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Procedia Technology 24 (2016) 232 – 239

Procedia Technology

International Conference on Emerging Trends in Engineering, Science and Technology (ICETEST - 2015)

# Biodegradation of Polyethylene using *Bacillus subtilis*

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**Abstract**

Polyethylene is among the major plastics being dumped in the environment. The study explores methods to enhance the rate of biodegradation of polyethylene using physical and biological means. Bacterial species –*Bacillus subtilis* – was tested for its potential in utilizing polyethylene as their sole carbon source. The microbial species produced surface active compounds (Biosurfactants) that enhance the degradation process. Pretreatment of polymer films with Ultraviolet radiation aids its accessibility as food for the microorganisms thus enabling a much faster rate of biodegradation. Inoculation of pretreated polyethylene films of thickness 18μ with *Bacillus subtilis* with the addition of its biosurfactant (surfactin) proved to be most efficient with a weight loss percentage of 9.26% in 30 days


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*Keywords: Polyethylene;Biodegradation;Biosurfactants*

## 1. Introduction

Biosurfactants are surface-active compounds synthesized by a wide variety of microorganisms. They are molecules that have both hydrophobic and hydrophilic domains, comprising an acid, peptide cations, or anions, mono-, di- or polysaccharides and a hydrophobic moiety of unsaturated or saturated hydrocarbon chains or fatty acids Due to their amphiphilic structure, biosurfactants increase the surface area of hydrophobic water-insoluble substances, increase the water bioavailability of such substances and change the properties of the bacterial cell

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Peer-review under responsibility of the organizing committee of ICETEST – 2015
doi:10.1016/j.protcy.2016.05.031

surface. Because of their potential advantages, biosurfactants are widely used in many industries such as agriculture, food production, chemistry, cosmetics and pharmaceutics.

There have been focuses on the recent hypotheses and experimental findings regarding the biodegradation of polyethylene. Ambika *et al*. (2009), made a review on different approaches to enhance the biodegradation of polyolefins. It discusses various physical, chemical and biochemical approaches that can be adopted to enhance their biodegradation. From this review it was inferred that biosurfactants can be used as an enhancing agent of biodegradation process [1]. Pretreatment of the polymer using physical means prior to biodegradation have been found to enhance the process considerably. UV radiation was used as a pretreatment by Mahalakshmi *et al.* (2012) and Sowmya *et al.* (2014) [2,3].

Research works on biosurfactants, its production, analysis and applications were also measured. There is ample research literature in the fields of polymer degradation and on the various aspects of biosurfactants such as its production, extraction from different microbes, its application in heavy metal removal and biodegradation of hydrocarbons. However, the use of biosurfactants in polymer degradation is an inadequate area of study.

The explicit objectives of this study are: To determine and compare the rate of biodegradation of Polyethylene (PE) films of two thicknesses

- Using mono culture of *Bacillus subtilis*
- With and without pretreatment of UV-rays on Polyethylene
- With and without addition of Biosurfactant

## 2. Materials and Methods

### *2.1. Collection of Polymer Sample*

Polyethylene (PE) films of two thicknesses – 18μ LDPE (Low density Polyethylene) and 41μ HDPE (High density Polyethylene)-were purchased. PE films were cut in required size of approx. 2 cm x 2 cm and they were subjected to UV treatment for 72 hours.

### *2.2. Microorganisms*

Bacterial species *Bacillus subtilis* was selected for the study based on their ability to produce biosurfactants. Agar slants of *Bacillus subtilis* MCC No. 2183 was obtained from Microbial Culture Collection, Pune. *Bacillus subtilis* produces biosurfactant known as surfactin. Bacterial species was cultured in nutrient broth (Nutrient Medium: Beef extract (10g), Peptone (10g), NaCl (5g), Distilled water 1L) and incubated for 24 hours at 32$^{o}$C [4, 5, 6].

