humans and other animals; as well as various environmental issues. It has been reported that plastic wastes caused over a million deaths of marine animals (Barnes et al.,2009). These wastes virtually choke the drainage system in the urban centres of the country, to such an extent that it takes only the slightest of rainfall to create flood like situations, as was recently witnessed in Patna and other parts of Bihar. Classification of plastics are usually done by the chemical structure of their polymer's backbone and side chains. Some of the important groups in these classifications include polyesters, silicones, acrylics, polyurethanes, and halogenated plastics. Worldwide, approximately 30% of plastics are used for packaging application among which polyethylene (LDPE, HDPE), polyvinyl chloride, polypropylene, polystyrene etc. are mostly used for these kinds of applications. The widespread applications of plastics are not only due to their favourable mechanical and thermal properties but also mainly due to the stability and durability (Rivard et al., 1995).

Biological degradation is a phenomenon in which living organisms particularly microbes are responsible for biological transformation of the organic compounds into simpler molecules. Organic material can be degraded aerobically or anaerobically. During aerobic biodegradation carbon dioxide and water are produced and during anaerobic biodegradation, carbon dioxide, water and methane are produced. Bio surfactants are surface-active compounds synthesized by a vast variety of microorganisms found in the nature. Pre-treatment of the polymer using physical means prior to biodegradation have been found to enhance the process to a significant level. UV radiation was used as a pre-treatment by Mahalakshmi et al. (2012) and Sowmya et al. (2014). There are ample of research literatures in the field of polymer degradation and on the various aspects of bio surfactants such as its production, extraction from different microbes (Vimala P. P 2016), its application in biodegradation of hydrocarbons and heavy metal removal. Arkatkar et al. (2009), made a review on different approaches to enhance the biodegradation of polyolefin. However, the use of bio surfactants in polymer degradation is an inadequate area of study. The comparative analysis of degradation of plastic with and without addition of bio surfactant was the main objective of this study.

Materials & Methods :

Soil was collected from a local area (landfill) of Patna. High density polyethylene (HDPE, 41 micron) and low-density polyethylene (LDPE, 18 micron) films were used.Pre-weighed HDPE and LDPE films were exposed to sunlight on the roof of a building for 1 month. Soil samples were collected from the upper 0-5 cm layer of the soil, stored in plastic crates and transported to the Central research laboratory of Patna Women's College, where it was kept at room temperature. The direct isolation process was carried out by adding 1 g of soil to 9 ml of sterile distilled water in a test tube to yield a 10-fold dilution. Next, a series of 10-fold serial dilutions were made in which 1 ml of each dilution was cultured on NA. Pure cultures were finally obtained by selecting a single colony of growth from highly diluted cultures. After incubation, the plates showing the best growth were chosen for enumeration.

Morphological and Biochemical tests were performed to confirm the microbial species.Colony morphology was done to determine the morphology of selected strains on the basis of shape, size and colour.Gram staining method showed that the species isolated from the soil was rod shaped and were purple in colour. Biochemical identification was done by using some manual biochemical methods. Catalase test indicated that the isolate was non motile and showed an effervescence confirming the stains was gram +ve.

Indole test indicated that the isolate was non motile and the stain was found to be gram–ve. Voges Proskauer test indicated that the isolate was non motile and the stains were found to be gram +ve. Methyl Red test indicated that the isolate was non motile and the stains were found to be gram–ve. Citrate test indicated that the isolate was non motile and the stains were found to be gram +ve.

Production of bio surfactant: Bacterial species were cultured in nutrient broth (Nutrient medium: Beef extract, peptone, NaCl, distilled water) and incubated for 24 hours at 32°C. For the production of bio-surfactants from each of the *Bacillus subtilis*, freshly prepared nutrient medium was inoculated with cultured broth and was incubated at 32°C for 24 hours. As the endogenous phase of bacterial growth was reached, olive oil was added(30 ml/L). Conical flask was kept in a shaking incubator for 3 and 7 days at 37 °C, 180 rpm. (Fig.1)

Estimation of bio surfactant: Screening test: -Oil spreading technique-10 ml of crude oil was added to the surface of 40ml of distilled water in a Petri dish to form a thin oil layer. Then 10 ml of culture or culture supernatant was gently placed on the centre of the oil layer. Presence of bio surfactant had displaced the oil and a clear zone was formed.

Quantification of bio surfactant: Biurettest: - In Biuret test, for the estimation of surfactant produced from *Bacillus subtilis*, 4ml of Biuret reagent was added to 400 ml of sample. The solution was then kept for 10 minutes and then absorbance was measured in UV-V spectrophotometer at 540nm.

