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Comparative Biodegradational Analysis of Poly (Butylene Succinate) using *Pseudomonas Fluorescens and Bacillus Species*

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Abstract

Poly (butylene Succinate) (PBS) is one of the synthetic aliphatic polyester with wide range of applications. It remains inert to degradation and deterioration leading to their accumulation and creating serious environmental issues. In this present investigation, we have isolated PBS degrading organisms *Pseudomonas fluorescens* and *Bacillus species* from the Municipal compost of Chennai metro, Tamilnadu, India. The biodegrading property of these organisms was established by weight loss analysis and Scanning Electron Microscopy. Weight loss analysis showed that 50% of PBS film was assimilated within 14 days when inoculated with *Pseudomonas fluorescens* and 33% with *Bacillus Species*. Studies with Scanning Electron Microscopy(SEM) reveals number of cracks and round holes on the eroded film surface. These observations are promising and suggest the faster degradation of PBS by these organisms.

Keywords: Poly (butylene Succinate) (PBS), Biodegradation, Microorganisms: *Pseudomonas fluorescens*, *Bacillus Species* and Scanning Electron Microscopy (SEM).

Introduction

Recent increasing laymen's concern on the environmental protection has roused public opinions to impose duties on the producers and the distributors to dispose the waste matters properly. (i). Every year several hundred thousand tons of non-degradable plastic products are discarded and filled in to marine and earth environments and creates very serious ecological and environmental problems. (ii). Therefore the design and development of materials capable of being degraded into safe components under specific environmental conditions have become increasingly important. The area of polymer research is focused on the development of a wide range of biodegradable polymer products with a predetermined life time. (iii). The biodegradable materials that can be completely degraded into water and carbon dioxide by microorganisms have been developed for the management of plastic waste. (iv). Aliphatic polyesters comprise an important family of polymers exhibiting such “green” Characteristics. (v). They are a class of artificial polymer that include poly(butylene succinate) (PBS), poly(Lactic acid), poly(caprolactone), poly(hydroxyl butyrate), poly(hydroxyl valerate) that can be degraded by microorganisms through simple chemical hydrolysis of ester bonds or by enzymatic attack or both (vi). The combined attack of water and microorganisms results in the rapid breakdown of these polymers into CO2 and water. Among aliphatic polyesters, the aliphatic diols and dicarboxylic acids have been extensively studied due to its simple preparation and also much cheaper than other degradable polymers such as poly(hydroxy alkonates) and Poly(caprolactone). (vii, viii).

PBS is a relatively new biodegradable polymer that shows high biodegradation rates due to its lower crystallinity and more flexible polymer chains that readily fits into the active sites of an enzyme (ix). This promising synthetic aliphatic polyester, is synthesized by the polycondensation of 1,4 butanediol with succinic acid. (x). The interesting properties of PBS like biodegradability, melt processability, thermal and chemical resistance makes it more suitable to be used in various biomedical applications, such as bioabsorbable surgical sutures, wound dressing, implants and drug delivery systems (xi,xii). PBS has excellent processability, therefore, it can be processed into melt-blown textile fibres, multifilament, monofilament, flat, and split yarn, and also into injection molded products, thereby making it versatile in many applications. (xiii,xiv). PBS is also used in mulch film, packaging film, bags and flushable hygiene products etc. It is estimated that 41% of biodegradable plastics are used in packaging and that almost half of that volume is used to package food products (xv). In modern society these type of polymers are available commercially in a variety of forms and used for several applications (xvi). Microbes have a very broad ability to utilize (catabolise) virtually all naturally occurring compounds as their sources of carbon and convert them in to harmless biomass and carbon-di-oxide. The general Biodegradation of polymers involves following steps: (a) Attachment of microorganisms to the surface of the polymer (b) Growth of microorganism utilizing the polymer as the carbon source. (c) Primary degradation of the polymer using enzyme. (d) Ultimate degradation. (xvii).

Microorganisms are able to consume the natural macromolecules within the plastic matrix. This leaves a weakened material, with rough open edges. (xviii). The polymer matrix is derived from natural resources (such as starch or microbially grown polymers), and the fiber reinforcements are produced from common crops such as flax or hemp. Microorganisms are able to consume these materials in their entity, eventually leaving carbon-di-oxide and water as by products. (ix).

Materials and Methods

Sample Collection

For the present study, soil samples were collected from different places of Chennai such as pallikaranai mash and Velachery (Tamil nadu). For this, 1.0g of soil was dissolved in 100ml of distilled water. Then it was serially diluted. The diluted samples were plated in a enrichment medium composed of Bacto - tryptone 5.0g, Yeast extract 2.5g, NaCl 5.0g, Surfactant Span-80 0.1g, Agar 15g & PBS 1.0g (pH 7.6) containing per litre.

