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Biodegradation Efficacy of *Bacillus cereus* and *Pseudomonas aeruginosa* on Polythene Bags

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ABSTRACT

Plastic pollution arises as the second obvious threat to the world environment after global warming. This is a descriptive cross-sectional research design aim to evaluate the efficiency of bacteria as biodegradable organisms on polythene bags. The selected bacterial isolate was identified by morphological characterization like shape, size, structure, texture, appearance, elevation and colors. Phenotypic characteristics such as microscopic characterization of gram reaction, motility were performed the standard protocols. *Bacillus cereus* degrades 1.000g of polythene bag in 30 days to 0. 975g. *Pseudomonas aeruginosa* degrades 1.000g of polythene bag in 30 days to 0. 955g. The percentage of polythene bags loss at day 30 was 3.10% weight loss when treated with *Bacillus cereus*, when treated with *Pseudomonas aeruginosa* at day 30, the percentage lost was 4.50%. The overall investigation can be concluded that *P. aeruginosa* and other species exhibited significant polythene degradation ability and in the near future, *Pseudomonas* sp. can be used to reduce the quantity of plastic waste, which is rapidly accumulating in the natural environment. Among the two isolates tested.

Keywords: Biodegradation, *Bacillus cereus*, *Pseudomonas aeruginosa,* Polythene Bag.

INTRODUCTION

Bacteria are the most ubiquitous and abundant microorganisms that can utilize both of the organic and inorganic materials as sources of nutrients. Some studies provided evidence that several microorganisms are capable of degrading synthetic plastics. Although several studies on the biodegradation of plastic have been carried out, many have focused on the biodegradation of a single kind of plastic and there is a need to identify those with the potential to degrade different kinds of plastics. The degradation of synthetic plastics by soil microorganisms is gaining the attention of different researchers (Ali *et al*., 2021). Beneficial and non-pathogenic soil microorganisms that degrade organic matter in the soil are potential degraders of different types of synthetic plastic wastes, which enter agricultural soils. To achieve these, it needs a careful isolation band and identification of effective species from different groups of fungi, bacteria, and actinomycetes from different soil types including those with low carbon content.

Plastic pollution arises as the second obvious threat to the world environment after global warming. Plastic debris finds its way from terrestrial environment into marine environment. The toxic effects of plastic pollution, even though it does not have any biological role, remain harmful to environmental organisms. The properties that makes plastic so useful such as durability, persistence and resistance to degradation also make it nearly impossible to completely degrade in nature (Haider *et* *al.,* 2019). Therefore, discharge of plastic waste pollution into the environment is of a major problem for the status of the ecosystem. This study aims to evaluate the biodegradation efficacy of *Bacillus cereus* and *Pseudomonas aeruginosa* on polythene bags.

MATERIALS AND METHODS

Sample Collection

Bacteria isolates were transported from National veterinary institute, Vom Plateau State to Adamawa State polytechnic. New Polythene bags were bought from Jimeta aultra-modern Market. This analysis took place in Adamawa state polytechnic, hospital's Laboratory.

Identification of Soil Bacteria

The selected bacterial isolates were identified by morphological characterization. In morphological characterization, macroscopic characteristics like shape, size, margin, arrangements, presence of spore and colors were studied. Phenotypic characteristics such as microscopic characterization of gram reaction and motility were performed according to the principle of comparative system. (Bergey's manual of systematics of archaea and bacteria), (Ariba *et al.,* 2015).

Surface sterilization of polythene bag

The collected plastics bags were cut into small pieces and cleaned with tap water and surface sterilized with ethanol. Then wash with distilled water, 0.1% mercuric chloride and again washed with distilled water.

Degradation of polythene bag

Nutrient broth was prepared and autoclaved at 121°C for 15 minutes. 200 ml of cooled, nutrient broth was poured into eight 250 ml sterile conical flasks. The sterile pre weighed polythene bag pieces were aseptically transferred into nutrient broth. A loopful of bacterial cultures such as *Bacillus cereus* and *Pseudomonas aeruginosa* was inoculated into nutrient broth. One 250 ml of flask containing the polythene bag pieces without bacterial cultures was maintained as control. These flasks were incubated at 37°C for 10, 20 and 30 days.

