Synergistic effect of calcium stearate and photo treatment on the rate of biodegradation of low density polyethylene spent saline vials

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The biodegradation of spent saline bottles, a low density polyethylene product (LDPE) by two selected Arthrobacter sp. under in vitro conditions is reported. Chemical and UV pretreatment play a vital role in enhancing the rate of biodegradation. Treated LDPE film exhibits a higher weight loss and density when compared to untreated films. Arthrobacter oxydans and Arthrobacter globiformis grew better in medium containing pretreated film than in medium containing untreated film. The decrease in density and weight loss of LDPE was also more for pretreated film when compared to untreated film indicating the affect of abiotic treatment on mechanical properties of LDPE. The decrease in the absorbance corresponding to carbonyl groups and double bonds that were generated during pretreatment suggest that some of the double bonds were cut by Arthrobacter species. Since Arthrobacter sp. are capable of degrading urea, splitting of urea group were also seen in FTIR spectrum indicating the evidence of biodegradation after microbial incubation. The results indicated that biodegradation rate could be enhanced by exposing LDPE to calcium stearate (a pro-oxidant) which acts as an initiator for the oxidation of the polymers leading to a decrease of molecular weight and formation of hydrophilic group. Therefore, the initial step for biodegradation of many inert polymers depends on a photo-oxidation of those polymers. The application in sufficient details with improved procedures utilizing recombinant microorganism with polymer degradation capacity can lead to a better plastic waste management in biomedical field. The present plastic disposal trend of waste accumulation can be minimized with this promising eco-friendly technique.

Keywords: Arthrobacter globiformis, Arthrobacter oxydans, Biodegradation, Calcium stearate, Plastic, Pro-oxidant solution, UV irradiation

Plastics are the product of 20th century. Originally, plastics were mimicking and replacing natural product but today they are largely synthetic materials made from extensively expensive but non-renewable resource like crude oil1. The use of plastic has become a part in all sectors of the economy. Plastics are extensively used in packaging of food products, pharmaceuticals, cosmetics, detergents and chemicals. The utilization is still expanding at high rate of 12% per annum2. The most widely used plastic are polyethylene, [low-density polyethylene (LDPE), high density polyethylene (HDPE) and low linear density polyethylene (LLDPE)], polystyrene (PP), polyvinyl chloride (PVC), polyethylene terephthalate (PET), and nylon. The widespread application of plastics is not only due to favorable mechanical and thermal properties but also mainly due to their stability and durability3.

Plastic degrade into smaller toxic parts, contaminating soil and waterways, where they can be accidentally ingested by animals and thereby enter the food chain4. The hazard of discarding waste plastic, so called “white pollution” is becoming more and more severe. Due to the harsh impact that plastics has on the environment, studying the biodegradation process of plastics has become increasingly important. The term "biological degradation" means that, materials are completely degraded by the microorganism such as bacteria, fungi and actinomycetes to form CO and biomass1,5. Processes inducing changes in polymer properties (deterioration of functionality) due to chemical, physical or biological reactions resulting in bond scission and subsequent chemical transformations (formation of structural in homogeneities) have

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been categorized as polymer degradation. There are various methods of degrading plastic which includes photo-degradation, thermal-degradation and o xo-biodegradation.

The molecular weight reduction and oxidative products are then responsible for a possible polymer biodegradation, the carbon-chain backbones being nutrients for the microorganisms once the molecular weight of the polymer is less than \( \sim 1000^6 \). Hydrocarbon chains are very resistant to biodegradation as the enzymes are not able to break the C-C bonds\(^7\). Therefore, the initial step for biodegradation of many inert polymers depends on a photo-oxidation of those polymers. During this photo-oxidation, hydroperoxides are incorporated through Norrish type I and II mechanisms which generate carboxylic within the polymer. Then the microorganisms attack the carboxylic parts of the polymer, releasing two carbon chain fragments that can be further used in anabolic reactions\(^6\).

