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Exploration on Biodegradability of Polyethylene Bags By *Bacillus Cereus* Isolated From Dumpsite Area

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Abstract:
Polythene plays an important role in packaging of goods, food material, medicine and garbage bags etc but its degradation is becoming a great threat and vital cause of environmental pollution. There are various polythene degradation methods available but the eco-friendly and acceptable method is by using microbes. The present study deals with the isolation, identification, screening and degradation of pretreated polythene by Bacillus cereus obtained from dumpsite soil. This organism was able to degrade both black and white polyethylene. Efficiency of Bacillus cereus to use autoclaved polyethylene as sole source of carbon was much better than UV treated polyethylene. Degradation was monitored by weight loss and FTIR studies. Bacillus cereus was able to degrade UV treated black and white polythene by 5% and 7.31% respectively, whereas autoclaved black and white polythene showed 9.61% and 12.69% of weight loss respectively. Hence the present studies showed that UV treatment which was given for 30 minutes was relatively ineffective in improving the biodegradability of both white and black polythenes. However autoclaving the polythenes at 121°C at 15lbs for 30 minutes helped in improving the biodegradability of both white and black polythene. Hence it can be concluded that autoclaving polythene bags is more effective than giving them UV treatment before biodegradation by Bacillus cereus. Therefore this method of autoclaving polythene bags may be used widely for biodegradation and serve as a promising tool for the elimination of polythene from the environment.
Keywords: Biodegradation, Bacillus cereus, polyethylene

Introduction:

Plastic is the most versatile synthetic 'manmade' substance created out of the fossil fuel resources that enable most of the industrial and technological revolutions of the 19th and 20th centuries. During the past 25 years, plastic materials have gained widespread use as they have been increasingly used in food, clothing, shelter, transportation, construction, medical and leisure industries Plastics are composed of petroleum based materials called resins (e.g., polythene and polypropylene) materials that are resistant to biodegradation. Due to this resistance, plastics that are disposed in landfills remain in their original form in perpetuity.

In the recent years there has been growing public concern over environmental deterioration associated with the disposal of conventional plastics. Discarded plastics, besides being highly visible are a rapidly increasing percentage of solid waste in landfills, resistant to biodegradation leading to pollution, harmful to the natural environment.

The degradation of polyethylene can occur by different molecular mechanisms such as chemical, thermal, photo and biodegradation [4]. Biodegradation of polyethylene is known to occur by two mechanisms: Hydro-biodegradation and oxo-biodegradation (Bonhomme *et al.*, 2003). These two mechanisms agree with the modification due to the two additives, starch and pro – oxidant, used in the synthesis of biodegradable polyethylene. Starch blend polyethylene has a continuous starch phase that makes the material hydrophilic and therefore, catalyzed by amylase enzymes. Microorganisms can easily access, attack and remove this part. Thus the hydrophilic polyethylene matrix continues to be hydro-biodegraded. In case of pro-oxidant additive, biodegradation occur following photo degradation and chemical degradation. If the pro-oxidant is a metal combination, after transition, metal catalyzed thermal per oxidation, biodegradation of low molecular weight oxidation products occurs sequentially (Bonhomme *et al.*, 2003; El-Shafei *et al.*, 1998; Yamada-Onodera *et al.*, 2001).

Biodegradability is evaluated by weight loss, tensile strength loss, changes in percent elongation and changes in polyethylene molecular weight distribution. Physicochemical distribution is initiated by treatment with acid at 70°C and UV irradiation of the polyethylene film. These pre-treatment favours the microbial degradation of polyethylene. The solid waste related problems pose threat to mega cities. El-Shafei *et al* (1998) investigated the ability of fungi and *Streptomyces* strains to attack degradable polyethylene consisting of disposed polyethylene bags containing 6% starch. He has isolated 8 different strains of *Streptomyces* and fungi Mucor rouxii NRRL 1835 and Aspergillus flavus.

Worldwide utilization of polyethylene is increasing at a rate of 12% per annum and approximately 140 million tonnes of synthetic

polymers are produced each year (Shimao, 2001). It takes thousand years for their efficient degradation. Huge amount of polythene getting accumulated in the environment, so their disposal creates a big problem in terms of ecology. Some possible methods are there for this purpose are biodegradation and biorecycling (Yang et al.,2005).

