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RECENT ADVANCEMENTS OF MICROBES IN PLASTIC DEGRADATION

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Abstract

Plastic/polyethylene is synthetic or human made polymers. Due to its hydrophobic backbone and complex structure it is non-biodegradable and inert in nature. The vital features of plastics that make them suitable for the production in wide range of products are light-weight, durability, strength. The ever increasing demand has led to a large-scale production that resulted in accumulation of plastics in hazardous quantities at breakneck speed in waste disposal. Plastics being non-biodegradable accumulate on the surface and persist for centuries causing a disruption in the normal soil, marine ecology thus encouraging research in development of effective and eco-friendly biodegradation materials. Biological degradation, here organic polymers substances like plastics are cracked down by living microorganism. In the recent years, certain strains of microorganisms have been isolated and studied by scientists all over the world to identify their plastic degrading capacity. Particular strains of bacteria and fungi have proved to have the most efficient degrading capacity among other microorganisms. Here in this study, we have analysed the studies conducted on various strains of bacteria like Brevibaccillus borstelensis, Genus pseudomonas, B.licheniformis and fungi strains like Aspergillus japonicus regarding their plastic degradation capacity. The studies were analyzed for their isolation processes, methodology or process of degradation and their proof of efficacy. Detailed summary of the most proficient microorganisms with their plastic degrading methods have been provided in this paper. The key benefits of using microorganisms for degrading plastics are no harmful side effects as it is a completely natural process, can be carried out in situ, therefore no transportation and due to its organic process, it is well accepted by the public.

Keywords – *Polyethylene degradation, bioremediation, microbial degradation, no side effects, public acceptance*

1. Introduction

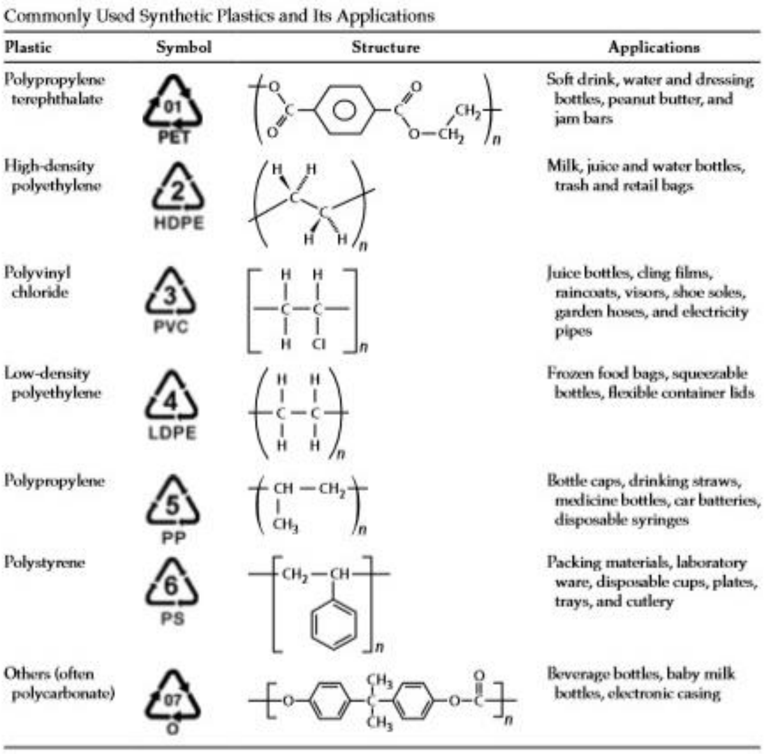
Identical to natural resins that are found in many plants and trees, plastics are human made synthetic or organic polymers. Plastics have swiftly moved into all pieces of everyday life with their ever-increasing demand due to their versatility [4]. The vital features of plastics that make them suitable for the production in wide range of products are:

- Lightweight
- Durability
- Strength
- Cheap/Cost effective
- Stability

The large-scale production has led to the accumulation of plastics in hazardous quantities in waste disposal. Plastics being non-biodegradable accumulate on the surface and persist for centuries causing a disruption in the normal soil ecology [5]. The division of plastics with respect to their physical characteristics is listed below:

- Thermosets- They are tight meshed, durable and once shaped can only be altered by further machining.
- Thermoplastics- They have branched or linear molecular structures which constitutes their thermal behavior and strength. They possess have moderate limit of flexibility and their service temperature is much lower than thermosets.
- Elastomers- These have cross linked structures giving a bit of elasticity and cannot be reshaped.

At present almost 20 diverse groups of plastics have been discovered with various grades and qualities [8]. Plastics are made through hydro-carbon monomers by modifying natural materials chemically or can also be produced through organic and inorganic raw materials.