### *2.3. Production of Biosurfactants*

For the production of biosurfactants from each of the B.subtilis, freshly prepared nutrient medium was inoculated with cultural broth and was incubated at 32$^{o}$C for 24 hrs. On reaching the endogenous phase of bacterial growth, olive oil was added (30 ml/L). Conical flasks were kept in a shaking incubator for 3 days and 7 days at 32$^{o}$C, 180rpm [7].

### *2.4. Estimation of Biosurfactants*

Screening test: oil spreading technique: Oil spreading assay, 10 μL of crude oil was added to the surface of 40 mL of distilled water in a petri dish to form a thin oil layer. Then, 10 μL of culture or culture supernatant were gently placed on the centre of the oil layer. The presence of biosurfactant would displace the oil and a clear zone would form [8].

Quantification of Biosurfactants: Biuret test: In Biuret test, for the estimation of surfactin produced from B.subtilis 2 mL of biuret reagent was added to 200 µL of sample. The solution was kept for 10 minutes and then absorbance was measured in UV-V Spectrophotometer at 540 nm [9].

### *2.5. Extraction of Biosurfactants*

Acid precipitation method: Incubated cultures were centrifuged at 4000rpm 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 [10].

### *2.6. Experimental Setup*

3.5 L of Mineral Salt Medium was prepared ($NaNO_3$ (2g), $MgSO_4$ (0.5g), KCl (0.5g), $Fe_2$ $(SO_4)_3$ (0.01g), $KH_2PO_4$ (0.14g), $K_2HPO_4$ (1.2g), Yeast extract (0.02g), Distilled water 1L) and 150 mL each was poured into 20 conical flasks. Polymer films were measured for their initial weight. The conical flasks were inoculated with bacterial species with the necessary combination (polymer films + microbes ∓ biosurfactant). Experimental setups were incubated at room temperature for 30 days with intermittent shaking at 180 rpm at 32$^o$C.

Combinations:

UP + B1; UP + B1 + BS; TP + B1; TP + B1 + BS;

Where, UP= Untreated PE film; TP= Treated PE film; B1= B.subtilis; BS= Biosurfactant

## 3. Results and Discussion

### *3.1. UV Pretreatment*

Gravimetric Analysis of the films after UV treatment was done (Table 1). Weight loss measured was not significant after the pretreatment.

Weight loss = (Weight of PE films before UV - Weight of PE films after UV).

Table1: Gravimetric Analysis of the films

| Polymer type | Before UV (g) | After UV (g) | Weight loss (g) |
|---|---|---|---|
| PE (18 µ) | 3.000 | 2.999 | 0.001 |
| PE (41 µ) | 3.000 | 3.000 | 0.000 |

### *3.2. Estimation of Biosurfactants*

Screening test (Oil Spreading Technique): As the supernatant from produced biosurfactants was poured on to the centre of oil layer spread over the layer of water, clear zone was formed displacing the oil layer, confirming the presence of biosurfactants (Fig. 1)

Quantification Test (Biuret test): Surfactin estimation (Fig.2) was found to be

After 3days of incubation: 0.454 abs = 3500 µg/mL

After 7days of incubation: 1.385 abs = 6400 µg/mL

Fig. 1 Clear zone formation of surfactin

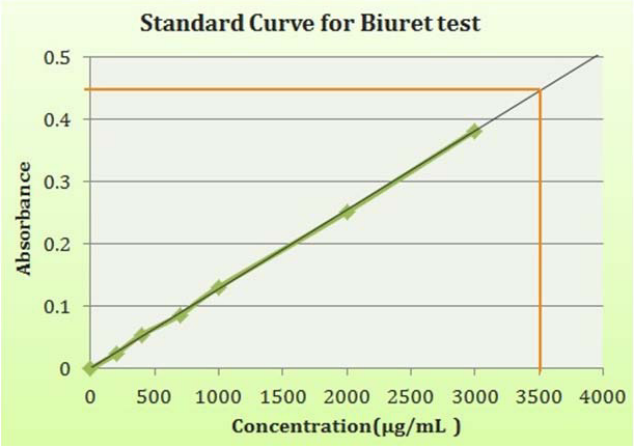


Fig.2 Surfactin estimation

### *3.3. Extraction of Biosurfactants*

Acid, Chloroform, ethanol mixtures were shaken well and were left overnight for evaporation. White coloured precipitate was seen at the interface between the two liquids proving the presence of biosurfactants. The mixtures were again centrifuged to obtain biosurfactants in pellet form and after drying weights were measured (Table. 2).