Currently enzymatic degradation is most widely used methods for plastics waste treatment. This method of biodegradation by microbial enzymes increases the rate of degradation of plastics without causing any harm to the environment (Bhardwaj et al, 2012). Polythenes in large amount get accumulated in the environment and thus create environmental issue. It is necessary to degrade polythene from atmosphere so, an attempt has been made in this investigation to isolate the potent bacterium i.e. Bacillus cereus that degrades polyethylene from the soil of dumpsite area.

MATERIALS AND METHODS:

1.Collection of soil sample: Soil sample was collected from a local dumpsite of Nagpur district and brought to the laboratory, preserved under laboratory conditions for further use.

2.Isolation and identification of *Bacillus cereus* from soil:

- **Serial dilution method:** 1.0 gram of soil sample was transferred into a conical flask having 99ml of sterile distilled water. The mixture was shaken and serially diluted (Cappuccino and Sherman., 1996).
- **Petri plate method:** Further the Isolation of microorganism were carried out by spreading the dilution and the polythene strips of 3×3cm were cut and placed on the nutrient agar plates. After the incubation the growth of microorganism were seen on the polythene strips.
- **Screening of polythene degrading microorganism:** This was carried out by zone of clearance method where the 0.5 concentrations of PEG were used in minimal media containing 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 polythene as C-source and degrade polythene (Sowmya et al., 2014)
- **Characterization and identification of microorganism:** After screening the isolates were characterized by various morphological and biochemical test, according to Bergey's manual of determinative bacteriology (Holt et al., 1994). Preliminary identified isolate was further confirmed by inoculating it on the Hi-Bacillus identification kit. Furthermore the identified isolate was confirmed by 16s rDNA sequencing.

3.Degradation of Polyethylene: The pre-weighed discs of Autoclaved, Surface sterilized and UV treated polyethylene of 1cm diameter prepared from polyethylene bags were aseptically transferred to the conical flask containing 50ml of Mineral Salt Medium. Loop full of organisms was added to medium. Control was maintained with polyethylene discs in the microbe free medium. Triplicates were maintained for each type of polyethylene and left on shaker. After three months of incubation, the plastic discs were collected, washed thoroughly using distilled water, dried in hot air oven at 50ºC over night and then weighed for final weight then weighed and percentage weight loss were calculated using below formula. (Usha, et al. 2011)(Kathiresan, K. 2003).

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

4.Confirmation of polyethylene degradation: Polyethylene degradation was confirmed by using Scanning Electron Microscopy (SEM) and Fourier Transform Infrared (FTIR) Spectroscopy.

RESULTS:

- **Screening of polythene degrading microorganism Primary screening:** In this procedure zone of clearance method was observed by staining with Coomassiee blue where the 0.5 concentrations of PEG was used in minimal media containing salts of ammonium and potassium.
- **Secondary screening:** In this process the zone of clearance was observed by adding 1.0 concentrations of PEG followed by staining with Coomassiee blue.
- **Identification and Confirmation of Bacillus cereus:** The Bacillus cereus was identified by Morphological, biochemical (Table 1 & 2)and cultural characteristics and confirmed by Hi-bacillus identification kit (Table 3), and 16s rDNA sequencing (Table 4).
- **Degradation of autoclaved polyethylene** *Bacillus cereus* was able to degrade autoclaved white and black polyethylene, indicating its capacity to use polyethylene as sole carbon source. The weight loss for autoclaved black polyethylene was 9.61% and for white polyethylene was 12.69% (Graph 1).
- **Degradation of UV treated polyethylene:** *Bacillus cereus* was able to degrade UV treated polyethylene. The weight loss for UV treated white and black polythene was found to be 7.31% and 5% respectively. Degradation was here because of pre treatment using UV light. UV light is known as initiator of polyethylene oxidation and enhances the bacterial

degradation when compared with its corresponding UV untreated control (Lee, B et al., 1991). The bacterial attachment was found on the surface of the plastic and it indicates possible utilization of plastic as carbon source. From the above results it was seen that Autoclaved black polythene underwent the maximum weight loss than that of autoclaved white and UV treated white and black polythene respectively. Hence Autoclaved White polythene showed the best results.

