



**DEBATE OVER PAPER VS PLASTIC BAGS AND CHARACTERIZATION OF CELLULOSE DEGRADERS:
AN OVERVIEW**

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ABSTRACT

The plastic vs paper debate is a frequently discussed issue. Paper and plastics debate attempts to determine which among the two has least ecological cost considering its large scale usage. Paper bags are the cornerstone of many foods packaging industry and available in various forms. They are mainly made up of cellulosic fibres and to improve the strength of their fibres, various chemicals are also added during manufacturing. It uses up enormous resources and is a highly energy consuming process. Due to its weight, transportation of paper items is found more expensive than transportation of plastic bags. On other hand, polyethylene is used for manufacturing of food storage containers, as well as to prepare bags to carry greasy, oily foods. It can withstand slightly higher temperature, i.e. 120^oC. Present research work was carried out in order to investigate scientific basis to determine which among paper and plastic is advantageous to use to minimize environmental cost. Paper bags were collected from food outlets like McDonald's and local eateries. Microorganisms which are capable of degrading paper bags within short period of time were isolated from soil. These organisms were identified on the basis of its morphological and biochemical characteristics. Enrichment of these microbial cultures was carried out to prepare consortium. Similarly the grocery bags and cereal box liners having High density polyethylene 2 (HDPE-2,) coating, as well as polypropylene, bags carrying thermoplastic polymer fibres were also checked for biodegradability. Screening of Microorganisms applied for these polymer degradation tests were from the soil of a dumping ground where different forms of plastic bags were dumped. The study reveals that in In-vitro conditions, within 15 days, decomposition of pieces of brown and a white paper bag had occurred. While in case of HDPE 2 bags, slight reduction in the weight of some samples had occurred, when applied for In-situ condition as well as In-vitro tests, applying selective consortium of bioculture for biodegradability.

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INTRODUCTION

Plastic products are produced on large scale worldwide. Globally, its production is more than 150 million tonnes/year. ⁽¹⁾ Currently, India generates around 56 lakh tonnes of plastic waste annually. Experts have estimated that annual waste generation in India will increase to 165 million tonnes by 2030. This means that around 66,000 hectares of land is needed to set up a landfill site which is 10 meters high and can hold up to 20 years' waste. ⁽²⁾ Plastic bags are made from a ubiquitous polymer substance known as polyethylene. ⁽³⁾ This begins as ethylene, commonly extracted from natural gases, then treated to become the polymer, forming long chains of carbon and hydrogen atoms. These chains can vary depending on what type of is being used. Plastic bags will never degrade in the short term and remains on landscape for lifetimes. Recycling of plastic bags can be done only for 2-3 times ⁽⁴⁾ and gives unaesthetic look.

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Burning of a plastic bag generate emission of toxic gases (carbon monoxide, chlorine, dioxins, acetaldehyde, furans, etc) while dumping makes the land infertile and some toxic elements like lead and cadmium are known to leach out. Also, substandard plastic bags (<40 microns) pose problem in collection and recycling. ⁽⁵⁾ They choke the drains, may cause floods in monsoon.

But there are some advantages of plastic bags. Plastic bags cost less to transport, require less energy to recycle and cause less global warming and all together take up less landfill space. These bags can be used several times.

On the other hand, paper bags are the cornerstone of many foods packaging industry. They are available in various forms. To improve the strength of fibres, various chemicals are added during manufacturing. Paper comes from a renewable resource i.e. trees. Manufacturing of paper is an energy consuming process. Greenhouse gases are produced during its production, at the same time reducing the number of trees left to absorb these gases. ⁽⁶⁾ Recycling of paper uses many chemicals to treat

it. Paper bags take more landfill space and due to its weight and size cost more during transportation. Paper bags cause more eutrophication than other bag types due to pollutants discharged to sewer at the material production stage. Paper bags manufacturers consume much larger quantities of water than other bag types during their production.⁽⁷⁾ At the same time they appear as advantageous due to their biodegradability and property of the recycling many times unlike plastic bags. When looking at plastic v/s paper and impact on the environment, it is important to take all aspects into account. Because both have their negative points and cause harm to the environment. Present research work was carried out in order to investigate scientific basis of the problem, Paper vs plastic and their biodegradability using selective consortia.