Commonly Used Synthetic Plastics and Its Applications

Plastic	Symbol	Structure	Applications
Polypropylene terephthalate	01 PET		Soft drink, water and dressing bottles, peanut butter, and jam bars
High-density polyethylene	2 HDPE		Milk, juice and water bottles, trash and retail bags
Polyvinyl chloride	3 PVC		Juice bottles, cling films, raincoats, visors, shoe soles, garden hoses, and electricity pipes
Low-density polyethylene	4 LDPE		Frozen food bags, squeezable bottles, flexible container lids
Polypropylene	5 PP		Bottle caps, drinking straws, medicine bottles, car batteries, disposable syringes
Polystyrene	6 PS		Packing materials, laboratory ware, disposable cups, plates, trays, and cutlery
Others (often polycarbonate)	07 O		Beverage bottles, baby milk bottles, electronic casing

Fig 1: Different types of plastics and their applications

2. Risks of Excess Plastic Usage

Every year nearly 140 million tons of plastics are being produced [6]. In USA and Australia roughly 20-25% of municipal waste is observed to contain synthetic polymers [7]. The widespread usage of plastic causes critical threats to marine as well as terrestrial eco-system, as these are non-biodegradable and dumped right after usage. Therefore, their disposal seems to cause a major problem. Adverse effects are reflected in the environment due to excess dumping of non-treated plastics. Some of them are listed below:

- Accumulation of chlorinated plastic in land, can affect the groundwater ecosystem.
- In the water bodies like rivers, lakes and oceans, plastics pose as severe threats as they can be mistaken as food and consumed by marine animals. Survey indicates that marine animals readily ingest trash and other plastic products leading to their death and disruption in the marine ecosystem.

- Polyethylene is a majorly consumed plastic that is believed to be carcinogenic along with causes various health problems like weakened immunity, infertility, sperm count.
- PVC is another form of synthetic polymer used in utility products, containers and mostly cosmetic appliances. The consumption of the same can cause deafness, skin diseases, digestion and vision problems.
- The thermal decomposition of plastics using fire releases harmful gases and cause increase in global warming.

3. Degradation of Plastics

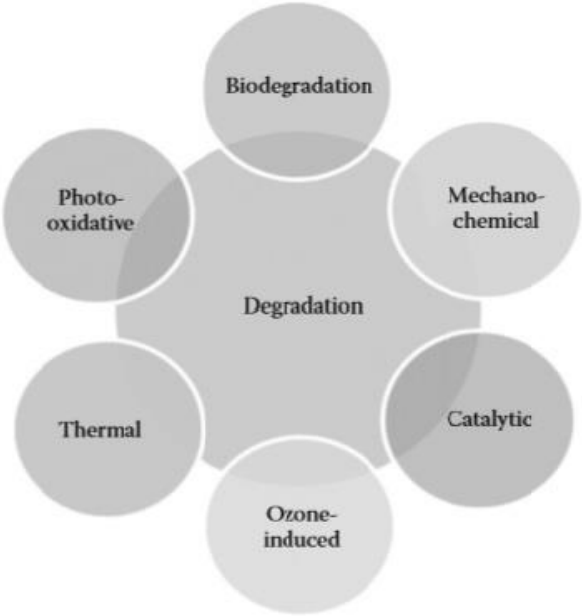


Fig 2: Degradation of plastics

One of the primary solid waste environmental pollutants are synthetic polymers and the other being their disposal. For better disposal and degradation, there has been a subsequent rise in interest on polymer biodegradation which can take place through environmental factors. Polymer degradation has been classified into Ozone-induced degradation, Thermal-induced degradation, Mechano-chemical degradation, Photo-oxidative degradation, Catalytic degradation and Biologically-induced degradation. Photo-oxidative degradation- Use of light energy, which causes scission of the polymer molecule which breaks the plastic into smaller fragments. Photo-degradation of polymer includes UV degradation and oxidation. Thermal degradation- Use of heat energy for rupture of bonds in radical sites in plastic for their degradation which mostly involves the support of atmospheric oxygen.

Biological degradation, here organic polymer substances are cracked down by living microorganism. Biodegradation is defined as the usage of micro-organism to interpret a-biotic degradation through chemical/physical/enzymatic action [9]. Both Synthetic and natural polymers can be degraded and deteriorated by microorganism. The primary process of breaking down polymer is called de-polymerization. Environmental conditions play a vital role in determining the degrading pathway for polymer degradation. Hydrolysis of ester, glycosidic and peptide bonds through nucleophilic attack on carbon atom is the most important type of degradation mechanism used here. Biodegradation therefore is a process that involves various steps carried out with the help of enzymes, microorganism, biotic availability of polymer structure and appropriate abiotic factors.

