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Research Paper

Biodegradation of polythenes by microbial isolates derived from soil samples in Rajshahi, Bangladesh

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ABSTRACT: *Isolation and characterization of microbes from random samples of sugar mill soil (SMS), power plant soil (PPS), sewage soil (SWS), grave yard soil (GYS) and agricultural land soil (ALS) were made from Katakhali under Rajshahi City Corporation, Bangladesh. Colony and cell characteristics revealed that the microbial isolates were of >2mm in size, either Gram +ve and motile rods, Gram –ve and non-motile rods or Gram –ve and motile spheres. Five isolates designated as T1, T2, T3, T4 and T5 from five types of soil samples respectively, were subjected to various biochemical tests including citrate utilization, oxidase, catalase, MacConkey's agar, KOH, carbohydrate utilization, sulfide indole mobility (SIM) and triple sugar iron (TSI) and the results were recorded. Antibiotic sensitivity tests using diffusion disc method showed that majority of the isolates were hypersensitive (HS) or sensitive (S), some were resistant (R) while others had intermediate (I) response towards 10 commonly used antibiotics under study. Weight loss method was used to assess biodegradation of experimental low-density polythenes (LDPE). In situ experiments with different soil samples showed that SWS was the most efficient biodegrader (20.4% weight loss in 45 days) while GYS was the least efficient one (13.4% weight loss in 45 days). The remaining soil samples showed 16.6% (PPS) > 15.4 % (ALS) > 15.2% (SMS) weight loss in connection with their biodegradation efficiencies. Results on biodegradation of LDPE by microbial isolates after an incubation period of 10 months under laboratory conditions revealed that isolate T3 from SWS had the most profound effect on the biodegradation of polythenes compared to the remaining four isolates, T5 from ALS being the least effective biodegrader. The remaining isolates showed T2 > T1 > T4 with respect to their biodegradation efficiencies.* ***Conclusion:*** *The present results therefore clearly demonstrated that biodegradation of synthetic LDPE and plastics by soil microbes could be one of the eco-friendly, inexpensive and innovative methods, which might serve as the most rewarding trouble-shooters for the local and national polythene and plastic waste contaminations.*

Keywords: *Biodegradation, Polythenes, Microbial Isolates, Soil Samples, Rajshahi, Bangladesh*

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I. INTRODUCTION

Polythenes and plastics are broad-spectrum man-made materials made up of elements extracted from the fossil fuel resources and have numerous valuable uses in industries belonging to food, clothing, shelter, transportation, medicine and construction, because they are of lightweight, low in cost, durable and unbreakable. They are high molecular weight polymers primarily synthesized from hydrocarbons and petroleum derivatives [1]. These are produced in huge quantities and are used widely for different purposes in our everyday life, and as a result, the global demand for these synthetic products is rapidly growing everyday [2].

It is a matter of great concern that various forms of polythenes and plastics nowadays disrupt soil, land and the aquatic eco-systems in a variety of ways [1]. It is estimated that all over the world, annual waste generation due to polythenes and plastics is about 57 million tons; and since large amount of these wastes is getting accumulated in the environment, their disposal evokes a major ecological issue [2]. Polythenes cause global warming and pollution not only as a major issue of waste disposal but also they release dioxides and CO_2 while burning [3]. Accumulation of polythene wastes in the environment is posing an ever increasing ecological threat throughout the globe [4]. Low-density polyethylene (LDPE) is one of the major categories of plastics that

are used extensively all over the world. It has wide range of inexpensive uses including supermarket carrying bags, outdoor furniture, siding, floor tiles, shower curtains and clamshell packaging.

