BIODEGRADATION OF BIOMEDICAL WASTE USING MICROBIAL CONSORTIUM FROM DUMPING YARD

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Abstract

Biodegradation of biomedical waste (LDPE) samples using microbial consortium of microorganisms was monitored as a function of number of days and the results were presented in Percentage weight loss due to degradation was determined by subtracting the weight of the sample taken out on a particular day i.e., 40 days of exposure from the initial weight ie., the weight of the sample at the start of degradation study each time. It was observed from the results that percentage weight loss by *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus subtilis* revealed 34.61, 26.92 and 15.38 %. Similarly, when the polymer LDPE was pretreated with UV exposure it was 46.15% for *Bacillus cereus* UV treated sample followed by *Bacillus licheniformis* exhibited 38.46% and *Bacillus subtilis* revealed 26.92 %. In the present study, bacteria cultured on nutrient broth medium until the mid - exponential phase was transferred to the test tube with constant volume of 1.2 ml to which increasing volumes of hexadecane were added. *Bacillus cereus* incubated in a liquid medium containing polyethylene as sole carbon source colonized the polyethylene and formed a sparse biofilm. BATH assay demonstrated that hydrophobicity of *Bacillus cereus* exhibited 89% and it was very high in compared to *Bacillus subtilis* and *Bacillus licheniformis*.

Key words:- Biodegradation, *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus subtilis*, Uv, BATH.

I. INTRODUCTION

Plastic usage is increasing day by day and the annual production is likely to exceed 300 million tons by 2010. The production of plastics has increased substantially over the last 60 years from around 0.5 million tons in 1950 to over 260 million tons today. In India alone every year 25 million tons of synthetic plastics are being accumulated in the sea coasts and terrestrial environment (Kaseem*et al*., 2012). Annually about 500 billion to trillion polythene carry bags are being consumed around the globe (Manisha sangale, *et al*., 2012).

Biomedical Waste, (BMW) or bio wastes are those potential hazardous waste materials, consisting of solids, liquids,sharps, and laboratory waste. Biomedical waste differs from other types of hazardous waste, such as industrial waste, in major sources of biomedical wastes is hospitals and nursing homes. Hospital is one of the complex institutions which are frequented by people from every walk of life in the society without any distinction between age, sex, race and religion. All of them produce waste which is increasing in its amount and type due to advances in scientific knowledge and iscreating its impact. The hospital waste, in addition to the risk for patients and personnel who handle these wastes poses athreat to public health and environment.

Classification

Approximately 75-90% of the biomedical waste is non-hazardous and as harmless as any other Municipal waste. The rest 10-25%, though mixed with non-hazardous waste, can be injurious to humans or animals and deleterious to environment. Biomedical wastes can be categorized based on their origin and physical, chemical or biological characteristics.

General waste, Pathological waste, Infectious waste, Sharps, Pharmaceutical waste, Chemical waste, Radioactive waste: It includes solid, liquid, and gaseous waste that is contaminated with radionuclides generated from in-vitro analysis of body tissues and fluid, in-vivo body organ imaging and tumour localization and therapeutic procedures. The main sources of biomedical waste are hospitals, medical clinics, laboratories and pharmaceutical factories. Other **Sources include: 1.** Blood donation camps, 2. Slaughter houses, 3. Cosmetic services, 4. Vaccination centres.

Management of Biomedical Waste

Due to the grave potential threats biomedical waste pose, managing and regulating its collection, storage, transportation, treatment and disposal method is essential. Safe disposal of biomedical waste is also a legal requirement in India. Polyethylene as a thin film, used as a packaging material, has found its place in usage in many industries and business houses because of its excellent tensile strength; it's resistance to microbes, low cost and easy availability (Ojeda *et al*., 2009). Most of the polyethylene, after serving was found on landfill sites. There it remains as it is due to its non-biodegradable nature (Albertson *et al*., 1987).

Polyethylene is classified into several different categories based mostly on its density and branching. Its mechanical properties depend significantly on variable such as the extent and type of branching, the crystal structure and the molecular weight. Plastic bags typically are made from one of three basic types: High Density Polyethylene (HDPE) Low Density Polyethylene (LDPE) and Linear Low Density Polyethylene (LLDPE). The thick glossy shopping bags are HDPE and garment bags from the dry cleaner are LDPE. The major difference between these materials is the degree of branching of the polymer chain; HDPE and LDPE are composed of linear, unbranched chain, while LDPE chains are branched. These synthetic polymers do not degrade in normal environmental conditions, this leads to the accumulation of polymeric waste in the environment is a cause for serious concern leading to long term environmental and waste management problems. One of the most destructive pollutants today is plastic waste accumulation. Thus an innovative solution to these problems is essential (Gnanavel, *et al.,* 2013).