- **Confirmation of polythene degradation using FTIR:** The white and black polythene pieces from the control and experimental sets were subjected to FTIR analysis. The results are presented in Graphs 2 to 9. The FTIR spectrum of all the samples showed four major peaks: cis-di substituted alkenes (675-730 cm^{-1}), mono substituted alkene (985-1000 cm^{-1}), nitrogroup (1870-1500 cm^{-1}), sp^3 C-H stretch (2800-3000 cm^{-1}).The UV treated experiments did not yield any major changes in both white and black polythene. Though UV treated White polythene showed 16% reduction is cis-di substituted alkenes but it was negligible while UV treated Black polythene did not show any change at all.

In Autoclaved experiments, on the other hand both the white and black polythene studies showed good degradation using *Bacillus cereus* as inoculum. The white polythene showed around ~59% reduction in the transmittance due to cis-di substituted alkene, ~67% reduction in the transmittance due to nitrogroup, ~58% reduction in transmittance at 1700 cm^{-1} and ~60% reduction in the transmittance in the range 3250-3750 cm^{-1}. Similarly, the black polythene showed around ~50% reduction in transmittance due to cis-di substituted alkene, ~64% reduction in transmittance due to nitro group, ~38% reduction in transmittance at 1700 cm^{-1} and ~60% reduction in transmittance at 3200-3700 cm^{-1}.

Table 1: Morphological and Biochemical Characterization of Isolate

Sr. No	Test	Bacillus Cereus
1.	Gram Staining	Gram Positive
2.	Motility	Motile
3.	Microscopic Character	Rod Shaped
4.	Glucose Fermentation	Positive
5.	Lactose Fermentation	Negative
6.	Mannitol Fermentation	Negative
7.	Indole Production	Negative
8.	Methyl Red Test	Positive
9.	Voges Proskauer Test	Positive
10.	Citrate Utilization	Positive

Table 2: Results shown by isolate on TSI slant

TSI	Acid	Gas	H_2S
Slant	Negative (Red)	Negative	Negative
Butt	Positive (Yellow)	Negative	Negative

Table 3: Biochemical Characterization of Isolate by Hi-Bacillus Identification Kit.

Sr. No	Test	Bacillus Cereus
1.	Malonate	Negative
2.	Voges Proskauer's	Positive
3.	Citrate	Positive
4.	ONPG	Negative
5.	Nitrate Reduction	Negative
6.	Catalase	Positive
7.	Arginine	Negative
8.	Sucrose	Negative
9.	Mannitol	Negative
10.	Glucose	Positive
11.	Arabinose	Negative
12.	Trehalose	Positive

