International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Biodegradation of Low-Density Polythene (LDPE) by Microbes Isolated from Plastic Polluted Sites Majorly Consisting of Laboratory Waste

Bhavya Tiwari

Department of Environmental Science, College of Basic Science And Humanities, Govind Ballabh Pant University of Agriculture And Technology, Pantnagar, Uttarakhand, India
Email: *bhavyatiwari334[at]gmail.com*

Abstract: ***The present study was conducted to assess the effect of bacterial strains, isolated from polluted sites, on low-density polythene (LDPE). Various bacterial colonies were isolated from two different sites of Pantnagar (Uttarakhand, India) that were almost similar to each other in terms of physicochemical properties but differed in the type of waste being dumped on the locations. Site A was dumped with biomedical waste from Veterinary College whereas Site B was dumped with laboratory waste like broken glassware, old stationary and other refuses. The isolated bacteria were tested for biochemical activity and then inoculated in flasks filled with minimal media and strips of low-density plastic (single use polythene) in them. After incubating for a period of 30 days, it was observed that isolate Ah8 exhibited maximum degradation efficiency of 8.43% (weight loss percentage). The isolate showed similarity of 99% with bacterial strain Bacillus altitudinis.***

Keywords: Low-density polythene (LDPE), Biodegradation, Bacterial isolates, weight-loss percentage, Bacillus altitudinis

1. Introduction

Polythene plays an important role in our day to day life, from carry bags, storage containers and food packaging to airplane construction, phone casing and other electronic bodies. It is the most consistent commodity that has penetrated its roots deep into our lives. Being so widely used, it also causes nuisance in the environment due to its complex structure, higher molecular weight and recalcitrant nature. Conventional methods of disposal cause further harm by releasing toxic chemicals, gases and leachates into the atmosphere, soil and water. Therefore, to minimize the addition of toxic by-products because of incineration and land filling, biological methods are being adopted to contain the explosion of plastic.

Plastic is basically a synthetic polymer which constitutes of carbon, oxygen, hydrogen, chloride, silicon and nitrogen. Organic polymers such as polyethylene, PVC (polyvinyl chloride), nylon, etc. are used to synthesize it, which can be molded into shape while soft, and set into a rigid or slightly elastic form. Coal, oil and natural gases are used to extract basic material for plastic production (**Seymour, 1989**). In terms of general formula, Polyethylene is expressed as C_nH_{2n}, ‘n’ signifies the number of carbon atoms. The first known natural polymers were shellac, resin, cobweb, animal glue and tortoise shell whereas first synthetic material was invented by Baekeland in 1907, ‘Bakelite’. The light weight, low cost, high durability, high strength, corrosion resistance with high thermal and electrical insulation properties of plastic material render them fit for daily use. Polythene plays an important role in various products like packaging material, carry bags, transportation, insulation, water proofing etc. Polyethylene [Low Density (LDPE), Moderate Density (MDPE), High Density (HDPE) and Linear Low Density (LLDPE)], Polybutylene terephthalate (PBT), Polypropylene (PP), Polyvinyl Chloride (PVC), Polystyrene (PS), Polyurethane (PUR) and Nylons are the most predominantly used plastics. There are two main categories of plastic that are in frequent use: thermoplastics and thermoset. Thermoplastics are plastics that are not subjected to chemical change in their composition when heated and can be molded repeatedly. Thermosets are assumed to have infinite molecular weight as they are made of repeating units derived from long chains of monomers. Thermosets can melt and be molded into various shapes but once solidified, they remain solid.

