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BIODEGRADATION ABILITY OF BACTERIA ON PLASTIC AND THERMOCOL CUPS

Sikha Dey, Ashok Kumar Singh and Gauri Singh*

Department of Microbiology, Dolphin (P.G) Institute of Biomedical and Natural Science, Manduwala, Dehradun, Uttarakhand, India.

***Corresponding Author: Dr. Gauri Singh**

Department of Microbiology, Dolphin (P.G) Institute of Biomedical and Natural Science, Manduwala, Dehradun, Uttarakhand, India.

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ABSTRACT
The use of plastic and thermocol is increasing day by day but its degradation is becoming a great ecological threat. Most ecofriendly and acceptable method of plastic and thermocol degradation is by using microbes The present study includes collection of sample, isolation, identification, screening and degradation of pretreated plastics and thermocols cups by microbes in laboratory and soil condition. Only four isolates showed positive results and identified as Bacillus sp. (S2), Staphylococcus sp. (S6), Pseudomonas sp. (S8), Pseudomonas sp (S9). Four isolates and one consortium prepared from these were used. Degradation was done at different time intervals i.e. 20, 30 and 40 days. it is observed that degradation is increase with the increase in the time interval hence effective observation was recovered after 40 days interval. In plastic cups, maximum degradation was shown by Consortium (S) which is 22.7 percent and in thermocols 35 percent was degraded by S2 isolates (*Bacillus sp).* under laboratory condition. This work also reveals that thermocol degrade faster as compared to plastics.

KEYWORDS: Bacteria, Biodegradation, Plastics, Thermocols.

INTRODUCTION
Plastics are defined as the polymers which become mobile on heating. Plastics are non metallic moldable compounds and the materials made from them, can be pushed into any desired shape.[1] "Plastikos" the greek word, from which the word plastic is actually originated, which means able to be molded into different shapes.[2] Today, the plastic that we use are made from inorganic and organic raw material, such as carbon, silicon, hydrogen, nitrogen, oxygen and chloride. For making plastics, the basic materials are extracted from oil, coal and natural gas. It is the mother industry to hundreds of manufactured components and products used in our daily life like automobile parts, television, refrigerator, packages, sanitary.

On the contrary Plastics and Thermocols cause the environmental pollution by getting accumulated in the environment because of their stable nature.[3] In most of the countries plastic pollution caused due to improper recycling and waste management system. The plastics sheets or bags also causes infertility of soil, preventing degradation of natural substances, depletion of underground water and danger to animal life. In seas, also plastics rubbish from ropes and nets to plastic bands from beer packs chokes and entangles marine mammals. Choking of drains due to carry bags is an another major ecological threat which is reported by municipal administrators.[4]

Degradation is defined as any physical and chemical changes in polymer because of environment factors such as light, heat, moisture where as Biodegradation is any changes in a material caused by biological activity of microorganisms like bacteria, yeast, fungi, actinomycetes.[5] In aerobic biodegradation, carbon dioxide and water are end products and carbon dioxide and water and methane in case of anaerobic. Microbial Biodegradation increases the rate of degradation of plastics without causing any harm to environment.[6]

MATERIALS AND METHODS
Sample Collection- Plastic samples were collected from the dumped areas.

Isolation
After the collection of plastics sample, these were taken and 1gm of sample was cut into pieces and added to 9ml of sterile water to make 1:10 dilution, adding 1ml of 1:10 dilution of 9ml of sterile water makes 1:100 dilution and so on.

Screening
Media was prepared having concentration of 0.5, 1.0, 1.5, 2.0 and 2.5 percent of PEG (Polyethylene Glycol). Screening of degrading bacteria was performed by methylene blue staining. Result was shown by zone of clearance.

Identification - Identification of isolates was performed according to their morphological, cultural and biochemical characteristics by Bergey's manual of systemic bacteriology.[7]

Degradation in laboratory condition

Determination of weight loss - Pre weighed disc of 1cm prepared from polythene bags were transferred to the conical flask containing 50 ml of culture broth medium, inoculated with different species of bacteria. Control was maintained with plastic discs in microbe free medium. Different flasks were maintained for each treatment and left in a shaker. After 1 month of shaking, the plastic discs were collected, washed thoroughly using distilled water, shade dried and then weighed for final weight. By using following formula, it was calculated.[8]

$$\text{Weight loss } \% = \frac{\text{(Initial weight – Final Weight)}}{\text{Initial weight}} \times 100$$

Degradation in soil condition

Plastic cups and polythene bags were buried at a depth of 5 cm in the sterilized soil, The materials were allowed to degrade naturally in the soil and checked under different time intervals using sterile forceps and transferred to laboratory aseptically.