Table 2: Measurement of extracted biosurfactant;
B1*: B.subtilis*; BS: Biosurfactant

| Microbe | Weight of petridish (g) | Weight of petridish + BS (g) | Weight of BS (g) |
|---|---|---|---|
| B1 | 45.65 | 45.92 | 0.27 |

### *3.4. Experimental Setup*

5 films of ~ 2cm x 2 cm of each thickness were used. B.subtilis (B1) culture at absorbance of 1.45 was used for inoculation. Extracted biosurfactant (BS) was dissolved in sterile water. (~ 0.3gm/L)

### *3.5. Gravimetric Analysis of PE films*

PE films were measured for their initial weight and weight after 30 days of incubation. The obtained values of gravimetric analysis are given in Table. 3 and Fig. 3.

Table3: Gravimetric Analysis of PE films
B1*: B.subtilis*; BS: Biosurfactant

| Treatment | Untreated(*g*) | | | | Treated (*g*) | | | |
|---|---|---|---|---|---|---|---|---|
| Polymer type/ Combination | PE (18 µ) | PE* (18 µ) | PE (41 µ) | PE* (41 µ) | PE (18 µ) | PE* (18 µ) | PE (41 µ) | PE* (41 µ |
| B1 | 0.064 | 0.063 | 0.084 | 0.084 | 0.054 | 0.053 | 0.079 | 0.077 |
| B1 + BS | 0.07 | 0.068 | 0.077 | 0.077 | 0.054 | 0.049 | 0.085 | 0.082 |