Plastics are a success story as the world plastic production totaled around 57 million tons (**Shristi Kumar et al., 2007**) in the year 2007 and presently about 322 million metric tons (**Statista, 2017**). Synthetic polymers started to take over natural polymers in almost every area, about half a century ago. With such an increasing amount of production, there is accumulation in the environment which evokes issues that are difficult to deal with. Currently, the world faces an increased flow of waste, energy exploitation and climate change accompanied by dumping of non-biodegradable plastic wastes. This ends up dumped at various sites around the globe leading to unmanageable conditions as it takes many years to degrade. Plastics are extensively used in a wide range of products from daily use containers, carry bags, bottles, cups and trays to construction material like tubing, insulation, seals and gaskets etc. It makes progress possible by making the electrical and electronic appliances around us a lot safer, light weight, attractive and less noisy. Plastic is versatile, sanitary, durable and light weight that renders it fit to be used for packaging applications that include baby products, vending packaging, drums and protection packaging. With the increasing advancements in the field of transporting, it has become the need of the hour for cost-effective and safe transportation of people as well as goods. Due to the non-corrosive, moldable and low-maintenance nature, plastic has become an integral part of transportation industry, being used internally for fittings as in dashboards, seats, flooring etc. and externally to give a flexible design and smooth curves to locomotive bodies.Recently, enzymatic degradation is most extensively being studied for plastic waste management. This method increases the rate of degradation without causing any harm to the environment (**Bhardwaj et al., 2012**). Soil contains a wide range of microbes with different enzymatic activities assisting the biodegradation process. Most frequently reported bacteria and fungi degrading plastic material are Pseudomonas, Acinetobacter, Arthrobacter, Brevibacillus and Aspergillus, Penicillin, Rhodococcus respectively. The efficacy of these organisms to degrade polymers has been documented to increase using consortia instead of a single species. In the past decades, breakthrough advancement in technology like addition of commercially available oxo-biodegradable polymer additive has helped in the isolation and identification of microbial populations suitable for the sole purpose of dealing with the proper disposal and management of plastic waste. Synthetic polymers like polyester, polyurethane, polyethylene with starch blend are found to degrade adroitly. Bioplastics (Biopolymers) obtained from plants or from growth of microorganisms; genetically-engineered to produce such polymers, are replacing currently used plastics at least in some of the fields (**Lee, 1996**). Many bacteria and archaea are being utilized to synthesize biodegradable plastics, polyhydroxyalkanoates is one of the biopolymers exhibiting properties like polyethylene and polypropylene (**Kim and Lenz, 2001; Rehm, 2003**).

2. Literature Survey

Waste management is de rigueur (a must) for the development of our country (**Narayana, 2009**). According to Central Pollution Control Board of India (**CPCB 2022-23**), the nation generates 2.26 million tonnes of waste plastic with an astonishing quantity of plastic waste generated in Telangana followed by Tamil Nadu, West Bengal & Uttar Pradesh in that order (**CPCB Report, 2020-21**). Recyclable polymers or thermoplastics pitch in about 80% of the total plastic waste produce, while thermoset or non-recyclable plastic fill the bill of the remaining 20%.

To combat the problem of proper waste disposal, India has been using conventional technologies for ages, which include the 3Rs (Reduce, Reuse and Recycle), incineration, land filling etc but these have not done such a commendable job so far. This brings us to the new age technologies encompassing fuel-from-waste, plasma pyrolysis, polymer blended bitumen and plastic waste in cement kiln.

Waste-to-energy industry has a huge advantage over all the other aforementioned technologies as it not only deals with the management of waste but also produces about 6.5 Megawatt of energy per day which in turn saves about 19 crore rupees per year spent on electricity generation (Power Sector). Polymer blended bitumen also called Plastone, became a buzz recently when it was used to construct roads in Tamil Nadu in 11 districts. Polymer bitumen is made by first shredding plastic into thin strips and heated to 165˚C and binded with bitumen, heated to 160˚C (**Fawcett and McNally, 2000**). This material is heat and cold resistant rendering the roads fit for any kind of weather and climate. Plastic waste management models have been adopted from countries like South Korea as they possess efficient technologies for the same. It comprises of processes like pelletization that is capable of converting plastic waste into fuel, substituting conventional sources of energy, by using waste plastic burner (**Makker et al., 2014**).