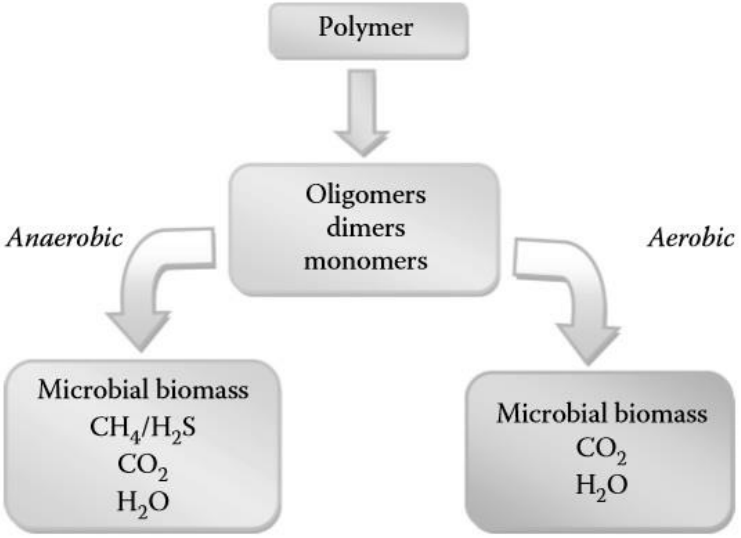


Fig 3: Biodegradation of polymers

4. Elements Affecting Plastic Degradation

Bio-degradation is a process that is ruled by various factors including the type of organism, nature of pre-treatment as well as polymer characteristic. The polymer characteristics include its type of functional groups, crystallinity, mobility, molecular weight and additives in the polymer all play a major role in its degradation [10]. Two important factors that affect biodegradability of organic/synthetic polymers are characteristic feature of polymer and exposure conditions. Exposure conditions are further divided into biotic and abiotic factors. The biotic factors include extra cellular enzymes, hydrophobicity, bio-surfactants and abiotic factors include temperature, moisture, pH, UV radiation. Multiple polymer characteristics have a vital effect on the biodegradability of plastics which are mentioned in the tabular below.

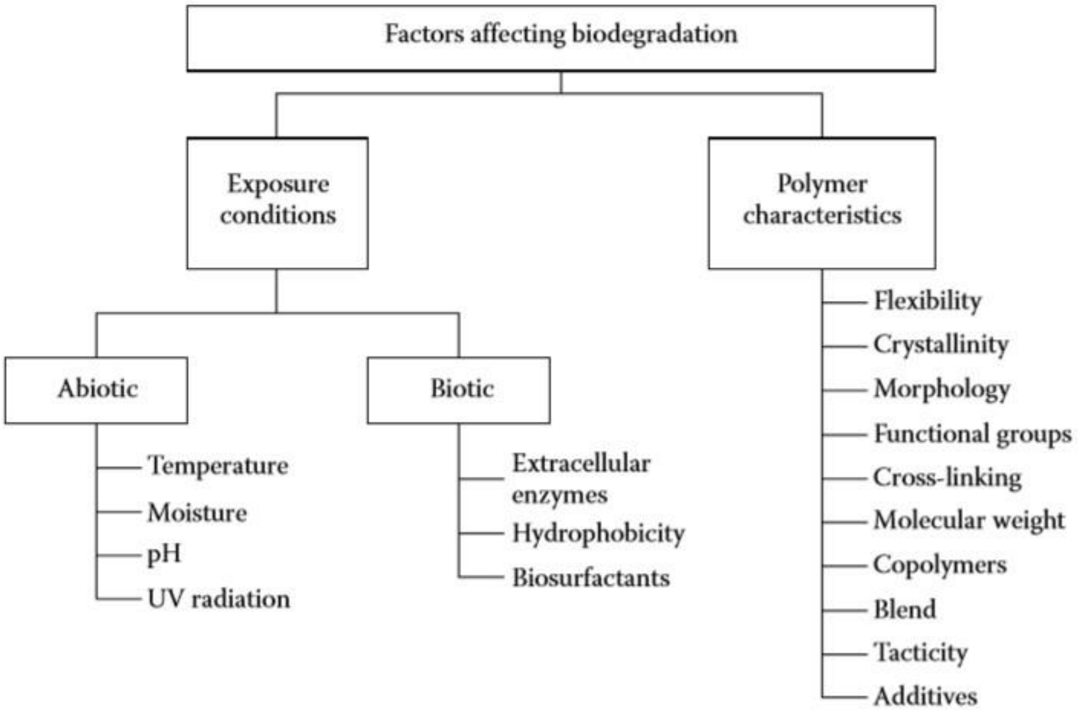


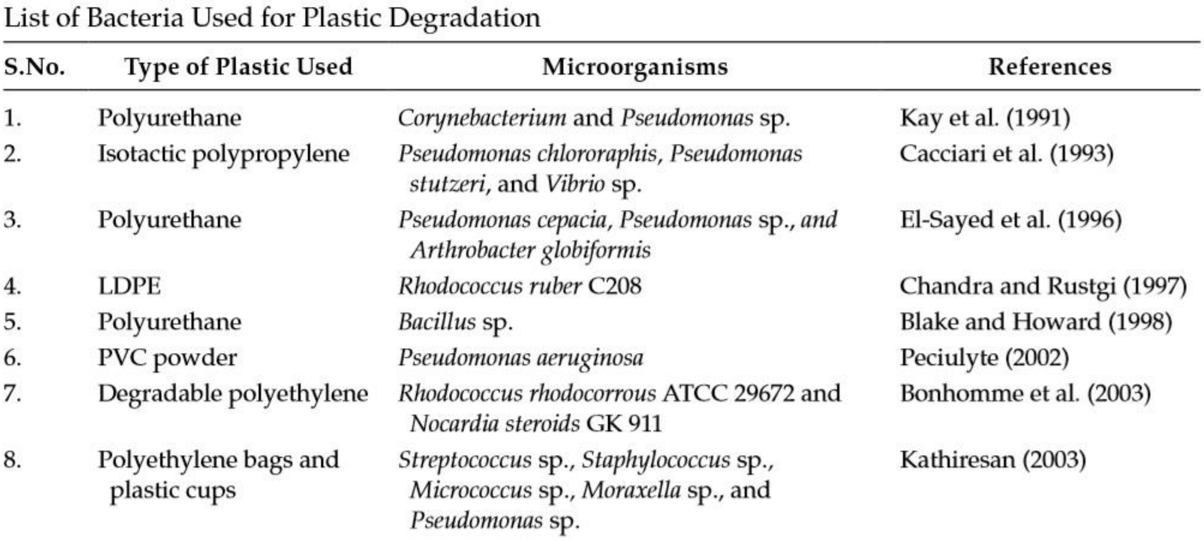
Fig 4: Factors affecting biodegradability of synthetic/organic polymers