PE*= Polyethylene films after 30 days of incubation

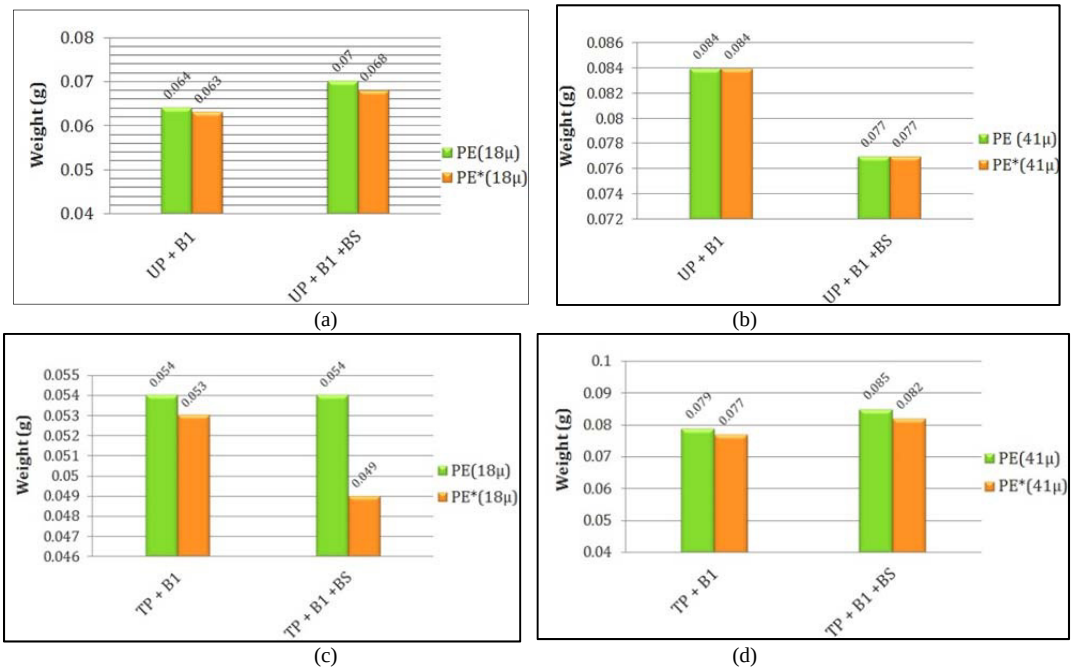


Fig. 3 Graphical representation of gravimetric analysis a) Untreated PE (18µ); b) Untreated PE (41µ); c) Treated PE (18µ); d) Treated PE (41µ)

### *3.6. Analysis of PE films*

Weight loss in percentage was calculated and compared with control after an incubation period of 30 days.

Weight loss (%) = ((Weight loss)/(Initial weight)) ×100 (2)

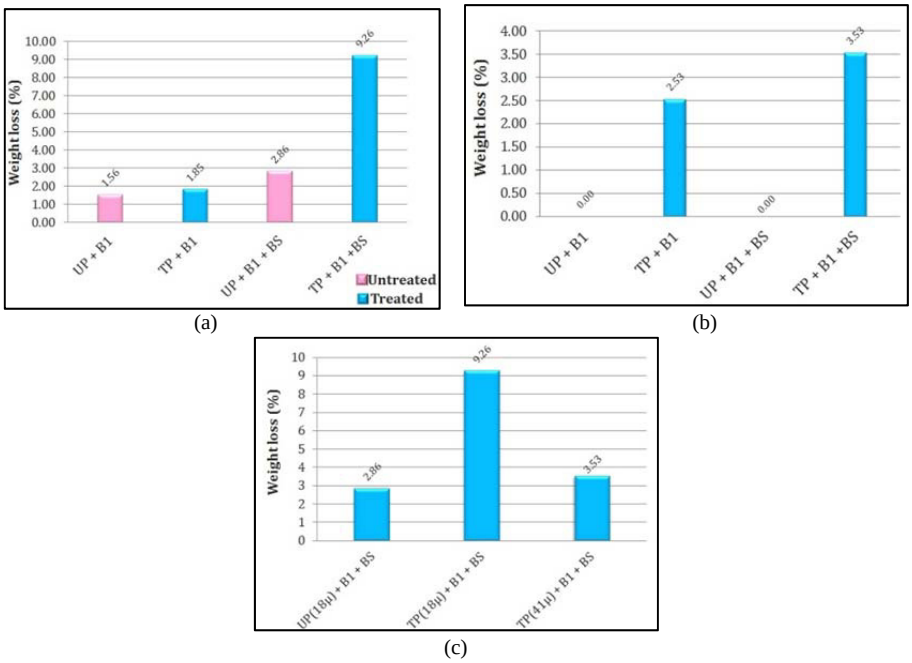


Fig 4 Comparison of weight loss measurement a) PE 18µ b) PE 41µ c) Both thicknesses

In the case of PE (18μ), weight loss of treated (TP) and untreated (UP) films with the inoculation of *B.subtilis* (B1) (Fig. 4a), showed weight loss percentage values of: UP + B1: 1.56%; TP + B1: 1.85%

With addition of biosurfactants (BS), treated and untreated films showed more weight loss percentage (Fig. 4a) than the control. But in treated polymers with the addition of biosurfactants, a better weight loss percentage was achieved: UP +B1 + BS: 2.86%; TP + B1 + BS: 9.26%;

In the case of PE (41μ), untreated films showed no weight loss with the inoculation of monoculture of *B.subtilis* (B1) (Fig. 4b), while treated polymer films indicated more weight loss: UP + B1: 0%; TP + B1: 2.53%

With addition of biosurfactants (BS) only treated films showed weight loss percentage than the control (Fig. 4b). UP + B1 + BS: 0%; TP + B1 + BS: 3.53%

Treated polymers were found to have more weight loss than untreated polymer because UV rays act as an initiator of polyethylene oxidation which enhances the bacterial degradation. Also degradation was found to increase in the presence of biosurfactants as it provides assistance in attachment of microbes to PE films (Fig. 4c).