Environmental impact of plastic waste

The striking increase in the annual production, use and the lack of biodegradability of these synthetic polymers have drawn our attention to the accumulation and pollution of environment that would scar our planet for centuries to come (**Albertsson et al., 1987**). Plastic waste is dealt with by land-filling, recycling and incineration which has detrimental effects on wildlife and the aesthetic appearance of dumping sites. Trace amounts of chemicals have been reported to leach out from these polymers causing noxious effects on human health. Not only humans but birds, animals and even marine biota are affected by plastic dump. If ingested mistakenly, pieces of plastic litter clog the intestines of animals resulting in their deaths.

Though plastic constitutes only 2.4% of total world municipal waste, it is considered a major threat. It accounts for 10% of solid waste (**Heap, 2009**) and has an alarming contribution of 80% to waste accumulation in the oceans, shorelines and land (**Barnes et al., 2009**). The additives that are mixed with different polymers to enhance their properties cause adverse effects to the end users viz. Humans. Plasticizers, compounds that increase the pliability of plastics, are added to polyvinyl chloride (PVC). Some of the plasticizers used are phthalate esters that are volatile compounds providing the vinyl their notable "new car smell". After sometime these esters vaporise and form an oily coating on the windshield and the vinyl starts to crack. Also, this vaporised compound stimulates health hazards in humans as the ester leaches and is incorporated into the blood. Between September 1967 and December 1973, a lethal form of liver cancer called angiosarcoma was spotted in workers of chemical company making PVC near Louisville, Kentucky (**Morbidity and Mortality Weekly Report, 1997**). Other than the environmental issues in hand, plastic polymers are a hazard in the sense that they catch fire easily and release toxic gases when burnt. Incidents have been reported where clothing made of polymers caught fire and resulted in numerous injuries. Plastics are also leading us towards an energy crisis as the raw material for production of plastic is the same as that needed for energy generation, i.e., petroleum. To avoid these problems, synthetic polymers are being modified by mixing them with starch blend and making them available for biodegradation. Research activities are carried out all around the globe to look for alternatives that are easily degraded and manageable. The most widely accepted solutions for the above mentioned problem are thermal degradation, photo degradation, environmental erosion and biodegradation (**Kawai, 1995**).

Degradation of low-density plastic

Degradation is the process of wearing down or disintegration of a material, caused by change in its chemical structure or its physiology, rending it unworthy of the use it was designed for. It is divided into various categories depending on the factors responsible for the deterioration; viz.

temperature, light or microbes. Thermal degradation of plastics is the result of heating the polymer to an extent that it causes molecular deterioration, consequently reducing ductility and cracking, chalking, colour transformation etc (**Olayan et al., 1996**). Photo degradation is the amendment of materials by light and it is related to the ability of the polymer to absorb part of solar insolation and degrade itself. Plastics absorb photons, particularly in the ultraviolet, infra-red and visible region to ensue breaking of polymer chains, generation of free radicals, oxidation, cleavage, and finally the complete alteration of the product (**Mark et al., 1986**) but low density polyethylene does not absorb this radiation unless there is presence of impurities (**Briassoulis et al., 2004**). Several degradable plastics have come into the market in the last decade but none of them have been efficiently degraded in landfills. An initial attempt to create degradable plastic was manufacturing shopping bags from Polylactic acid, although not in use today because these compounds have their own effect on the environment due to the processes involved in their production. Eventually, the burden lies on developing microbes that are efficacious to solve this earthly issue (**Kathiresan, 2003**). Processes that actuate change in polymer properties by virtue of physical, chemical or biological reactions resulting in subsequent chemical modifications are categorized as polymer degradation. Degradation is carried out under the influence of abiotic factors namely wind, light, water, temperature etc and also biotic factors can have effect on the deterioration of plastic products. This process governed by living organisms like fungi, bacteria, yeast is termed as biodegradation.