MATERIALS

1. Polyethylene, low density as well as high density polyethylene bags and paper bags from grocery stores, food outlets.
2. Garden soil, compost manure and soil from the dumping sites where plastic bags, paper bags were dumped.
3. Beakers
4. Thermometer
5. pH paper
6. St. Nutrient agar plates, slants.
7. St. Cellulose – Congo red agar plates

Minimal media for enrichment of microorganisms

- NH_4NO_3 - 1g/lit
- $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.2 g/lit
- K_2HPO_4 -1 g/lit
- $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ -0.1 g/lit
- KCl -0.15g/lit
- Yeast extract-0.1 g/lit
- $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ - 1mg/lit
- $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ - 1mg/lit
- MnSO_4 - 1mg/lit
- Requirements for biochemical analysis.

METHOD

Enrichment of cellulose degraders and polyethylene degraders: Initial enrichment is performed in the soil. Second step is conducted by using liquid synthetic medium. Soil samples were collected from different sites rich in plastic waste were used as a source of microbial inoculums for enrichment culture. Each soil sample, 1 to 3 gram diluted to 10ml distilled water and used as inoculum for enrichment culture. 6 sets of minimal media were prepared i.e. 6 flasks each containing 100 ml was sterilized.

Set 1 which comprises of 3 flasks for cellulose degraders, were inoculated with the inoculum prepared from soil sample and pieces of 3 different types of paper bags were added separately into it. In case of Set 2 which was to check biodegradability of plastic comprises of 3 flasks, were inoculated with same inoculum as described earlier. Weighed and measured sizes of 3 samples of plastic bag pieces were added into it.

These flasks were incubated at room temperature initially for 1 week on shaker. Sample of broth was taken from both sets and incubation was continued for one month for set 1 and 3 months

for set 2. Collected sample of broth from both sets after one week's incubation was diluted adequately with sterile saline and spread on nutrient agar plates. Incubation of plates was carried out at 37°C for 2 days. Individual colonies were sub cultured on sterile nutrient agar slants. These isolates were identified as per Bergey's Manual of Determinative Bacteriology.⁽⁸⁾

Morphological Characteristics: Gram staining is the procedure which was used to distinguish among two main types of bacterial cells that are gram positive and gram negative. It is used for preliminary analysis of unidentified isolates. The motility test was carried out by the hanging drop technique.

Cultural Characteristics: Cultural characteristics were observed on Nutrient media.

Biochemical Characteristics: Total 10 isolates were tested. The isolates are further subjected to standard biochemical tests such as –

Triple sugar iron (TST) test to confirm results on fermentation of sugar⁽⁹⁾, urease test for the detection of urease positive organisms. Catalase test is performed to determine the breakdown of hydrogen peroxide. Nitrate test to detect the formation of nitrite and mannitol to conclude fermentation of mannitol. Oxidase test was performed to detect the production of cytochrome c oxidase. Gelatin hydrolysis to find organisms that can liquefy gelatin was carried out. Cellulose hydrolysis was carried out to detect cellulose degrading organisms.⁽¹⁰⁾

Other tests for identification were – Indole test, Methyl red test, Voges-Proskauer test & Citrate utilization (IMVIC) test to identify utilization of Tryptophan, formation of acidic, basic end product & utilization of citrate respectively.

Based on the results, organisms were identified. Consortium of these organisms for set 1 and set 2 were prepared and again tested for biodegradability.

In situ method with garden soil, compost and landfill soil was also carried out where pieces of papers and plastic bags were separately buried in the soil mixture in beaker. Appropriate moisture content and pH with temperature was maintained throughout the process. The beakers for paper bags were allowed to stand for 1 month and plastic bag pieces for 3 months.

RESULTS

Consortium of microorganisms degrading cellulosic material of paper bags was prepared using primary screening and secondary screening techniques. Microorganisms such as *Micrococcus varians*, *Acetobacter aceti*, *Micrococcus lylae*, *Listeria denitrificans*, *Pseudomonas*, *Bacillus lentus*, *M. agilis*, *Bacillus acidocaldarius* were identified using Bergey's Manual of systematic bacteriology, as shown in table 1

Enrichment of these microorganisms was carried out. These consortia were applied to check biodegradability of paper bags as well as plastic bags. To detect cellulose hydrolysis, cellulose – congo red agar was used as screening media, on which clear zone was observed upon hydrolysis of cellulose as shown in fig: 1. The study reveals that in In-vitro conditions, within 15 days to one month, decomposition of pieces of brown and a white paper bag had occurred. While in case of Polyethylene/ HDPE 2 bags, slight reduction in the weight of

some samples had occurred, when applied for In-situ condition as well as In-vitro tests.

