms play a pivotal role in biological decomposition of materials, Kenneth. Et al (1993), Glass and swift, (1998), Gu, (2003), and Ritmann, (2001) have assessed the biodegradability of some of the polymer films by measuring changes in physical properties or by observation of microbial growth after exposure to biological or enzymatic environments, and by CO_2 evolution. Kenneth et al(1993) Reported that the biodegradation of plastics proceeds actively under different soil conditions according to their properties, while Kathiresan and Salvam (2006) found that Mangrove soil is a good source of different microbes capable of degrading plastics and polythenes.

MATERIAL AND METHODS

Materials

Soils from three different environs were used for the study and were collected from the sites as described below:

1 *Mangrove Soils:* from Coringa Mangrove sanctuary, Kakinada;
2 *Petroloeum Soils:* from a site contaminated with crude oil at Visakhapatnam;
3 *Molasses Soils:* from a site contaminated with molasses at Govada Sugar Factory, Chodavaram.

Two species of bacteria *[Pseudomonas putida* (gram –ve), *Staphylococcus* (gram +ve)] and two species of fungi [*Aspergillus niger* and *Aspergillus glaucus*] isolated from the test soils were used as agents for biodegradation of plastic and polythene material.

Methods

Microorganisms were identified from morphological and physiological characters, (Barrow and Feltham 1993, and Buchanan and Gibbons, 1974) The bacterial and fungal colonies were isolated and sub-cultured repeatedly for obtaining pure colonies and then preserved in slant tubes for further use. An electronic *Colony Counter* was used to estimate the microbial counts.

1 cm diameter discs were prepared from the cleaned plastics and polythene samples. These discs were dried and sterilized in hot air oven, and weighed for 200mg and were used for assessing their degradation under different soil media. Weight loss of the plastics and polythene discs kept in the test media or soils, was calculated separately after every stipulated time, and the rate of biodegradation was estimated. Physicochemical characteristics of the test soils were carried out following the standard methods. (APHA, 2002).

Biodegradation of plastic and Polythene were studied under two conditions (*Laboratory conditions* and *field conditions*).Under Laboratory conditions, individual conical flasks containing 50 ml of media was inoculated by bacteria (*Pseudomonas putida*, *Staphylococcus)* and fungi (*Aspergillus niger*, *Aspergillus glaucus*). Nutrient broth media was prepared for bacteria and Rose Bengal broth media was prepared for fungi separately. Separate flasks were maintained for each treatment and were incubated at room temperature in an incubatory rotary shaker at 10 rpm for 2, 4, 6, 9, months. Four sets of Controls were maintained with plastic and polythene discs in the microbe-free medium.

Under field conditions, separate pots with mangrove soil, petroleum soil and molasses soil were taken and inoculated with bacteria and fungi. The pots were kept exposed to natural conditions under sunrays. The pots were watered once in 2 days for a period of 2, 4, 6, 9 months. Pots with test soils and without soil amendments were considered as controls. Separate sets were maintained for each of the test species.

RESULTS AND DISCUSSION

Microorganisms play a very significant role in biological decomposition of materials, including synthetic polymers in natural environments and the growth of microorganisms depends on several physico chemical factors of the soils, and is species specific. Oxygen, microbial activity of the disposal environment, surface area, and presence of other nutrient materials are also factors which influence the Biodegradation (Lee,1996). The physicochemical characteristics of the test soils used in the study are presented in Table 1.

Microbial counts in the controls and soils amended by microbial inoculation after different time periods are presented in Fig. 1 for bacteria and Fig. 2 for fungi. Rapid growth of microbes were observed in all the three test soils under amended conditions, where in the counts of microbes in Control soils after 9 months period were e" than those of amended soils of 2 months age. This indicates microbial inoculation enhanced the growth of populations.

Compared to Mangrove soils, the microbial counts were relatively lower in Petroleum and Molasses soils under both the Control and amended conditions. The Bacterial (*Pseudomonas putida* and s*taphylococci)* counts in Mangrove, Petroleum and Molasses soils were recorded at 45.68×10^3 /g, 26.70×10^3 /g and 23.49×10^3/g, respectively. Similarly, the fungal (*Aspergillus niger* and *Aspergillus glaucus*) counts were recorded at 33.33×10^2/g, 20.22×10^2 /g and 22.33×10^2/g, respectively for the three test soils.