In the present study municipal soil amended with polyethylene (Saline bottles) was collected for enrichment studies, isolate microorganisms from municipal garbage waste, screen the resistant bacteria isolates by mineral salt medium amended with polyethylene powder, and the isolates were characterized morphologically and biochemically, and to assess the degradation by determining the weight loss of residual polyethylene before and after degradation. Evaluation by the bacterial hydrophobicity by BATH assay to degrade the polymer in synthetic medium by pre-treatment methods such as UV treated polyethylene strips and to analyze the polyethylene degradation and to monitor functional group changes caused due to degradation by FTIR.

2. MATERIAL AND METHODS

2.1. Polyethylene

Branched low density polyethylene and linear high density polyethylene plastic bags was obtained commercially. Besides, LDPE bags amended in biomedical waste collected from municipal solid wastes and used in the present study.

2.2. Enrichment Culture technique

Bacterial strains isolated from compost mixture assayed for their ability to utilize polyethylene as the sole source of carbon and energy. Soil samples amended with polyethylene plastic bags isolated from municipal solid waste samples, was used as a source for the isolation of potential polyethylene degrading microorganisms. Plastic strips from saline bottles were cut (2x2 cm) and placed in an oven at 70^{o}C for 20 days in order to stimulate the thermophilic phase of a full scale composting process and to achieve material disinfection. Then, these strips were mixed with compost soil. This film was buried for 30 days at room temperature with 50% water holding capacity.

2.3. Isolation of polyethylene degrading bacteria from enriched soil sample:

Biomedical waste polyethylene degrading microorganisms were serially diluted and spread plated on nutrient agar and incubated for 24 hours at 37^{o}C. After incubation, individual bacterial colonies with different morphological characteristics were selected and restreaked on nutrient agar plates to obtain the pure culture of the isolates. The pure cultured strains were maintained in 20% glycerol stock.

2.4. Preparation of polyethylene powder for screening:

Biomedical waste polyethylene (LDPE) sheets were cut into small bits and immersed in xylene and boiled for 15 minutes. Xylene was added to dissolve the polyethylene and the residue was crushed while it was warm by hand with the help of gloves. The polyethylene powder obtained was washed with ethanol to remove residual xylene and allowed to evaporate [approximately 2 to 3 hours] to remove ethanol. The powder was dried in hot air oven at 60^{o}C overnight. The polyethylene powder was stored in closed containers in room temperature.

2.5. Screening of polyethylene Degrading Microorganisms by clear zone method:

The polyethylene degrading microorganisms were screened by using mineral salt medium amended with 0.1% of LDPE powder. After which the medium was sterilized at 121^{o}C and pressure for 15lbs for 20 minutes. About 20ml of sterilized medium was poured before cooling into the plates. The isolated organisms were inoculated on polymer containing agar plates and then incubated at 25 – 30^{0}C for 2- 4 weeks. The organisms producing zone of clearance around their colonies were selected for further analysis.

2.6. Morphological and Biochemical characterization of Isolates:

The morphological and biochemical characterization of the isolates from biomedical waste effluent was performed. The morphological characterization such as Gram staining, spore staining, Motility and colony morphology were studied. Biochemical characterization like IMViC, Triple sugar iron test, starch hydrolysis, nitrate reduction test, gelatin test and carbohydrate fermentation test were carried out.

2.7. Consortium Preparation

About 1 ml of the inoculum of isolated Biomedical waste polyethylene degrading microorganisms (BW-1, BW-2, BW-4) or loopful of microbes was inoculated in nutrient broth and incubated at 37ºC. These mixture of microbes was centrifuged at 3000 rpm and the pellet was used as consortium inoculum. Biodegradation of biomedical waste polyethylene bags by a mixed bacterial consortium in two different systems.

2.7.1. Biodegradation in synthetic medium - studies of UV treated LDPE

Biodegradation test were performed with polyethylene film biomedical waste polyethylene cut into 1x1 cm. The polyethylene LDPE strips were subjected to UV exposure (UV light, 254 nm wavelength for about 60 hours). The polyethylene films were disinfected in 70% ethanol and air dried for 15 minutes in laminar flow hood after which films were transferred to mineral salt medium containing 5% bacterial inoculum and incubated at 30 ±7ºC for 60 days. Simultaneously a set of control experimental flask were performed without bacterial culture. Biodegradation is assessed by estimating the changes in weight and compared with control. Biodegradation is assessed by estimating the changes in weight and by using FTIR.

2.8. Analysis of Biomedical waste polyethylene (LDPE)Biodegradation

2.8.1. Determination of weight loss of residual Biomedical Waste polyethylene

The Residual polyethylene particles were recovered from the broth cultures by passing through a coarse filter paper. To facilitate accurate measurement of the residual polyethylene, the bacterial biofilm adhering to the polyethylene surface was washed with 2% (v/v) aqueous sodium dodecyl sulphate (SDS) solution for 2-3 hours and then with distilled water. The washed polyethylene was then dried in an oven at 60 ± 2ºC to observe a constant weight. The dry weight of recovered polyethylene indicated the rate of biodegradation.

$$\text{Percentage of weight loss} = \frac{\text{Initial weight at the beginning} - \text{final weight after 20 days}}{\text{Initial weight at the beginning}} \times 100$$

2.8.2. Evaluation of bacterial hydrophobicity

Bacterial cell-surface hydrophobicity will be estimated by the bacterial adhesion to hydrocarbon (BATH) test (Rosenberg et al, 1980).

