



ECOFRIENDLY, LOW COST BIODEGRADATION OF PLASTICS- RESEARCH STUDY

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ABSTRACT

A low-cost, environmentally acceptable approach of plastic biodegradation is something that this research study intends to investigate. One of the main environmental concerns is plastic garbage, and conventional disposal techniques like landfilling and incineration have shown to be ineffective and damaging to the environment. A viable alternative to disposal is biodegradation, in which waste plastic is broken down into harmless components by microbes. The study will look into many aspects of the biodegradation process, such as temperature, moisture level, and the kind of plastic utilised. The efficiency of several microorganisms in degrading the polymers will be evaluated, and the ideal biodegradation circumstances will be found. By estimating the cost of production and contrasting it with conventional disposal options, the study will also look at the method's economic viability. This technique's potential for expansion for industrial usage will also be investigated. The results of this investigation will aid in the creation of a long-term plastic waste management strategy and assist in minimizing the negative environmental effects of plastic trash. In our research study we collected various waste materials from different sources and subjected to microbial degradation and detected waste degradation using ecofriendly methods.

Keywords: Waste; Ecofriendly; Bacteria; Fungi; Biodegradation.

INTRODUCTION

Recent years have seen a rise in global awareness of the serious environmental issue of plastic pollution. Traditional plastics cannot disintegrate biologically and can take hundreds of years to do so, leaving behind a lingering plastic waste problem in the environment. Researchers have been looking into alternative plastic disposal strategies, like biodegradation, to address this problem. An inexpensive, environmentally benign approach for the biodegradation of plastics is the focus of this research investigation. The primary goal of the study is to evaluate the efficacy of various biodegradable substances on various plastic materials, such as polyethylene (PE), polypropylene (PP), and polystyrene (PS). To quantify the rate of plastic deterioration under various settings, including temperature, humidity, and exposure to biodegradable agents, laboratory tests will be conducted as part of the study. Both manmade chemicals and naturally occurring components including enzymes, bacteria, and fungi will be examined as biodegradable agents. To determine the rate of deterioration of plastics under various conditions, including temperature, humidity, and exposure to biodegradable chemicals, the study will involve conducting laboratory experiments. In addition to synthetic molecules, the biodegradable agents being investigated will also contain elements from the natural world including enzymes, bacteria, and fungi. The results of this study's research will aid in the creation of a more environmentally friendly method of managing plastic trash, lessening the harmful effects of plastics on the environment. Additionally, this research will shed light on the possibility of expanding this technology for industrial application, opening it available to many industries and communities. The usage of plastics is widespread across a number of industries, but because they are not biodegradable, there are rising concerns about how to dispose of them. The buildup of plastic garbage in the environment has emerged as a major problem that threatens ecosystems and species worldwide. Finding eco-friendly, affordable, and effective processes for the biodegradation of plastics is therefore urgently needed. The purpose of this study is to explore the possibility of employing microbial deterioration in the biodegradation of plastics. The study will concentrate on readily accessible and inexpensive microbes that can degrade various types of polymers. To increase the effectiveness of the biodegradation process, the microbial degradation process will be optimized by changing variables including temperature, pH, and nutrition availability. Due to its affordability and environmental friendliness, microbial degradation is a technology that holds a lot of promise for the biodegradation of plastics. To improve the degrading process and comprehend how it affects the ecosystem, more study is necessary. Over time, in tandem with current economic development, particularly in emerging nations, human responsibility for the environment has increased. However, there are a number of contemporary challenges that allay their concerns, like global warming and the disappearance of the diversity of creatures in the world's ecosystem and habitat (Skogen et al., 2018). Plastic trash has emerged as one of the biggest global problems in terms of environmental consciousness (Klein et al., 2019). Plastics have low heat conductivity, are corrosion-resistant, and are insulating and flexible materials. Because of this, it is commonly understood that plastics are essential to every area of human life (Napperand Thompson, 2019). In-depth research into plastics' potential

