IJASRM
International Journal of Advanced Scientific Research and Management, Volume 4 Issue 2, Feb 2019
www.ijasrm.com
ISSN 2455-6378

Microbial degradation of Polyethene from Dumped Soil in and around Thirukalukundram

J. Sivakumar[1], S. Illanjiam[2] and C. ShanmugaSundaram[2]

[1] PG & Research Department of Biotechnology
Hindustan College of Arts & Science, Padur, Chennai – 603 103

[2] PG & Research Department of Microbiology
Hindustan College of Arts & Science, Padur, Chennai – 603 103

Abstract

The polluted soil samples were collected from five different locations in Thirukalukundram, Aruna Matriculation Higher Secondary School, Annai Theresa Arts & Science, College, Soap Company, Government Hospital and Garbage Dumping Yard. Microbes were isolated by serial dilution and spread plate method from five different locations. Characterization and identification of microbes was done by Gram staining, catalase, oxidase and motility. *Bacillus sp.*, *Staphylococcus sp.*, *Corynebacterium sp.*, *Clostridium sp.*, and *Actinomycetes sp.* was identified from the collected samples. The biodegradation potential of microbes on different kinds of Plastics like10, 20 and 40 microns thickness was studied about 40 days period of time interval. The degradation potential of microbes on Polyethenes was calculated once in a ten days. Biodegradation potential was characterized by FTIR analysis. Among the isolated microbes, *Bacillus sp* showed the high potential on degradation activity on Polyethenes. The current research proves that *Bacillus sp* could be effectively used to degrade Polyethene interrestrial environments. Utilization of the *Bacillus sp* may be the best solution to solve the land pollution by Polyethene and also to conserve the natural environment.

Keywords: *Actinomycetes sp, Bacillus sp, Clostridium sp, Corynebacterium sp, Staphylococcus sp* and Polyethenes.

1. Introduction:

Plastics can be said as building materials as they are being used for several purposes in our everyday life [1]. On the opposite they are responsible for the environmental pollution by getting amass in the environment this takes place since its constant character [2]. Plastic is an artificial polymer. It consists of carbon, nitrogen, oxygen, silicon, hydrogen and chloride. It is derived from various sources such as coal, oil and natural gas. Plastics are widely used since its firmness and robustness. They are many types such as Polyethene (PE), nylons, Polystyrene (PS), Poly Ethylene Terephthalate (PET), Polyurethane (PUR) and Polyvinyl Chloride (PVC)[3]. Due to the deficiency of proficient methods for protected clearance of these artificial polymers, they often end up dumped in the environment, pretentiousness rising environmental threat to vegetation and animals [4].

Plastic deprivation by microorganisms due to the action of certain enzymes that breaks the polymer chains into monomers and oligomers. Plastic that has been enzymatically busted down further fascinated by the microbial cells to be metabolized. Aerobic metabolism forms CO_2 and H_2O. Anaerobic metabolism produces carbon dioxide, water, and methane as end products [5].

In the normal circumstance degradable or nondegradable organic materials are considered as the main environmental problem, e.g. plastics. The accretion of these plastic wastes created severe hazard to environment and wild life [6]. The environmental concerns include water, soil and air pollution. The dispersion of rural, urban and industrial effluents pollutes the soil. The soil contaminations are mainly formed by anthropological actions [7]. Environmental pollution is caused by synthetic polymers, such as water-soluble synthetic polymers and wastes of plastic in wastewater [8]. Being an adaptable, lightweight, sturdy and potentially translucent substance, Plastics are finest matched for a diversity of applications. [9].

More than a couple of decades, plastic materials have increased prevalent use as they have been progressively more in shelter, clothing, construction, food, transportation, therapeutic and relaxation industries [10]. The stability, light heaviness and process capacity of these polymers causes them to longer in the environment for several years and end up in landfills and natural water resources producing a cruel hazard to the environment and its ecosystems [11].

As the microbes have diverse character, so the deprivation diverges from one microorganism to another. Microorganisms degrade the polymers like Polyethene, polyurethane by using it as a substrate for their growth. A variety of factors which are accountable for biodegradation are the type of treatment required, organism characteristics and type of polymers [12, 13]. Biosurfactant, an extracellular surfactant concealed by microbes, enhances the biodegradation progression [14]. Microorganisms can degrade plastic over 90 genera, from bacteria and fungi, among them; *Pseudomonas sp*., *Bacillus megaterium*, *Ralstonia eutropha*, *Azotobacter*, *Halomonas sp*., etc. [15].