2.9. FTIR Analysis

The changes in the polyethylene structure following UV irradiation and heating and subsequent incubation with bacterial was analyzed by FTIR spectroscopy. LDPE/HDPE samples degraded by microorganisms were collected after 60 days of incubation. The LDPE / HDPE residue was air dried and used for FTIR analysis. LDPE / HDPE samples were milled with potassium's bromide (KBr) to form a very fine powder. This powder was then compressed into a thin pellet which can be analyzed. KBr is also transparent in the IR.

Two types of polyethylene samples were analyzed

i) Untreated control
ii) UV-irradiated PE films in synthetic medium and then incubated with bacterial consortium.

3.0. RESULT AND DISCUSSION

Polyethylene as a thin film, used as a packaging material, has found its place in usage in many industries and business houses because of its excellent tensile strength; it's resistant to microbes, low cost and easy availability (Ojeda *et al*., 2009). Most of the polyethylene, after serving was found on landfill sites. There it remains as it is due to its non-biodegradable nature (Albertson *et al*., 1987) and therefore creating serious environmental problems.

Hence, the present investigation was focused with much attention to isolate potential polyethylene degrading microorganism from municipal solid waste soil.

3.1. Enrichment and Isolation of microorganisms from municipal solid waste soil

The municipal solid waste soil samples were collected in sterile polythene bags from the municipal solid waste. The polyethylene strips (2 x 2 cm) which were incubated with municipal solid waste soil were enriched in the laboratory for two weeks. About 1 gram of the enriched soil sample was subsequently serially diluted and it was spread plated on to nutrient agar plate for the isolation of single colonies.

3.2.Screening of polyethylene degrading bacteria

The polyethylene degrading bacteria were screened in mineral salt agar amended with 0.1% of polyethylene powder and incubated the plates at 25 ºC–30ºC for 2 weeks. The organisms producing clear zone around their colonies after incubation were selected for further characterization and degradation studies. Among the four isolates only three efficient polyethylene degrading strains were selected and denoted as BW-1, BW-2 and BW-4.

3.4. Identification of the efficient polyethylene degrading microorganisms

On nutrient agar plate, BW-1 produced large, white colored raised opaque colonies with irregular margins. The organism was a gram positive, short rod shaped, spore forming organism. Further biochemical tests were conducted to ascertain the genus of the bacteria.

The results were depicted in (Table.1). Indole was not produced. It oxidized glucose with the production of acid end products in methyl red test. Acetoin and butane diol were not produced in analysis of voges proskauer test. Citrate was utilized as its sole carbon and energy source. Nitrates were reduced to nitrites. It hydrolyzed Gelatin and starch. The strain fermented sugars like dextrose and sucrose. From the results obtained, the strain BW-1 was confirmed as *Bacillus cereus*. The results were compared in accordance with the Bergey's manual of determinative bacteriology.

The strain BW-2 colonies become opaque with dull to rough surface. The aged cultures may become brown in color. BW-2 appeared as straight or slightly curved rods, gram positive and endospore forming organism. The isolate revealed negative results for Indole, methyl red and voges proskauer test. It utilized citrate as its sole carbon source. It reduced nitrates to nitrites. Starch and gelatin were hydrolyzed. The strain ferments sugars like dextrose, sucrose and mannitol. From the results observed, the strain –BW-2 was identified as *Bacillus licheniformis*. The feature agreed with description of the Bergey's manual of systematic Bacteriology.

The strain BW-4 was a gram positive, short rod shaped spore forming organism. The colonies are cream coloured, opaque, flat dry colonies with undulate margins on nutrient agar. Further biochemical tests were conducted to ascertain the genus of the bacteria. The results were depicted in (Table.2). Indole was not produced by the organism. Glucose was not oxidized to produce acid end products in methyl red test. Acetoin and butane diol were not produced in voges proskauer test. Citrate was utilized as its sole carbon source. Nitrates were reduced to nitrites. The organism utilized starch and gelatin. The strain fermented sugars like dextrose, Mannitol, fructose and sucrose. From the results, BW-4 was confirmed as *Bacillus subtilis*.