There are numerous microbes such as bacteria, fungi, microalgae and invertebrate associated microbiota that can break down LDPE. These microbes usually contain enzymes called oxygenases, which can add oxygen to the long carbon chain of the polythenes and destabilizes the local electric charge, and the polythenes can then be broken down [5]. Degradation of polythenes by microbes is one of the eco-friendly and innovative methods available today. Majority of the microbial strains that are able to degrade polythenes belong to different taxa such as Gram-positive and Gram negative bacteria, *Streptomyces* and fungi [5, 6]. Following sites are reported to be rich sources of polythene degrading microbes: rhizosphere soil of the mangroves; polythenes and plastics buried in the soil; soil at the polythene/plastic dumping sites; and marine water [7]. According to a recent estimate, the most prevalent wild-type plastic- and polythene-degrading microbes include bacteria (56.3%, 36 genera), followed by fungi (32.4%, 30 genera), microalgae (1.4%, 1 genus), and microbiota (2.8%) [8]. Since polythenes contain a carbon-carbon backbone structure, microbes can utilize polythenes as a source of food, energy, and reproduction, resulting in their breakdown into inorganic components. Biodegradation therefore seems to be a promising solution to tackle the polythenes as well as plastic waste issue but it requires a thorough understanding of the efficient microbes, gene clusters, pathways, and enzymes involved in the process [1].

Biodegradation is defined as decomposition of contaminant molecules by the action of the enzymatic machinery of biological system. In other words, biodegradation is the process by which organic substances are broken down by living organisms [6]. Microbes release the extracellular enzymes such as lignin and manganese peroxidases to degrade the plastics and polythenes but the detailed and exact characterization of these enzymes in relation to the degradation of these synthetic products is still needed to be investigated [1].Various plastic and polythene degradation methods are available in the literature but the cheapest, eco-friendly and acceptable method is the microbial degradation using microbes either *in situ* or under laboratory conditions [3, 9].

Recently, several researchers reviewed the occurrence and mechanisms of microbial degradation of polythenes and plastics in different environmental niches such as soil, landfill or aquatic systems in Punjab, Pakistan [1], Punjab, India [4], Columbia [8], Chile [10], Libya [11] and Morocco [12]. In this report, an attempt has been made to isolate polythene degrading microbes from five different soil samples collected from Katakhali under Rajshahi City Corporation, Bangladesh. Here the isolated microbes were characterized using both morphological and biochemical analyses and biodegradation of the experimental LDPE was quantified both *in situ* and under laboratory conditions using standard analytical methods [6, 13-16].

II. MATERIALS and METHODS

Collection of soil samples:

Soil samples were collected from five different places of Katakhali under Rajshahi City Corporation **(Fig. 1)**, which were designated as follows: 1. Harian Sugar Mills soil (SMS); 2. Katakhali Power Plant soil (PPS); 3. Katakhali Sewage soil (SWS); 4. Katakhali Graveyard soil (GYS); and 5. Samsadipur Agricultural Land soil (ALS). Soil samples were collected from 5-10 cm depth, thus avoiding surface soil exposed to sunlight. Soon after collections, the samples were transported to Genetics and Molecular Biology Laboratory, Department of Zoology, University of Rajshahi, for the isolation of microbes, which were subsequently tested for their ability to degrade the experimental polythenes *in situ* as well as under laboratory conditions. The investigation was conducted during December 2020 through November 2021.

Figure 1: Different soil samples from Katakhahi, Rajshahi

Isolation of microbes from soil samples:

The collected soil samples were first categorized into five treatment groups designated as T1, T2, T3, T4 and T5 from SMS, PPS, SWS, GYS and ALS, respectively (**Fig. 2**). They were then homogenized by passing through a 2-mm sieve to remove rubbish and gravels associated with them. Accordingly, five microbial isolates (T1-T5) were separated and characterized based on their morphological and biochemical characteristics as described earlier [6, 14, 16]. Briefly, microbes were isolated from one gram of soil sample by serial dilution

method. The nutrient agar media (NAM) were used for microbial cultures and then isolation of the microbes was performed by spread plate technique (**Fig. 3**). The isolates were sub-cultured repeatedly for getting pure colonies. All the plates were incubated at 37° C and pH 7 for 24 to 48 hrs. The identification of the microbes was performed on the basis of microscopic examinations and biochemical tests [17].