There are too poisonous monomers, which have been associated to tumor and reproductive troubles. Due to consciousness of wastes complexity among public and its collision on the environment has awakened new interest in the area of degradable polymers. [16]. Moreover, these artificial polymers are usually non-biodegradable until they are degraded into little molecular mass fragments that can be incorporated by microorganisms [17].

In addition, eco-friendly plastics recommend set of reward such as low gathering of massive synthetic resources in the environment (which invariably minimize injuries to wild animals) increased soil fertility and reduction in the cost of waste management [18]. Hence the present manuscript aims to isolate and investigate on the different microbial species that are capable to degrade polymer bags. Recent years, environmental problem related to nonbiodegradable thermoplastics and research to modify them to biodegradable materials is of great interest [19]. As per a research study, plastic played a role about 8% of the entire solid waste generated [20]. From early 20th century, the environmental concern regarding to its slow degradation started as it takes thousands of years for its efficient degradation. To prevent accumulation of plastic it should be properly disposed. Abundant plastic wastes have often been found during deep-sea investigation using research submersibles [21]. From the foregoing accounts and also on the literature surveys there are controversial reports on the degradation of plastics, hence the present investigation has been undertaken to present an authenticated report.

2. Materials and Methods:

2.1 Sample collection

Samples were collected from different soil polluted areas of Thirukkalukundram, located 52 kms away from Kanchipuram, Aruna Matriculation Higher Secondary School, Annai Theresa Arts & Science College, Soap Company, Government Hospital and Garbage dumping yard. The sample was brought to the laboratory in sterile condition and kept in laboratory condition for further use. Polyethene bags of different densities such as 10 micron, 20 micron and 40 micron were purchased from local market of Thirukkalukundram.

2.2 Serial dilution method

1 gm of soil sample was transferred into a clean conical flask having 10ml of sterile distilled water. The combination was mixed and serially diluted [22].

2.3 Spread plate method

The isolation of microbes was carried out by scattering the dilution and the Polyethene pieces of 4×4 cm were cut and located on the nutrient agar plates. Later than the incubation the growth of microorganisms were seen on the Polyethene strips [23].

2.4 Screening of Polyethene degrading microorganisms

This was performed by zone of clearance technique where the 0.5 concentrations of Poly Ethylene Glycol was used in minimal media having salts of ammonium and potassium and the zone of clearance around the colonies were observed by staining with Coomassiee blue this indicate its capacity to utilize Polyethene as C-source and degrade Polyethene [24]..

2.5 Characterization and identification of microorganisms

Subsequent to screening the isolates were characterized by different morphological and biochemical analysis, according to Bergey's manual determinative bacteriology [25].

2.6 Pre-treatment of Polyethene

The Polyethene covers were incise into the tiny strips then transferred to a clean solution having 70ml Tween 80, 10ml bleach, and 984ml distilled water and mixing for 40 to 60 minutes [26]. Then the strips were taken onto a beaker with distilled water and mixed for 1 hour. Additional, they were aseptically kept in the ethanol solution 70% [23].

2.7 Degradation of Pre-treated Polyethene

At first weighed strips of 4×4-cm size of 10, 20 and 40 micron Polyethene were aseptically taken to the conical flask having 50 ml of nutrient broth medium and loaded with bacteria (0.5ml). Control was kept with plastic discs in the germ-free medium. Various flasks were placed in a shaker for 10, 20 and 40 days respectively. After the respective duration of shaking, the Polyethene strips were taken, washed carefully using distilled water, shade-dried and then weighed for final weight and percentage of weight loss were calculated using following formula [5].

Formula for calculating the degradation activity of microbes:

$$\% \text{ of Biodegradation} = \frac{\text{Final weight}}{\text{Initial Weight}} \times 100$$

2.8 FTIR:

Fourier Transform Infrared analysis (FTIR) spectroscopy was carried out by using the degraded Polyethene mixture with *Bacillus sp.* Fourier Transform Infrared spectrophotometer was used to determine the chemical nature of the degraded compound after 40 days of treatment.

3. Results:


a. Aruna Matriculation Higher Secondary School b. Annai Theresa Arts & Science College


c. Soap Company d. Government Hospital

e. Garbage Dumping Yard.