5. Role of Micro-organisms in Degradation of plastics

Use of microorganisms in cleansing the environment from contaminants is called bioremediation. The perks of microorganism use are their ability to utilize environmental contaminants as nutrients/food for their metabolic reactions and their micro structure which allow them to cause an efficient effect on same. Genetically engineered microorganisms are being developed by researchers all over the world to degrade specific contaminants at required in-situ places. Microorganisms are most suitable for bioremediation due to two important factors, one being the contaminants are seen as a source of carbon which can be used for building proteins and enzymes and second being the use of electrons from the contaminants as an energy producing material. The method of destroying organic compounds in the presence of oxygen is known as aerobic respiration. Therefore, carbon dioxide, H_20 and an enlarged population of organisms are considered as the key by-products of aerobic respiration [11]. Microorganisms can also exist in the absence of oxygen and this type of process is termed as anaerobic respiration. Here nitrate, iron, manganese, sulfate can play the substitute for oxygen in degrading the contaminants by accepting the electrons. Bioremediation is commonly used all over the world where human activity has left the location unstable, damaged and unusable. With the ever increasing population, there are fewer landfills for polluted material. Thanks to advancing science, bioremediation has been made economical.

The key benefits of using microorganisms for degrading plastics are:

- No harmful side effects as it is a completely natural process
- Can be carried out in situ, therefore no transportation
- Process time is less
- Economical and cost effective l
- Skilled labour and complex equipment is not required
- Due to its organic process, it is well accepted by the public
- Lower liability and less damage to the environment



List of Bacteria Used for Plastic Degradation

S.No.	Type of Plastic Used	Microorganisms	References
1.	Polyurethane	*Corynebacterium* and *Pseudomonas* sp.	Kay et al. (1991)
2.	Isotactic polypropylene	*Pseudomonas chlororaphis*, *Pseudomonas stutzeri*, and *Vibrio* sp.	Cacciari et al. (1993)
3.	Polyurethane	*Pseudomonas cepacia*, *Pseudomonas* sp., and *Arthrobacter globiformis*	El-Sayed et al. (1996)
4.	LDPE	*Rhodococcus ruber* C208	Chandra and Rustgi (1997)
5.	Polyurethane	*Bacillus* sp.	Blake and Howard (1998)
6.	PVC powder	*Pseudomonas aeruginosa*	Peciulyte (2002)
7.	Degradable polyethylene	*Rhodococcus rhodocorrous* ATCC 29672 and *Nocardia steroids* GK 911	Bonhomme et al. (2003)
8.	Polyethylene bags and plastic cups	*Streptococcus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Moraxella* sp., and *Pseudomonas* sp.	Kathiresan (2003)

Fig 5: List of microorganisms for plastic degradation

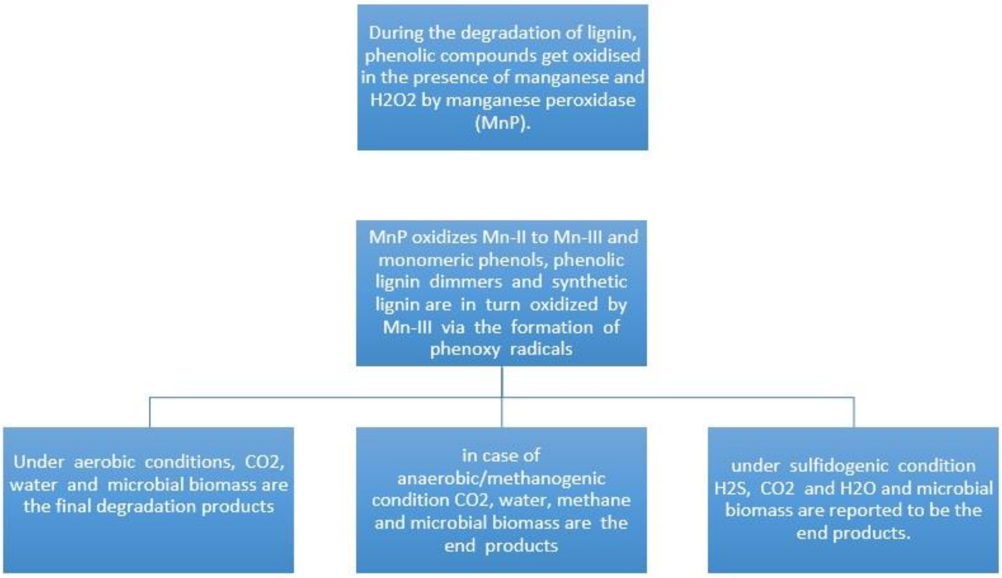
6. Mechanism of Poly-ethylene Biodegradation