Biodegradation of plastic

The process that leads to deterioration in quality of a material due to the action of living entities like fungi, yeast, bacteria etc, is called biodegradation. Biodegradation is a multi-step process and is carried out utilizing various mechanisms: biosorption, bioaccumulation, biomineralisation, biotransformation and bioleaching (**Sood et al., 2016**). Microbes accomplish the task of breakdown of polymers by bond breaking enzymatic activities following a sequence: bio-deterioration (change in the chemical or physical properties of a polymer), bio-fragmentation (breaking down of long chain polymers into smaller fragments through enzymatic cleavage), bio-assimilation (microbes engulf the small fragments of broken down polymers), bio-mineralisation (production of simpler compounds like CO2, H2O, CH4) (**V. M. Pathak and Navneet, 2017**). To enhance the efficiency of degradation of plastic various parameters can be altered such as blending polymers with biodegradable additives, using genetically modified microorganisms suitable according to the environmental conditions of the place of dumping the waste, by pre-treatment of plastic including thermal treatment (drying in an oven), exposure to UV etc. Since the process of natural degradation of plastic is time taking, efforts have been made like the aforementioned to accelerate the process. Majorly studied organisms for biodegradation are the minute members of the biota; microbes (bacteria, fungi, yeast etc). Heterotrophic microorganisms use plastic as a substrate for their growth (**Glass and Swift, 1989**).

3. Method/ Approach

Soil samples were collected from sites dumped with waste around Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India, and was analyzed for physico-chemical features such as soil moisture, texture, pH, nitrogen content, phosphorus content, organic carbon and electrical conductivity.

After the sample collection, serial dilution was carried out by taking 1gm of soil in test tubes and adding 9 ml of distilled water to make 1:10 dilution. Then adding 1ml of 1:10 dilution to 9ml of distilled water making 1:100 dilutions and so on till a dilution of 10^{-8} was reached. These diluted samples were then cultured for bacterial growth and purified, isolated colonies were obtained with repeated inoculation.

Procedures mentioned in the Bergey's Manual of Systematic Bacteriology (**Kandler and Weiss, 1986**) were used to identify the isolates of bacteria. The basis of identification was difference in morphology, cultural and biochemical characteristics. Observation under the microscope determines the morphology of isolated strains based on shape, size and colour of colonies formed.

Biochemical identification test kits of HiMedia Laboratories (Figure 1) were used to identify the bacterial isolates based in their specific characteristics and the reference provided by the laboratory manual.

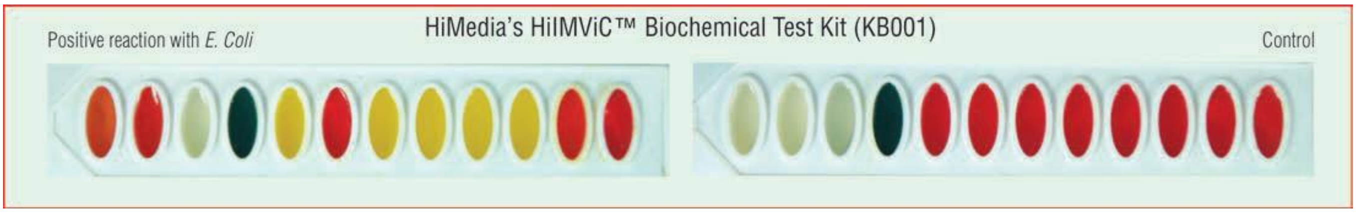

Figure 1: KBM001 HiMotility Biochemical kit

Shake Flask Experiment: After the isolation and identification of bacteria, strips of commercially used polythene bags were set up for shake flask experiment after their pre-treatment. Small squares of size 3X3 cm were cut from plastic bags and transferred to a fresh solution used for surface sterilization containing 7ml Tween-20 (Polysorbate 20), 10ml bleach and 983ml distilled water then stirred for 30-60 minutes **(El-Shafei *et al.*, 1998).** The pieces were then shifted to a beaker filled with distilled water and stirred for an hour. Next, the polythene pieces were placed in ethanol solution (70% v/v) for 30 minutes with the help of sterile forceps. Finally they were placed on Petri plates to shade dry overnight.