Note* The left side plate is in the concentration of 10^{-3}

The centre plate is in the concentration of 10^{-5}

The right side plate is in the concentration of 10^{-7}

Plate. 1 Colony morphology of isolated organisms from different locations

3.1 Colony Morphology

The sample collected from the Aruna Matriculation Higher Secondary School showed the colony morphology on the plates were pale-white, circular, flat, opaque, round, medium and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large In the case of Annai Theresa Arts & Science College was observed as white, entire, convex, opaque or translucent, circular, medium and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large colonies were observed. Whereas in Soap Company colonies were seen like creamish-white, entire, raised, translucent, irregular, large and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large. The sample collected from the Government Hospital showed the colony morphology on the plate was observed as white, circular, raised, opaque, round, large and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large. Whereas in Garbage Dumping Yard showed white-dull, wavy, umbonate, opaque, irregular, large, motile and the white-dull, entire, pulvinate or umbonate, opaque, irregular, large colonies (Plate. 1).

3.2 Preliminary tests

As for as the gram staining is concerned the organisms isolated from Aruna Matriculation Higher Secondary School, Soap Company, Government Hospital and Garbage Dumping Yard showed gram-positive rods. Whereas in Annai Theresa Arts & Science College showed gram-positive coccus. Whereas in motility the organisms isolated from Aruna Matriculation Higher Secondary School, Soap Company and Government Hospital showed motile results**.** Annai Theresa Arts & Science College and Garbage Dumping Yardnon-motile organisms were found. Soap Company showed negative results for catalase rest of the locations showed appearance of gas bubbles and resulted as positive results. As for the Oxidase test was concerned all the locations showed negative results except Aruna Matriculation Higher Secondary School (Table. 1).

3.3 Degradation of Polyethene

The degradation activity of microbes on the Polyethenes in Aruna Matriculation Higher Secondary School showed the moderate. Annai Theresa Arts & Science College showed the lower rate of degradation. The efficient activity was observed in Soap Company. The moderate activity was observed in Government Hospital, whereas in Garbage Dumping Yard it showed the higher degradation activity (Plate. 2).

The isolated microbial strains have shown degradation potential on Polyethene. The *Bacillus sp* showed interesting facts in the degradation activity. The initial weight of the 10 micron Polyethene was 1.2 mg, 20 micron 2.4 mg and 40 micron 4.5 mg respectively. At the end of the 60th day the weight of the 10 micron was decreased into 0.5 mg (0.41%), 20 micron into 1.8 (0.75%) and 40 micron into 3.9 mg (0.86%). On 60th day the *Staphylococcus sp* showed the 10 micron was decreased into 0.7 mg (0.58%), 20 micron into 2mg (0.83%) and 40 micron into 4.1 mg (0.91%). Whereas *Corynebacterium sp* showed the 10 micron was decreased into 0.8 mg (0.66%), 20 micron into 2.1 mg (0.87%) and 40 micron into 4.1

IJASRM International Journal of Advanced Scientific Research and Management, Volume 4 Issue 2, Feb 2019
www.ijasrm.com
ISSN 2455-6378

mg (0.91%). *Clostridium sp* degraded the 10 micron Polyethene cover into 0.6 mg (0.50%), 20 micron into 1.9 mg (0.79%) and 40 micron into 4 mg (0.88%). Actinomycetes degraded the 10, 20, and 40 microns Polyethene covers into 1 mg (0.83%), 2.2 mg (0.91%) and 4.2 mg (0.93%) respectively (Fig. 1, 2,3,4,5 and Table. 2).

4. Discussion:

Biodegradation based on the polymer characteristics, organism category and nature of pretreatment [16]. The pre-treatment of polyethene is extremely important for its biodegradation. Physical breaking of the polyethene and chemical cleaning by ethanol might have added advantage to its degradability. To sustain this, Volke-Sepulveda [27] showed that addition of ethanol to fungal cultures containing Polyethene improved the biodegradation rate of the polymer. In this current research, only the Polyethene degrading microorganisms are screened and used to observe their potential of degradation of Polyethenes. The impact of the weight loss of the Polyethenes is measured within the particular time interval of period.