The polythene bag pieces were carefully removed from the culture (by using forceps) after different days of incubation. The collected pieces were washed thoroughly with tap water, ethanol and then distilled water. The pieces were shade dried and weighed for final weight. The data was recorded. The same procedure was also repeated for all the treated samples.

Determination of degradation of polythene bag

The percentage of degradation of polythene bag pieces by *Bacillus cereus* and *Pseudomonas aeruginosa* was determined by calculating the percentage of weight loss of plastics. The percentage of weight loss will be calculated by the following formula.

$$\text{Percentage of weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

RESULTS

General Morphological Characterization of the Isolated Bacteria

Tables 1 denote the morphological and biochemical characterization of the *B. cereus* and *P. aeruginosa.* The result obtained showed that *B. cereus* is 1-4um in size, Rod-shape, bluish-green in color, Motile in motility, with wavy/irregular margin and also have Gram-negative as gram staining. It has a single arrangement with a presence of spore while *P. aeruginosa* is 1-5um in size, Rod-shape/cucci in shape, Pantone transparent white in color, smooth/regular margin, motile in motility with Gram-positive as

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Gram staining. It also has a single/pair/ chain arrangement with no spore present.

Table 1: General Morphological Characterization of the Isolated Bacteria.

Size	Shape	Color	Margin	Motility	Grain Staining	Arrangement	Presence of any spore	Bacteria isolate
1-4 µm	Rod-shape	Bluish-green	Wavy/irregular	Motile	Gram-negative	Single	Spore	*Pseudomonas aeruginosa*
1-5 µm	Rod-shape/cocci	Pantone transparent White	Smooth/Regular	Motile	Gram-positive	Single/Pair/Chain	No spore	*Bacillus cereus*

Biodegradation of Polythene Bag by *Bacillus cereus*

The initial weight of the polythene bags at day 10 was 1.000g, *Bacillus cereus* degrades 0.987g of polythene bag, at day 20, *B. cereus* degrades 0.982g of polythene bag, and at day 30, it was degraded to 0. 975g of polythene bag (Table 2).

Biodegradation of Polythene Bag by *Pseudomonas aeruginosa*

The initial weight of the polythene bags recorded was 1.000g, at day 10, *Pseudomonas aeruginosa* degrades 0.976g of polythene bag at day 20, *P. aeruginosa* degrades 0.968g of polythene bag, and at day 30, *P. aeruginosa* degrades 0. 955g of polythene bag (Table 3).

Table 2: Biodegradation of polythene bag by *Bacillus cereus.*

	Day 10		Day 20		Day 30	
	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)
1	1.000	0.989	1.000	0.983	1.000	0.964
2	1.000	0.985	1.000	0.982	1.000	0.987
3	1.000	0.969	1.000	0.982	1.000	0.973
Mean Weight	1.000	0.981	1.000	0.982	1.000	0.975

Table 3: Biodegradation of polythene bag by *Pseudomonas aeruginosa.*

	Day 10		Day 20		Day 30	
	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)	Initial weight (g)	Final weight (g)
1	1.000	0.982	1.000	0.987	1.000	0.964
2	1.000	0.976	1.000	0.975	1.000	0.987
3	1.000	0.969	1.000	0.966	1.000	0.973
Mean Weight	1.000	0.976	1.000	0.974	1.000	0.975

Percentage of Biodegradation of Polythene Bag

The percentage of polythene bags loss at day 10 when treated with *Bacillus cereus* was 1.30%, at day 20, it was 1.70% weight loss, and at day 30, it was 3.10% weight loss, the mean weight loss was 2.03%. The percentage of polythene bags loss at day 10 when treated with *Pseudomonas aeruginosa* was 2.40%, at day 20, it was 3.20% weight

loss, and at day 30, it was 4.50% weight loss.
The mean weight loss was 3.37% (Table 4).