TABLE.1. MORPHOLOGICAL, PHYSIOLOGICAL AND CULTURAL CHARACTERISTICS OF THE ISOLATED POLYETHYLENE DEGRADING MICROORGANISMS

S. No	Characteristics	OBSERVATION		
		BW-1	BW-2	BW-4
1	**Gram's staining**	Gram positive rods	Gram positive, straight or slightly curved rods	Gram Positive Short rods
2	**Colony morphology**	Colonies are large raised opaque with irregular margins	Colonies become opaque with dull to rough surface. Aged cultures may become brown.	Cream coloured, opaque, flat dry colonies with undulate margins
3	**Motility**	Motile	Motile	Motile
4	**Spore staining**	Present	Present	Present

TABLE. 2. BIOCHEMICAL CHARACTERIZATION OF THE EFFICIENT POLYETHYLENE DEGRADING MICROORGANISMS *Bacillus cereus, Bacillus licheniformis* and *Bacillus subtilis*

S. No	Biochemical tests	*Bacillus cereus*	*Bacillus licheniformis*	*Bacillus subtilis*
1	**Indole production**	Negative	Negative	Negative
2	**Methyl red test**	Positive	Negative.	Negative.
3	**Voges Proskauer test**	Negative	Negative	Negative
4	**Citrate utilization**	Positive	Positive	Negative
5	**Triple sugar iron**	Alkaline slant	Alkaline slant	Alkaline slant
6	**Nitrate reduction**	Positive	Positive	Positive
7	**Gelatin hydrolysis**	Positive	Positive	Positive
8	**Starch hydrolysis**	Positive	Positive	Positive
9	**Utilization of sugars**			
	a) Glucose	Acid formation	Acid formation	Acid formation
	b) Sucrose	Negative	Acid formation	Acid formation
	c) Mannitol	Negative	Acid formation	Acid formation
	d) Lactose	Negative	Negative	Negative
	e) Fructose	Negative	Acid formation	Acid formation

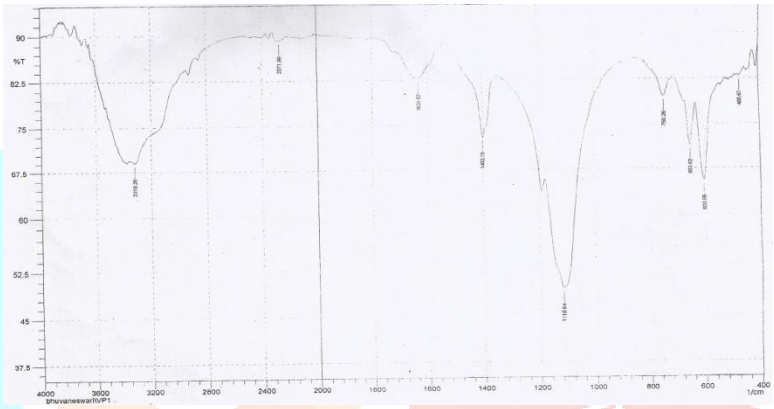
3.5. Consortium preparation for enhanced degradation

The microorganisms isolated from the compost mixture were mixed together and inoculated in nutrient broth and incubated at 37ºC. These mixture of microbes were centrifuged at 3000 rpm and the pellet was used as inoculums for bacterial consortium.

3.5.1. Analysis of Biodegradation of LDPE by Fourier Transform Infrared Spectroscopy

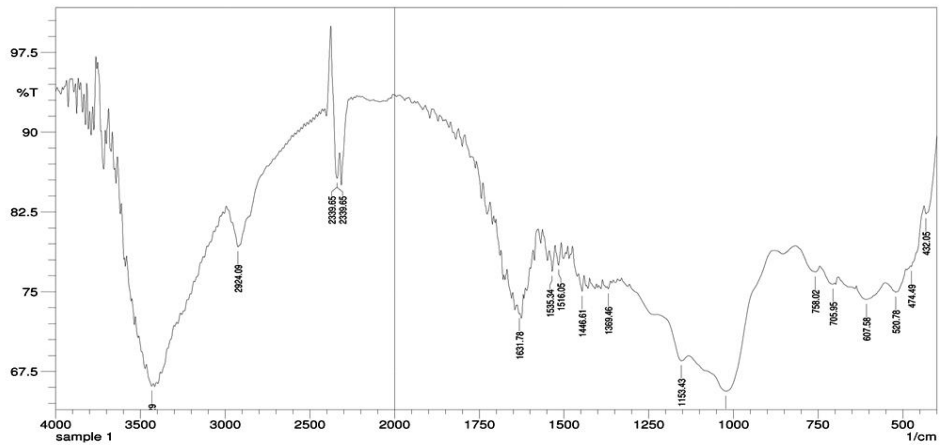
In order to enhance the LDPE degradation rate, was monitored in control flask. The FTIR spectra of LDPE strips with synthetic media and plastic strips (control) under laboratory condition cleaved the LDPE polyethylene strips into new groups. From the Figure. 1,The functional group modification occurred due to degradation of LDPE strips were performed from 400 to 4000 cm^{-1} wave number region. The absorption bands appeared at 3319.26 cm^{-1} has been attributed to O-H bond stretching. The band at 1631.67 cm^{-1} corresponds to the stretching of C=C bond which is due to the presence of benzene ring. The absorption band recorded at 1402.15 cm^{-1} is due to the stretching of C-H bonds which indicated the presence of methylene groups. Another absorption peak at 1118.64 cm^{-1} is due to the C – O bond stretching which leads to the formation of other groups (Fig. 1).