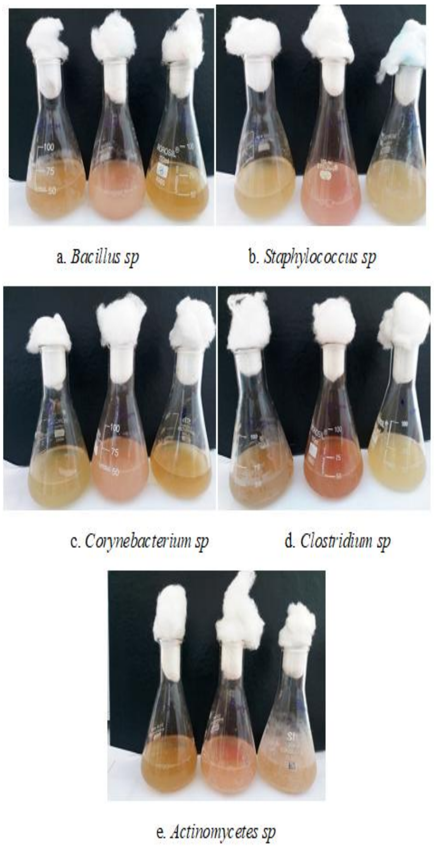
Table. 1 Colony morphology of the microbial strain on the basis of serial dilution from different locations

S.No	Colony Morphology & Preliminary Tests	**Isolate 1** *Bacillus sp*	**Isolate 2** *Staphylococcus sp*	**Isolate 3** *Corynebacterium sp*	**Isolate 4** *Clostridium sp*	**Isolate 5** *Actinomycetes sp*
1	Colony colour	Pale-white	White	Creamish-White	White	White-dull
2	Margin	Circular	Entire	Entire	Circular	Wavy
3	Elevation	Flat	Convex	Raised	Raised	Umbonate
4	Opaque / Translucent	Opaque	Opaque / Translucent	Translucent	Opaque	Opaque
5	Shape	Round	Circular	Irregular	Round	Irregular
6	Size	Medium	Medium	Large	Large	Large
7	Motility	Non-Motile	Non-Motile	Motile	Motile	Non-Motile
8	Gram Staining	Gram-positive rods	Gram-Positive coccus	Gram-Positive rods	Gram-negative rods	Gram-Positive Rods
9	Catalase	Positive	Positive	Positive	Positive	Positive
10	Oxidase	Positive	Negative	Negative	Positive	Positive

Table. 2 Potential of biodegradation by isolated microbial strains on Polyethene covers

S.No	Density of the Polyethene Covers	**10 Microns**				**20 Microns**				**40 Microns**			
	Name of the isolated Bacteria	Initial wt (mg)	Final wt (mg)	Difference	Weight Loss/month (in %)	Initial wt (mg)	Final wt (mg)	Difference	Weight Loss/month (in %)	Initial wt (mg)	Final wt (mg)	Difference	Weight Loss/month (in %)
1	*Bacillus sp*	1.2	0.5	0.7	0.41%	2.4	1.8	0.6	0.75%	4.5	3.9	0.6	0.86%
2	*Staphylococcus sp*	1.2	0.7	0.58	0.58%	2.4	2	0.4	0.83%	4.5	4.1	0.4	0.91%
3	*Corynebacterium sp*	1.2	0.8	0.4	0.66%	2.4	2.1	0.3	0.87%	4.5	4.1	0.4	0.91%
4	*Clostridium sp*	1.2	0.6	0.6	0.50%	2.4	1.9	0.5	0.79%	4.5	4	0.5	0.88%
5	*Actinomycetes sp*	1.2	1	0.2	0.83%	2.4	2.2	0.2	0.91%	4.5	4.2	0.3	0.93%

Biological degradability of polymers by microbes reduce with enhance in the molecular weight of the polymer. With raise in molecular weight, there is decline in polymer solubility which makes it adverse for microbial assault as the polymer needs to be incorporated into the bacterial cell covering and busted down by cellular enzymes. Replicating units of polymers like monomers, dimers and oligomers are simply tainted and mineralized [16]. Biodegradation is improved by abiotic hydrolysis, photo-oxidation and physical disintegration. These processes increase the exterior region of the polymer and lessen its molecular weight; assisting microbial degradation [28].