*Arthrobacter* are basic soil bacteria (Gram-positive obligate aerobes) and have the ability to degrade pesticides. They also show capability of utilizing hydrocarbons as nutrition and energy source. Plastics are made from petroleum based hydrocarbon. Microorganisms like other living things need to eat in order to obtain energy. The hydrocarbons in plastic serve as food for micro organism thus playing important role in degradation\(^15\). Chemicals and UV pretreatment play a vital role in enhancing the rate of biodegradation\(^6\). Keeping these information in view the present study has been undertaken to observe biodegradation of low density polyethylene product —Spent saline vials by two *Arthrobacter* sp., pretreated by calcium stearate and UV radiation. The hypothesis of the study is that microorganisms will utilize chemically pretreated UV-irradiated plastic strips as sole carbon source and energy. The proxidant used was calcium stearate (C\(_{36}\)H\(_{77}\)CaO\(_{3}\)) — an insoluble calcium salt of stearic acid and palmitic acid; it is formed when soap is mixed with water that contains calcium ions. It is insoluble in water and does not lather well. Commercially it is sold as 50% dispersion in water or as a spray dried powder. In plastics, it can act as an acid scavenger or neutralizer at concentrations up to 1000 ppm, a lubricant and a release agent. It may be used in plastic colorant concentrates to improve pigment wetting. In rigid PVC, it can accelerate fusion, improve flow, and reduce die swell\(^9\).

### Materials and Methods

**Sample collection and preparation**—Spent saline vials (LDPE Plastic sample) were collected from hospital disposed waste. The polyethylene films (density 0.893g/cm) were cut into pieces (2.5cm × 3cm each), weighed, disinfected in absolute ethanol and air dried under aseptic conditions for 15 min in a laminar–flow head\(^11\).

**Proxidant treatment**—About 1% calcium stearate (CaSt) was used in the present study. Since CaSt is insoluble in water, ethanol was used for preparing the solution\(^10\). The treatment was done as per Mohan et al\(^11\).

**FTIR spectroscopy**—Fourier Transform Infrared analysis (FTIR) was performed using Spectrum 400 IR system. The entire spectral region between 400 and 4000 cm\(^{-1}\) was scanned with a resolution of 2 cm\(^{-1}\).

**Mechanical test**—The density of each pretreated film was measured by CML/WP/077 test method\(^11\).

**Determination of weight loss**—Recovered plastic samples were analyzed for degradation by weight loss before and after microbial treatment using Electronic balance. The percentage weight loss of the inoculated plastic samples is given by the formula

\[
\text{Weight loss (\%)} = \left( \frac{\text{Final weight} - \text{Initial weight}}{\text{Original weight}} \right) \times 100
\]

**UV hood fabrication**—Chamber made of wood (16 cm long × 27 cm wide) with a 6WATTA UV lamp was used for UV irradiation. The chamber was covered by black carbon paper so that the radiation is not emitted out and is completely absorbed by the sample placed inside\(^11\).

**UV treatment**—The proxidant treated polyethylene were subjected to a programme of continuous exposure to UV (312 nm) for 15 days. (Accelerated weathering tests are widely used to assess the weatherability of polymers. The short-wavelength emission of UV-B lamps can cause unnatural acceleration or degradation whereas UV-A lamps have no output below the normal solar cut-off of 295 nm and may allow enhanced correlation with actual outdoor weathering\(^9\).) Partially photolyzed samples were later subjected to biodegradation. After incubation (15 days) the treated plastic film samples were taken for characterization by FTIR (3000MX Bio-Rad Ex) analysis and density change was also assessed\(^11\).

**Media preparation**—Nutrient broth (minimal media) was used for degradation study. Media sterilization was performed by autoclaving at 121 °C and 15 lbs pressure for 20 min.
Biodegradation assay – Microorganism used were Arthrobacter oxydans (ATCC 3699) and Arthrobacter globiformis (ATCC 1010) supplied by Microbial Type Culture Collection And Gene Bank (MTCC), CSIR-Institute of Microbial Technology (CSIR-IMTEH), Chandigarh, India. Biodegradation assay was performed in 100 mL conical flasks by adding 1µL of pure Arthrobacter culture into 50 mL of minimal nutrient broth containing treated and untreated plastic samples as a carbon source in separate conical flasks. The assay was performed with respective positive (minimal media + Arthrobacter oxydans + pretreated plastic film and minimal media +Arthrobacter globiformis + pretreated plastic film) and negative (minimal nutrient broth+ treated plastic films) controls. The flasks were incubated at 27 ºC for 30 days. The growth rate of Arthrobacter sp. was assessed by turbidity method (OD measured at 660 nm)\(^{12}\).