Change in the physical characters of plastic and Polythene discs

Both plastic and polymer samples forms multi-cellular microbial communities known as bio-films on the surface. Development of biofilms contain layered aggregations of microorganisms attached to a solid surface (Ritmann and Mc carty, 2001) and are found to be powerful degrading agents in nature. After each time interval, the plastic and polyethene discs were removed carefully and observed for any physical change. Microorganisms present in the soil samples appeared to settle on the plastic and polythene discs and formed bio-films (Fig: 3(a) and Fig: 3(b).

After careful removal of bio-films from the disc through sterile wash and drying, the discs were examined for surface features under the scanning microscope; it was observed that changes like roughness and cracks appeared indicating biodegradation of plastic and polythene. The change from without degradation and after degradation is presented in Fig. 4(a) and 4(b).

Table 1. Physicochemical characteristics of the test soils

Parameter	Mangrove Soil	Petroleum Soil	Molasses Soil
Physical:			
Color	Dark Brown	Dark Brown	Brown
pH	6.9		6.8 6
Temp	30^0C	32^0C	33^0C
Moisture	60%	52%	35%
Texture	Silt and loamy	Loamy	Loamy
Chemical			
Phosphorus as p	86980µg/kg	86781µg/kg	2534µg/kg
Sulphates as S	598µg/kg	416µg/kg	432µg/kg
Total organic carbon	10.91%	4.75%	4.44%
Iron as Fe	4.59%	2.93%	2.21%
Potassium as K	3738µg/kg	2803µg/kg	2090µg/kg
Magnesium as Mg	5624µg/Kg	4531µg/kg	3599µg/kg
Calcium as Ca	6169µg/kg	5729µg/kg	8223µg/kg
Total nitrogen as N	2840µg/kg	2685µg/kg	2701µg/kg

Table 2. Weight of loss of the test sample

Time (Month)	% Weight Loss								Microbe
	Plastics				Polythenes				
	L	M	PS	MS	L	M	PS	MS	
2-4th	3.00	4.25	2.00	0.50	3.50	4.00	4.37	0.87	*P. putida*
4-6th	6.50	7.75	2.75	1.25	7.75	9.00	4.87	2.12	
6-9th	8.25	13.25	4.00	2.25	12.75	16.25	6.37	2.12	
2-4th	1.00	4.25	0.88	0.00	1.75	2.50	1.25	0.00	*Staphylococci*
4-6th	3.00	7.75	3.00	1.00	4.50	8.25	3.25	1.00	
6-9th	6.00	13.25	4.00	2.00	9.25	15.00	5.25	2.00	
2-4th	3.75	4.75	1.00	0.25	4.75	5.50	1.13	1.25	*A. niger*
4-6th	8.50	8.50	3.37	1.37	9.25	8.00	1.88	1.75	
6-9th	13.25	15.50	4.62	3.37	14.75	10.75	6.75	3.25	
2-4th	6.50	5.75	0.75	0.00	6.25	5.50	0.87	0.88	*A. glaucus*
4-6th	10.25	7.50	1.75	1.00	9.25	8.00	1.87	2.00	
6-9th	17.25	12.00	3.50	2.25	16.00	11.00	6.37	2.25	

(L=lab Conditions, M= mangrove soil, PS= petroleum soil, MS=Molasses Soil)