Identification

Based on the Morphological, Cultural and Physiological characteristics, growth on nutrient broth and nutrient agar as well as several biochemical tests, the bacterial isolate was identified as *Pseudomonas fluorescence* and *Bacillus Species* table (i). The features agreed with the description of *Pseudomonas fluorescence* and *Bacillus Species* in Bergey's manual of systematic bacteriology. (xx).

Table i: Taxonomical Characteristics.

Characteristics	*Pseudomonas fluorescens*	*Bacillus species*
Morphological Characteristics:		
Morphology	Rod	Rod
Gram – staining	Negative	Positive
Biochemical characteristics:		
Fluorescent pigment	Produced	Not produced
Starch hydrolysis	Positive	Positive
Catalase	Positive	Positive
Methyl red	Negative	Negative
Voges – Proskaeur	Negative	Negative
Indole	Negative	Negative
Citrate utilization	Positive	Positive
Nitrate reduction	Positive	Positive
Lipolytic activity	Positive	Positive
Sugar fermentation:		
Fructose	A	A
Glucose	A	A
Xylose	NA	A
Mannitol	A	A
Sucrose	NA	A
Lactose	NA	NA

A – Acid production

NA – no acid production

Determination of degradation of PBS

To determine the degradation of Polybutylene succinate *Pseudomonas fluorescens and Bacillus species* were cultivated at 37°C with reciprocal shaking (130 rpm) in liquid Polybutylene succinate medium containing Bacto-tryptone - 5.0g, Yeast extract -2.5g, NaCl - 5.0g, Surfactant span 80 - 0.1g, Polybutylene succinate-1.0g (pH-7.0).

As control, Polybutylene succinate was incubated under the same condition but without *Pseudomonas fluorescens and bacillus species.* After 14 days the culture broth was used for various analysis.

Extraction of degraded products

After the period of incubation, the degraded products were extracted with chloroform from the culture broth. The chloroform extract was evaporated to dry using rotatory evaporator and used for analysis.

Weight loss Analysis

After 14 days of incubation the residual Polybutylene succinate films were taken out and washed with distilled water for several times. Then the films were dried to construct weight under vacuum at 50°C. The weight loss percentage was calculated as,

$$D = \frac{Mo-Mt}{Mo} \times 100\%$$

Where, M0 is the weight of the original films
Mt is the weight of the residual films

SEM observations

The surface morphologies of PBS films left, after incubation in case of Control, *Pseudomonas fluorescens* and *Bacillus species* inoculated were observed using HITACHI S-4300 Scanning Electron Microscope with 15Kv acceleration.

Results

Identification of bacterial isolate

The results of the Taxonomical and biochemical analysis of the bacterial isolate indicates that the organisms are *Pseudomonas fluorescence* and *Bacillus Species* Table : No.(i). The features agreed with the description of *Pseudomonas fluorescence* and *Bacillus Species* in Bergey's manual of systematic bacteriology.

Growth on PBS enrichment medium confirmed that the isolated organisms (*Pseudomonas fluorescence and Bacillus species)* were able to degrade PBS (A Polymer of plastics).

Weight loss analysis

Weight loss analysis is the preliminary study which gives the positive effect on the rate of biodegradation using the enzyme producing microorganisms.

Figure No. (i) Shows the visualized picture of PBS films for control, *Bacillus species* and *Pseudomonas fluorescens* inoculated.

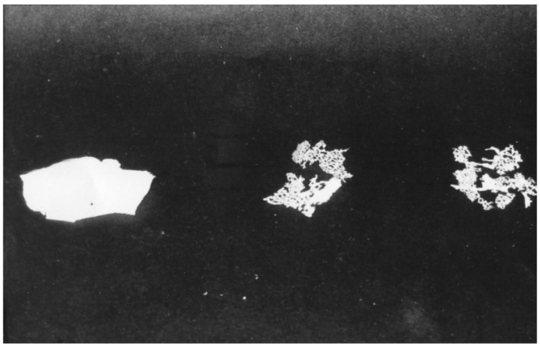

Figure i: Weight loss analysis. This figure Shows the visualized picture of PBS films for control, *Bacillus species* and *Pseudomonas fluorescens* inoculated.