FIG.1. FTIR ANALYSIS OF CONTROL LDPE STRIPS TREATED WITH UV



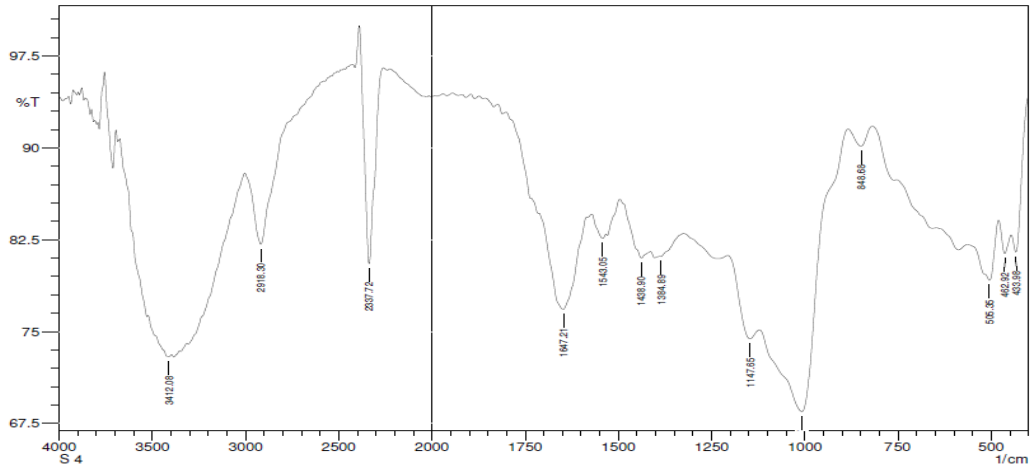
The UV irradiated pretreated LDPE strips on treatment with the bacterial consortium *Bacillus cereus* and *Bacillus subtilis* also exhibited characteristic absorption bands at 2924.09 cm^{-1} (C-H bond stretching), 2351.23 $cm^{-1}$and 2318.44cm^{-1} to O-C-O bond stretching 1668.43cm^{-1}, 1639.49cm^{-1} and 1541.12cm^{-1} to C=C bond stretching (Benzene ring), 1450.47 to C-H bond stretching (Methylene group). A prominent peak was observed at 1151.50cm^{-1} which indicated the C-O bond stretching formation of (ester group). The absorption band at 1014.56cm^{-1} indicated the C-H bond stretching. From the above FTIR spectrum, it was evident that the LDPE polymer is cleaved into various groups by the enzymatic machinery of consortium of microorganisms (Fig.2).

FIG.2. FTIR ANALYSIS OF (UV) LDPE STRIPS TREATED WITH CONSORTIUM OF ORGANISMS (*Bacillus cereus* and *Bacillus subtilis*)



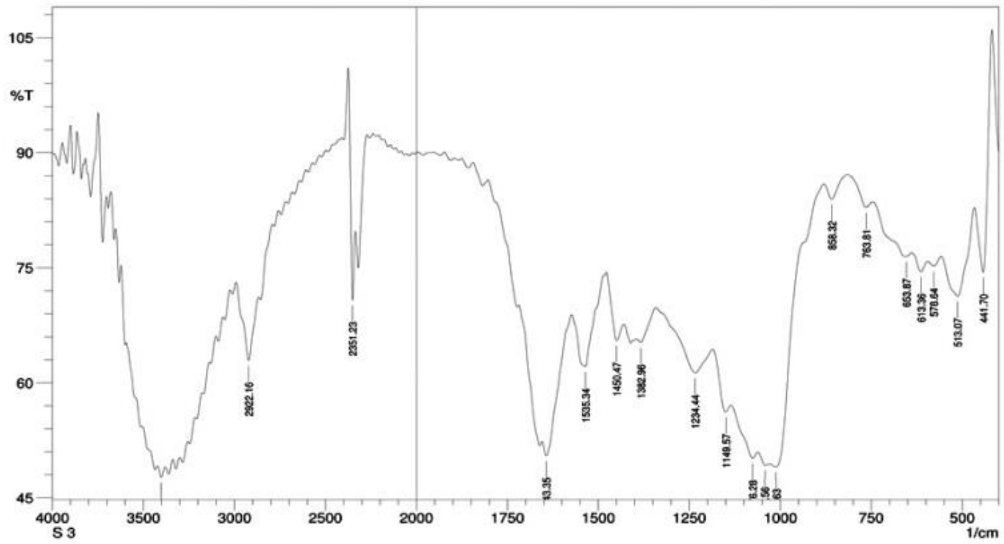
The UV irradiated pretreated LDPE strips on treatment with the bacterial consortium *Bacillus cereus* and *Bacillus subtilis* also exhibited characteristic absorption bands at 2924.09 cm^{-1} (C-H bond stretching), 2351.23 $cm^{-1}$and 2318.44cm^{-1} to O-C-O bond stretching 1668.43cm^{-1}, 1639.49cm^{-1} and 1541.12cm^{-1} to C=C bond stretching (Benzene ring), 1450.47 to C-H bond stretching (Methylene group). A prominent peak was observed at 1151.50cm^{-1} which indicated the C-O bond stretching formation of (ester group). The absorption band at 1014.56cm^{-1} indicated the C-H bond stretching. From the above FTIR spectrum, it was evident that the LDPE polymer is cleaved into various groups by the enzymatic machinery of consortium of microorganisms (Fig.3).