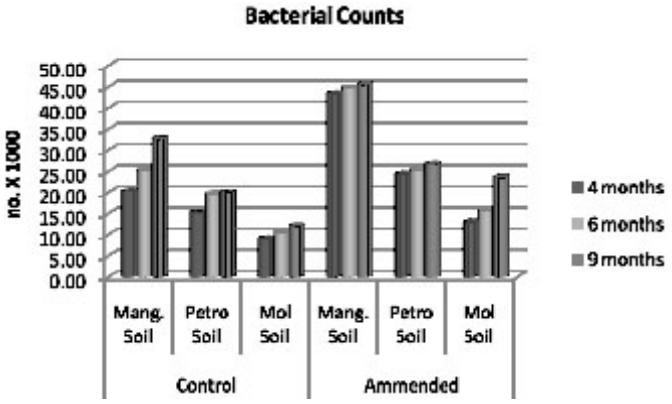


Fig. 1. Bacterial Microbial Count after different time periods

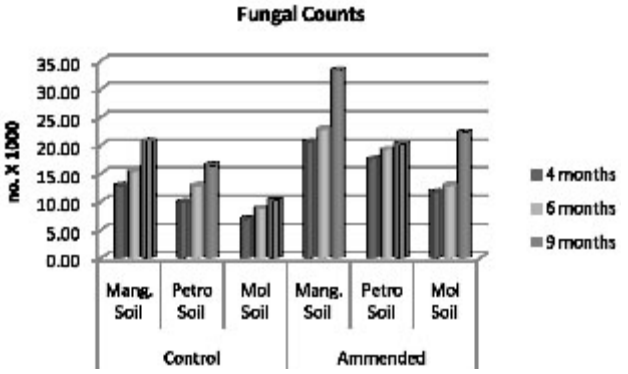
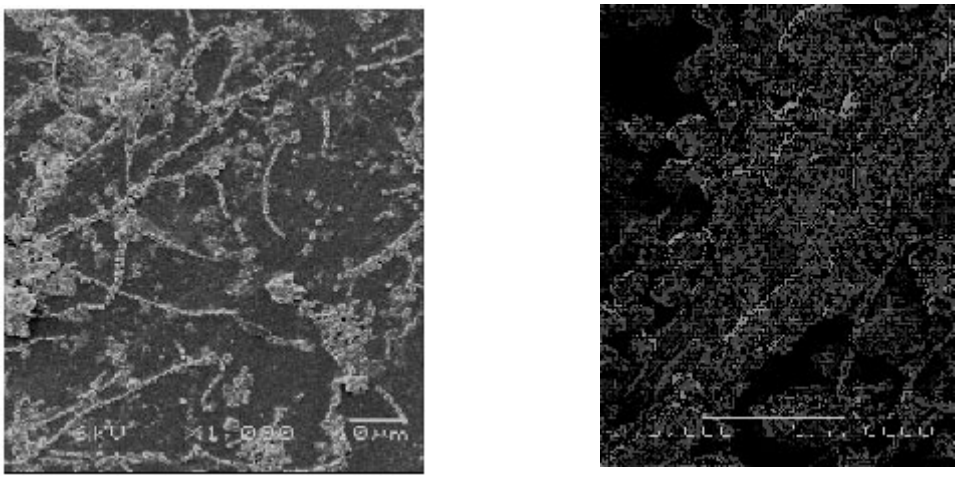


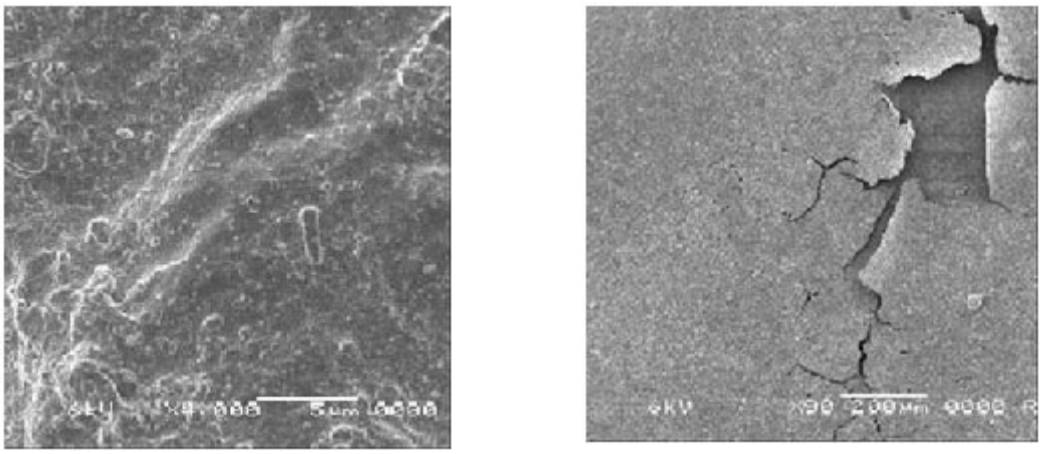
Fig. 2. Fungal Microbial Count after different time Periods