applications in a variety of sectors has been sparked by their advantageous properties. Disposable plastics are frequently used in supermarkets, mulch film, and packaging due to their affordability and versatility. Evidence indicates that worldwide yearly plastics manufacturing surpassed 3.59 million tonnes in 2018 and will continue to rise, that the production of plastics increased to 3.59 million tonnes in 2018 and will do so going forward (Zhu and Wang, 2020). For the most part, hydrocarbons and petroleum derivatives are used to create the high molecular weight (HMW) polymers that make up plastics(Zheng et al., 2005). Numerous HMW polymers, including polycaprolactone (PCL), polyethylene (PE), polyurethane (PUR), polyhydroxybutyrate (PHB), polyhydroxyalkanoate (PHA), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polybutylene succinate (PBS), and polylactic acid (PLA), are produced for various uses (Andrady and Neal, 2009). In recent decades, both the demand for and use of plastic has grown tremendously. Annual production of plastic is anticipated to be 367 million metric tonnes, up from 1.5 million tonnes in 1950(Tiseo, 2021). In soil, compost, and batches treated with microorganisms in a lab setting, this quantification technique has been widely utilized to evaluate the decomposition of plastic(Singh and Sharma, 2008; Chamas et al., 2020). *Bacillus cereus* may more effectively breakdown UV-treated polyethylene (14%) than autoclaved (7.2%) or surface sterilized (2.4%) polyethylene, according to an analysis based on weight loss of polyethylene after treatment(Sowmya, 2014). Hundreds or thousands of organic subunits (also known as "monomers") are joined together to form synthetic polymers by use of powerful covalent chemical bonds. Bakelite, the first fully synthetic polymer, was created in the early 20th century by the condensation reaction of phenol and formaldehyde, but actual industrial production of polymers didn't start until the 1950s. Global manufacturing has risen rapidly since then, reaching 380 Mt annually in 2015.It has become a huge concern how much plastic debris is getting into the oceans. Both the Eastern Pacific Ocean gyre and the South Pacific subtropical gyre feature large-scale concentrated accumulations of plastic waste(Eriksen2013, Law, 2014, Kaiser, 2010). Petrochemicals are the primary source of most plastics, which are made of monomers polymerized to create organic polymers(Cole, et al.,2011, Geyer, Jambeck, et al.,2017). Biodegradable polymers are being created as a replacement for non-biodegradable polymer materials in a range of applications(Zhong, et al.,2020).

MATERIALS & METHODS:

In present research, we used several chemicals and strains to carry out the experiments and we purchased the chemicals, namely, Methanol, acetone, distilled water, Magnesium Sulfate Di potassium Phosphate, Ammonium Nitrate, Ferric Chloride from the Krishna Raman Chemicals Pvt. Ltd from Chennai, Tamilnadu. A few of the plastic samples utilized in this experiment were culled from industrial waste, medical waste, and household waste. These are the plastic scraps that we collect, and we intend to break the long chain polymers in the plastic using bacteria that have the ability to penetrate the long chain polymers, a process known as biodegradation.

Microbial cultures for bio-degradation process:

Aspergillus niger and *Pseudomonas aeruginosa* are two examples of the test organisms used to measure the breakdown of plastics by microbes. Bio -degradation of plastics in an ecofriendly, cheap manner the characteristics that allowed for the microorganism's identification in the experiment. The ability and composition of the culture medium is particularly important because it determines how the bacteria and high media break down long chain polymers and produce extra cellular material as a byproduct.

SAMPLING ORGANISM: *Pseudomonas aeruginosa*

The gram's staining procedure identifies it as a gram's negative bacteria, and when the slide is examined under a microscope, a pink red rod-shaped object is visible. All across the world, it can be found in the soil, water, and most artificial habitats. An opportunistic human pathogen is *P. aeruginosa*. 90% of deaths from cystic fibrosis are complicated by *P. aeruginosa*, a prominent gram-negative bacterium that can also cause the worst visual problems. A very significant soil bacterium called *P. aeruginosa* has the ability to degrade polycyclic aromatic hydrocarbons.

For testing fuels for microbial contamination and researching how microbes deteriorate hydrocarbons, Bushnell Haas Broth is advised. Range of chemotherapeutic drugs and antibiotics, making it difficult to eradicate. *P.aeruginosa*.