FIG. 3. FTIR ANALYSIS OF (UV) LDPE STRIPS TREATED WITH CONSORTIUM OF ORGANISMS (*Bacillus cereus* and *Bacillus licheniformis*)



The FTIR spectra of LDPE strips after inoculation with the consortium of *Bacillus subtilis* and *Bacillus licheniformis*revealed a prominent adsorption peak at 3402.43 cm^{-1} indicated the O-H bond stretching. The peaks observed at 2922.16 cm^{-1} which has been attributed to C-H bond stretching (methylene group), 2351.23 cm^{-1} to O-C-O bond stretching, 1643.35 cm^{-1} and 1535.34 cm^{-1} to C=C stretching, 1450.47 cm^{-1}, 1382.96 cm^{-1}, 1076.28 cm^{-1} and 1012.63 cm^{-1} corresponds to C-H bond stretching, 1149.57 cm^{-1} to C-O bond stretching and 858.32 cm^{-1} to C=C bond stretching (Benzene ring) (Fig. 4).

FIG. 4. FTIR ANALYSIS OF NORMAL LDPE STRIPS TREATED WITH CONSORTIUM OF ORGANISMS (*Bacillus subtilis* and *Bacillus licheniformis*)



3.5.2.Determination of weight loss of LDPE polyethylene after 60 days of exposure

Biodegradation of LDPE samples using individual microorganisms and consortium of microorganisms was monitored as a function of number of days and the results were presented in Table. 3. Percentage weight loss due to degradation was determined by substracting the weight of the sample taken out on a particular day i.e., 60 days of exposure from the initial weight ie., the weight of the sample at the start of degradation study each time. It was observed from the table that percentage weight loss by *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus subtilis* revealed 34.61, 26.92 and 15.38 %. Similarly, when the polymer LDPE was pretreated with UV exposure it was 46.15% for *Bacillus cereus* UV treated sample followed by *Bacillus licheniformis* exhibiting 38.46% and *Bacillus subtilis* revealed 26.92 %.

TABLE: 3: WEIGHT LOSS OF LDPE POLYETHYLENE BEFORE AND AFTER BIODEGRADATION (EXPOSED TO 60 DAYS)

Organisms	Before Degradation (In mg)	After Degradation (In mg)	Weight of PE degraded (In mg)	Percentage of PE degraded (In %)
BIODEGRADATION BY BACTERIAL CONSORTIUM				
B.C+B.L	0.026	0.015	0.011	42.31
B.C+B.S	0.026	0.017	0.009	34.61
B.L+B.S	0.026	0.018	0.008	30.77
B.C+B.L(UV)	0.026	0.013	0.013	50
B.C+B.S(UV)	0.026	0.016	0.010	38.46
B.L+B.S(UV)	0.026	0.017	0.009	34.62

B.C = *Bacillus cereus,* B.L = *Bacillus licheniformis*, B.S = *Bacillus subtilis,* UV =ultraviolet radiated LDPE strips.

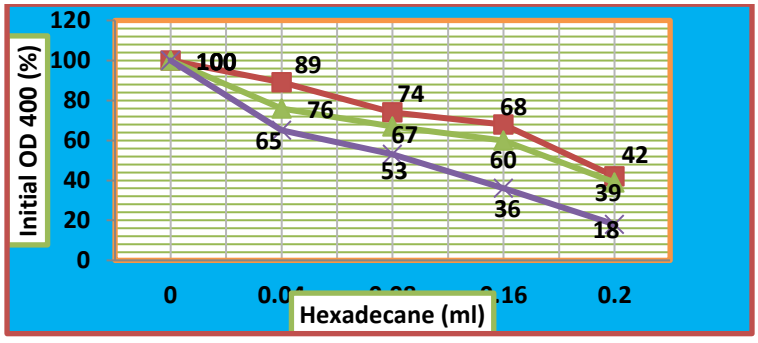
3.6. Determination of bacterial hydrophobicity by BATH assay