Results of biochemical test

Table 1 Tests for indentification of cellulose degarders

Isolate No.	Indole	Methyl Red	VP	Citrate	Oxidase	Catalase	TSI	Nitrate	Urease	Cellulose degradation	Gelatine hydrolysis	Organism
3B	+	+	-	-	-	+	A/A	-	-	+	-	<i>Acetobacter acetii</i>
3C-1	+	-	-	-	-	+	A/A	+	-	+	-	<i>Acetobacter</i>
4A	+	+	-	-	-	+	K/A	+	-	+	+	<i>Micrococcus variens</i>
6A	-	-	-	-	+	+	K/NC	-	-	-	-	<i>M.lavlae</i>
6B	-	+	-	+	-	+	A/A	-	-	+	-	<i>Listeria denitrificans</i>
7A	+	+	-	+	+	+	K/A	-	-	+	-	<i>Pseudomonas</i>
7B	+	+	-	+	+	+	K/A	-	-	+	-	<i>Bacillus lentus</i>
9B	+	-	-	-	-	+	A/A	+	-	+	-	<i>Bacillus</i>
11A	+	+	-	+	+	+	K/A G	+	-	+	+	<i>M.agilis</i>



Fig 1 Cellulose – Congo red agar with clear zone of hydrolysis of cellulose



Fig 2 Cellulose hydrolysis test using CMC and bromothymol blue.

Table 2 Results of biodegradability of paper in In-vitro conditions

Set1	Initial wt	Degradation
Sample1	0.04 gm	Complete degradation after 15 days.
Sample2	0.05 gm	Complete degradation after one month.
Sample3	0.07 gm	Complete degradation after 24 days.

Table 3 Results of biodegradability of plastic in In-vitro conditions

Set 2	Initial wt	Weight after 3 months
Sample 1	0.08	0.07
Sample 2	0.04	0.04
Sample 3	0.08	0.06

DISCUSSION

The time required for degradation of paper bags when appropriate consortium of cellulose degrading bacteria applied was found maximum of 30 days. To reduce load of domestic waste containing such paper bags and other cellulosic material on dumping ground/landfill sites, it will definitely be considered as advantageous. On other hand, plastic will unquestionably create lasting litter for years. The consortium developed using screening methods were found to be poorly effective against degradation of plastic bags compared to paper bags. There was insignificant reduction in the weight of plastic pieces when in situ and invitro conditions applied. Consequently preliminary observation suggests that paper bags always originate as triumphant in the debate ‘plastic vs paper’ in the ecological debate.

References

1. Overview of Plastic Waste Management, Central Pollution Control Board, Parivesh Bhawan, East Arjun Nagar, Delhi-110032 June, 2013
2. <https://thewire.in/environment/landfill-solid-waste-cpcb>
3. Geyer, Roland; Jambeck, Jenna R.; Law, Kara Lavender (2017-07-01). "Production, use, and fate of all plastics ever made". *Science Advances*. **3** (7): e1700782.
4. Ignatyev, I.A.; Thielemans, W.; Beke, B. Vander (2014). "Recycling of Polymers: A Review". *Chem Sus Chem*. **7** (6): 1579–1593.
5. https://www.wfindia.org/about_wwf/enablers/cel/national_green_tribunal/case_summaries/?10221/Sandeep-Lahariya-Vs-State-of-MP-Ors
6. Merrild H¹, Damgaard A, Christensen TH. 2009 Recycling of paper: accounting of greenhouse gases and global warming contributions. *Waste Manag Res*; **27**(8):746-53.
7. <Http://publications.environment-agency.gov.uk/PDF/SCHO0711BUAN-E-E.pdf>
8. Bergey’s Manual of Determinative Bacteriology
9. Hajna A.A., 1945, *J. Bacteriol*, 49:516.
10. Pratima Gupta, ¹ Kalpana Samant,² and Avinash Sahu²-2012. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential, *International Journal of Microbiology*, **2**, Article ID 578925, 5 pages. <http://dx.doi.org/10.1155/2012/578925>