COMPOSITION:

Ingredients	Grams/Litre
Magnesium Sulfate	0.2
Calcium Chloride	0.02
Mono potassium Phosphate	1.0
Di potassium Phosphate	1.0
Ammonium Nitrate	1.0
Ferric Chloride	0.05

Controlled quality appearance

Homogenous powder that is white to cream and flows freely Color and Clarity of. Prepared Medium Clear to slightly opalescent colorless solution in tubes. pH 6.80-7.20, Responding to Culture, After a week of incubation at 25–30°C, cultural traits were noticed.

Cultural Reaction

ATCC Organisms,	Plain Growth,	Hydrocarbon-Induced Growth
<i>Pseudomonas aeruginosa</i>	-/+	+++

**FIG. 1: BHA GROWTH MEDIA****FIG. 2: ASPERGILLUS NIGER: FUNGI**

Aspergillus niger is a type of fungus that grows on citrus fruits like lemons, oranges, and others. We recently cultured *Aspergillus niger* from lemons after a black cluster formed on the fruit after being grown in the proper culture medium. From the mother culture, the organism was removed and grown in additional medium to produce a pure culture of the *niger* species. The purity of the culture is next confirmed using the grams staining method, and last it is confirmed by looking at it under a microscope where the *niger* is found to be cone-shaped with a wiped tail out. Colonies start white to pale yellow but quickly form jet-black conidia; the reverse is usually buff or yellow-gray, consideration is frequently excessive, resulting in the development of conidia around the circumference. Conidia are round, 3-5, and become rougher as they age, typically a contaminant; may be disease-causing agent.

**Fig. 3: Microscopic view of *Aspergillus niger***

Strong mycotoxins known as ochratoxins are reportedly produced by some *A. Niger* strains. Recent research indicates that *A. niger* also generates isoflavane orobol and ochratoxins A. Black moulds on onions and ornamental plants can be brought on by *A. niger*. Black conidia are detected between the scales of the bulb when onion seedlings infected with *A. Niger* develop the prevalent postharvest disease of onions. *Aspergillus niger* grown from gold mining solution contains cyano metal complexes, including gold, silver, copper, iron, and zinc. Heavy metal supplied solubilization is aided by this fungus. Thus, this fungus is capable of breaking down both hydrocarbon compounds and long chain polymers. It will be cultivated in a suitable culture medium, such as Czapek dox agar, which has been made in our laboratory by adding the proper ingredients. For the general cultivation of fungus, it serves as a semi-synthetic medium.

Composition

Ingredients	Gms/litre
Sucrose	30.00
Sodium nitrate	2.00
Dipotassium Phosphate	1.00
Magnesium sulphate	0.500
Potassium chloride	0.500
Ferrous sulphate	0.010
Agar	15.00

Final pH (at 25°C) 7.3±0.2

Put 49.01 grams in 1000 ml of distilled water and stir to dissolve. To completely dissolve the medium, heat it until it boils. By autoclaving for 15 minutes at 121°C and 15 lbs of pressure, sterilize. Mix thoroughly, and then transfer to sterile petri dishes. There are many different types of fungi in nature, including yeasts and filamentous forms or *moulds*. A semi-synthetic medium called Czapek Dox Agar is used to grow fungus, and the only source of nitrogen is sodium nitrate. The formula created by Thom and Church, which has a known chemical composition, is used to make this medium. For the isolation of *Aspergillus*, *Penicillium*, and several other funguses with comparable physiological needs, Czapek Dox Agar is advised. The single source of carbon is sucrose, and the sole source of nitrogen is sodium nitrate. Di-potassium phosphate buffers the medium. Magnesium sulphate, potassium chloride, ferrous sulphate serves as sources of essential ions.

Presentation Quality Control: Homogenous, freely flowing yellow powder. Comparable to 1.5% Agar gel, gelling firm. Clarity and colour of the prepared medium. In Petri dishes, a clear to slightly opalescent gel with a faint precipitate in a light yellow hue appears. Reaction 7.3 ± 0.2 :pH 7.10-7.50

Cultural Reaction: Observed cultural traits following a 48–72 hour incubation at 25–30°C.