Polythene degradation is initiated with micro-organisms being attached to its outer surface. Streptomyces viridosporus, streptomyces setonii, streptomyces badius as well as wood degrading fungi would degrade polythene by producing extracellular enzymes. In wood degrading fungi oxidases, peroxidases and laccases form the ligninolytic system also known as the extracellular enzymatic complex. Enzyme system characteristics vary depending on various factors like the culture conditions, its strain and the particular type of organism used amongst many others. [15]

Degradation of lignin is carried out by three enzymes -

- Lignin-peroxidase
- Manganese-peroxidase
- Laccase

These ligninolytic enzymes are used in several industries, chemical, agricultural, fuel, food, cosmetic, textile, paper etc. It is also observed that they are involved in degradation of dyes and other xenobiotic compounds. Depending upon the conditions of degradation, the end products kept varying. [1] [14]



7. Determination of Poly-ethylene Degradation

Various mechanisms are employed to determine the polythene degradation. At topographical level, SEM (Scanning-Electron Microscope) is utilized to check the scission level along with the microbe-attachment to the polythene outer surface before and after the microbial attack.

The small samples' micro-destruction is analysed by Fourier Transform Infrared Spectroscopy, also the recognized composites on the surface could be documented by collecting FT-IR spectra. Various parameters are used in determining the change in tensile strength, percentage of elongation, weight loss to measure physical changes after the microbial attack on polythene. Polythene degradation products can also be characterized using techniques like HPLC and TLC.

Extracellular enzymes are released by the microbes such as manganese peroxidase, lignin peroxidase etc. for polythene degradation. It has been observed that microorganisms from different sources can be held responsible for polythene degradation. Efficient microbes for polyethylene degradation are yet to be screened from all the sources that can be multiplied at large scale to commercialize degradation of polyethylene. [1]

8. Summary of plastic degrading bacteria.

Here, the review of the competency of species *Pseudomonas* Bacillu Brevibacillus, Lysinibacillusbacteria in degrading and metabolizing synthetic plastics is done

8.1 Species of genus pseudomonas

Micro-plastics are formed by long term disclosure to sun-rays along with physical abrasion. Microorganisms can further degrade micro-plastics. Plastics can be eliminated from the contaminated environment by biological degradation and subsequent microbial metabolism.

Genus *pseudomonas* species are generally ubiquitous in both terrestrial and aquatic environments and have been found effective in bioremediation of naphthalene, simple hydrocarbons, toluene, crude oil and other hydrophobic polymers. *Pseudomonas* species are considered valuable for biotechnology due to their genetic plasticity and diverse metabolic capabilities, they are also of great potential in synthetic biology [15]. A particular *pseudomonas* strain is engineered through genetic manipulations to oxidize terpenic, aromatic, aliphatic and poly-aromatic hydrocarbons. When it comes to degrading plastics, Genus *pseudomonas* is one of the most cited degraders amongst various plastic polymers [18]. To completely eliminate plastic polymers via biological means first requires polymer breakdown into smaller oligomers so that the monomers will pass through cell-membrane eventually which will be followed through by intracellular metabolism assimilation. In various papers we analysed, it is proved that *Pseudomonas sp.* has the ability to degrade PES maximally at a constant rate of 165 mg. Due to the presence of hydroxyl end groups and ether bonds, PEG is found to be more biologically degradable than PE. PEG is still found in a variety of products such as cosmetics, pharmaceuticals, inks polystyrene and lubricants which is both stiff and lightweight, and is found to serve as a viable thermal insulation in packaging materials. The polystyrene structures are identified by phenyl functional groups attached to a continuous chain of hydrocarbons [17]. Through the thorough survey conducted, we have identified that *pseudomonas sp.* is able to degrade hetero-polymer which were made of polystyrene. [15]

8.2 Species of genus Brevibacillus

A thermophilic bacterium was isolated from the soil called *Brevibaccillus borstelensis* which was identified to degrade low density polythene as its sole C source. In the paper we analysed, when polyethylene was incubated with B. *Borstelensis* for a period of 30 days at 50^0C, it was found to reduce its molecular weight by 30% [2]. Presence of mannitol also helped to degrade polyethylene. Biodegradation of polyethylene increased with the irradiation time-period when UV photo-iodised. A strain of *Rhodococcus* rubber when isolated and colonized was found to degrade polyethylene. The hydrophobic nature of bacteria's cell surface plays a major role in in the formation of a biofilm on top of the plastics. Two observations were made from this experiment conducted in the paper analysed:

- To this culture medium, addition of mineral oil would enhance bio-film formation and thereby the bio-degradation of polythene.
- Another reason which was identified for the increasing the bio-degradation of polyethylene was the increased hydro-phobic interactions between bacterial bio-film and polythene.