Bacterial cell adhesion to hydrocarbon (BATH test) (Rosenberg *et al*., 1980), which is based on the affinity of bacterial cells for an organic hydrocarbon such as hexadecane. The more hydrophilic the bacterial cells, the greater affinity for the hydrocarbon, resulting in transfer of cells from aqueous suspension to the organic phase and a consequent reduction in the turbidity of the culture. In the study, bacteria cultured on Nutrient broth medium until the mid - exponential phase and was transferred to the test tube with constant volume of 1.2 ml to which increasing volumes of hexadecane were added. *Bacillus cereus* incubated in a liquid medium containing polyethylene as sole carbon source colonized the polyethylene and formed a sparse biofilm. BATH assay demonstrated that hydrophobicity of *Bacillus cereus* exhibited 89% and it was very high when compared to *Bacillus subtilis* and *Bacillus licheniformis*. The increasing volume of hexadecane led to decrease in the percentage of bacterial hydrophobicity. (Table:-4 and Fig: 5).

TABLE.4 : HYDROPHOBICITY OF BACTERIAL ISOLATES DETERMINED BY BACTERIAL ADHESION TO HYDROCARBON TEST

S. No	Volume of culture (ml)	Volume of hexadecane (ml)	Bacterial hydrophobicity (%)		
			Bacillus cereus	*Bacillus licheniformis*	*Bacillus subtilis*
1	1.2	0	100	100	100
2	1.2	0.04	89	76	65
3	1.2	0.08	74	67	53
4	1.2	0.16	68	60	36
5	1.2	0.2	42	39	18

FIG. 5. HYDROPHOBICITY OF BACTERIAL ISOLATES DETERMINED BY BACTERIAL ADHESION TO HYDROCARBON TEST



BW-1: *Bacillus cereus*, BW-2: *Bacillus licheniformis*, BW-4: *Bacillus subtilis*

Plate: 1. Sample collection from Biomedical waste

Plate: 2. Sample collection of Biomedical waste (saline bottles)

Plate: 3. Plastic strips of Biomedical waste

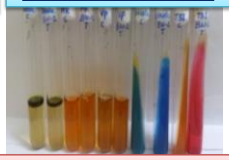
Plate: 4. Isolation of BMW Microorganisms

Plate:5. Pure culture of BMW Microorganisms

Plate: 6,7,8:-Biochemical characterization of BMW polyethylene Degrading Microorganism -BW-1 BW-2 BW-4

IMViC and TSI -Test

IMViC and TSI -Test



IMViC and TSI -Test

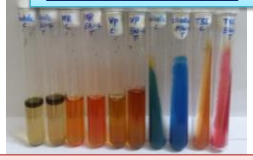
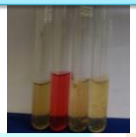


Plate: 9,10,11: Biochemical characterization of Biomedical waste polyethylene degrading Microorganisms

Nitrate and Gelatin Test- BW-1



Nitrate and Gelatin Test- BW-2

Nitrate and Gelatin Test- BW-2

Plate: 12. Biodegradation of biomedical waste polyethylene using microbial consortium (*Bacillus cereus* + *Bacillus licheniformis*)

Plate:13. Biodegradation of Biomedical Waste Polyethylene Using Microbial Consortium (*Bacillus Cereus* + *Bacillus Subtilis*)

Plate: 14. Biodegradation of Biomedical waste Polyethylene using Microbial consortium (*Bacillus licheniformis* + *Bacillus subtilis*)

In the present study, with a focus of attention to investigate the biodegradative potential of bacteria isolated from biomedical waste LDPE is known for being a remarkably resistant polymer to degradation. In the present study serial dilution of enriched soil sample was performed and spread plated on nutrient agar plate after incubation. The colonies with different morphological characteristic study were selected and re-streaked on nutrient agar are obtained new colonies. The colonies was found gram positive rod with endospore, motile, white the regular colonies.

The polyethylene degrading bacteria were screened in mineral salt agar amended with 0.1% of polyethylene powder and incubated the plates at 25 – 30°C for 2 weeks. The organisms producing clear zone around their colonies after incubation were selected for further characterization and degradation studies. Among the four isolates only three efficient polyethylene degrading strains were selected and denoted as BW-1, BW-2 and BW-4. The bacterial cultures were maintained in nutrient agar slants and 20% glycerol stock for further use.

The isolated microorganisms were characterized by morphological and biochemical characterization results were discussed with Ausubel, *et al*., 1992.

The microorganisms isolated from the compost mixture were mixed together and inoculated in nutrient broth and incubated at 37ºC. These mixtures of microbes were centrifuged at 3000 rpm and the pellet was used as inoculums for bacterial consortium. In order to enhance the LDPE degradation rate, was monitored in control flask. The FTIR spectra of LDPE strips with synthetic media and plastic strips (control) under laboratory condition cleaved the LDPE polyethylene strips into new groups. Similar result was obtains which similar to the studies made by (Anjana Sharma and Amitabh Sharma, 2003).

The UV irradiated pretreated LDPE strips on treatment with the bacterial consortium *Bacillus cereus* and *Bacillus subtilis* also exhibited characteristic by FTIR. Our findings were similar with Esmaeili, *et al.,* 2013.