METHODOLOGY

First, the procedure for preparing high media must be followed for both bacterial and fungal species, such as bushnell hass broth and czapek agar, in which they must be prepared in the above-mentioned composition and at the proper pH. The high media must also be prepared in a well-maintained, sterilised conical flask. To prevent contamination, the medium must first be cleaned with distilled water before being wrapped in cotton, blocked, or sealed inside the conical flask opening. The conical flask was then placed in the autoclave for 15 minutes at 1.5 lbs of pressure to sterilize it. The needed amount of distilled water must then be mixed with the measured amount of the media composition, the mouth must be sealed tightly, and aluminium foil must be wrapped around the container. In the autoclave, fix the mixture for 20 minutes at 1.5 lbs of pressure. Bacterial medium Bushnell hass broth needs to be placed into four individual conical flasks of roughly 100 ml each for the required four samples once the medium has been removed from the autoclave and fungal medium Czapek agar has been poured onto the sterilized petriplates. The medium must be incubated, and the incubator's ideal temperature range is 22–25 °C, which must be maintained to prevent contamination.

MICROBIAL GROWTH

In petri dishes with a thin layer of agar-based growth medium and a variety of sizes, microbiological cultures can be cultivated. After the desired bacteria or fungi have been added to the growth medium in the petri dish, the plates are incubated at the ideal temperature for the bacteria or fungi to grow (for instance, typically at 37 degrees Celsius for cultures from humans or animals, or lower for environmental cultures).

Using liquid culture, which suspends the desired bacteria in liquid broth, a nutrient medium, is another way to cultivate bacteria. For creating an antibacterial assay, these are perfect. In order to ensure equal development, the researcher may use a shaker to inoculate liquid broth with bacteria before allowing it to grow overnight. Picking choosing a single person to start a culture makes it easier to isolate pure multicellular organism cultures. Developing pure culture techniques is essential to the observation of the subject specimen, and this method is helpful for fungus. Preparing a streak plate is the most typical way to isolate individual cells and create a pure culture. To physically separate materials, use the streak plate technique. *Pseudomonas aeruginosa*, the microbe used in the experiment, is collected from the soil where the plastic waste was put, where it has detached and being cultivated. The fungal species *Aspergillus niger* is obtained from citrus fruit and onion peels; it was discovered as a black mould and has since been isolated and grown to produce pure cultures of the species. And the appropriate medium that is being

incubated has been infused with the germs from the mother culture. Insertion of Plastic and Microbes

Plastics gathered from home, medical, and industrial waste must be washed with distilled water and dried in a hot air oven for five minutes. Additionally, each sample's dry plastics must weigh roughly 1 g. We attempted to use fungi and bacteria to break down the plastics. Therefore, eight plastic samples are sliced into discs and fixed with culture medium.

The microorganisms must then be incubated using an inoculating loop in the laminar air flow chamber in order to prevent contamination. The mother culture of the microbes must be used to inoculate the conical flask (for bacteria) and petriplates (for fungi). The *P.aeroginasa* in the conical flask must next be incubated in a shaken-well incubator for even and quicker bacterial growth at a temperature of around 21–23°C. The plastic and fungi are inoculated into the petriplates for *A. niger*, and the temperature should be incubated and maintained at 25 to 30°C.

Every 24 hours, the samples should be checked to see if any bacterial or fungal growth has occurred. Additionally, it can prevent contamination from subcultures of bacteria. After one week of incubation, there will be a massive fungus growth in the petri dishes and an extracellular enzyme layer will be seen at the petriplate margins. It demonstrates how long chain polymers break down. Additionally, it has been determined how carbon, sulphur, and nitrous oxide are produced.

RESULT

After being incubated for a month, polyethylene plastic is cleaned with sterile distilled water, sprayed with alcohol, dried, and weighed (final weight). Measurement of the percentage of bacterial decomposition of polyethylene plastic by %degradation is equal to $1 \times \frac{\text{final weight}}{\text{initial weight}} \times 100$. After a month has passed since the plastic samples were removed from the cultured medium, they are weighed. There will be a difference in how samples removed from bacterial and fungal growth media are reduced. More fungi than bacteria have decomposed. The weight of plastic samples at the initial and final weights, as well as the difference in weight lowered between bacteria and fungi are shown in the table below. However, these differences only make a slight difference.

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one month period taken off from the cultured medium. There will be difference between the reduction of samples taken off from bacterial and fungal growth media. Fungi have degraded more than the bacteria. But these difference makes only a little different, the below given table shows the weight of plastic samples of the initial and final weight and the difference of weight reduced between bacteria and fungi.