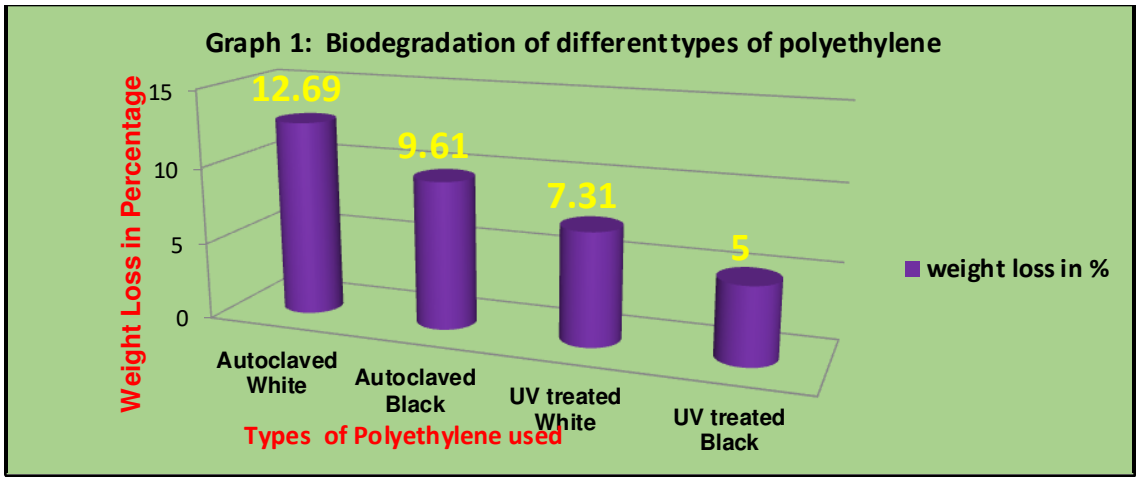
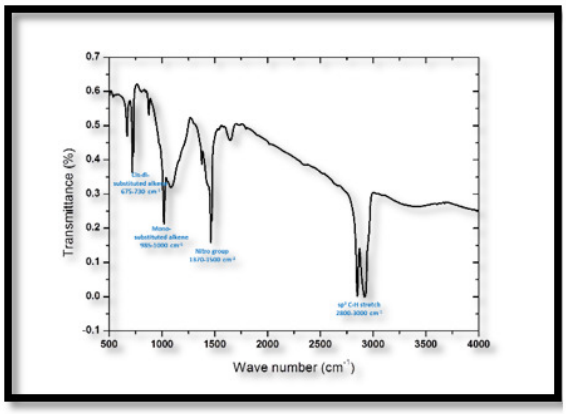
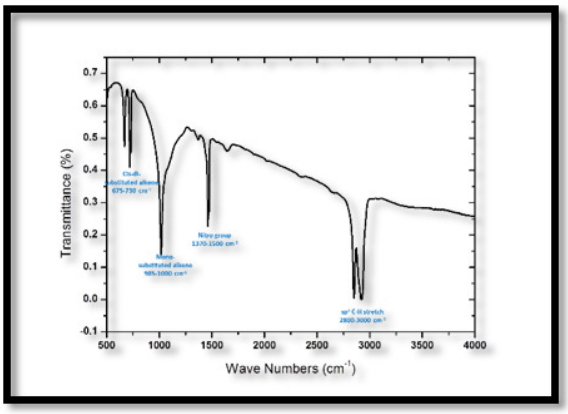


Table 4: 16s rDNA Sequencing confirms *Bacillus cereus*

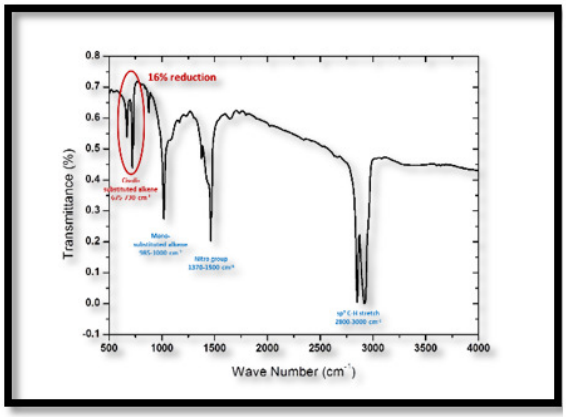
Sr. No.	Isolate Id/ Remark	Lab Id	Sequence data	Query cover	Identity (%)	BLASTidentity
1.	**Bacillus** (Initially, it was contaminated; purified and used for identification)	**VBN 1**	>TCAGCGCCTCCCAGGCGGAGTGCTTATGCGTTAACTTCAGC ACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCG TTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCC CCACGCTTTCGCGCCTCAGTGTCAGTTACAGACCAGAA AGTCGCCTTCGCCACTGGTGTTCCTCCATATCTCTACGCATT TCACCGCTACACATGGAATTCCACTTTCCTCTTCTGCA CTCAAGTCTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCG TGGGCTTTCACATCAGACTTAAGAAACCACCTGCGCGC GCTTTACGCCCAATAATTCCGGATAACGCTTGCCACCTACGT ATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTT CTGGTTAGGTACCGTCAAGGTGCCAGCTTATTCAACTAGCAC TTGTTCTTCCCTAACAACAGAGTTTTACGACCCGAAAG CCTTCATCACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCC ATTGCGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGT CTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTC AGGTCGGCTACGCATCGTTGCCTTGGTGAGCCGTTACCT CACCAACTAGCTAATGCGACGCGGGTCCATCCATAAGTGACA GCCGAAGCCGCCTTTCAATTTCGAACCATGCGGTTCAA AATGTTATCCGGTATTAGCCCCGGTTTCCCGGAGTTATCCCA GTCTTATGGGCAGGTTACCCACGTGTTACTCACCCGTC CGCCGCTAACTTCATAAGAGCAAGCTCTTAATCCATTCGCTC GACTTGCATGTATTAGGCACGCCGCCAGCGTTCATCCT GAGCCATGATCCAAACTACTGGG	98	99	*Bacillus cereus*