A): Biofilm formed on discs by Bacteria

B): Biofilm formed on discs by fungi

Fig. 3. Biofilms with Bacteria and Fungi



A) Disc Surface before Degradation **B)** Disc Surface after Degradation

Fig. 4. Changes before and after degradation

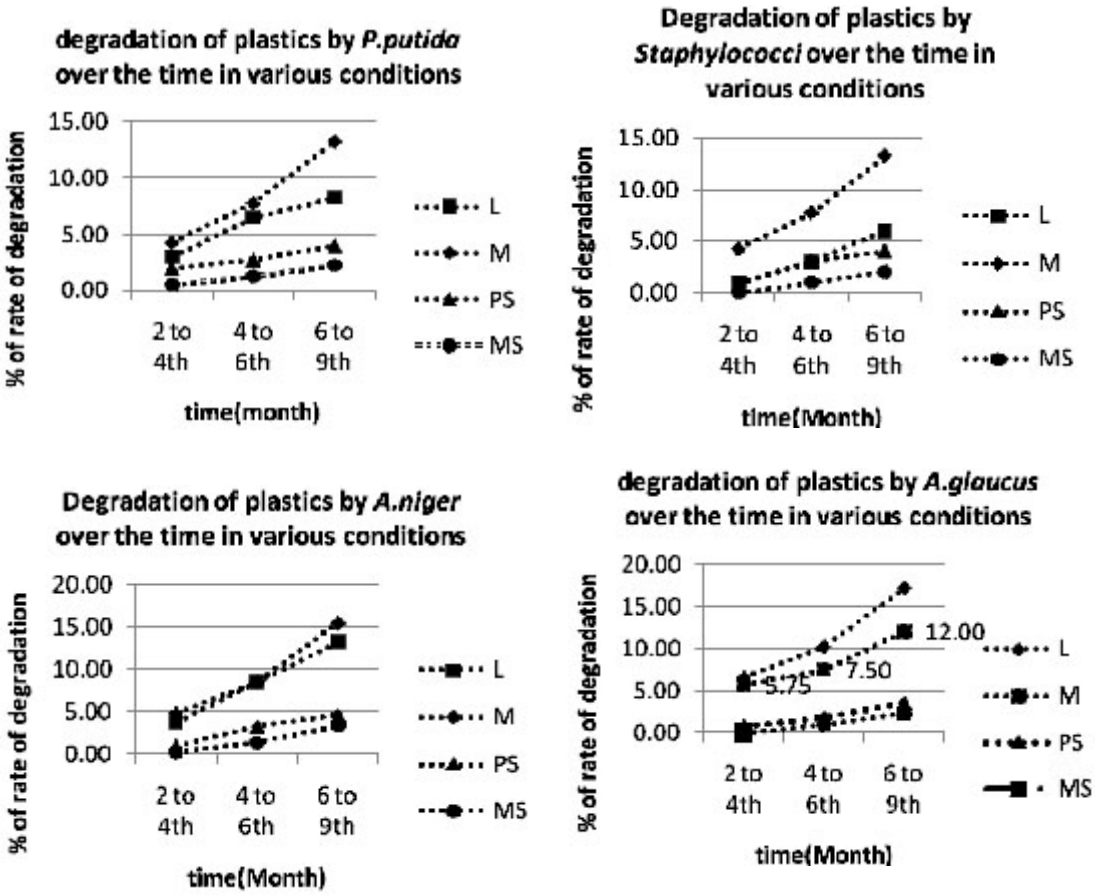


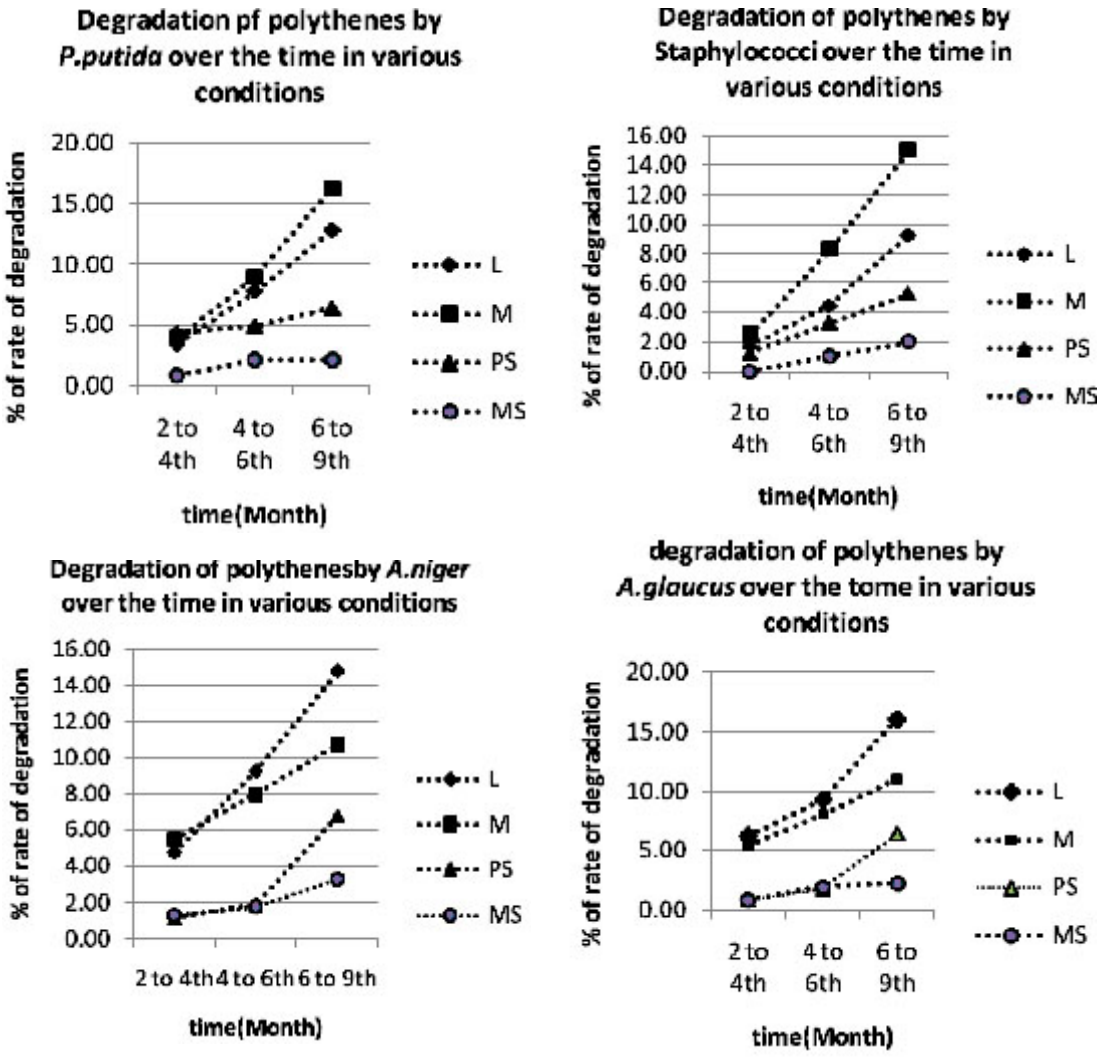
Fig. 5. Degradation of plastics by various microbes

Biodegradation Rates

Raghavan (1995) stated that biodegradation is the conversion of the constituents of a polymer to carbon dioxide/methane, microbial cellular components and miscellaneous by-products, by microorganisms. Microorganisms break down the long polymer chains into shorter chains at the c-c bonds. These shorter carbon chains pass through the cell walls of the microbes and are used as an energy source and finally converted into water, biomass, carbon dioxide or methane. Hence, biodegradation is calculated by weight loss of the test sample.

Loss in the weight of the test discs was considered as Biodegradation. After each time interval the weight of the discs were recorded after careful removal of the biofilms and other material aggregations. The weight loss was expressed in terms of percent weight loss from the initial time and the results for each time interval for different microbes are presented in Table 2.

Irrespective of the microbes used for degradation, both plastic and polythene have very high degradation in Mangrove soil, which were followed by Petroleum soil and Molasses soil. In case of plastic, *A. niger* demonstrated greater



(L- lab conditions. M-mangrove soil , PS- Petroleum Soil , MS- Molasses Soil)

Fig. 6. Degradation of polythenes by various microbes

degrataion ability, with a maximum rate of 15.5% weight loss after 6-9th month period in Mangrove soil, and in other soils also the microbe has relatively higher degradation rates compared to other microbes. In case of polythene, *P. putida* demonstrated greater degrataion ability, with a maximum rate of 16.25% weight loss after 6-9th month period in Mangrove soil, while in case of Petroleum and Molasses soils, *A. niger* had shown greater degradation with 6.75% and 3.25% weight loss, respectively.