Degradation of pre-treated polythene: Initially weighed strips were placed in 200ml conical flasks containing 50 ml minimal media containing Dipotassium phosphate, Monopotassium phosphate, Sodium citrate, Magnesium sulphate, Ammonium sulphate and inoculated with bacteria. Control was retained with microbe free media in the flasks. Flasks were kept in a shaking incubator for 30 days at 35˚C, 120rpm. After the incubation period, the strips were aseptically collected and rinsed with distilled water. Finally, strips were weighed and percentage weight loss was calculated using the formula mentioned below **(Usha, et al. 2011):**

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

Sequencing of isolated bacteria: The bacterial strand that showed maximum degradation efficiency was plated in triplicates and sent to Chromous Biotech Pvt. Ltd, Bangalore for 16s RNA sequencing and phylogenetic analysis. The results obtained from the lab were then analyzed on BLAST (Basic Local Alignment Search Tool) that looks for local regions of similarity between sequences of living entities. This program calculates significant match between sequences of known and unknown strain. After the BLAST analysis, phylogenetic tree of the unknown is obtained to see for similarity with the closest analogue in the sequence.

4. Data Analysis

The data from shake flask experiment was recorded and analyzed using MS-Excel. Standard deviation (SD), standard error (SE) and average of the data was calculated and the final values were presented as ± SE. Significant difference between the data was calculated using two way analysis of variance (Two-way ANOVA) either in SPSS 16.0 or in MS-Excel (ANOVA with replication). All the values were calculated and summed up in a table.

5. Result and Discussion

Two areas were selected for the purpose of isolation, i.e., dumping sites of College of Veterinary and Animal Sciences (CVAS) labeled as site A and College of Basic Sciences and Humanities (CBSH) labeled as site B. The physicochemical analysis of both the sites was done to determine the difference between the two in their composition and texture and the results obtained were as follows:

Table 1: Physicochemical analysis of various parameters of Site A (CVAS) and Site B (CBSH)

Parameters	Values	
	Site A	Site B
Texture	Sandy-Loam	Sandy-Loam
Electrical Conductivity (dS m^{-1})	0.79 ± 0.70	0.82 ± 0.75
pH	6.02 ± 0.36	5.92 ± 0.50
Nitrogen (% wt)	0.04 ± 0.02	0.06 ± 0.06
Moisture (%)	20.25 ± 0.04	19.53 ± 0.03
Organic Carbon (%)	4.01 ± 0.50	3.12 ± 0.50
Phosphorus (mg kg-1)	8.1 ± 0.01	7.30 ± 0.08

The physicochemical analysis of the soil from the two sites conclude that their texture was same but other parameters like electrical conductivity, pH, moisture content, carbon, nitrogen and phosphorous content differ. Although the values are close to each other but differ due to the different type of waste being dumped at the sites.

The colony morphology of the isolates was different and is listed in the table below (Table 2). The isolates were coded on the basis of the site they were found at (A or B) and their replicates (a-z).

Table 2: Colony Morphology of bacterial strains

Isolates	Form	Elevation	Margin	Source
Ae1	Circular	Raised	Entire	Dumping site of CVAS
Bl2	Circular	Flat	Undulate	Dumping site of CBSH
Ai3	Circular	Crateriform	Curled	Dumping site of CVAS
Bb4	Circular	Flat	Undulate	Dumping site of CBSH
Ad5	Circular	Flat	Entire	Dumping site of CVAS
Bc6	Punctiform	Raised	Entire	Dumping site of CBSH
Bf7	Irregular	Flat	Lobate	Dumping site of CBSH
Ah8	Circular	Convex	Entire	Dumping site of CVAS

The results for KBM001 HiMotility Biochemical kit were interpreted using the identification index provided with the kit which is a standardized colorimetric identification system and the results are indicated by a change in color after inoculation and incubation of the sample. Positive results are indicated by a change in color and recorded as (+) for partial color change and (++) for complete color change whereas negative result is indicated by no change in color and recorded as (-) (Table 3).