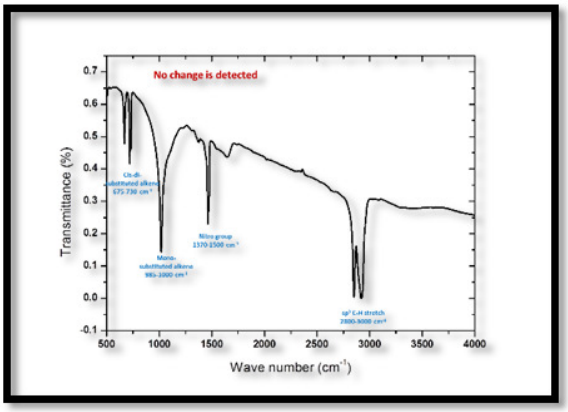
Graph 2: UV treated white polythene CONTROL



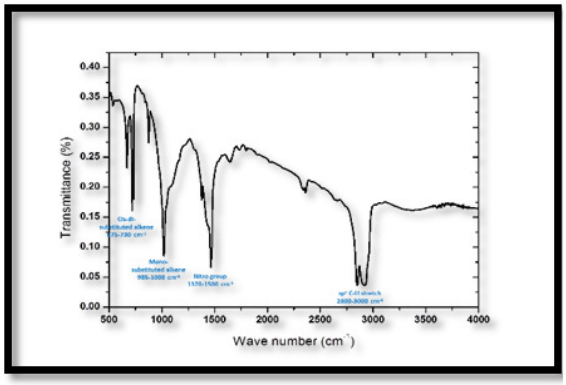
Graph 4 : UV treated black polythene CONTROL



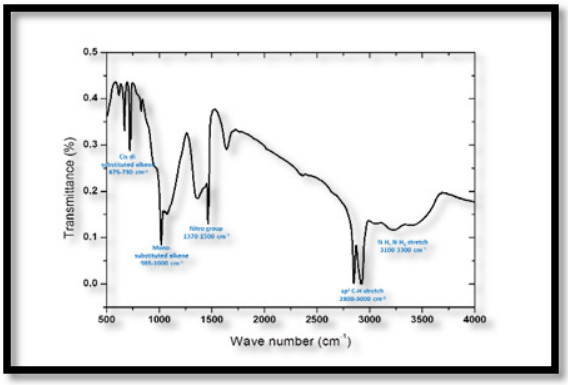
Graph 3 : UV treated white polythene EXPERIMENTAL



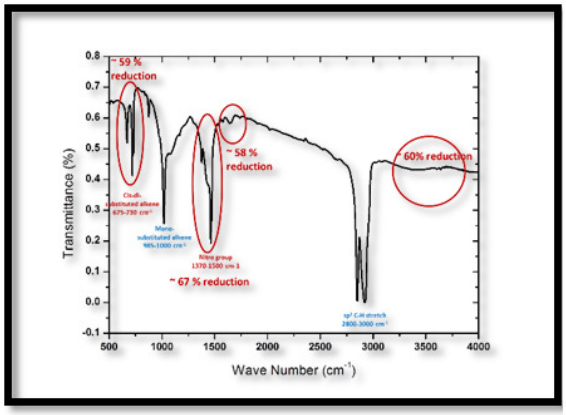
Graph 5 : UV treated black polythene EXPERIMENTAL