Note* The left side conical flask contains 10 micron Polyethene cover strips

The centre conical flask contains 20 micron Polyethene cover strips

The right side conical flask contains 40 micron Polyethene cover strips

Plate. 2 Biodegradation of Polyethene covers by isolated organisms

Considerable number of researches supports such degradation capacity of micro-organisms. *Pseudomonas sp* can be degrading the Polystyrene; no toxic pre-treatment was not given to these polystyrene samples. *Pseudomonas sp* was isolated from the areas which were polluted with polyolefin's and then sub cultured on artificial media in the lab [29].

A. niger and *Rhodococcus fascians* biodegrade PS at a quicker rate as compared to *B. subtilis, Pseudomonas aeruginosa* and *Micrococcus luteus*. Umamaheswari and Murali, [30], discovered that PET and PS bubbles obscured in soil, cow-dung, and sewage can be degraded by fungi breaking bonds of PET and PS foam polymer and these putrefaction can be confirmed by FTIR spectroscopy which showed stretching between the constituent bonds like C=C, CH, OH, C-O, and C=O of polymer. Studies also support the degradation of other plastics by various soil microorganisms [31, 32].

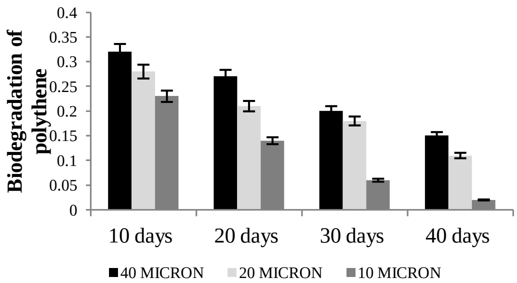


Fig. 1 Biodegradation of Polyethene by *Bacillus sp*

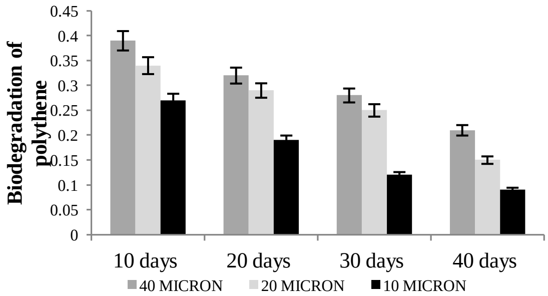


Fig. 2 Biodegradation of Polyethene by *Staphylococcus sp*

After one week to ensure the capability of loaded microbe to humiliate the LDPE strips were taken out, a greasy development on the outside of Polyethene was reported; it is a type of biofilm creation. Study of biofilm produced by *Penicillium* created by *Penicillium frequentans* and *Bacillus mycoides* showed that *Penicillium frequentans* had Polyethene (DPE-chemical or photo initiator added Polyethene) deprivation capability and since it can colonize on Polyethene [33].

Microbes capable of Polyethene degradation are native to many sites like Mangrove forests [34] Polyethene dumped garbage soil [35] and garden soil [36].On sequencing, the isolate was found to be *Staphylococcus arlettae*. This organism showed vast changes in the intensity of absorption by Polyethene films representing the degradation of the Polyethene.The researchers screened Polyethene degrading microorganism from garbage soil which

ISSN 2455-6378

revealed greater potential of *Streptomyces sp* when compared to other bacteria and fungi [27].
Similarly, gram positive non-sporing rods from landfills were found to degrade Polyethene efficiently [37]. The present study shows result is in accordance with [34], where *Staphylococcus sp* was found to degrade plastic effectively on the basis of weight loss of upto 52%. Other studies were also conducted to isolate fungi with ability to degrade Polyethene. Study by Singh and Gupta [28]; Saminathan [35] showed common soil fungi like *Aspergillus*, *Mucor*, *Penicillium*, *Fusarium* etc., has the ability to degrade LDPE upto 36% (*Aspergillus*).

