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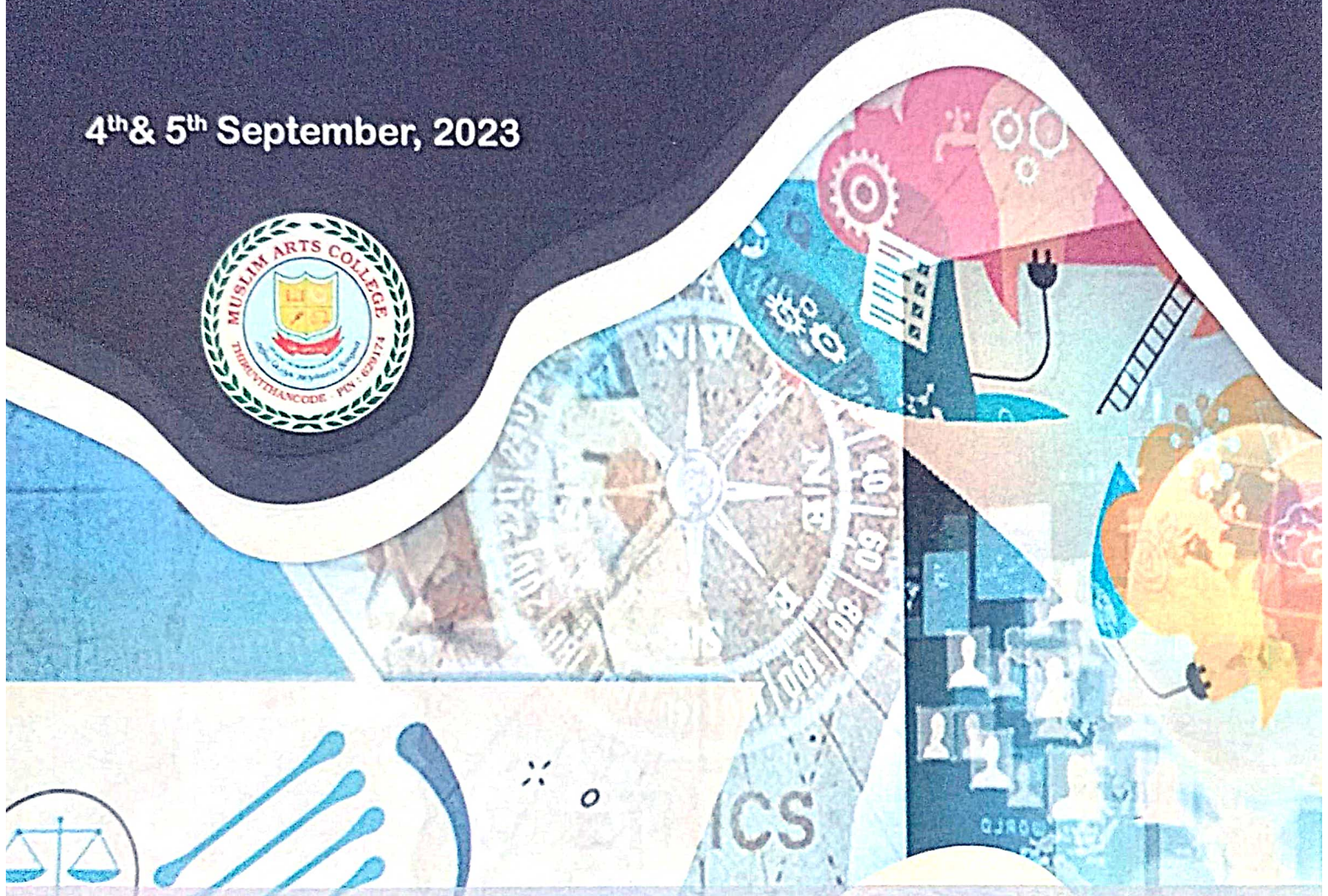

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BIODEGRADATION OF POLYTHENES BY PSEUDOMONAS AND BACILLUS SPECIES ISOLATED FROM DUMPED SOIL AREA

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Abstract

The biodegradation involves microbial agents and does not require heat. Organic Material can be degraded in two ways, either aerobically or anaerobically. In landfills and sediments, plastics are degraded anaerobically, while in composite and soil, aerobic Biodegradation takes place. Aerobic biodegradation produces water and CO_2, and Anaerobic biodegradation results in the formation of water, CO_2 and methane as end Products. Pseudomonas species possess greater potential to degrade Polythene when compared with Bacillus species. Microbes has enough potential to Degrade polythene. Hence biodegradation serve as a promising tool for the elimination of polythene from the environment.