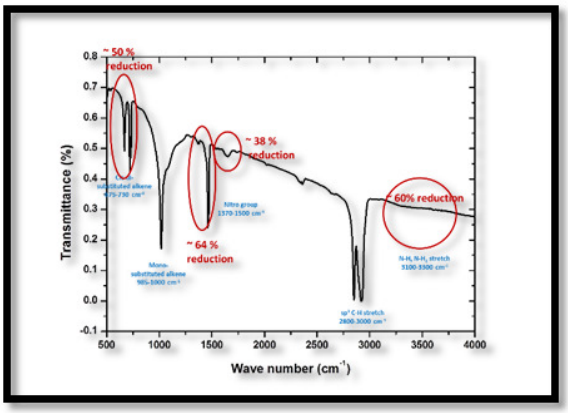
Graph 6 : Autoclaved white polythene CONTROL



Graph 8 : Autoclaved black polythene CONTROL



Graph 7 : Autoclaved white polythene EXPERIMENTAL



Graph 9 : Autoclaved black polythene EXPERIMENTAL

- **DISCUSSION:**

Microorganisms play a vital role in biological decomposition of materials, including synthetic polymers in natural environments. In the depolymerization process two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerises (Gu et al., 2000). During degradation, exo-enzymes from microorganisms break down complex polymers yieldingsmaller molecules of short chains, e.g., oligomers, dimers, and monomers, and are smaller that can pass the semipermeable outer membranes of the microbes, and then utilized as carbon and energy sources (Frazer, 1994; Hamilton et al., 1995).

In the current study, two types of polythenes were used for the observing the degradation percentage and those were white polythene and black polythene.

In present investigation *Bacillus cereus* was isolated and identified as Gram positive rod. Biochemical test performed also confirmed organism. Further tests were done by using HI-Bacillus identification kit and later on for confirmation purpose the isolate was sent for 16s rDNA testing. This confirmed the isolate to be *Bacillus cereus*. After the growth of *Bacillus cereus* on polyethylene containing medium, it was screened for degradation of autoclaved and UV treated both black and white polythene bags. *Bacillus cereus* was able to degrade UV treated black and white polythene by 5% and 7.31% respectively, whereas autoclaved black and white polythene showed 9.61% and 12.69% of weight loss respectively. After the treatment of polyethylene with by autoclaving, *Bacillus cereus* was able to degrade it more efficiently.

The present study showed that UV treatment was ineffective in improving the biodegradability of both white and black polythene bags as it showed negligible changes in the FTIR spectrum. However autoclaving both the polythenes helped in improving the biodegradability of both white and black polythene.

In previous findings it was seen that *Bacillus cereus* was isolated and identified as Gram positive rod. Biochemical test performed also confirmed organism. *Bacillus cereus* was grown on medium containing polyethylene and agar.

After the growth of *Bacillus cereus* on polyethylene containing medium, it was screened for degradation of autoclaved, UV treated and surface sterilized polyethylene. *Bacillus cereus* was able to degrade UV treated polyethylene (14%) more efficiently than autoclaved (7.2%) and surface sterilized (2.4%). After the treatment of polyethylene with UV light, *Bacilluscereus* was able to degrade it more efficiently. Carbonyl groups are produced by UV light or oxidizing agents and these groups are the main factors at the beginning of the degradation, being attacked by microorganisms that degrade the shorter segments of polyethylene chains. (Albertsson, et al., 1987).

Nanda S, et al in 2010 carried a study on degradation of natural and synthetic polyethylene using 3 species of *Pseudomonas*. Natural polymer contained 6% of vegetable starch. Degradation was monitored by weight loss. *Pseudomonas* sp. isolated from sewage sludge sump was found efficient in degradation with 46.2% for natural and 29.1% for synthetic polyethylene. In contrast *Pseudomonas* sp. from household garbage dump was lowest in degradation with 31.4% of natural and 16.3% of synthetic. *Pseudomonas* sp. from textile effluent drainage site degraded 39.7% of natural and 19.6% of synthetic polyethylene.