One set of sample was thoroughly washed using distilled water, shade-dried and then weighed for final weight. The degradation was determined in terms of percent weight loss of the materials over a period of 40 days. After performing this, following formula was applied for calculating the weight loss.[9]

$$\text{Weight loss } \% = \frac{\text{(Initial weight – Final Weight)}}{\text{Initial weight}} \times 100$$

RESULTS

Isolation of bacteria- A total of twelve isolates of bacteria were recovered from different dump areas.

Primary screening of plastic and thermocol degrading bacteria

Minimal media having composition of 0.3g of NH_4NO_3, 0.5g of K_2HPO4, 0.1g of NaCl, 0.02g of $MgSO_4.7H_2O$, 2g of Agar was prepared. Polyethylene glycol (PEG) was also prepared. Then primary screening of isolates were performed by Methylene blue staining. Only 5 isolates showed positive result by showing zone of clearance.

Secondary Screening: Out of 5 isolates only 4 isolates showed positive result in secondary screening. This was performed by adding 1% PEG concentration.

Identification- After secondary screening, only 4 isolates were identified and named as S2, S6, S8, S9. The identified bacteria are S2=*Bacillus sp.*, S6=*Staphylococcus sp.*, S8= *Pseudomonas sp.*, S9= *Pseudomonas sp.* Then, Consortium was also prepared and named as 'S'.

Table-1: Morphological and Biochemical Characterization of isolates

Sl. No.	Characteristics	S2	S6	S8	S9
01	Gram staining	Gram+ve	Gram+ve	Gram-ve	Gram-ve
02	Shape	Bacilli	Cocci	Bacilli	Bacilli
03	Nitrate Reduction	+	+	+	+
04	Urease activity	-	-	-	-
05	H_2S Production	-	-	-	-
06	Citrate Test	-	-	+	+
07	Voges Proskauer test	+/-	+/-	-	-
08	Methyl red test	-	-	-	-
09	Oxidase test	-	-	+	+
10	Catalase test	+	+	+	+
11	Starch Hydolysis	+	-	-	-

Degradation of Plastic under laboratory condition

After 40 days maximum degradation was shown by Consortium (S) which is 22.7 percent under laboratory condition and minimum degradation was shown by S9 isolate, which is a *Staphylococcus sp. (*Table 2 and Fig 1). Here after 20 days no degradation was observed by S8 and S9 isolates. In case of control, no changes occurred even after 40 days.

Table 2 Weight loss of Plastic sample under laboratory condition

SL. NO.	ISOLATES	INITIAL WEIGHT (g)	20 days	30 days	40 days
			FINAL WEIGHT (g)		
1.	Control	0.018	0.018	0.018	0.018
2.	S2	0.028	0.027	0.026	0.022
3.	S6	0.024	0.023	0.022	0.020
4.	S8	0.022	0.022	0.021	0.019
5.	S9	0.021	0.021	0.020	0.018
6.	CONSORTIUM	0.022	0.021	0.020	0.017

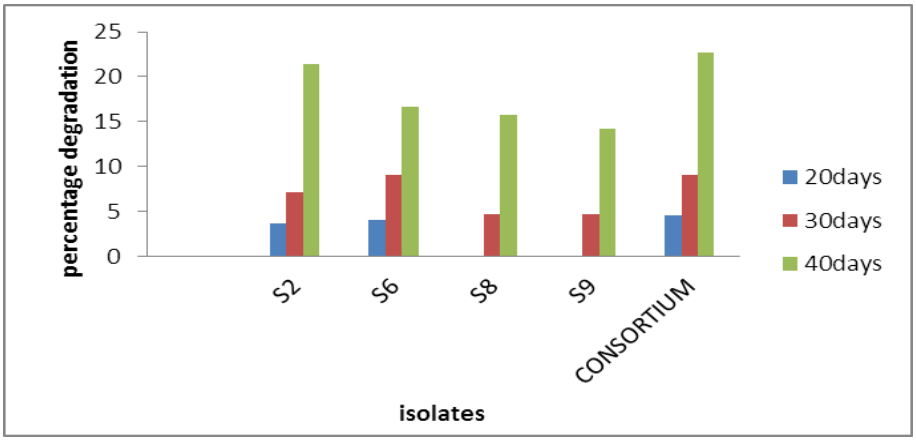

Fig1- Percentage degradation of Plastic sample under laboratory condition

Degradation of Plastic under soil condition

Under soil condition, plastic cups were degraded maximum by *Staphylococcus sp*. and consortium where as least degradation was shown by *Pseudomonas sp*. after the interval of 40 days(Table3 and Fig2).