Figure 2: Microbial isolates (T1-T5) derived from soil samples; Tc = Control

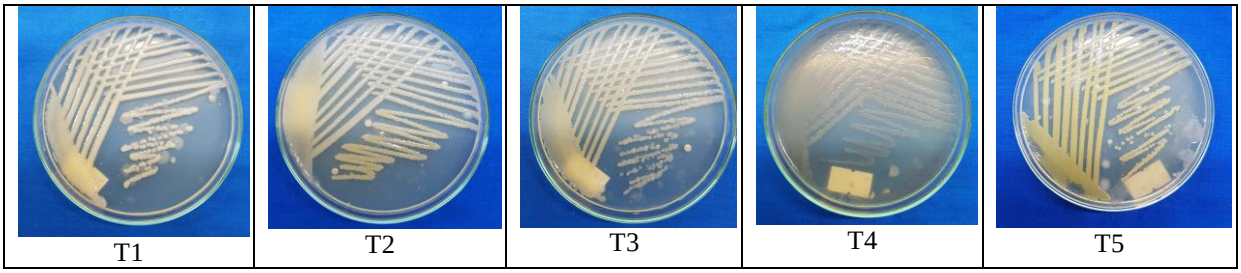


Figure 3 Microbial isolates grown on nutrient agar media (NAM)

Characterization of microbial isolates from soil samples:

Characterization of microbial isolates was performed by three major procedures: (a) colony and cell characteristics [11]; (b) various biochemical tests [9]; and (c) antibiotic sensitivity tests [18]. In short, the microbial isolates were identified by marking their colony and cell characteristics grown on MacConkey's and Voges-Proskauer (VP) agar media, where shape, size, colour, consistency, surface, margin, elevation, opacity, motility and Gram positive/negative properties (**Fig. 4**) were observed. In addition, the isolates were subjected to conventional biochemical tests such as utilization of carbohydrate, citrate, oxidase, catalase, KOH, sulfide indole mobility (SIM) and triple sugar iron (TSI) test following the standard methods (**Fig. 5**). Ten commonly used antibiotic discs *viz.* Ampicillin (AMP), Azithromycin (AZI), Cephradine (CEP), Doxycycline (DOX), Erythromycin (ERY), Gentamicin (GEN), Kanamycin (KAN), Neomycin (NEO), Penicillin (PEN) and Tetracycline (TET) were used for antibiotic sensitivity tests by standard disc diffusion method (**Fig. 6**).

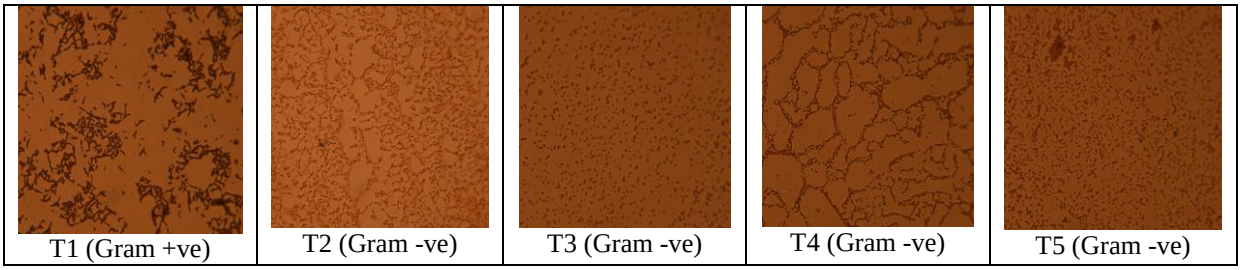
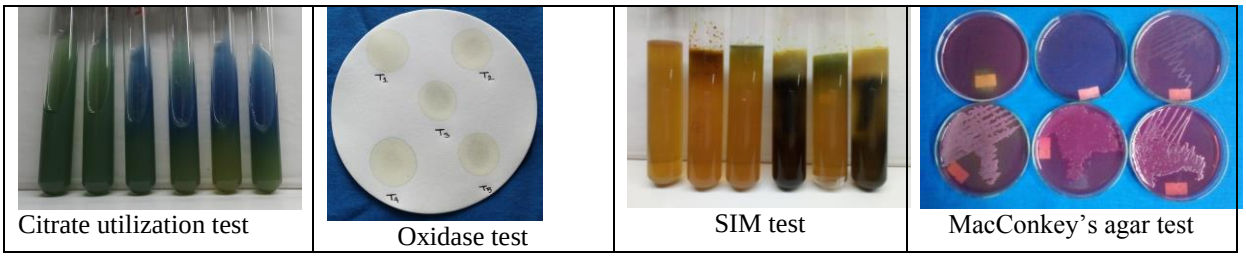
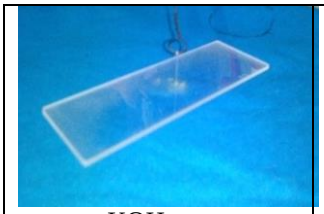
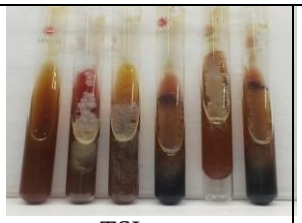
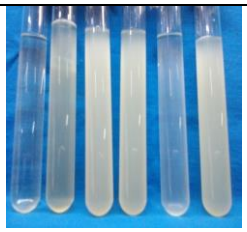