Keywords: Pseudomonas, Bacillus, biodegradation.

Introduction

The word "plastic" derived from the Greek word "Plastikos", that means which Can be molded into different shapes. From different hydrocarbons and petroleum Derivatives high molecular weight organic polymers are obtained. These polymers are Known as plastic (Al-Thawadi 2020). Plastics stated as the polymers which start moving On heating so can be casted into moulds (Kale et al., 2015). Generally, plastic materials Are derived from petrochemicals except biodegradable bioplastic (Getachew et al., 2016). Plastic consists of chloride, oxygen, hydrogen, carbon, Silicon and nitrogen. Polyethylene consists of 64% of total plastic and its general Formula is CnH2n. Plastic is a man-made hazardous long-chain synthetic polymer. Being a versatile, lightweight, strong and potentially transparent material, plastics are Ideally suited for a variety of applications (Kale et al., 2015).

One of the major environmental problems today is contaminations resulting from Various human activities e.g., petroleum industry, petrochemical sector, agriculture Sector, nuclear technology. The release of wastes generated due to those activities are of A particular concern in the environment. Biodegradation is nature's way of recyclingwastes, or breaking down organic matter into nutrients that can be used by other Organisms. By harnessing these natural forces of biodegradation, some types of Environmental contaminants can be reduced and completely removed (Ahmed et al.,2008).

The microbial species identified from the sample polythene bags tested were Bacillus sp., Staphylococcus sp., Streptococcus sp., Diplococcus sp., Micrococcus sp., Pseudomonas sp. and Moraxella sp. The microbial species associated with the degrading materials were identified as bacterial genus like (Pseudomonas, Streptococcus, Staphylococcus, Micrococcus, Moraxella). Biodegradation of plastic waste using plastics degrading bacteria is a valuable plastic waste treatment that must be implemented to maintain the environmental quality of the problems caused by plastic waste. This process has no side effect that pollutes the environment. Biodegradation is very important because excessive use of polythene poses a serious threat to the ecosystem and human life on the planet. There is a need to use adequate biodegradable Methods to reduce the burden of plastic on the environment. In this study, the polythene-Degrading microbes were isolated from the soil, and the bacterial isolates' Biodegradation efficacy was evaluated (Chee et al., 2010).

Materials and Methods

Sample Collection:

Soil samples were collected from plastic contaminated places in Kanyakumari And polythene bags were collected from a stationary shop in Nagercoil, Kanyakumari District.

Isolation of Bacteria:

1 g of soil sample was transferred into a conical flask having 99 ml of sterile Distilled water. The mixture was shaken and serially diluted upto 10-7 dilutions. Isolation of microorganism was carried out by pour plate technique. Sterile petriplates were Labelled with selected dilutions (10-4, 10-5 , 10-6). 1 ml of the

dilutions were transferred To respective petriplates and 15-20 ml of molten skim milk agar media was added. The Plates were rotated in clockwise and anti-clockwise direction for proper mixing. After Mixing, they were incubated at 370C for 24 hours. After incubation, zone forming colonies were observed and sub-cultured. Further Isolation was carried out by spreading the sub-cultured microbes and the polythene Strips of 1X1 cm were cut and placed on the nutrient agar plates. After incubation at 370C for 24 hours, the growth of microorganisms was seen on the polythene strips. The Microbes grown on polythene strips were isolated and subjected to gram staining and specific biochemical tests.