The FTIR spectra of LDPE strips after inoculation with the consortium of *Bacillus subtilis* and *Bacillus licheniformis* were confirmed by FTIR analysis. The above result were in total conform ting to the finding of (Sowmiya, 2014).

Biodegradation of LDPE samples using microbial consortium of microorganisms was monitored as a function of number of days and the results were presented in Percentage weight loss due to degradation was determined by subtracting the weight of the sample taken out on a particular day i.e., 60 days of exposure from the initial weight ie., the weight of the sample at the start of degradation study each time.

It was observed from the results that percentage weight loss by *Bacillus cereus, Bacillus licheniformis* and *Bacillus subtilis* revealed 34.61, 26.92 and 15.38 %. Similarly, when the polymer LDPE was pretreated with UV exposure it was 46.15% for *Bacillus cereus* UV treated sample followed by *Bacillus licheniformis* exhibiting 38.46% and *Bacillus subtilis* revealed 26.92 %. Similar result was also reported by (Gu, 2000).

Several methods were employed to monitor the biodegradation of polyethylene. Rosenberg et al, 1980 have described BATH to estimate the bacterial cells surface hydrophobicity that can be directly related to their ability to form an effective biofilm over any hydrophobic surfaces. This test was followed by Hadad *et al*, 2005 wherein the results show low reduction in turbidity of the bacterial suspension.

Bacterial cell adhesion to hydrocarbon (BATH test) (Rosenberg *et al*., 1980), which is based on the affinity of bacterial cells for an organic hydrocarbon such as hexadecane.

4. CONCLUSION

In the present study was focus to investigate biodegradation potential of microorganism isolated from municipal solid waste (LDPE) was carried out by serial dilution, plated on nutrient agar, colonies obtained was characterized morphologically and biochemically, which was found to be *Bacillus cereus, Bacillus licheniformis,* and *Bacillus subtilis.* The study was extended with respect to degradation by flask a culture experiment which was carried for 60days of incubation to analyze the degradation pattern of LDPE by determining the weight variables. However, measurement itself cannot be a reliable indication of material degradability. Since both increase in weight and weight loss of polymer chains may occur. Besides, deterioration of polymers can also be evaluated by change in rheological properties. Among them, FTIR spectroscopy is most widely used in determining structural changes in macromolecules due to functional group modification during biodegradation. The UV irradiated pretreated LDPE strips on treatment with the bacterial consortium *Bacillus cereus, Bacillus licheniformis* and *Bacillus subtilis* were studied.

REFERENCES

[1] Albertson, A., Andersson, S.O. and Karlsson, S. 1987. The shape of the biodegradation curve for low and high density polyethylenes in prolonged series of experiments. EurPolym J**,** 16:623-630.

[2] Gnanavel, V. Mohana JeyaValli, M., Thirumarimurugan, P. and Kannadasan, T. 2013. Degradation of plastics waste using microbes. Elixir Chem. Engg. 54, 12212-12214.

[3] Hadad, D., Geresh and Sivan, A. 2005. Biodegradation of polyethylene by the thermophilic bacterium *Brevibacillusborstelensis.* Journal ApplMicrobiol. 98: 1093-1100.

[4] Kaseem, M., Hamad, K. and Deri, F. 2012. Thermoplastic starch blends. A review of recent works. Polymer SciSer A Chem Mat Sci. 54: 165-176.

[5] Manisha Sangale, K., Mohad Shahnawaz and Avinash Ade, B. 2012. A review on biodegradation of polythene: the microbial approach. Bioremediation and Biodegradation. 3(164): 1-9.

[6] Ojeda, T., Dalmolin, E., Strong, M., Jacques, R., Benedict, F., Camargo, F. 2009. Abiotic and biotic degradation of oxo-biodegradable polyethylenes. *Polym Degrad and Stab*, 94:965-970.

[7] Rosenberg, M., Gutnik, D., Rosenberg, E. 1980. Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity. FEMS MicrobiolLett 9:29–33

[8] Anjana Sharma and Amitabh Sharma 2003. Degradation of LDPE and polythene by an indigenous isolate of Pseudomonas stutzeri. Journal of Scientific and Industrial Research, 66(1): 293-296.

[9] Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K. 1992. Short protocols in molecular biology, 2nd edn. Wiley, New York

[10] Esmaeili, A., Pourbabaee, A.A., Alikhani, H.A., Shabani, F., Esmaeili, E. 2013. Biodegradation of Low-Density Polyethylene (LDPE) by Mixed Culture of Lysinibacillus xylanilyticus and *Aspergillus niger* in Soil. Plos one. 8(9): e71720. doi:10.1371/journal.pone.0071720

[11] Gu, J.D. 2003. Microbiological deterioration and degradation of synthetic polymeric material:recent research advances. Int Biodeterior Biodegrad 52:69-91.