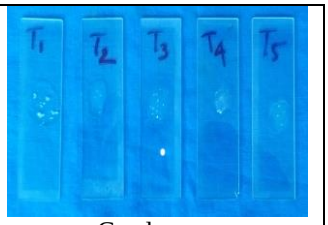
Figure 4 Gram characteristics of the microbial isolates (T1-T5)




KOH test


TSI test


Carbohydrate utilization



Catalse test

Figure 5 Various biochemical tests for characterizing the microbial isolates

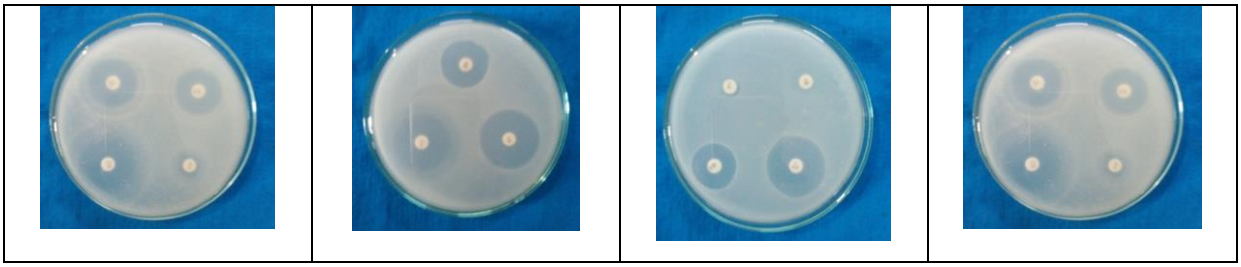


Figure 6 Antibiotic sensitivity patterns of the microbial isolates

Collection of polythene samples:

Low-density polyethylene (LDPE) having 20% starch, donated by the China-Bangladesh Alliance Company, were used in this study. For the biodegradation study, LDPE samples were cut into small pieces and then sterilized by 70% ethanol.

***In-situ* biodegradation of plastics by sampled soils:**

For *in-situ* biodegradation LDPE samples were cut in small pieces and then washed with 70% alcohol first and then with distilled water. The pieces were oven dried and the initial weights were recorded. The polythene strips were then incubated in the containers with five different soil samples in liquid culture or shaker method. After 15, 30 and 45 days, the polythene strips were removed from the soil samples, washed in distilled water, dried in oven and the final weights was recorded. Biodegradation of the polythene samples was estimated by measuring the weight loss of the samples due to microbial activities of the sampled soils by using the following formula [9, 16, 19]: Percentage of weight loss = (Initial wt – Final wt) ÷ Initial wt × 100.