Table3- Weight loss of Plastics in soil condition

SL.NO.	ISOLATES	INITIAL WEIGHT (g)	20 days	30 days	40 days
			FINAL WEIGHT (g)		
1.	Control	0.02	0.02	0.02	0.02
2.	S2	0.021	0.0210	0.021	0.020
3.	S6	0.024	0.0235	0.0221	0.02
4.	S8	0.022	0.0215	0.0210	0.02
5.	S9	0.021	0.0210	0.0210	0.02
6.	CONSORTIUM	0.022	0.0212	0.0209	0.02

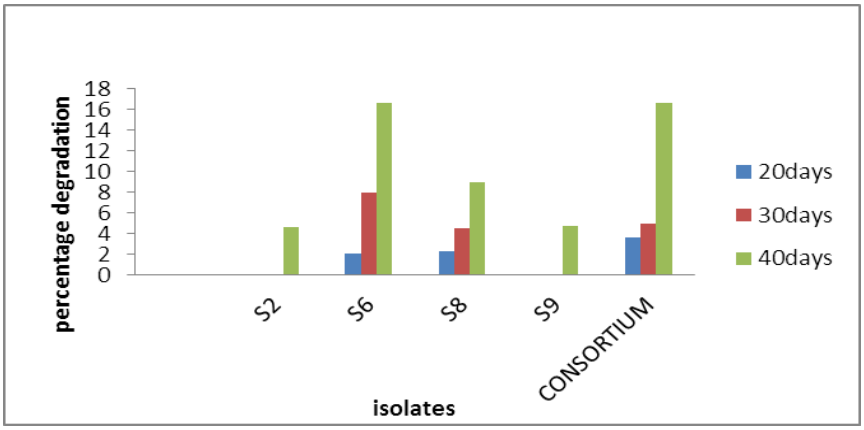

Fig2- Percentage degradation of Plastics under soil condition

Degradation of Thermocol under laboratory condition

it was noted that it showed maximum degradation rather than plastic cups in laboratory as well as in soil condition. Maximum degradation of thermocol was done by *Staphylococcus sp*. (S6) and consortium (S)(Table4).

Table 4- Weight loss of Thermocol sample under laboratory condition

SL.NO.	ISOLATES	INITIAL WEIGHT (g)	20 days	30 days	40 days
			FINAL WEIGHT (g)		
1.	Control	0.02	0.02	0.02	0.02
2.	S2	0.02	0.019	0.018	0.013
3.	S6	0.02	0.018	0.016	0.014
4.	S8	0.02	0.019	0.018	0.016
5.	S9	0.02	0.019	0.017	0.015
6.	CONSORTIUM	0.02	0.018	0.016	0.014

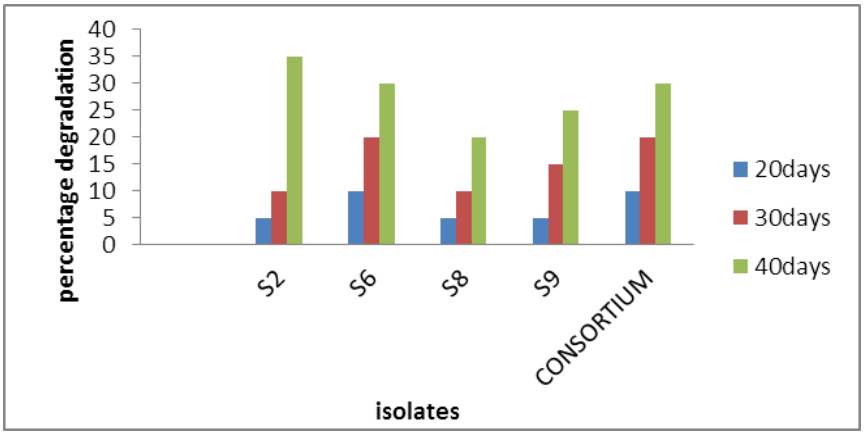

Fig 3-Percentage degradation of Thermocol sample under laboratory condition

Degradation of Thermocol under soil condition

Under soil condition and 35 percent was degraded by S2 isolates which is a *Bacillus sp.* under laboratory condition. Under laboratory condition least degradation was observed by *Pseudomonas sp*. (S8 isolate) after 40 days(Table5).

Table 5- Weight loss of Thermocols under soil condition

SL.NO.	ISOLATES	INITIAL WEIGHT (g)	20 days	30 days	40 days
			FINAL WEIGHT (g)		
1.	Control	0.025	0.025	0.025	0.025
2.	S2	0.025	0.025	0.024	0.023
3.	S6	0.025	0.024	0.023	0.022
4.	S8	0.025	0.024	0.0245	0.023
5.	S9	0.025	0.025	0.024	0.023
6.	CONSORTIUM	0.025	0.024	0.024	0.022