Table 4: Biodegradation of polythene bag by *Pseudomonas aeruginosa.*

Days of treatment	Initial weight	Final weight	weight loss (%)
Bacillus cereus			
10	1.000	0.987	1.30
20	1.000	0.983	1.70
30	1.000	0.969	3.10
Mean	1.000	0.980	2.03
Pseudomonas aeruginosa			
10	1.000	0.976	2.40
20	1.000	0.968	3.20
30	1.000	0.955	4.50
Mean	1.000	0.966	3.37

DISCUSSION

Biodiversity and the occurrence of polymer-degrading microorganisms vary depending on the environment, such as soil, sea, compost, activated sludge, etc (Oliveira *et al*., 2021). Microorganisms play a vital role in the biological decomposition of materials, including synthetic polymers in natural environments. In the depolymerization process, two categories of enzymes are actively involved in the biological degradation of polymers: extracellular and intracellular depolymerase (Urbanek *et al*., 2020). 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 than can pass the semipermeable outer membranes of the microbes, and then utilized as carbon and energy sources (Broker *et al*., 2021). Generally, the adherence of microorganisms on the surface of plastics followed by the colonization of the exposed surface is the major mechanism involved in the microbial degradation of plastics (Tokiwa *et al.,* 2009).

Determination of the Morphological Characterization of the Isolated Bacteria

Bacterial species importantly involved in the biodegradation process include *Bacillus* (capable of producing thick-walled endospores that are resistant to heat, radiation and chemical disinfection), *Pseudomonas*, *Klebsiella*, *Rhodococcus*, *Flavobacterium*, *Comamonas*, *Escherichia*, *Azotobacter* and *Alcaligenes* (Tiwari *et al.,* 2018). The sizes bacteria reported in the present study agrees with the findings of Palleroni (2015), but differ from what Bottone (2010) reported, he recorded *B. cereus* as a large (1 by 3 to 5 µm), *Pseudomonas aeruginosa* size ranges from 0.5–0.8 µm by 1.5–5.0 µm in size (Stover, 2000). The size of these bacteria can be influenced by many factors such as Nutrient availability, Environmental stress and Presence of spores (which are generally smaller than vegetative cells). The shape of the bacteria isolated, which is rod-shaped (bacillus/cocci), has several important effects on its biology and pathogenicity. They go a long way to determine the major functions of the bacteria, particularly in the aspect of motility and colonization which agrees with Bergey's manual of systematics of archaea and bacteria.

Lau *et al*. (2004) said the pigmentation (colour), margin and motility of the bacteria particularly *Pseudomonas aeruginosa* has significant effects on its biology and pathogenicity. *P. aeruginosa* produces a blue-green pigment called pyocyanin, which is responsible for its characteristic color and they also act as a potent virulence factor, contributing to the pathogen's ability to cause infections.

Determination of Biodegradation Efficacy of polythene bag by *Bacillus cereus*

The ability of *Bacillus* species to utilize polyethylene, with and without pro-oxidant additives, was also evaluated (Abrusci *et al.,* 2011). Previous study reports that *Bacillus sp.* is capable of producing extracellular enzymes such as oxido-reductase could be one of the key factors for the biodegradation of pre-treated LDPE and BPE 10 effectively in this present study. The increase in surface energy and decrease in contact angle indicates the polymer turns to more hydrophilic, thus facilitating the bacterial attachment on the surface of polymer.

In the present study, *Bacteria cereus* increase in the incubation period shows a dramatic increase in weight loss of polythene bags. This is in agreement with Sowmya *et al.* (2014) in which they reported that *Bacillus cereus* was able to grow on minimal medium containing polyethylene as the sole carbon source. This showed its capacity to utilize polyethylene as a carbon source and to degrade polyethylene. Degradation of polyethylene was carried out by *Bacillus cereus* which was isolated. The decrease in mechanical strength of the additive-added polymer after incubation with *B. cereus* (C1) was due to the initial absorption of energy in the form of light and the pro-oxidant catalyzes the chain session by forming free radicals and thereby facilitating the microbe to degrade the polymer backbone (Abrusci *et al.,* 2011).