Bravibacillus borstelensis was found to be more effective when comes to degrading low density poly-ethylene. The experiment was conducted in the following manner:

a. Waxes obtained from the bacterial samples was initially screened due to their capacity to grow and degrade a polyethylene product.
b. From polyethylene waste disposal sites, soil samples along with SM medium and 10ml of polyethylene oligomers of intermediate size which were in liquid wax forms.
c. The intention was to obtain mesophilic and thermophilic bacteria separately, hence 2-week incubation was done at either 30^{o}C/50^{o}C.
d. Wax-degrading bacteria could be easily recognized by the presence of clear-zones surrounding the colony which were subjected to grow in an opaque wax containing medium.
e. Further testing was done on these colonies of bacteria which were able to degrade the wax to inspect whether they can grow in SM medium which contained polythene as their sole carbon-source.
f. The concentration of protein that was extracted is used to calculate the population density of the biofilm on the polythene surface.

When the results showed that highest degree of biodegradation of polyethylene was observed after incubation in a medium that is mannitol free for 30 days. This was because carbon deprivation tends to promote the utilization of poly-ethylene as it's carbon source. For *Bravibacillus borstelensis*, the bio-degradation rate of poly-ethylene was measured by calculating the molecular weight loss, in this case which was found to be up to 30%. [2]

8.3 Species of genus Lysinibacillus bacteria

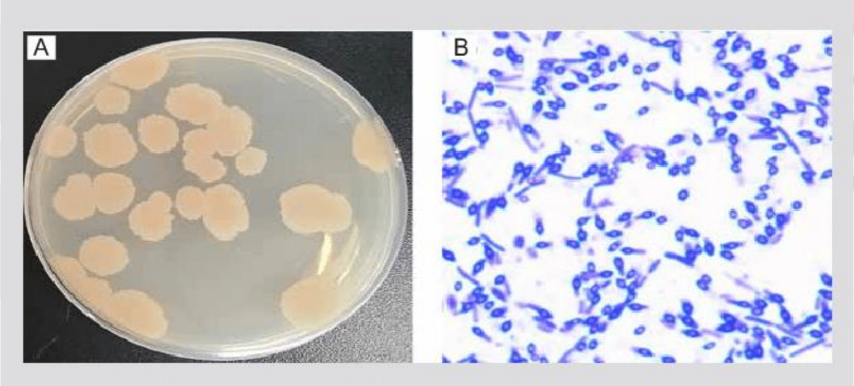


Fig 6. (a) *Lysinibacillus* bacterium culture plate (b) *lysinibacillus* bacterium under microscopic view [12]

a. In the paper we analysed, an distinctive method of bio-degradation was investigated where commercial poly-ethylene was degraded using a particular biosurfactant manufactured by *B.licheniformis* as well as *Lysinibacillus* bacterium. The following observations were made-
b. Bio-surfactant that was manufactured by *B. licheniformis* didn't have any anti-adhesive property for the formation of biofilm unlike the various other forms of bio-surfactant which was created by other strains of the bacteria.
c. Pre-oxidation step was not needed for the *lysinibacillus* for the formation of the bio-film on the polyethylene surface and simultaneously oxidize it.
d. A significant amount of weight loss was observed in the poly-ethylene samples when treated with bio-surfactant and *lysinibacillus.*
e. Maximum weight-loss achieved was of 2.97± 0.05% when polyethylene samples were treated with *lysine-bacillus* for one month, followed by treatment with biosurfactant for one month, that was then followed by treatment of *lysine-bacillus* for one month.

f. Poly-ethylene degradation generally takes place by transfiguration of carbonyl groups to un-saturated hydrocarbons by *lysinibacillus* bacterium and bio-surfactant.

Lysinibacillus bacteria has the ability to degrade poly-ethylene chiefly because of its oxidative property. This oxidation by *lysinibacillus* on polyethylene was observed usually when the oxidized or un-oxidized level of used polyethylene is very low. *Lysinibacillus* oxidises polyethylene which is used by the same bacteria for the bio-film formation. Anti-adhesive properties were absent in the bio-surfactant which was produced by *B.licheniformis*. oxidation properties. The oxidation level of other two polyethylene samples which were treated with bio-surfactant and aged under sunlight was found to be comparatively higher. Hence *lysinibacillus* was found to be responsible for the polyethylene biodegradation and this process was able to advance through the conversion of carbonyl group into unsaturated hydrocarbons. Therefore, the bacteria *lysinibacillus* species is used for bio-deterioration and bio-degradation commercial polythene. Thus this method was found to be very environmental friendly. [3]

9. Summary of plastic degrading fungi

Fungi belonging to genus aspergillus are called as polyethylene degrader. Fungi are generally considered to be better polyethylene degraders than bacteria. The highest fungal degradation (weight reduction activity) was found to be of 36.4±5.53%, this belongs to the fungi aspergillus oryzae strain A5(MG779508).