Gram Staining:

A clean grease free slide was taken and a smear of the bacterial culture was made on it with a sterile loop. The smear was air-dried and then heat fixed. Then it was subjected to the following staining reagents:

i. Flooded with Crystal violet for 1 min. followed by washing with running distilled water.
ii. Again, flooded with Gram's Iodine for 1 minute followed by washing with running distilled water.
iii. Then the slide was flooded with Gram's Decolourizer for 30 seconds.
iv. After that the slide was counter stained with Safranin for 30 seconds, followed by washing with running distilled water.
v. The slide was air dried and cell morphology was checked under microscope.

Biochemical Tests:

Biochemical identification of the isolated strains were done by using some manual biochemical methods. The test is based on the principle of change in pH and substrate utilization. Organisms undergo metabolic changes on incubation which are indicated by a colour change in the media that is either interpreted visually or after addition of a reagent.

1)Catalase Test:

The catalase test was performed to detect the presence of catalase enzyme by inoculating a loopful of culture in a sterile glass slide containing 3% of hydrogen peroxide solution. Positive test was indicated by formation of effervescence or appearance of bubbles, due to the breaking down of hydrogen peroxide to O_2 and H_2O.

2)Citrate Utilization Test:

This test determines the ability of bacteria to convert citrate (an intermediate of the Krebs' cycle) into oxaloacetate (another intermediate of the Kreb's cycle). Citrate is the only carbon source available to the bacteria in this media. If bacteria cannot use citrate, it will not grow. Positive result is seen if the bacteria grows and the media turns into bright blue colour as a result of an increase in the pH of the media.

3)Methyl Red Test:

In the methyl red test (MR test), the test bacteria are grown in a broth medium containing glucose. If the bacteria have the ability to utilise glucose with production of a stable acid, the colour of the methyl red changes from yellow to red, when added into the broth culture.

4)Voges Proskauer Test:

In VogesProskauer Test, the test bacteria are grown in a broth medium containing glucose. If the bacteria have the ability to produce acetyl methyl carbinol from glucose fermentation, the colour of the Barrit's reagent changes to red when added into the broth culture.

Pre-Treatment Of Polythene:

The polythene bag was cut into small strips and transferred to a fresh solution having 70 ml Tween-80, 10 ml bleach and distilled water and stirring for 30-60 minutes. The strips were transferred onto a beaker with distilled water and stirred for 1 hour. Further, they were aseptically placed in the 70% ethanol solution for 30 minutes. Finally,the polythene strips were transferred to a petri dish.

Degradation Of Pre-Treated Polythene:

Initially weighed strips of 1X1 cm size of 1 g polythene were aseptically transferred to the conical flask containing 50 ml of nutrient broth medium and Inoculated with bacteria (1 ml). Control was maintained with polythene strips in Microbe-free medium. Different flasks were kept in incubator for 10, 20, 30 and 40 days respectively. After the respective duration of incubation, the polythene strips were Collected, washed thoroughly using distilled water, shade-dried and then weighed for Final weight and percentage weight loss were calculated using below formula.

Weight loss %= Initial weight – Finalweight x 100

Initial weight

Result

Prilimanary Isoaltion Of Bacteria:

A total 10 bacteria recovered from different places, areas selected from Place Contaminated places.

ISOLATION OF BACTERIA:

In the present investigation two soil bacteria were isolated from plastic Contaminated soil and identified its morphological and biochemical characterization(Table.1).

Table.1 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF ISOLATED BACTERIA

S. No.	Gram Staining	Methyl Red Test	Voges Proskauer Test	Citrate Utilization Test	Catalase Test	Bacterial Isolates
1	Gram Negative	Negative	Negative	Positive	Positive	*Pseudomonas* species
2	Gram Positive	Negative	Positive	Negative	Positive	*Bacillus* species

Degradation Of Polythene Bags:

The biodegradation efficacy of two isolates such as Pseudomonas and BacillusSpecies were investigated by using polythene bag. The degrading ability of bacterial Cultures showed variability and the results of degrading ability were presented in table 2&3