Table:

Plastics Samples	<i>P.aeuroginasa</i> Initial/final(g/mg)	<i>A.niger</i> Initial/final(g/mg)	Diff . reduced	
			Bacteria	Fungi
Household	1/0.87	1/.84	0.13	0.16
Medicinal	1/0.92	1/89	0.08	0.11
Industrial- 1	1/84	1/80	0.16	0.20
Industrial-2	1/82	1/78	0.18	0.22

DISCUSSION

This study has examined the main issues related to the types, applications, and degradability of natural and manufactured polymers as well as disposal techniques and criteria for measuring polymer degradation. The biodegradation of plastics via the liquid culture approach has been another subject of study. It is evident that most refractory polymers can be partially broken down in the right conditions and concentrations. The current study focuses on the isolation, identification, and capacity of soil-based bacteria capable of digesting plastic. During morphological and biochemical investigation, the microorganism produces a variety of modifications. In this investigation, synthetic plastic samples were taken from industrial, medical, and domestic garbage. This plastic was utilised to research how bacteria degrade it. Microbial degradation of a solid polymer like polyethylene requires the formation of a biofilm on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by degradative enzyme processes. It has been discovered that the growth of multicellular microbial communities, or biofilm, attached to the surface of synthetic wastes act as potent natural degraders. The establishment of a microbial colony is essential for the start of biodegradation when the entire biodegradation process of any organic substrate is taken into account. Therefore, a significant element affecting the entire degradation period is the length of the microbial colonization. Up to 0.0278 10⁹ total heterotrophic bacteria were found in one gramme of the materials that were undergoing degradation. In the experiment, a Gram-negative bacteria and a fungal species were utilized as the microbial species.

The goal of the current investigation was to determine the percentage of weight loss by bacteria by inoculating pieces of plastic in a liquid culture medium containing bacterial isolates and keeping them there for a month. The outcome demonstrates the microorganisms' capacity for degradation after one month of incubation. *Pseudomonas aeuroginasa* & *Aspergillus niger*

discovered a greater percentage of weight loss resulting from degradation. This demonstrates that it has a higher capacity for degradation than other microorganisms.

The three primary purposes of this programme are as follows:

- It locates an unidentified isolation.
- If identification is unsuccessful, more tests are chosen to distinguish between potential strains.
- Results retrieval and storage.

In order to employ sustainable, biodegradable plastics, it is necessary to take into account a variety of elements, including social, economic, biophysical, technological, and a set of all-encompassing, process-oriented principles (Taofeeq D. Moshood et al., 2022). The goal of social sustainability was to raise the standard of living by implementing training and adopting fair and equitable social costs of biodegradable plastics in the pursuit of intergenerational equity and cultural diversity in plastics manufacture. A sustainable framework for strengthening the long-term viability of biodegradable plastics as well as the elements that influence the adoption of biodegradable plastics. The results assess the framework's utility, which consists of seventeen principles dispersed across three sustainability levels. The social dimension has nine, the environmental dimension has eight, and the economic dimension has seven. This study offers a comprehensive and practical way for evaluating and choosing the best options for businesses using biodegradable polymers. As a result, a variety of microbial biodegradation approaches have the ability to handle the plastic waste problem in a way that is both practical and advantageous for the environment (Shilpa, Nitai Basak et al., 2022). Microbial deterioration may also be accelerated and combined with physicochemical methods to achieve notable outcomes. This article includes a list of the numerous microbial species, their genes, metabolic pathways, and enzymes that are involved in the biodegradation of plastic. This study presents a comprehensive yet simple theoretical design for biodegradable plastics. The findings of the research and planned studies create a new area for study and development.

CONCLUSION:

Comparatively greater than bacteria, *Aspergillus niger* deteriorates plastic. *Pseudomonas aeruginosa* has a lower capacity to break down plastic than the other microorganisms employed. The isolated microorganisms were local to the disposal site for polyethylene and showed some degradability there. However, they also demonstrated biodegradation in lab settings on both synthetic media and high media. These microorganisms managed to crack the long-chain polymer, which they then broke through. The polymers' hydrocarbon is then released, resulting in the emission of carbon and hydrogen as a byproduct of the sample.

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