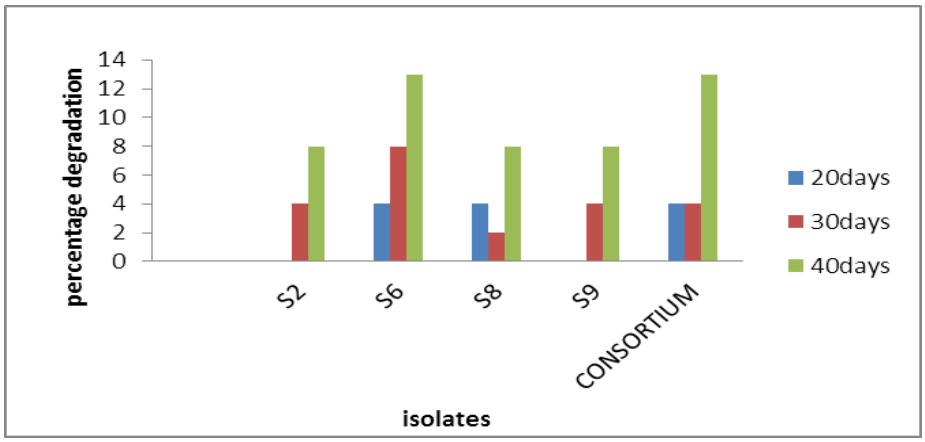

Fig 4- Percentage degradation of Thermocol under soil condition

DISCUSSION

Uncontrolled use of the plastics and thermocols in our daily life for packaging like automobile parts, television, refrigerator, sanitary, transportation, industry and agriculture in rural as well as urban areas, has elevated serious issue of plastic waste disposal and its pollution.[10]

A very general estimate of worldwide plastic waste generation is annually about 57 million tons.[11] Due to their excessive molecular mass, high number of aromatic rings, unusual bonds they do not break down in the environment easily because they are resistant to microbial attack.[12] They remain in the environment for a very long time without any deterioration and the large scale accumulation of waste plastics in the biosphere has given rise to the problem of severe environmental pollution.[13]

In the current study, plastic cups and thermocols were used for observing the degradation percentage and comparison of rate of degradation between them. Similar work has been done by Singh *et al*.[14] that degradation of two types of polythenes (10 µ and 40 µ) by bacteria. In that case maximum degradation was shown by 40 µ

polythene where as 10 µ showed the minimum degradation.

In the current study 12 isolates of bacteria were recovered from different dump areas. Further screening of isolates were done by zone of clearance method. Augusta *et al.* (1993)[15] reported that the zone of clearance around the colony is due to extracellular hydrolyzing enzymes secreted by the target organism into suspended polyesters agar medium.

Among them only 4 isolates showed positive results and named as S2, S6, S8, S9 isolates. Later, by morphological and biochemical tests, they were identified as Bacillus sp.(S2), Staphylococcus sp.(S6), Pseudomonas sp.(S8), Pseudomonas sp. (S9). Further pretreated plastics and thermo cols were degraded under laboratory and soil condition at different time interval like 20, 30 and 40 days. In our study, thermocol showed faster degradation as compared to plastic cups. Shivashankari and Vinetha (2014)[16] also reported that longer time is needed for biodegradation of polythenes than plastics. Their result shows that *M. Luteus* degrade 32% plastic cups in 55 days.

The results of our work were compared with earlier research studies done by Sowmya *et al* (2014)[17] in which they reported that *Bacillus cereus* was able to grow on minimal medium containing polythene as sole carbon source. Degradation by bacteria was monitored by screening which was followed by weight loss. On the other hand, Tolker - Nielsen *et al*. (2000)[18] who reported that *Pseudomonas sp*. have slow rate of micro colony formation when compared to other organisms.

There are lots of reports demonstrating the potential of plastic degrading microbes, but none of them found to have practical application, thus there is a strong need to screen efficient organisms and developing technologies capable of degrading plastic efficiently without affecting environment.

The study was published in the journal JAMA Internal Medicine in 2015 reported that Thermocol contains an important thermoplastic compound, called polystyrene which is obtained by the polymerisation of styrene or phenylethene. The chemical properties of phenylethene are identical to polyethene. It responds very slowly to bacterial decomposition in the soil, thus making the soil infertile. It also releases poisonous gases on burning, which can cause respiratory problems, or even death, when inhaled. Hence Thermocol is harmful to environment.

CONCLUSIONS

- The bacteria were identified to be Bacillus sp., Staphylococcus sp., Pseudomonas sp.
- Staphylococcus sp. degrades plastic more than that of other bacteria.
- Pseudomonas has less capacity to degrade plastic as compared to other bacteria.
- The isolated microbes were native to the site of polyethylene disposal and shown some degradability in natural conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media.
- Hence, result demonstrated that Thermocol cups shows maximum degradation than plastic cups by using bacteria. So Thermocol cups should be use instead of plastic cups for better environment.

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