9.1 Biodegradation of polythene by aspergillus spp.

Heterotrophic fungi is obtained for polyethylene degradation from polluted sites followed by isolation and identification of the same by staining techniques. The identified and isolated strain was identified to be *A. japonicus, A. niger, A. flavus, A. terreus, A. mucor* so. amongst which the predominant fungal strains were *A. japonicus* and *A. niger*. Both the strains were selected for degradation of polythene under laboratory conditions. Over a period of 4 weeks the effectiveness on the biodegradation process of commercial polythene bags having low density polythene (LPDE) were studied in a shaker culture under normal laboratory conditions. SEM (Scanning electron microscope) analysis was used which revealed the presence of fragility of polythene surface after the fungal degradation and also the porosity of the same. The biodegradation was measured as the amount of mean weight-loss, that was approximately 8-12% over the 4 weeks' time period.

As seen earlier the microorganisms were capable of degrading numerous types of organic as well as inorganic materials. In a study by Kambe et al. (1995) where isolation and characterisation of bacteria from soil was accomplished that utilised polyester poly-urethane as its nitrogen-carbon source. Here it was observed that numerous bacteria were able to degrade polycaprolactone (PCL). Similarly, another study [20] where the fungal colonisation as well as the bio deterioration of plasticised PVC (polyvinyl chloride) showed that an increased microbial growth generally occurred during a long time exposure at in-situ conditions.

Kathiresan revealed in his study that a high variety of microorganisms in mangrove soil were capable of plastic degradation, at a slower rate. Almost 60% of the total plastics produced are low density polyethylene (LPDE) which accounts for the most commonly found solid waste among the plastics.

Estimation of the isolated fungi from the given polythene trials:

- The polyethylene bags were taken from the contaminated sites of Chennai, Tamil Nadu in a sterile box. Serial dilution of samples was done followed by pour plating it in potato dextrose Agar in sterile conditions to isolate heterotrophic fungi.
- Incubation of plates was carried out at 37°C for 48h.

- Post incubation, plates having between 25-250 colonies were selected and total plate count was calculated.
- Characterisation of heterotrophic fungi which were morphologically different were selected.
- The selected colonies were transferred by using sterilized forceps to sterilized PDA agar-slants.
- The chosen fungi were identified by microscopic and macroscopic observations by staining techniques.

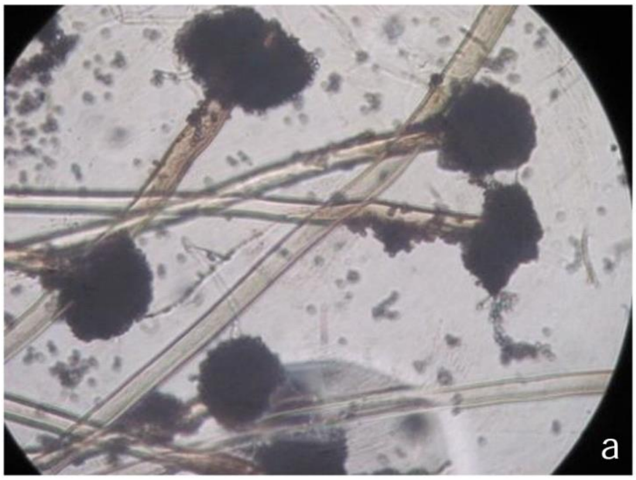


Fig 7: Microscopic view of isolated fungi culture – Aspergillus niger [13]

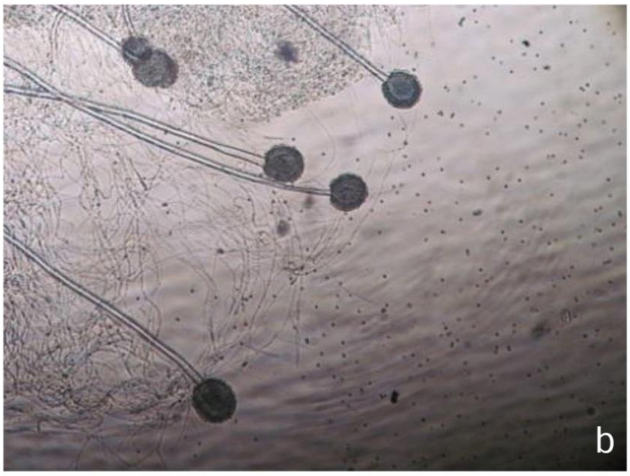


Fig 8: Microscopic view of isolated fungi culture - Aspergillus japonicas [13]

9.2 Screening and identification of fungi strains (Aspergillus spp.):

Agar with substrates like 1% gelatin, 1% starch, 1% tween-80 was found to be an appropriate medium to initiate the enzyme action of fungi existing in the culture samples.

About 5-20ml of substrate was transferred to the sterilized petri-dish.

After drying the plates over-night, aseptical cuts were made in the wells to load the culture filtrates of fungi which was isolated.

Incubate the plates for 3-4 hours at 37°C.

Opacity can be identified surrounding the well surface after incubation, this indicates positive result for the presence of substrates.

Further quantitative assay is conducted.