Determination of Biodegradation Efficacy of polythene bag by *Pseudomonas aeruginosa*

Sivan *et al.* (2006) isolated a biofilm-producing strain of *Rhodococcus ruber* (C208) that degraded polyethylene at a rate of 0.86% per week. In the present study, *Pseudomonas aeruginosa* degrades 2.4, 3.2, and 4.5% of polythene bag at 10, 20, 30 days incubation respectively. An increase in the incubation period shows a dramatic increase in weight loss of polythene bags. This is in agreement with Deepika and Jaya, (2015) which recently reported that *Pseudomonas* sp. have significant plastic degradation capacity and it degrades up to 24.22% for the period of 6 months. Similarly, Khan *et al.* (2017) studied the biodegradation of low-density polythene (LDPE) by *Pseudomonas* species. They reported that after 120 days of the incubation period, the percentage of weight reduction was 20% in *Pseudomonas aeruginosa* (PAO1), 11% in *Pseudomonas aeruginosa* (ATCC) strain, 9% in *Pseudomonas putida,* and 11.3% in *Pseudomonas syringae* strain. The overall investigation can be concluded that *Pseudomonas aeruginosa* and other species exhibited significant polythene degradation ability and in the near future, *Pseudomonas sp.* can be used to reduce the quantity of plastic waste, which is rapidly accumulating in the natural environment. Various polythene degradation methods are available in the literature but the cheapest, eco-friendly and adequate method is degradation using microbes.

Comparison of Biodegradation Efficacy of *Bacillus cereus* and *Pseudomonas aeruginosa*

In the present investigation, two bacteria isolate such as *Bacillus cereus* and *pseudomonas aeruginos* were used and their morphological characterization were identified. The results of this work were compared with earlier research studies done by Sowmya *et al.* (2014) who reported that *Pseudomonas aeruginosa* was able to grow on minimal medium containing polyethylene, which showed its capability to utilize polyethylene as carbon source and to degrade polyethylene. Degradation of polyethylene was carried out by Bacillus cereus which was isolated from dumpsite soil. Kathiresan (2003), Usha *et al.* (2011) and Deepika and Jaya, (2015) who also reported that Pseudomonas aeruginosa exhibited significant polythene degradation ability, the plastic associated soils are rich in bacterial species. Previously, Tadros *et al.* (1999) and Norman *et al.* (2002) have reported on the biodegradability potential of *Pseudomonas fluorescens and P. aeruginosa* on synthetic plastics. Hadad *et al.* (2005) also isolated a thermophilic bacterial strain, identified as *Brevibacillus borstelensis*, which utilized standard and photo-oxidized polyethylene. Among the two isolates tested, *Pseudomonas aeruginosa* was found to be more effective in the degradation of polythene bags.

REFERENCE

Abrusci, C., Pablos, J.L., Corrales, T., López-Marín, J., Marín, I., and Catalina, F. (2011). Biodegradation of photo-degraded mulching films based on polyethylenes and stearates of calcium and iron as pro-oxidant additives, *Int. Biodeterior. Biodegrad*., 65: 451–459.

Ali, S. S., Elsamahy, T., Al-Tohamy, R., Zhu, D., Mahmoud, Y. A., and Koutra, E. (2021). Plastic wastes biodegradation: Mechanisms, challenges and future prospects. Sci. Total Environ. 780, 146–590. doi: 10.1016/j.scitotenv.2021.146590

Ariba, M.B., Varalakshmi, B. and Umamagheswari, K. (2015). Biodegradation of Polythene Bag using Bacteria Isolated from Soil 4 Number 11 pp. 674-680 http://www.ijcmas.com

Broker, S. S., Prashant, S. P., Kumari, A., Kumari, S., Devi, R., and Kumar, R. (2021). Metabolic Pathways in Biodegrading Psychrotrophic Bacteria Under Cold Environments. In *Extreme Environments* (pp. 217-233). CRC Press.

Bottone, E. J. (2010). Bacillus cereus, a volatile human pathogen. Clinical microbiology reviews, 23(2), 382-398.