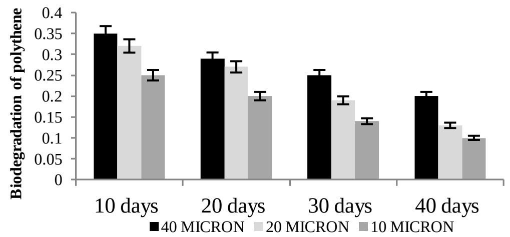


Fig. 3 Biodegradation of Polyethene by *Corynebacterium sp*

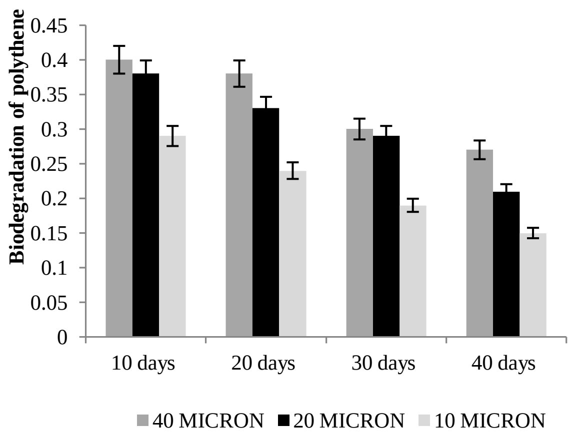


Fig. 4 Biodegradation of Polyethene by *Clostridium sp*

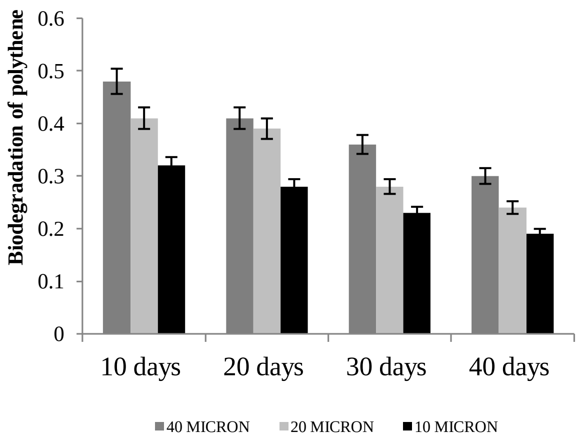


Fig. 5 Biodegradation of Polyethene by *Actinomycetes sp*

FTIR:

Fourier transform infrared spectroscopy was performed to illuminate the chemical constitution of degraded Polyethene covers by *Bacillus sp* to identify the type of functional groups. The infrared absorption bands confirm the biodegradation of Polyethene as some chemical changes were seen in surface of the polymer (Fig. 6). Since the *Bacillus sp* showed better degradation hence the FTIR performed for this species alone.

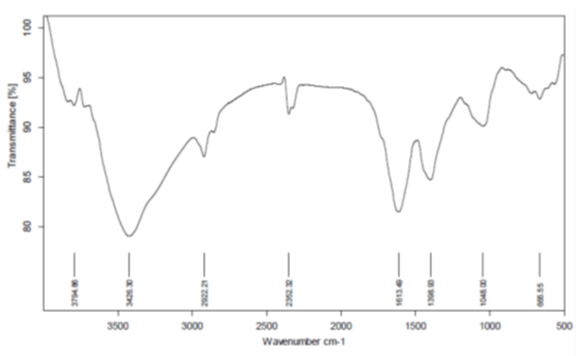


Fig. 6 FTIR Analysis shows the frequency peaks of degraded sample by *Bacillus sp*

Previous study has explored that the isolated *Staphylococcus arlettae* strain is capable of degrading Polyethene films effectively and the change in intensities of alkane group was observed through FTIR analysis [38]. During the incubation period, the system was maintained undisturbed with no supplementation of exogenous nutrients into the medium. Hence, it may be recognized that after the mineral nutrients from the media were totally utilized by the bacteria, Polyethene served as the carbon and energy source [39].
In present study, the isolated microbes from different locations show the sufficient activity of degradation of Polyethenes. Since, the *Bacillus sp*, shows the high level degradation amount of Polyethenes which has been isolated from the garbage dumping yard. Though it has wide variety of organisms, the growth of the *Bacillus sp* would be high in the cultured plate. It proves that the dumping yard contains the high amount of *Bacillus sp* where it can degrade the Polyethenes in some sort of time interval.

5. Conclusion:

Polyethene bags are used world-wide, since it is easy to carry out and easy to dispose everywhere. But the degradation of those Polyethene bags was so hard now-a-days. It creates soil pollution and the nutrient amount present in the soil. Also these Polyethene covers are making a thick layer over the earth surface; due to this the percolation of rain water in the underground is disturbed. The microbial flora in the soil also gets disturbed. The current research will help to degrade the Polyethene bags and also without forming any harmful activities to the environment. Based on the potential of the microbes may be used in the degradation process. This research work reduces the time requirement for the degradation of Polyethene bags and also does not affect the human health. Burning plastics releases the toxic substance called diaxin, which causes in born error in human beings. The large scale production of the culture and it may be spread over on the Dumping yard may show the high degradation activity on the degradation of Polyethenes.