Biodegradation of plastics by microbial isolates under laboratory conditions:

Five microbial isolates were cultured on nutrient broth for 24h. Then 100µl of the broth was spread on fresh nutrient agar plate and incubated at 37° C and pH 7 for overnight. Pre-weighed pieces of polythenes were transferred into cultured nutrient agar plates. Control was maintained with polythene pieces in a microbe-free medium. The cultures were incubated at 37° C and pH 7 for a period of 10 months. In the end, the polythene pieces were collected, washed thoroughly in distilled water, dried in oven and then the final weights were recorded. The percentage of weight loss of the polythene samples was calculated as described above for *in situ* biodegradation.

III. RESULTS AND DISCUSSION

Colony and cell characteristics of the microbial isolates:

Colony and cell characteristics of five microbial isolates have been presented in Table 1. All the isolates were circular in shape and >2 mm in size. Isolates T1-T3 were creamy white, T4 shiny and T5 was yellow in colour. T1 and T5 were non-sticky but the remaining isolates had sticky consistency. All the isolates exhibited smooth surface, entire margin and raised elevations. Isolate T4 was transparent, whereas the rest isolates were opaque. T1 isolate was motile and Gram positive rod, T2 was non-motile and Gram negative rod, but the remaining isolates were spherical, motile and Gram negative (**Fig. 4**).

Table 1: Colony and cell characteristics of the microbial isolates from experimental soil samples at Rajshahi, Bangladesh

Colony Morphology	**Microbial isolates**				
	T1	T2	T3	T4	T5
Size	>2 mm	>2 mm	>2 mm	>2 mm	>2 mm
Shape	Circular	Circular	Circular	Circular	Circular
Colour	Creamy white	Creamy white	Creamy white	Shiny	Yellow
Consistency	Non-sticky	Sticky	Sticky	Sticky	Non-sticky
Surface	Smooth	Smooth	Smooth	Smooth	Smooth
Margin	Entire	Entire	Entire	Entire	Entire
Elevation	Raised	Raised	Raised	Raised	Raised
Opacity	Opaque	Opaque	Opaque	Transparent	Opaque
Cell Morphology	Gram +ve rod, motile	Gram -ve rod, non-motile	Gram -ve spherical, motile	Gram -ve spherical, motile	Gram -ve spherical, motile

Biochemical characteristics of the microbial isolates:

Table 2 shows the biochemical characteristics of five microbial isolates derived from the experimental soil samples. Citrate utilization test was negative for T1, all the isolates were negative for oxidase, isolates T2 and T5 were negative for catalase, and all the isolates except T1 showed lactose fermentation and positive on MacConkey's agar and KOH test. Majority of the isolates showed positivity towards carbohydrate utilization tests, while T3 and T5 were H_2S producers and only T1 produced indole. On triple sugar iron (TSI) test, isolates T1, T3 and T5 were red but T2 and T4 exhibited yellow colouration (**Fig. 5**).

Table 2: Biochemical characteristics of the microbial isolates from experimental soil samples at Rajshahi, Bangladesh

I. Biochemical Tests	**Microbial isolates**				
	T1	T2	T3	T4	T5
Citrate utilization	-ve	+ve	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve	-ve	-ve
Catalase	+ve	-ve	+ve	+ve	-ve
MacConkey's Agar	-ve	+ve	+ve	+ve	+ve
KOH	-ve	+ve	+ve	+ve	+ve
II. Carbohydrate utilization					
Glucose	+ve	+ve	+ve	+ve	+ve
Fructose	-ve	+ve	+ve	+ve	+ve
Galactose	+ve	+ve	+ve	+ve	-ve
Maltose	+ve	+ve	+ve	+ve	-ve
Sucrose	-ve	-ve	+ve	+ve	+ve
Lactose	-ve	+ve	+ve	+ve	+ve
Xylose	-ve	+ve	-ve	+ve	+ve
Dextrose	+ve	+ve	-ve	-ve	-ve
III. Sulfide indole mobility (SIM)					
H_2S production	-ve	-ve	+ve	-ve	+ve
Indole production	+ve	-ve	-ve	-ve	-ve
Motility	Motile	Non-motile	Motile	Motile	Motile
IV. MacConkey Agar					
MacConkey growth	-ve	+ve	+ve	+ve	+ve
Lactose fermentation	-ve	+ve	+ve	+ve	+ve
V. Triple sugar iron (TSI)					
Reaction in slant	Red	Yellow	Red	Yellow	Red
Gas production	-ve	-ve	-ve	+ve	-ve
H_2S production	-ve	-ve	+ve	-ve	+ve