### *3.7. FTIR Spectroscopic Analysis of PE Films*

FTIR spectral analysis of treated PE films - 18μ and 41μ with the addition of biosurfactant are shown in Fig. 5 and 6 respectively.

FTIR analysis of PE 18μ indicated that films without the addition of biosurfactant (Fig. 5a) have greater intensity peaks than films with the addition of biosurfactants (Fig. 5b). Greater peak intensity means that there is more of that particular type of bond. The main band of 2920-2851 $cm^{-1}$ was indicative of the C-H stretch (Table 4). The intensities were reduced from 15.8 to 3.7 for the wavenumber 2920 $cm^{-1}$ and from 16.9 to 4 for wave number 2851 $cm^{-1}$. From the IR spectroscopy it can be stated that the bacterial degradation led to a substantial increase in the C-H stretch band of the polyethylene at 2920-2851 $cm^{-1}$ (Fig. 5c).

FTIR analysis of PE 41μ indicated different peak positions for films with and without the addition of biosurfactants (Fig 6a and 6b). The main band of 2920-2851 $cm^{-1}$ was indicative of the C-H stretch. The intensities were reduced from 89 to 40 for the wavenumber 2920 $cm^{-1}$ and for wavenumber 2851 $cm^{-1}$. For film treated with biosurfactants showed lesser intensity for all wave numbers and peak numbers 4, 5, 6 (1470-1450 $cm^{-1}$) were entirely shifted to a smooth trough 7 both indicating wave numbers corresponding to alkanes, their intensities were also reduced from 89 to 40.

FTIR results of PE films showed formation of ketone, aldehyde, carboxylic acids, and alcohols after biodegradation. The increase in carbonyl absorption band at 1750 $cm^{-1}$ region was primarily due to the formation of carbonyl bond through oxidation of the polyethylene moieties during the UV treatment.

Table 4: Characterization peak in FTIR

| Sl No. | Wave number ($cm^{-1}$) | Bond | Functional group |
|---|---|---|---|
| 1 | 3000-2850 | -C-H Stretch | Alkanes |
| 2 | 2830-2695 | H–C=O: C–H stretch | aldehydes |
| 3 | 1710-1665 | -C=O Stretch | Ketones, Aldehydes |
| 4 | 1470-1450 | -C-H Bend | alkanes |
| 5 | 1320-1000 | -C-O Stretch | Acohols, Carboxylic acid, esters, ethers |
| 6 | 1000-650 | =C-H Bend | alkenes |