References:

[1] Gnanavel G, Mohana VP, Valli J, Thirumarimurugan M, and Kannadasan T, Degradation of Plastic Wastes using Microbes. Elixir International Journal 54: 12212-12214, (2012).

[2] Hemashenpagam N, Growther L, Murgalatha N, Raj VS, and Vimal SS, Isolation and characterization of a bacterium that degrades PBSA. International Journal of Pharma and BioSciences, 4: 335–342, (2013).

[3] Strong AB, Plastics: materials and processing 3rd edition. Pearson Prentice Hall, Upper Saddle River, NJ. (2006).

[4] Bhardwaj H, Gupta R, and Tiwari A, Microbial Population Associated with Plastic Degradation. Open Access Science Reports, 5: 1-4, (2012).

[5] Usha R, Sangeetha T, and Palaniswamy M, Screening of Polyethene degrading microorganisms from garbage soil. Libyan Agriculture Research Centre Journal International, 2(4): 200-204, (2011).

[6] Chua ASM, Takabatake H, Satoh H, and Mino T, Production of poly hydroxy alkanoates (PHA) by activated sludge treating municipal waste water: Effect of pH, Sludge Retention Time (SRT), and Acetate Concentration influent. Water Research, 37(15): 360-361, (2003).

[7] Ghosh M, and Singh SP, A review on phytoremediation of heavy metals and utilization of it's by products. Applied Environmental, 3:1-18, (2005).

[8] Premraj R, and Mukesh CD, Biodegradation of polymer. Indian Journal of Biotechonology, 4: 186-193, (2005).

[9] Andrady AL, Microplastics in the marine environment. Marine Pollution Bulletin, 62:1596–1605, (2011).

[10] Shristi Kumar K, Hatha AM, and Christi KS, Diversity and effectiveness of tropical mangrove soil microflora on the degradation of Polyethene carry bags. International Journal of Tropical Biology, 55: 777-786, (2007).

[11] Hoffmann KL, Renickova J, Kozakova K, Ruzicka P, AlexyBakos D, and Precnerova L, Polymer Degradation and Stability. 76: 511-519, (2003)

[12] Gu JD, Ford TE, Mitton DB, and Mitchell R, Microbial degradation and deterioration of polymeric material In: Review, W. (Edition). The Uhling corrosion handbook. 2nd Edition. Wiley, New York, USA, pp: 439-460, (2000).

[13] Artham T, and Doble M, Biodegradation of Aliphatic and Aromatic Polycarbonates. Macromolecular Bioscience, 8: 14- 24, (2008).

[14] Ali AM, Nakajima-Kemble T, Nomura N, and Nakahara T, Applied Environmental Microbiology, 64 (1): 32-36, (1998).

[15] Chee JY, Yoga, SS, Lau NS, Ling SC, Abed RM, and Sudesh KL, Bacterially Produced Poly hydroxyl alkanoate (PHA): Converting Renewable Resources into Bioplastics, edited by Mendez Vilas A. Applied Microbiology and Biotechnology. (2010).

[16] Shah AA, Hasan F, Hameed A, and Ahmed S, Biological degradation of plastics: A Comprehensive review. Biotechnology Advances, 26: 246-265, (2008).

[17] Francis V, Raghul SS, Sarita GB, and Eby TT, Microbial degradation studies on linear low-density Polyethene - polyvinyl alcohol blends using Vibrio sp. International Conference on advances in polymer Technology, pp 26–27, (2010).

[18] Tokiwa Y, Calabia BP, Ugwu CU, and Aiba S, Biodegradability of Plastics. International Journal of Molecular Sciences, 10(9): 3722–3742, (2009).

[19] Zheng Y, Yanful EK, and Bassi AS, A review of plastic waste biodegradation. Critical Reviews in Biotechnology, 25: 243– 250, (2005).

[20] Sreedevi S, Solid Waste Generation and its Management- A Case Study, International Research Journal of Environmental Sciences, 4(1): 90-93. (2015).