The biodegradation rate change from one time interval to another varied in different soils with different microbes. Cell surface and hydrophobicity were reported to be an important factor in the formation of bio-film on the polyethylene surface and which consequently enhanced biodegradation of the polymer (Orhan, 2004). These microbes' acts on the bonds and breaks bonds present in the plastic and polythene.

The rate change during the 1st time interval (2-4th month) was maximum in case of plastic and polythene in Petroleum soil with *P. putida*; in Mangrove soil with *A. glaucus*; while in case of polythene in Mangrove soil with *A. niger*. During the 2nd time interval (4-6th month) the rate change was maximum in case of plastic in Petroleum soil with *Staphylococci* and *A.niger;* and in case of polythene in Molasses soil with *P.putida* and *A.glaucus*. The biodegradation rate changes for different species are illustrated in Fig. 5 for plastic and Fig. 6 for polythene.

A good number of bacteria like *Pseudomonas, Staphylococcus* (Robert, 2002,) , *Pearson et al* 2008) and fungal strains like *Aspergillus niger, A.flavus, A.glaucus (*Adekunle, and Adebambo, (2007); were reportedly capable of degrading petroleum components, including plastic and polythene. The tests in the present study were carried out with individual microbe species under isolation. However, under a microbial consortium the effects may alter because of competition and dominance of the microbes(Kumar *et al*, 2007).

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REFERENCES

1. Albertsson, A.C. *J. Appl. Polym. Sci.*, 1987; **25**: 1655-1671.
2. Gu, J. D. *Int. Biodeterior. Biodegrad.*, 2003, **52**: 69-91.
3. Glass, J. E. and Swift, G. *Agricultural and Synthetic Polymers, Biodegradation and Utilization*, ACS Symposium Series 433, American Chemical Society, Washington DC, 1989, pp. 9-64.
4. Ritmann B E and Mc Carty P L. ,Environmental Biotechnology : Principles and Applications [M]. McGraw-Hill Book Co, 2001, New York.
5. Kenneth, E. J., A. L. Pometto, Z. L. Nikolov, *J. App. Env. Mic.* 1993, 59: 1255-1261.
6. Kathiresan , K. and Salvam M. M. Evaluation of beneficial bacteria from mangrove soil. *Botanica Marina*, 2006, **49**(1).
7. Barrow, G. I. and Feltham, R. K. A. (eds), *Cowan and Steel's Manual for the Identification of Medical Bacteria*, Cambridge University Press, 1993, Cambridge.
8. Buchanan, R. E. and Gibbons, N. E. (eds), (1974) *Bergey's Manual of Determinative Bacteriology*, Williams and Wilkins, Baltimore, Kenneth, E. J., Pometto, A. L. and Nikolov, Z. L., *J. App. Env. Mic.*, 1993; **59**: 1255-1261.
9. APHA publication, Standard Methods for the Examination of Water and Wastewater , 20th Edition, 2001.
10. Lee , S. Y., Bacterial Polyhydroxyalkanoates, *Biotechnol. Bioeng*. 1996, **49**: 1-14.
11. Raghavan, D. Characterization of Biodegradable Plastics. *Polymer-Plastic Technology Engineering*. 1995; **34**(1): 41-63.
12. Orhan Y, Hrenovic, J and Buyukgungor, H. Biodegradation of plastic compost bags under controlled soil conditions, *Acta Chim Slov*, 2004, **51**: 579-588.
13. Robert, U., OTU Proceedings of International Symposium on Environmental Pollution control and waste Management, 7-10, Tunis (EPCOWN'2002), p. 2002; 118-122.
14. Pearson, A., Kraunz, K.S. and Alex, L. Sessions, Anne E. Dekas, William D. Leavitt and Katrina J. Edwards, Quantifying Microbial Utilization of Petroleum Hydrocarbons In salt marsh Sediments by using the ^{13}C content of Bacterial rRNA; Applied and Environmental Microbiology, 2008; p.1157-1166.