Table No. (ii) Shows the weight loss analysis

Table No. (ii) Weight loss analysis

Organism Used	Weight of PBS before incubation(mg)	Weight of PBS after incubation(mg)	% of weight loss
Pseudomonas fluorescens	30	15	50
Bacillus species	30	20	33.3
Control	30	30	-

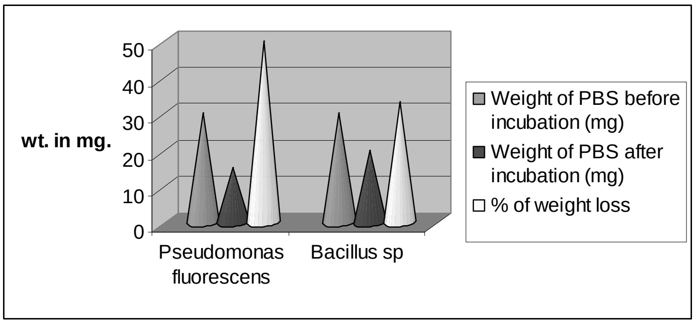

Figure ii: Weight loss analysis. Figure No. (ii), shows the 50% weight loss with *Pseudomonas fluorescens* and 33.3% weight loss for *Bacillus species* with respect to control.

SEM (Scanning Electron Microscopy) Analysis of PBS films

Figure No. (iii), shows the Scanning electron micrographs of the surfaces of PBS films in an enlarged pattern before and after the microbial degradation.

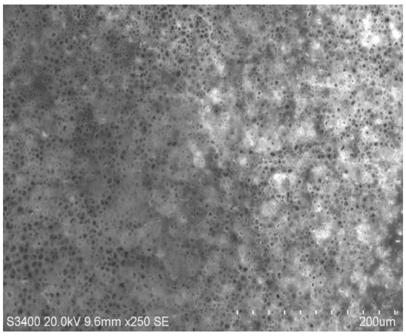

Figure: (iii a) PBS film (Control)

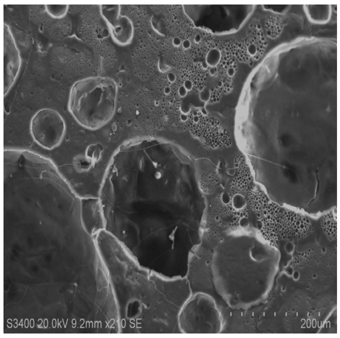

Fig: (iii b) PBS film (*Pseudomonas fluorescens*) inoculated

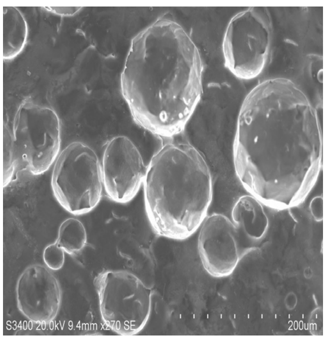

Fig: (iii c) PBS film (*Bacillus*) inoculated

Figure iii: Scanning Electron Micrograph of PBS Figure [(iii: a)] control - *The* PBS film is clear. Figure (iii: b)] *Pseudomonas fluorescens inoculated* (14 days) –Large size holes and ruptures in the film. [(Fig iii: c)] *Bacillus species inoculated* (14days) – Smaller size holes and ruptures in the film.

Discussion

The results of the biochemical and taxonomical analysis clearly reveals that the isolated microorganisms are *Pseudomonas fluorescens and Bacillus species*. The growth of these organisms in the enrichment medium confirms its role in biodegradation by utilizing PBS as one of its growth source.

The possible methods of treating biodegradable PBS polymers are (i) disposing them directly in the environment and (ii) treating them in a closed system before disposal. The closed system treatment can be achieved by cultivating these microorganism which has good potential for the treatment of PBS.

The finding of the weight loss analysis gives a clear picture on the rate of biodegradation by these microorganisms. The high degree of degradation is exhibited *by Pseudomonas flourescens* (50%) than that of the *Bacillus species* (33%). The structural morphology of the films also provides clear evidence that the destruction is more in the films inoculated with *Pseudomonas fluorescens* than the films with *Bacillus* species with respect to control.

The reduction in the weight and altered structural morphology is due to the ability of the microorganisms to utilize the residual PBS films as its carbon source.

The confirmatory evidence comes from the pictures of the SEM analysis. The films clearly shows the different size holes (larger and Smaller) that has been made by the enzymatic secretion of these microorganisms that cleaves the polymeric bonds and causes erosion. The high degree of destruction by *Pseudomonas fluorescens* is evident from the larger holes and ruptures produced during incubation than that of the *Bacillus species* which produced only small holes with respect to the clear control film. This observation is suggestive that the possible mechanism of the biodegradation by these organisms may be similar and the extent of degradation is varying with different organisms.

Conclusion

The results of the present study is encouraging that the synthetic polymers like PBS can be effectively degraded by isolated microorganism *Pseudomonas fluorescens* and *Bacillus species.* Though the mechanism of degradation by these microorganisms may be similar, the degree of the degradation is varied with different organisms. This investigation clearly reveals that *Pseudomonas fluorescens* has more potential to degrade PBS than that of the *Bacillus species.*

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