Table 3: Biochemical Test of isolated strains using KBM001 HiMotility Biochemical kit

No.	Test	Ae1	Bl2	Ai3	Bb4	Ad5	Bc6	Bf7	Ah8
1	Motility	-	-	++	-	-	-	-	++
2	Motility	-	-	+	-	-	-	-	+
3	Indole	-	-	-	+	-	-	-	-
4	Citrate Utilization	+	+	-	+	-	+	-	-
5	Glucuronidase	-	-	-	-	-	-	-	-
6	Nitrate Reduction	+	+	+	++	+	+	+	+
7	ONPG	+	-	+	+	+	+	+	++
8	Lysine Utilization	+	-	+	-	-	-	-	+
9	Lactose	-	-	+	-	-	+	+	++
10	Glucose	-	-	-	-	+	++	++	++
11	Sucrose	+	+	+	+	+	+	-	++
12	Sorbitol	++	++	++	-	++	+	-	++

Table 4 consists of results from KB002 HiAssorted Biochemical Test Kit. The various reactions included in the kit are citrate utilization, lysine and ornithine utilization, urease test, phenylalanine deamination, nitrate reduction and H_2S production.

Table 4: KB002 Hi Assorted Biochemical Test Kit

No	Test	Ae1	Bl2	Ai3	Bb4	Ad5	Bc6	Bf7	Ah8
1	Citrate Utilization	+	+	-	+	-	+	-	-
2	Lysine Utilization	+	-	+	-	-	-	-	+
3	Ornithine Utilization	-	-	-	-	-	-	-	-
4	Urease	+	+	-	+	+	+	+	-
5	Phenylalanine Deamination	-	-	-	-	-	-	-	-
6	Nitrate Reduction	+	+	+	++	+	+	+	++
7	H_2S Production	-	-	-	-	-	-	-	-
8	Glucose	-	-	-	-	+	++	++	++
9	Adonitol	-	-	+	-	+	+	+	++
10	Lactose	-	-	-	-	-	+	+	++
11	Arabinose	++	-	++	-	+	-	-	+
12	Sorbitol	+	+	+	-	-	++	-	-

Result of degradation of low-density plastic by bacterial isolates in one month

Figure 2 represents weight loss percentage by different isolates in a month. It is clear that isolate Ah8 had the maximum degradation efficiency and could further be used for large scale projects of plastic degradation. Further studies would refine the work to find out the optimum conditions for growth and create an entire workforce for degradation of plastic, be it low-density or high-density. The most efficient microbe can further be tested for degrading other resins used for the manufacturing of various synthetic products.

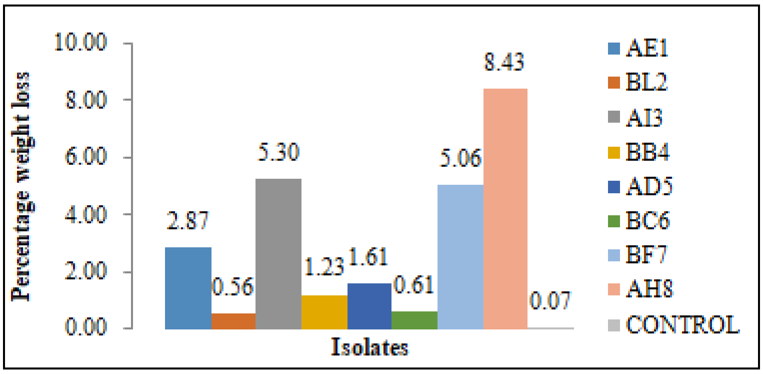

Figure 2: The degradation percentage of polythenes by different isolates

Table 5 represents the final data for the degradation of commercially used, low-density polythene. The values were recorded and calculated using MS-Excel giving the final table in the form of ± SE (Standard Error). The replicates were averaged to give precise results. After calculating the maximum efficiency of the bacterial isolates, most suitable microbe was sent for 16s RNA sequencing and phylogenetic analysis to Chromous Biotech Pvt. Ltd., Bangalore. The results obtained were run in BLAST (Basic Local Alignment Search Tool) that provides identification of the unknown against the known bacterial strain. Sequence of sample is run in BLAST and it compares the sequence with a recorded database of sequences and gives out the closest analogue strain. In the present study, the partial sequencing of unknown showed 99% similarity with *Bacillus altitudinis* which is a gram positive bacterium, motile in nature, found at higher altitudes, hence the name.