Deepika, S. and Jaya, M.R. (2015). Biodegradation of low density polyethylene by micro-organisms from garbage soil. *J. Exp. Biol. Agricult. Sci.*, 3(1): 15 21.

Hadad, D., Geresh, S., Sivan, A. 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillus borstelensis*. *J. Appl. Microbiol.,* 98: 1093 1100.

Haider, T. P., Völker, C., Kramm, J., Landfester, K., and Wurm, F. R. (2019). Plastics of the Future? The Impact of Biodegradable Polymers on the Environment and on Society. *Angewandte Chemie* (international edition), 58 (1): 50-62.

Kathiresan, K. (2003). Polythene and plastic degrading microbes in an Indian mangrove soil. *Rev. Biol. Trop.*,51(3-4): 629 633.

Khan, S., Nadir, S., Shah, Z.U., Shah, A.A., Karunarathna, S.C., Xu, J., Khan, A., Munir, S., and Hasan, F. (2017). Biodegradation of polyester polyurethane by *Aspergillus tubingensis*. *Environ Pollut* 225: 469–480.

Lau, G. W., Hassett, D. J., Ran, H., and Kong, F. (2004). The role of pyocyanin in Pseudomonas aeruginosa infection. *Trends in molecular medicine*, 10(12), 599-606.

E-ISSN: 3115-4662 DOI: 10.64290/bima.v9i2B.1299

Norman, R.S., Frontera-Suau, R. and Morris, P.J. (2002). Variability in *Pseudomonas aeruginosa* 680 lipopolysaccharide expression during crude oil degradation. *Appl. Environ. Microbiol*., 68(10): 5096-5103.

Oliveira, J., Belchior, A., da Silva, V.D., Rotter, A., Petrovski, Ž., Almeida, P.L. and Gaudêncio, S.P. (2020). Marine environmental plastic pollution: mitigation by microorganism degradation and recycling valorization. *Frontiers in Marine Science*, 7: 567126.

Palleroni, N. J. (2015). Burkholderia. Bergey's Manual of Systematics of Archaea and Bacteria, 1-50.

Sivan, A., Szanto, M. and Pavlov, V. (2006). Biofilm development of the Polyethylene-degrading bacterium *Rhodococcus ruber*. *Applied microbiology and biotechnology*. 72. 346-52. 10.1007/s00253-005-0259-4.

Stover, C.K., Pham, X.Q., Erwin, A.L., Mizoguchi, S.D., Warrener, P., Hickey, M.J. and Olson, M.V. (2000). Complete genome sequence of *Pseudomonas aeruginosa* PAO1, an opportunistic pathogen. *Nature*, 406(6799): 959-964.

Sowmya, H.V., Ramalingappa., Krishnappa, M. and Thippeswamy, B. (2014). Biodegradation of Polyethylene by *Bacillus cereus, Advances in Polymer Science and Technology: An International Journal,*4(2): 28-32.

Tadros, R.M., Noureddini, H., and Timm, D.C. (1999). Biodegradation of thermoplastic and thermosetting polyesters from Z-protected glutamic acid. *J. Appl. Polym. Sci*., 74(14): 3513-3521.

Tiwari, A. K., Gautam, M., and Maurya, H. K. (2018). Recent development of biodegradation techniques of polymer. *Int. J. Res. Granthaalayah*, *6*(6), 414-452.

Tokiwa, Y., Calabia, B.P., Ugwu, C.U., and Aiba, S. (2009). Biodegradability of plastics. *International Journal of Molecular Sciences*, 10(9): 3722-3742.

Urbanek, A.K., Mirończuk, A.M., García-Martín, A., Saborido, A., de la Mata, I., and Arroyo, M. (2020). Biochemical properties and biotechnological applications of microbial enzymes involved in the degradation of polyester-type plastics. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, 1868(2): 1403-15.

Usha, R., Sangeetha, T. and Palaniswamy, M. (2011). Screening of polythene degrading microorganisms from garbage soil. *Libyan Agricult. Res. Center J. Int.,* 2: 200-204.