[21] Fugikura K, Okutani S, and Maruyama T, Deep-sea Life-Biological observations using research submersibles. Tokai University Press, 57-80, (2008).

[22] Cappuccino JG, and Sherman N, Microbiology - a Laboratory Manual,The Benjamin/Cummings Pub. Co. Inc. NewYork, USA, 137–49, (1996)

[23] Gauri S, Ashok KS, and Kalpana B, Biodegradation of Polyethenes by Bacteria Isolated From Soil. International Journal of Research and Development in Pharmacy and Life Sciences, 5 (2): 2056-2062, (2016).

[24] Sowmya HV, Ramalingappa K, Thippeswamy MB, Biodegradation of Polyethene by Bacillus cereus. Advances in Polymer Science and

Technology, an International Journal, 4(2): 28-32, (2014).

[25] Holt JG, Krieg PA, Sneathm JT, Staley A, and Williams ST, Bergey's Manual of Determinative Bacteriology, 9thedn.Baltimore, MD: Williams and Williams. (1994).

[26] El-Shafei HA, El-Nasser HA, Kansoh AL, Ali AM, Biodegradation of disposable Polyethene by fungi and Streptomyces sp. Polym Degrad Stab 62: 361365, (1998).

[27] Volke-Sepulveda T, Saucedo-Castaneda G, Gutierrez-Rojas M, Manzur A, and Favela-Torres E, Thermally treated low density Polyethene biodegradation by Penicillium pinophilum and Aspergillus niger. Journal of Applied Polymer Science, 83: 305-314, (2002).

[28] Singh J, and Gupta KC, Screening and identification of low density Polyethene (LDPE) degrading soil fungi isolated from Polyethene polluted sites around Gwalior city (MP). International Journal of Current Microbiology and Applied Sciences, 3(6): 443-448. (2014).

[29] Rawat A, Satish N, Sita S, and Raju R, Biodegradation of polystyrene (B. Tech. Thesis). Gokaraju Rangaraju Institute of Engineering and Technology, Hyderabad, India. (2011).

[30] Umamaheswari S, and Murali M, FTIR spectroscopic study of fungal degradation of Poly (ethyleneterephthalate) and polystyrene foam. Elixir Chemical Engineering, 64: 19159-19164, (2013).

[31] Yoon MG, Jeon HJ, and Kim MN, Biodegradation of Polyethene by a Soil Bacterium and AlkB Cloned Recombinant Cell, Journal of Bioremediation Biodegradation, 3: 145, (2012).

[32] Arutchelvi J, Sudhakar M, Arkatkar A, Doble M, Bhaduri S, and Uppara PV. Biodegradation of Polyethene and polypropylene. Indian Journal of Biotechnology, 7: 9-22, (2008).

[33] Kathiresan K, and Bingham BL, Biology of mangroves and mangrove ecosystems. Advances in Marine Biology, 40: 812 - 817, (2001).

[34] Vatseldutt S, and Anbuselvi A, Isolation and characterization of Polyethene degrading bacteria from Polyethene dumped garbage. International Journal of Pharmaceutical Sciences Review and Research, 25(2): 205-206, (2014).

[35] Saminathan P, Sripriya A, Nalini K, Sivakumar T, and Thangapandian V, Biodegradation of plastics by Pseudomonas putida isolated from garden soil samples. Journal of Advanced Botany and Zoology, 1(3): 1-4, (2014).

[36] Mahdiyah BH, and Mukti A, Isolation of Polyethene plastic degrading bacteria. Biosciences International, 2(3), 29-32, (2013).

[37] Gupta SB, Amrita G, and Tapas C, Isolation and Selection of Stress Tolerant Plastic Loving Bacterial Isolates from Old Plastic Wastes, World Journal of Agricultural Sciences 6(2): 138-140, (2010).

[38] Kavitha R, Anju KM, and Bhuvaneswari V, Biodegradation of low density Polyethene by bacteria isolated from oil contaminated soil. International Journal of Plant, Animal and Environmental Sciences, 4(3): 601-610, (2014).

[39] Elisangela F, Andrea Z, Fabio DG, Cristiano RM, Regina DL, and Artur CP, Biodegradation of textile azo dyes by a facultative Staphylococcus arlettae strain VN-11 using a sequential micro aerophilic/aerobic process. International Biodeterioration and Biodegradation, 63(3): 280-288, (2009).