T1 = Gram +ve rod, motile; T2 = Gram –ve rod, non-motile; T3 = Gram –ve spherical, motile; T4 = Gram –ve spherical, motile; T5 = Gram –ve spherical, motile

Antibiotic sensitivity of the microbial isolates:

Data on the antibiotic sensitivity tests (**Fig. 7**) against microbial isolates revealed that majority of the T1 and T3 isolates were either hypersensitive (HS) or sensitive (S), and only 20% were resistant (R) to 10 antibiotic discs under study. Isolate T2, on the other hand, were 90% HS, isolate T4 were 60% resistant (R), and isolate T5 showed 50% R and 30% intermediate (I) response towards the experimental antibiotic discs.

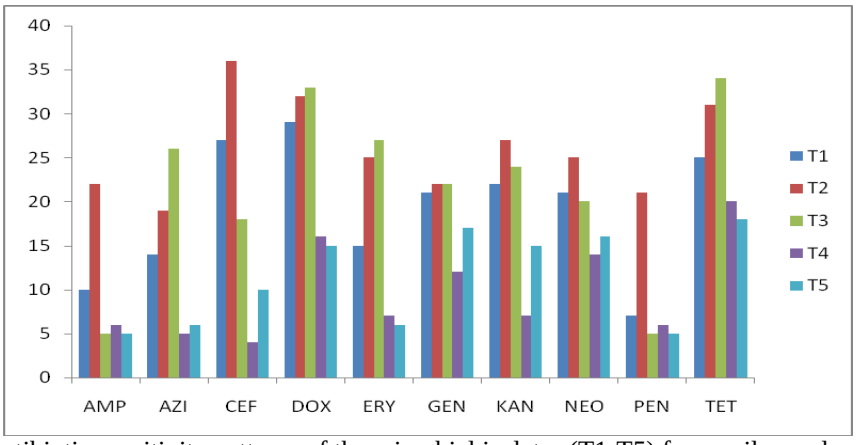


Figure 7: Antibiotic sensitivity patterns of the microbial isolates (T1-T5) from soil samples at Rajshahi, Bangladesh.

***In-situ* biodegradation of polythenes by sampled soils:**

Percentage of weight loss owing to *in situ* biodegradation of LDPE by different soil samples (Table 3) showed that sewage soil (SWS) was the most efficient biodegrader while grave yard soil (GYS) was the least efficient one. The remaining soil samples showed PPS > ALS > SMS with regard to their biodegradation efficiencies.

Table 3: *In situ* biodegradation of LDPE by different soil samples at Rajshahi, Bangladesh

Soil samples	**Percentage of weight loss after days of treatment**		
	15 days	30 days	45 days
SMS (Sugar Mill soil)	8.3	11.5	15.2
PPS (Power Plant soil)	9.8	12.2	16.6
SWS (Sewage soil)	10.5	15.8	**20.4**
GYS (Graveyard soil)	4.2	7.6	**13.4**
ALS (Agricultural Land soil)	5.2	9.3	15.4

Figures indicate % weight loss at treatment days; % wt loss = (Initial wt – Final wt) ÷ Initial wt × 100.