Table 5: Comparative analysis of polythene weight loss with different microbial species under laboratory conditions

Isolate	Initial Weight (mg)	Weight after 15 days (mg)	Weight after 30 days (mg)	Weight Loss (%)	
				After 15 days	After 30 days
Ae1	7.85 ± 0.05	7.75 ± 0.05	7.625 ± 0.025	1.27	2.87
Bl2	9.00 ± 0.0	8.98 ± 0.01	8.95 ± 0.05	0.22	0.56
Ai3	7.55 ± 0.25	7.3 ± 0.20	7.15 ± 0.15	3.31	5.3
Bb4	8.15 ± 0.25	8.12 ± 0.20	8.05 ± 0.15	0.37	1.23
Ad5	7.775 ± 0.02	7.755 ± 0.03	7.65 ± 0.05	0.26	1.61
Bc6	8.25 ± 0.05	8.23 ± 0.01	8.20 ± 0.10	0.24	0.61
Bf7	8.11 ± 0.01	7.95 ± 0.02	7.7 ± 0.30	1.97	5.06
Ah8	8.6 ± 0.10	7.93 ± 0.025	7.875 ± 0.025	7.79	8.43
Control	7.455 ± 0.10	7.452 ± 0.02	7.45 ± 0.01	0.04	0.07

6. Conclusion

Microbes play a vital role in the natural degradation of material. They are capable of deteriorating even the most recalcitrant of materials like polythene because of their ability to use the carbon backbone as a source of energy to grow. As the production and consumption of plastics is growing day by day, so is the improper disposal and management. For this, new methods and techniques are being incorporated with the conventional ones to increase the efficiency of degradation. It is not just a matter of toxic pollutants being released into the environment due to the dumping and leaching of chemicals but also an issue of aesthetic importance. Since the physical and chemical methods of waste management are either slow or expensive, biological methods are being emphasized for the purpose which includes utilizing microorganisms.

Earlier studies have been compared to the present research and the results procured were in accordance to those found previously. Bacillus altitudinis was the identified bacteria that exhibited maximum degradation efficacy during the present work. The isolate was capable of utilizing all types of carbohydrates from the test kit indicating its ability to accumulate carbon from any source available nearby making it fit for degradation under laboratory conditions as well as under natural conditions (soil burial). Fewer studies have been done employing B. altitudinis for plastic degradation; hence, this study opens up new avenues for research on this bacterium. Since the bacterium is capable of degrading hydrocarbon backbone easily and rapidly, it makes it suitable for the deterioration of low as well as high density plastics.

7. Future Scope

From the findings of this research we can conclude that the isolates were able to degrade plastic under laboratory conditions in a short span of time Although, *B. altitudinis* hasn't been studied extensively and so its pathogenicity is still under question but can be revealed through more advanced study. The above-mentioned results are indicative of the fact that if studied further, this microbe could be the missing piece of the puzzle that is plastic waste management. Predominantly tested weight loss along with physico-chemical changes are insufficient to prove the real biodegradation of polyethene (PE) . There is a need for providing concrete and reliable evidence for biodegradation of PE in order to minimize artifacts formed from degradation of additives rather than PE. Hence, upcoming research should be performed using additive-free PE.

References

[1] **Albertsson, A.C.; Andersson, S.O. and Karlsson, S. 1987**. The mechanism of biodegradation of polyethylene. Polymer Degradation and Stability, 18: 73–87.

[2] **Bhardwaj, H.; Gupta, R and Tiwari, A. 2012.** Communities of Microbial Enzymes Associated with Biodegradation of Plastics. Journal of Polymers and the Environment-Springer. 21(2): 575–579.