Extraction of bio surfactant: Acid precipitation method:- Incubated cultures had been centrifuged at 4000 rpm at room temperature for 30 minutes. To the supernatant obtained, 1M $H_2SO_4$was added to adjust the pH at 2. Chloroform: Ethanol was added in the ratio of 2:1. These mixtures were shaken well to ensure proper mixing and were left overnight for evaporation (Table 2).

Experimental setup: 5 Films from *Bacillus subtilis* culture of thickness 2cm x 2cm of each were used. 500 ml of mineral salt medium was prepared $NaNO_3$(0.42g), $MgSO_4$(0.107g), KCl (0.107g), $Fe_2(SO_4)_3$ (0.0021g), KH_2PO_4 (0.029g), K_2HPO_4 (0.257g), Yeast extract (0.0042g), Distilled water (750 mL) and 150ml each were poured into 5 conical flasks. The polymer films were measured for their initial weight. The conical flask were labelled accordingly i.e.; in the 1st conical flask untreated High Density polyethylene (HDPE) film was immersed; in the 2nd conical flask untreated Low Density Polyethylene films (LDPE) film was immersed; in the 3rd conical flask treated High Density polyethylene (HDPE) film was immersed; in the 4th conical flask treated Low Density Polyethylene (LDPE) film was immersed and in the 5th conical flask both HDPE & LDPE were immersed along with the above prepared medium and bio-surfactant which was earlier dissolved in sterile water-(0.1g/100ml) extracted after screening test. The conical flasks were inoculated with bacterial species with the necessary combination (polymer films + microbes + bio-surfactants). Experimental setup was incubated at room temperature for 40 days with intermittent shaking at 180 rpm at 32°C. PE Films were measured for their final weight after 40 days of incubation. The obtained values of gravimetric analysis are given in Table 3.

Results and Discussion :

Gravimetric analysis of the films after UV treatment was done.Weight loss measured was not significant after the pre-treatment (Table1). Weight loss = Weight of PE films before UV - Weight of PE films after UV. The quantification test (Burette test) was done after 3 days and 7 days of incubation respectively**.** After 3 days of incubation the surfactant estimation was found to be 1.328abs whereas after 7 days of incubation the surfactant estimation was found to be1.45 abs.

Table 1. Gravimetric Analysis of the films

Polymer type	Before UV (g)	After UV(g)	Weight loss (g)
PE (18micron)	0.0067g	0.0060g	0.0007g
PE (41micron)	0.0250g	0.0210g	0.004g

Fig. 1. Top layer indicating the presence of bio surfactant after 7 days of incubation

Measurement of the extracted bio surfactants was done and it was found to be a production of 0.0515 g bio-surfactant after 7 days of incubation.

Table 2. Measurement of extracted bio surfactants

Microbe	Weight of petridish (g)	Weight of petridish + Bio surfactant (g)	Weight of Bio surfactant (g)
B.subtilis	28.7023	28.7538	0.0515

PE Films were measured for their final weight after 40 days of incubation (Table. 3).PE films (18 micron) inoculated with *B.subtilis* with the addition of bio surfactants showed a weight loss of 0.0025g i.e. 41.66% whereas PE films (41micron) showed a weight loss of 0.0057gi.e. 27.14%.

Table 3. Analysis of plastic degradation after 40 days

TREATMENT	UNTREATED(g)				TREATED(g)			
POLYMER	PE	PE*	PE	PE*	PE	PE*	PE	PE*
TYPE	18µ	18µ	41µ	41µ	18µ	18µ	41µ	41µ
	0.0067	0.0060	0.0250	0.0236	0.0060	0.0035	0.0210	0.0153