9.3 Microbial degradation of plastics by fungal strain (Aspergillus spp):

The LPDE strips of 1cm diameter that were transferred to a conical flask which contained rose Bengal broth media of 50ml were inoculated with particular fungi strains. LPDE strips were in the microbe free medium in a controlled environment.

- For each treatment 4 flasks were preserved and placed in a shaker for a month.
- The strips were further treated with distilled water and shade-dried then assessed to find the closing weight.
- Closing weight was noted down from which weight loss of polyethylene strips was to be calculated.
- To analyse the surface degradation of low-density polyethylene (LPDE), SEM analyzation was carried out. [13]

9.4 Final Observations of Experimentation (Aspergillus spp).

Fungi identified were - *Aspergillus Niger and Aspergillus japonicas*. *Aspergillus Niger* showed LPDE degradation upto 5.8% per month. *Aspergillus j.* showed LPDE degradation upto 11.11% in a month both under laboratory conditions. These changes to polythene surface were a result of fungal enzymes and fungal extra cellular metabolites. When both the polythene strips were heated at a rate of 10°C per min, the melting point of polythene strips which was treated with fungi had reduced to 161°C when compared to control which was found to be 162.2ºC. [13]

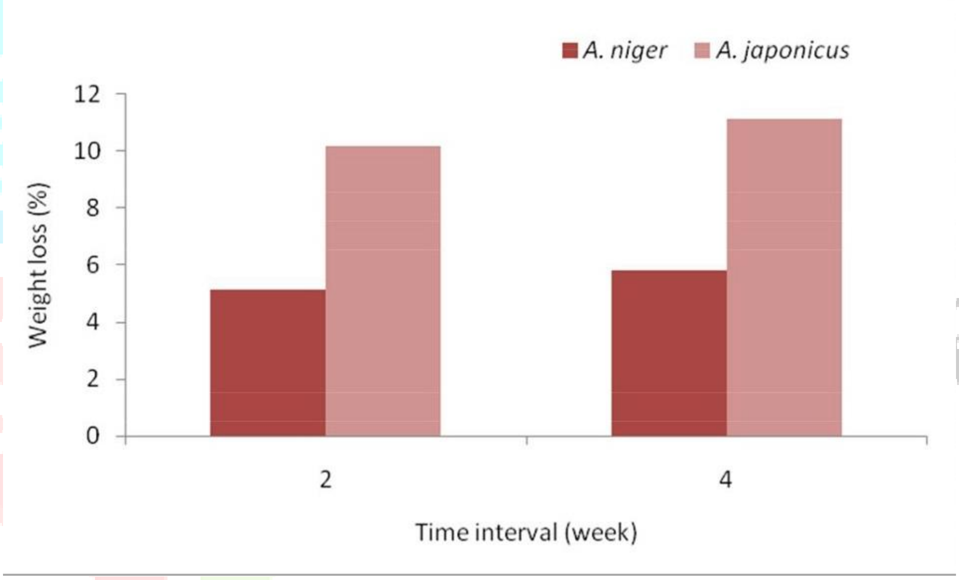


Fig 9: Plastic degradation percentage by fungal strains

10. CONCLUSION

Based on the survey conducted here, it is evident that plastic plays a vital role in our day to day life. Due to its good quality, accessibility, affordability and versatility in applications the use of plastic is increasing in our everyday life in a breakneck speed. Since the usage of plastic is growing tremendously, the need for its degradation has become a noticeable and hazardous issue. Various methods of degradation are being explored among which the most affordable and eco-friendly one can be attained by biodegradation using microorganisms. In this paper, we have analyzed studies and summarized some of the highly competent microorganisms and their polythene degrading capacity.

Amongst bacteria, three microbial species were found which proved to degrade polythene to a great extent. They are *pseudomonas aeruginos, brevisbacillus borstelensis and lysinibacillus fusiformis*. *Pseudomonas* has great potential of degradation of plastic, it degraded 7.6% to 8.2% of plastic at 30^o C. pseudomonas species enhances bio-film formation and induced molecular changes on the plastic surfaces and degrades LPDE. Enriched culture methods proved effective when identifying a thermophillic bacteria such as *brevibacillus borstelenesis* which utilized polyethylene as their nitrogen-carbon source. Maximum degradation was observed when combined with photo-oxidation. The third type of bacteria that we studied was *lysinibacillus*, which had the ability to form a bio-film on the uppermost polyethylene surface therefore oxidizing and degrading the polyethylene. The degradation of polyethylene was effectively carried out with the help of both bio-surfactant as well as the bacterium, hence it was proved to be the most efficient one amongst all the bacteria. An overall analysis confirmed that fungal strains are better plastic degraders than bacteria. The two fungal strains *A.niger* and *A.japonicus* were found to be highly efficient in degrading plastic or polythene of low density. The mean weight loss was observed to be around 8% to 12% after 4 weeks from the study we analyzed. This was proved by the fragility and porosity of fungal surface. The key benefits of using microorganisms for degrading plastics are no harmful side effects as it is a completely natural process, can be carried out in situ, therefore no transportation and due to its organic process, it is well accepted by the public. It also has lower liability and is eco-friendly.

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