Biodegradation of polythenes by microbial isolates under laboratory conditions:

Results on biodegradation of polythenes by microbial isolates after an incubation period of 10 months under laboratory conditions are presented in **Fig. 8**. It is apparent from the data that isolate T3 had the most profound effect on the biodegradation of polythenes compared to the remaining four isolates, T5 being the least effective biodegrader. The remaining isolates showed T2 > T1 > T4 with respect to their biodegradation efficiencies.

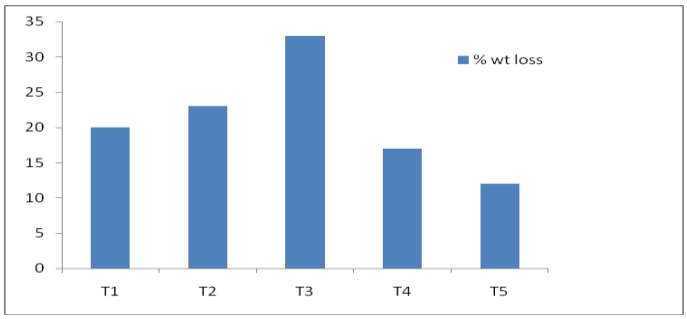


Figure 8: After an incubation period of 10 months under laboratory conditions, weight loss (%) of LDPE through degradation by the microbial isolates (T1-T5) collected from different soil samples at Rajshahi, Bangladesh. T1 refers to Gram +ve rod, motile; T2 Gram –ve rod, non-motile; T3 Gram –ve spherical, motile; T4 Gram –ve spherical, motile; and T5 = Gram –ve spherical, motile microbes.

The most commonly found LDPE and plastic waste is single-use products such as polythene bags, plastic bottles, and packaging and cutlery items. The major sources of these wastes in the environment have been identified as household and industrial wastes, landfills, and human activities [1]. Literature review on the characteristics and identity of various plastic degrading microbes around the world show diversified results. Thus, soil samples were collected from polythene and plastic dumping site wastes in the mangrove area, Tamil Nadu, India [6]. The rich sources of polythene degrading microbes were mangrove soil, polythene buried in the soil, plastics and soil at the dumping sites and marine water in Maharashtra, India [7]. Microbial species found associated with polythene bags and plastic cups degradation were identified as five Gram positive and two Gram negative bacteria, and eight fungal species from the mangrove soil in the estuary of south-east India [13]. In Bhopal, India, biodegradation of polythene and plastic by five different types of soil samples from medicinal garden, sewage water, energy park, sludge area and agricultural land was estimated [14], where various species of bacteria and fungi were found to degrade these wastes efficiently. The biodegradation of polythene was relatively faster and earlier than that of the plastics. Microbial biodegradation of polythene bags and plastic cups was analyzed in Tamil Nadu, India, where bacterial and fungal species such as *Pseudomonas*, *Bacillus*, *Staphylococcus*, *Aspergillus* and *Streptomyces* were found to be associated with the synthetic materials [20].

Soil bacteria from Tamil Nadu, India, were isolated from plastic contaminated soil samples, where two bacterial isolates were identified by their morphological and biochemical characterization [9]. Microbes from agricultural soils capable of degrading polythenes and plastics in Tanzania have been isolated and identified [12]. Plastic degrading microbes from five different soil samples *viz.* garden soil, mangrove soil, forest soil, soil near petrol pump, and garbage soil in Mumbai, India, were isolated [15]. Microbes from garbage soil in Andhra Pradesh and Telangana areas, Hyderabad, India, were isolated and identified [16]. Plastic biodegradation by employing bacterial and fungal species isolated from dumped soil samples in Chennai, India [19], and different plastic degrading soil bacteria from dump site, garbage and mangrove soil and their efficiencies in Tamil Nadu, India, were investigated, where the microbial genera such as *Bacillus*, *Pseudomonas*, *Azotobacter*, *Ralstonia* and *Halomonas* were identified [21]. LDPE-degrading bacterial strains have been isolated and identified from landfill soil containing large amounts of plastic materials in Iran [22]. Moreover, degradation of PVC and PE by *Bacillus* species isolated from the marine ecosystem has been demonstrated in India [23].