Film harvest—After exposing to Arthrobacter isolates for one month the polyethylene pieces were harvested, washed in 70% ethanol, dried at 45 ºC, equilibrated, and the weight was determined. Each of the films with and without chemical and UV treatment was compared with the corresponding uncultured material (negative control) as well as with the cultured material. After incubation for one month the treated plastic film samples were taken for characterization by FTIR analysis, density change and weight loss was also assessed.

Results and Discussion
The Plastic strip was confirmed to belong to LDPE Class based on FTIR characterization. The IR spectra when thoroughly examined revealed subtle but definite differences among the various forms of polyethylene. The IR regions between 1400-1350 cm\(^{-1}\) and 1000-800 cm\(^{-1}\) were examined. Main differences are in the 1380 cm\(^{-1}\). In LDPE, band in region 1400-1330 cm\(^{-1}\) consists of 3 peaks while HDPE consists of two peaks. The characteristic band at 1377 cm\(^{-1}\) (which is assigned to CH\(_3\)-gp) terminating the short and long–chain branching and the main polyethylene chain is indicative that the sample is surely a LDPE\(^{13}\) (Fig. 1).

Effect of chemical treatment—Chemical treatment of prepared plastic film with calcium stearate which act as photo inducer the resultant structural changes, weight loss and density which were observed and recorded as follows: Under mechanical changes, density of each pretreated polyethylene piece were observed by CML/WP/077 test method which resulted as 0.903 g/cc for calcium stearate treated, showing minor variation when compared to untreated LDPE with a density of 0.89 g/cc. Chemical pre-treatment before UV treatment and incubation with Arthrobacter oxydans and Arthrobacter globiformis resulted in weight loss of LDPE by 2.5%. 

Fourier transform infrared spectroscopy (FTIR) analysis of calcium stearate treated plastic strips showed increase in number of bands in the region of 1330cm\(^{-1}\)-990cm\(^{-1}\) indicating the evidence of break in the polyethylene chain.(Figs 1 and 2)\(^{13}\).
Effect of UV irradiation—Chemical treatment was followed by UV radiation for 15 days before incubation with Arthrobacter strains, the resultant structural changes, mechanical properties and weight loss were observed and recorded as follows: pretreated UV irradiation of LDPE for 15 days showed decrease in the density of each pretreated polyethylene strip which was around 0.86 g/cc for calcium treated. UV irradiation of pretreated LDPE for 15 days showed a weight loss of about 31.5% for calcium stearate. Structural changes in the polymer were determined by FTIR. It was observed that in case of chemically pretreated and UV irradiated LDPE, bands appeared in the region of 1710-1740 cm\(^{-1}\) (corresponding to carbonyl compound), 1640 cm\(^{-1}\) (corresponding to -c=c-) which are not found in un-pretreated film (control) and also the peak at 1377 cm\(^{-1}\) (control) were reduced to 1373 cm\(^{-1}\), this indicates that chemical pre-treatment and UV irradiation of LDPE resulted in generation of carbonyl groups and double bonds. (Fig. 2)

LDPE degradation using A. oxydans and A. globiformis—When pretreated UV irradiated strips were incubated with A. oxydans and A. globiformis for one month the resultant structural changes, mechanical changes and weight loss were observed and recorded as follows: density of each pretreated UV irradiated polyethylene piece after microbial treatment were observed by CML/WP/077 test method which resulted as 0.803 gm/cc and 0.823gm/cc for calcium stearate treated. The weight loss of LDPE after microbial incubation was observed as 52 and 42.1%. The peaks that were generated (1740 cm\(^{-1}\)) by chemical pretreatment and UV irradiation were later reduced to 1738 cm\(^{-1}\),1712 cm\(^{-1}\) for A. oxydans and at 1738 cm\(^{-1}\), 1711 cm\(^{-1}\) for A. globiformis indicating breakdown of double bond after microbial treatment and also disappearance of some peaks were observed. A. oxydans and A. globiformis were used in the study were capable of utilizing polyethylene as the sole carbon source. LDPE samples used in the experiment were treated by exposing them to pro-oxidant (Calcium stearate) which acts as a UV sensitizer and also to UV light\(^{14,15}\). Polyethylene needs to undergo some non-biotic degradation before microbial attack because of its hydrophobicity and its large molecular dimensions \(^{16}\). UV light or oxidizing agents such as a UV-sensitizer are needed at the beginning of biodegradation of inert materials such as polyethylene\(^{8}\). These pretreated polymers were then applied to microbial treatment for 1 month using all the Arthrobacter strains in a nutrient broth containing treated LDPE as a sole source of carbon. The Arthrobacter sp. grew well in the medium containing abiotically treated LDPE compared to untreated LDPE.