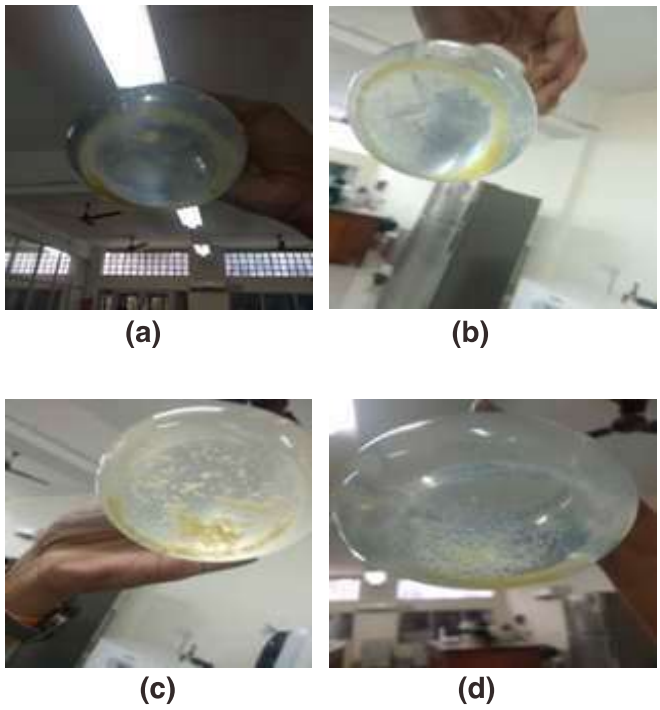

Fig. 2. (a) Treated HDPE, (b) Treated LDPE, (c) Untreated HDPE, (d) Untreated LDPE after 40 days of incubation.

Biodegradation of polyethylene was carried out using B.subtilis. In this study UV treatment was chosen as the physical means of pre-treatment and it was found to enhance the ability of microbes to assimilate PE films. Amphiphilic nature of bio surfactant is responsible for the attachment of microorganisms on hydrophobic surfaces. Consequently, addition of bio surfactants helped in attachment of microbes to PE films and hereby enabling them to use polymer as a carbon source at a faster rate. The bacterial species were capable of utilising PE as a carbon source. PE films of lesser thickness was noted to be degrade faster indicating more weight loss. It was found that treated PE showed more weight loss. PE films (18 micron) inoculated with B.subtiliswith the addition of bio surfactants showed a weight loss percentage of 41.66% on the other hand PE films (41micron) showed a weight loss percentage of 27.14%. It was found that addition of bio surfactant enhanced the ability of Bacillus subtilis to utilise PE.

Conclusion:

The present study deals with the isolation, identification and degradative ability of plastic degrading microorganisms from soil. Different types of changes are produced by the microorganism during morphological and biochemical analysis. Soil sample collected from local area of Patna was used in this study. The plastic was used to study their biodegradation by microorganisms isolated from soil. Microbial degradation of a solid polymer like polyethylene requires the formation of a bio surfactant on the polymer surface to enable the microbes to efficiently utilize the non-soluble substrates by enzymatic degradation activities. Production of bio surfactant, by the bacterial species have been found to be powerful degrading agents in nature. In the present study pieces of plastics were inoculated in the liquid culture medium containing bacterial isolates and kept for 40 days to observe the percentage of weight loss by bacteria. The result shows the degradative ability of the microorganisms after one month of incubation. A sharp loss in the weight was observed due to presence of bio surfactant produced by *Bacillus subtilis.*

Acknowledgement:

We express our sincere thanks to our respected Principal, Dr. Sister M. Rashmi A.C. for giving opportunity to conduct this research work. We are thankful to Mr. Sumeet Ranjan, our laboratory incharge for his constant help and support. Above all we are grateful to the Research Committee for providing the facilities and financial support.

References:

Arkatkar A, Arutchelvi J, Sudhakar M, Bhaduri S, Uppara PV, Doble M. (2009). Approaches to Enhance the Biodegradation of Polyolefins. *The Open Environmental Engineering Journal*. 2:68-80.

Barnes DKA , Galgani F, Thompson RC, Barlaz M. (2009).Accumulation and fragmentation of plastic debris in global environments. *Philos Trans R Soc Lond B Biol Sci*.364(1526):1985-98.

Mahalakshmi V, Andrew SN (2012). Assessment of Physicochemically treated plastic by fungi. *Annals of Biological Research*. 3(9): 4374-4381

Rivard C., Moens L., Roberts K., Brigham J., Kelley S. (1995). Starch esters as biodegradable plastics: Effects of ester group chain length and degree of substitution on anaerobic biodegradation. *Enz Microbial Tech*. 17(9): 848–52.

Shimao, M. (2001). Biodegradation of plastics. *Curr. Opin. Biotechnol*. 12(3): 242-247.

Sowmya HV, Ramalingappa MK, Thippeswamy B. (2014). Biodegradation of Polyethylene by *Bacillus cereus*. *Advances in Polymer Science and Technology: An International Journal*. 4(2): 28-32.

Vimala PP, Lea, Mathew (2016). Biodegradation of Polyethylene using *Bacillus subtilis*. *Procedia Technology*. 24: 232—239.