Table.2 BIODEGRADATION OF POLYTHENE BAG BY *PSEUDOMONAS* SPECIES

S. No	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1	10	1.000	0.8830	11.7
2	20	1.000	0.8558	14.4
3	30	1.000	0.8243	17.6
4	40	1.000	0.8032	19.7

Table.3 BIODEGRADATION OF POLYTHENE BAG BY *BACILLUS* SPECIES

S. No	Days of treatment	Initial weight of polythene bag (g)	Final weight of polythene bag (g)	% of weight loss
1	10	1.000	0.8890	11.1
2	20	1.000	0.8579	14.2
3	30	1.000	0.8283	17.2
4	40	1.000	0.8074	19.3

Discussion

Microorganisms play a vital role in biological decomposition of materials, Including synthetic polymers in natural environments. In the depolymerization process Two categories of enzymes are actively involved in biological degradation of polymers: Extracellular and intracellular depolymerases. During degradation, Exo-enzymes from microorganisms play a vital role in biological decomposition of Materials, including synthetic polymers in natural environments. During degradation exo-enzymes from microorganisms break down complex Polymers yielding smaller molecules of short chains, e.g., oligomers, dimers, and Monomers, and are smaller that can pass the semi-permeable outer membranes of the Microbes, and then utilized a carbon and energy sources (Hamilton et al.,1995).

In the present study, Bacillus species degrade 11.1, 14.2, 17.2 and 19.3 % of Polythene bag at 10, 20, 30 and 40 days incubation respectively. At the same time Pseudomonas degrade 11.7, 14.4, 17.6 and 19.7 % of polythene bag at 10, 20, 30 and 40 days incubation respectively. An increase in incubation period there is a dramatic Increase in weight loss of polythene bag. Among the two isolates tested, Pseudomonas Was found to be more effective in degradation of polythene bag at 40 days. Previously, Norman et al., (2002) have reported on the biodegradability Of potential of *Pseudomonas fluorescens* and Pseudomonas aeruginosa on synthetic Plastics. Hadad et, al., (2005) also isolated a thermophilic bacterial strain, identified as Brevibacillusborstelensis, which utilized standard and photo-oxidises polythene.

In the current study a total of 15 bacteria were recovered from different sites and After primary and secondary screening 2 of them showed the positive results and Identified as Bacillus species and Pseudomonas species through morphological and Biochemical test. Further study was continued by degradation of pretreated polythene By obtaining degradation percentage where Degradation of initially weighed pretreated polythene was done with respective intervals of time and final weighed of polythene Was observed. Isolate Pseudomonas shows maximum degradation in 40 days, followed By Bacillus.The results of this work were compared with earlier research studies done by Sowmya et, al., (2014) in which they reported that Pseudomonas was able to grow on Minimal medium containing polyethylene as sole carbon source. This showed its Capacity to utilize polyethylene as carbon source and to degrade polyethylene. Degradation of polyethylene was carried out by Pseudomonas which was isolated from Dumpsite soil. Further Degradation was monitored by screening which was followed By weight loss. In Screening procedure, Augusta et al., (1993), reported that the zone Of clearance around the colony is due to extracellular hydrolysing enzymes secreted bythe target organism into suspended polyesters agar medium.

References

- Kale SK, Deshmukh AG, Dudhare MS, Patil VB, 2015. Microbial degradation of plastic: a review. J. Biochem. Technol. 6(2), 952-961.
- Getachew, A., Woldesenbet, F., 2016. Production of biodegradable plastic by polyhydoxybutyrate (PHB) accumulating bacteria using low-cost agricultural waste material. BMC Res. Notes 9(1), 509.
- Al-Thawadi S, 2020. Microplastics and nano plastics in aquatic environments: challenges and threats to aquatic organisms. Arab.J.Sci. 45(6), 4419-4440.
- Chee JY, Yoga SS, Lau NS, Ling SC, Abed RMM and Sudesh KL. Bacterially Produced Polyhydroxyalkanoate (PHA): Converting Renewable Resources into Bioplastics, edited by Mendez Vitas A. Applied Microbiology and Biotechnology, 2010, p1395.