Among the two strains, LDPE treated with A.oxydans showed maximum weight loss i.e. 52% for calcium stearate treated and density of 0.803g/cc. This decrease in weight loss and density is due to exposure of polymer to photo sensitizer before UV irradiation which later weakened the bonds present in the polymer and thereby making the groups present in the LDPE available for A.oxydans. In the biodegradation of polyethylene, an initial abiotic step involves oxidation of polymer chain due to the dissolved oxygen or that which is present in the ambient leading to formation of carbonyl groups. These eventually form carboxylic groups, which subsequently undergo β-oxidation and are totally degraded via the citric acid cycle resulting in the formation of CO\(_2\) and H\(_2\)O. β-oxidation and citric acid cycle are catalyzed by the microorganisms which was observed by Albertson et al\(^{1,3}\).

Monitoring the formation or disappearance of carbonyl groups (1710–1740 cm\(^{-1}\)) and double bonds (840–890 cm\(^{-1}\)) using FTIR is necessary to elucidate the mechanism of biodegradation process. In chemically pretreated and UV irradiated LDPE peaks appeared at, 1710–1740 cm\(^{-1}\)(corresponding to carbonyl compound), 1640 (corresponding to -c=c-) and 840–890 cm\(^{-1}\) which were absent in unpretreated film. This indicates that chemical pre-treatment and UV irradiation of LDPE resulted in generation of carbonyl groups and double bonds. The peaks that were generated (1740 cm\(^{-1}\)) by chemical pretreatment and UV irradiation were later reduced to 1738 cm\(^{-1}\), 1638 cm\(^{-1}\), 840 cm\(^{-1}\) respectively after microbial treatment and also disappearance of some peaks were observed (Figs 3 and 4). It is similar to result obtained during biodegradation of polyurethane which indicated that polyurethane biodegradation was due to hydrolysis of ester bond as reported by Howard et al\(^{17}\) and Nakajima-Kambe et al\(^{2}\). In the present study the decrease in IR absorption bands indicated the splitting of urea groups (1638 cm\(^{-1}\))^2. This decrease in peaks is due to consumption of carbonyl and double bond groups by the microorganisms indicating the breakdown of polymer chain. FTIR as a tool for differentiating between abiotic and biotic degradation of LDPE\(^{9}\) and
Fig. 3—IR Spectra of pre-treated LDPE strip after Arthrobacter oxydans treatment

Fig. 4—IR Spectra of pre-treated LDPE strip after Arthrobacter globiformis treatment

many other similar studies under in vitro conditions have also observed a continuous increase in amount of carbonyl compounds with exposure in an abiotic environment as against a decrease in the biotically aged sample, it is also observed that the amount of carbonyl groups decreased with prolonged exposure to a biotic environment.

Conclusion

The potential application areas in which plastics play an important role include agriculture, horticulture, packaging, domestic, hospitals. The degradation of most synthetic plastics in nature is a very slow process that involves environmental factors, followed by the action of wild microorganisms. In the present study, the vital use of incorporated light-sensitive chemical additives or copolymers for the purposes of weakening the bonds of the polymer in presence of ultraviolet radiation has been elucidated. The spent saline vials (LDPE) which are generated as routine waste from hospitals can be biodegraded by the synergistic activity of chemical pre-treatment, UV irradiation and microbial degradation by microbes belonging to the genera of Arthrobacter.

References