In another study it was seen that degradation of polyethylene using microorganisms isolated from compost soil was studied. Degradation was studied by inoculating isolated organisms into Mineral salt medium containing 1 gram of polyethylene films as sole carbon source. Degradation was studied using SEM and FTIR. Degraded products were analyzed by Gas Chromatography.(Mahalakshmi, V et al., 2012). SEM and FTIR were also used to evaluate biodegradation. SEM results showed formation of cavities and erosions. Degradation of low - molecular - weight polyethylene (LMWPE) was carried out using thermophilic bacterium *Chelatococcus*sp. Degradation was studied by using FTIR. The FTIR peaks corresponding to alkenes also were more intense, indicating that dehydrogenations occurred concomitantly with microbial induced oxidation. (Jeon, H.J. and Kim, M.N. 2013). These FTIR results also showed formation of alkenes. *Pseudomonas* sp. was more efficient in degrading polyethylene when compared to *Rhodococcus* and *Brevibacillus*. *Pseudomonas* sp. degraded 40.5% of polyethylene after 3 weeks of incubation. (Nanda S. et al., 2010). This isolated species was able to degrade 9.5% of autoclaved polyethylene; it degraded 35% of UV treated and 6.2% of surface sterilized polyethylene after 3 months of incubation. Degradation of high molecular weight polyethylene was carried out with partially purified manganese peroxidase from *Phanerochaete chrysosporium*. Experiment was carried out under nitrogen limited and carbon limited condition.(Shimao, M. 2001).

In our present research we got maximum reduction of alkenes and nitro group peaks in the FTIR of both white and black autoclaved polythene. FTIR spectrum of white and black UV treated polythenes showed very less amount of degradation. In our study the polythene samples were kept in mineral salt medium in the shaker incubator for a continuous period of 2 months. Hence the result yielded by FTIR showed that autoclaved white polythene was the best to be biodegraded by *Bacillus cereus* for the period of two months.

As per the previous study High-density and low-density polythenes are the most commonly used synthetic plastics and they degrade slowly in natural environment, causing serious environmental problems. (Lee et al., 1991; Gu, 2003). As per the study of All India Plastic Manufacturers' Association (AIPM) (2014), recently decided that plastic carry bags, of width below 40 micron would not be produced, nor imported for supply to consumers to avoid pollution hazards. This is because the thickness of the bag determines the strength of the bag to break into smaller pieces. The thinner the bag is the higher is the probability of its breakdown and mixing with the soil which seriously deteriorates the soil fauna.

In another study different types of organisms were used to degrade polythene bags like a total of 15 bacteria were recovered from different sites and after primary and secondary screening 3 of them showed the positive results and identified as *Bacillus sp, Pseudomonas sp* and *Staphylococcus sp* through morphological and biochemical test. Further study was continued by degradation of pretreated polythene by obtaining degradation percentage where degradation of initially weighed pretreated polythene was done with respective intervals of time and final weighed of polythene was observed. By observing these results we can conclude that, *Bacillus sp* posses greater potential to degrade polythene when compare with other bacteria. This was shown by Gauri singh et al., 2016.

The results of this work were compared with earlier research studies done by Sowmya, et al (2014) in which they reported that *Bacillus cereus* was able to grow on minimal medium containing polyethylene as sole carbon source. This showed

its capacity to utilize polyethylene as carbon source and to degrade polyethylene. Degradation of polyethylene was carried out by Bacillus cereus which was isolated from dumpsite soil. Further Degradation was monitored by screening which was followed by weight loss. It was seen that *Bacillus cereus* is the best polythene degrading bacteria it takes less time for biodegradation.

But it was observed that the results of the above that is Sowmya, et al., (2014) are in contradiction to the current findings. As per the findings of the Research Paper, UV treated polythene showed higher amount of biodegradation as compared to Autoclaved polythene. However, as per our findings, Autoclaved white and black polythene showed higher potential of biodegradation when compared to UV treated white and black polythene.

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Gauri Singh, Ashok Kumar Singh and Kalpana Bhatt 2016 Department of Microbiology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Manduwala, Dehradun (Uttarakhand) India DEGRADATION OF POLYTHENES BY BACTERIA ISOLATED FROM SOIL.

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