Recently, microbes and their enzymes capable of degrading a wide range of commonly used synthetic plastics including LDPE trashes have been reported in Punjab, Pakistan, where microbial strains of *Bacillus*, *Staphylococcus*, *Streptococcus*, *Streptomyces*, *Pseudomonas*, *Comamonas* and *Ideonella* have been identified [1]. In West Bengal, India, majority of the bacterial strains that were able to degrade plastics belonged to Gram-positive and Gram negative bacteria, Streptomyces and fungi [5]. Many soil microbial genera such as *Bacillus*, *Aspergillus* and *Spirulina* have demonstrated their biodegradation potential to degrade various types of plastics in Columbia [8]. In Libya, microbial species associated with the degrading plastic materials were identified as two Gram positive and five Gram negative bacteria belonging to the genera *Bacillus* and *Arthobacter* [11]. The present study corroborate nicely to those mentioned above except that the microbial isolates were not identified up to generic level, further research on which is under consideration. Bacterial species *Bacillus subtilis* was tested for its potential in biodegrading polyethylene in Trivandrum, India [24]. Microbial strains used in the biodegradation of plastic wastes in Nigeria belonged to the genera *Aspergillus*, *Penicillium*, *Moraxella*, *Nocardia*, *Brevibacterium*, *Streptomyces*, *Streptococcus*, *Staphylococcus*, *Pseudomonas*, and *Bacillus*, in which the most prevalent wild-type plastic-degrading microbes included bacteria (56.3%, 36 genera), followed by fungi (32.4%, 30 genera), microalgae (1.4%), and invertebrate associated microbiota (2.8%) [25].

Biodegradation of polythenes and plastics can be studied either *in situ* or under laboratory conditions by screening and isolating microbes from the environmental reservoirs which are further quantified using various analytical methods [26]. Municipal or landfill dumping soil samples were isolated and screened for plastic degrading microbes, which showed the evidence of a substantial amount of mineralization, thus suggesting an approach that has a profound scope to cope with the plastic waste problem in a cost-effective and environmental friendly manner [4]. The mangrove soil was found to be a good source of microbes capable of degrading polythene and plastics, where biodegradation of polythene bags was significantly higher (4.21%) than that of plastic cups (0.25%) during a period of nine months [13]. In another experiment, polythenes were degraded relatively faster than that of the plastics [14]. Utilizing the liquid culture or shaker method, the bacterial species *Pseudomonas* degraded 37.09% of polythene and 28.42% of plastics in 6 months incubation period. Fungal species, however, degraded 20.96% of polythene and 16.84% of plastics and *Streptomyces* species degraded 46.16% of polythene and 35.78% of plastics during the same period [20]. These findings are very much similar to our present findings where sewage soil sample (SWS) showed the highest degradation of 20.4% of LDPE over a period of 45 days *in situ*, while microbial isolate from the sewage soil (T3) degraded 33% LDPE over a period of 10 months under laboratory conditions.

IV. CONCLUSIONS

Considering the detrimental effects of the existing physical and chemical methods for plastic waste management on the environment, biological methods have emerged as the best option to mitigate plastic pollution around the globe. Biodegradation of synthetic LDPE and plastics by microbes therefore could be one of the eco-friendly, inexpensive and innovative methods. On a large scale, LDPE waste treatment technology through microbes could be the most rewarding trouble-shooters for the local and national polythene and plastic waste problems. Findings of the present work demonstrate that microbes from the natural habitats are capable of degrading LDPE efficiently. The present results also suggest that there is a need to complement the existing knowledge on the biodegradation and biotransformation of synthetic LDPE and plastics to enable effective bioremediation strategies to mitigate the effects of environmental polythene and plastic contamination in the country as a whole. Further fundamental and applied research, such as development of successful biotechnological strategies to improve degradation through enzyme engineering and plastic waste bioconversion into value-added products, would be most promising for biodegradation of synthetic LDPE and plastics in the natural habitats.

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