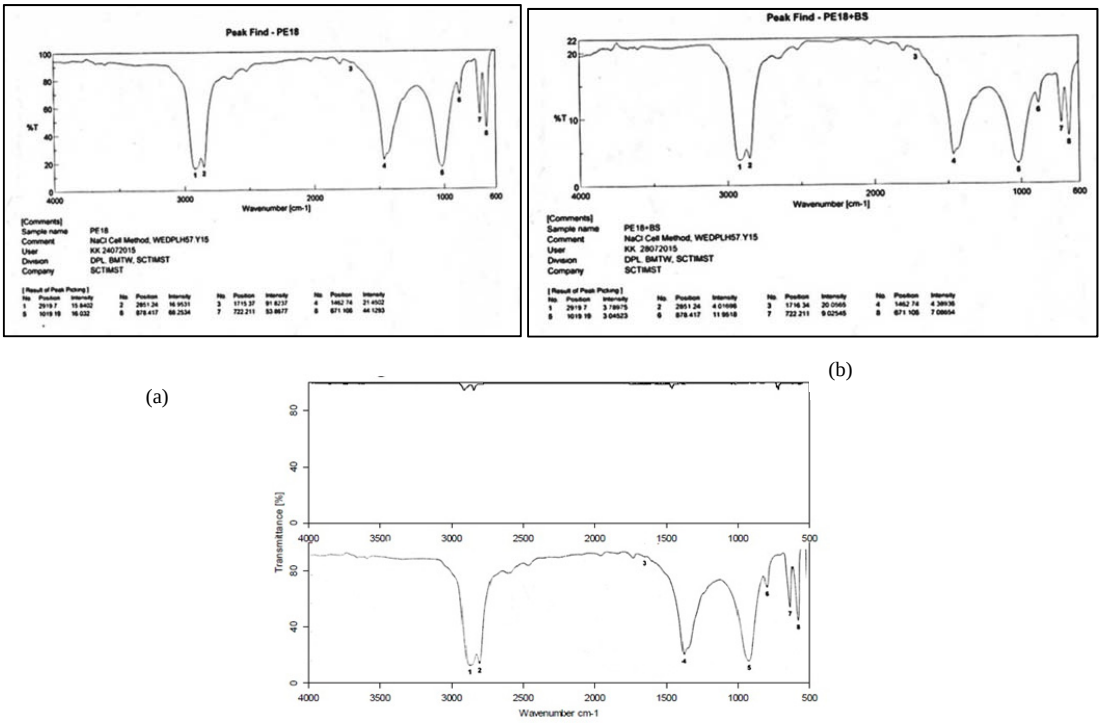


Fig 5 FTIR spectrum of PE (18μ) a) without addition of biosurfactant b) with the addition of biosurfactant c) comparison with virgin PE 18μ

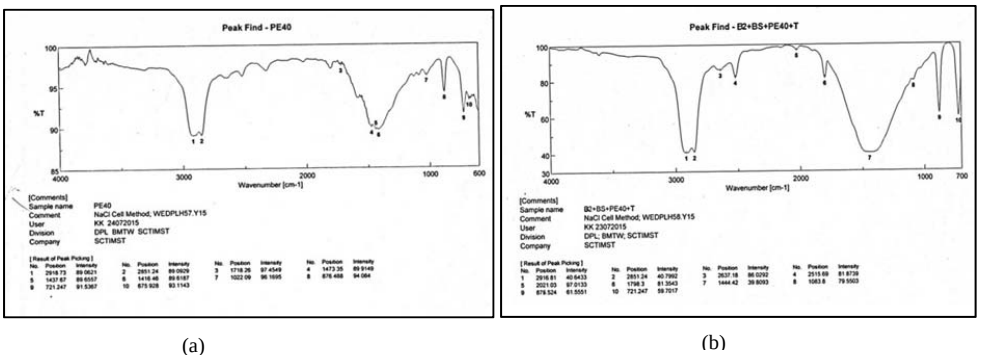


Fig. 6 FTIR spectrum of PE (41μ) a) without addition of biosurfactant b) with the addition of biosurfactants

## 4. Conclusion

Biodegradation of polyethylene was carried out using *B.subtilis*. In this study UV treatment was chosen as the physical means of pretreatment and it was found to enhance the ability of microbes to assimilate PE films. Amphiphilic nature of biosurfactant is responsible for the attachment of microorganisms on hydrophobic surfaces. Consequently addition of biosurfactants helped in attachment of microbes to PE films and thereby enabling them to use polymer as a carbon source at a faster rate. The bacterial species were capable of utilising PE as the carbon source. PE films of lesser thickness were noted to degrade faster indicating more weight loss. Following points may be summarized.

- Treated PE showed more weight loss
- Addition of biosurfactant enhanced their ability to utilise PE.
- PE films of thickness 18μ showed more weight loss

- PE films (18μ) inoculated with *Bacillus subtilis* with the addition of its biosurfactant (surfactin) showed a weight loss percentage of 9.26% in 30 days.
- FTIR Analysis showed lesser intensity peaks in films treated with biosurfactant. Formation of ketone, aldehyde, carboxylic acids, and alcohols were noted after biodegradation

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