[3] **Glass, J.E and Swift, G. 1989.** Washington, July 13, 1990. Agricultural and synthetic polymers, biodegradation and utilization. Glass, J.E and Swift, G. Washington, American Chemical Society, p: 9–64.

[4] **Gupta, A.; Joia, J.; Sood, A.; Sood, R.; and Sidhu, C. 2016**. Microbes as Potential Tool for Remediation of Heavy Metals: A Review. Journal of Microbial and Biochemical Technology, 8:364-372.

[5] **Kandler, O. & Weiss, N. 1986**. Genus Lactobacillus Beijerinck 1901, 212AL. In Bergey's Manual of Systematic Bacteriology, vol. 2, Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins. p: 1209–1234.

[6] **Kathiresan, K. 2003**. Polythene and plastics-degrading microbes from the mangrove soil. Revista de biologia tropical Journal. 51: 3-4.

[7] **Kawai, F. 1995.** Breakdown of plastics and polymers by microorganisms. Advances in Biochemical Engineering / Biotechnology, 52: 151–194.

[8] **Kim, Y.B. and Lenz, R.W. 2001.** Polyesters from microorganisms. Advances in Biochemical Engineering/Biotechnology. 71: 51-79.

[9] **Kumar, S.; Hatha, A.A.M. and Christi, K.S. 2007**. Diversity and Effectiveness of Tropical Mangrove Soil Microflora on the Degradation of Polythene Carry Bags. International Journal of Tropical Biology and Conservation, 55: 777-786.

[10] **Makker, A.; Gupta, A. and Gupta, L. 2014**. Plastic Waste in India- Management and Utilisation In Energy Technologies, Climate Change and Environmental Sustainability: Innovative Perspective, New Delhi, July 2014. Editor: Prof. (Dr.) Govind Chandra Mishra. Kishangarh, Vasant Kunj, Excellent Publishing House. p:79-86.

[11] **Mark, H.F.; Bikales, N.M.; Overberger, C.G. & Menges, G. 1986**. Encyclopedia of Polymer Science and Engineering. Volume no.15. 2nd Edition. New York, USA. Wiley Interscience Publication; 839-p.

[12] **Olayan, H.B.; Hamid, H.S. and Owen, O.D. 1996**. Photochemical and thermal crosslinking of polymers. Journal of Macromolecular Science - Reviews in Macromolecular Chemistry & Physics, 36: 671–719.

[13] **Pathak and Navneet. 2017.** Review on the current status of polymer degradation: a microbial approach. Bioresources and Bioprocesing. 4:15

[14] **Rehm, B.H. 2003**. Polyester synthases: natural catalysts for plastics. Biochemical Journal. 15;376(Pt 1):15-33.

[15] **Seymour, R.B. 1989**. Polymer Science Before and After 1899: Notable Developments during the Lifetime of Maurtis Dekker. Journal of Macromolecular Science:Part A- Chemistry, 26: 1023-1032.

[16] **Usha, R.; Sangeetha, T. and Palaniswamy, M. 2011.** Screening of Polyethylene Degrading Microorganisms from Garbage Soil. Libyan Agriculture Research Center Journal International. 2 (4): 200-204.

Author Profile

Bhavya Tiwari, the authoress of this manuscript was born on 27th June, 1994 in Khatima, Uttarakhand, India. She passed her High School from Saraf Public School, Khatima (India) and Intermediate from Nirmala Convent Senior Secondary School, Haldwani (India) in 2010 and 2012 respectively.. She obtained her graduate degree in B.Sc. (H.Sc.) from G.B. Pant University of Agriculture and Technology, Pantnagar, India, in 2016. In 2016-17 (1st Semester), she was admitted for M.Sc. degree programme in Department of Environmental Science, G.B. Pant University of Agriculture and Technology, Pantnagar, India. She was the recipient of Graduate Teaching/ Research Assistance provided by the University. Email ID: bhavyatiwari334@gmail.com
Ph. No. : +918958557704