# Screening for Polypropylene Degradation Potential of Bacteria Isolated from Mangrove Ecosystems in Peninsular Malaysia

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**Abstract:** Polypropylene (PP) is a versatile polymer that is widely used globally and plays an important role in many commercial applications. Its recalcitrance to degradation by microorganisms makes it persist in the environment, causing environmental pollution. In the present studies, *Bacillus cereus* and *Sporosarcina globispora* isolated from mangrove sediments in Peninsular Malaysia were screened for ability to degrade polypropylene using mineral salt media. The bacteria isolates were able to grow on media infused with polypropylene. The extents of biodegradability of the polypropylene granules by the isolated bacterial strains were assessed *in vitro* in the medium containing polypropylene as sole carbon source. After 40 days of incubation, the biodegradation of polypropylene granules was measured in terms of weight loss and rate of polymer reduction. *Bacillus cereus* showed 12% and *Sporosarcina globispora* showed 11% degradation by weight loss in 40 days. The rate of reduction of the polypropylene polymer granules by the isolates was 0.003g $day^{-1}$ and 0.002g $day^{-1}$ for *B. cereus* and *S. globispora*, respectively. The designated isolates can degrade the microplastic material and indicate positive potential towards remediation of polypropylene-contaminated environment.

**Key words:** Biodegradation, mangrove, polypropylene, screening.

## 1. Introduction

It has been estimated that more than 4.8 million tons of plastic waste get deposited into the ocean from land [1] and constitute an increasing source of anthropogenic litter in aquatic environments [2]. Pollution of the environment by plastics occurs due to inadequate waste management and disposal. Most plastics dumped on land, shorelines or landfills are carried into the aquatic environment by wind and through runoff [3]. These plastics are not degradable and therefore, persist in the environment and are resistant to degradation by microorganisms as most lack catabolic genes that can break them down [4], [5]. In particular, polypropylene, a versatile polymer that has different applications and serves as both plastic and fibre is one of such. As a polymer that is favored due to its numerous advantages including processability, barrier properties, brilliance, and dimensional stability. it is used in many commercial applications such as packaging and labelling, and as a fibre, it is used in the production of indoor and out-door carpeting. As the use of the polymer, increases so does the amount of waste disposed into the environment [6].

Preventing the release of plastic polymers into the environment is almost impossible. It is therefore important to discover ways to biodegrade these compounds [4].

Only very few results on the biological degradation and utilization of polypropylene polymer has been reported. Rare examples include members of *Rhizopus arrhizus* [7], Vibrio and Pseudomonas species [8]. Most microorganisms are opportunistic and have an inherent ability to adapt in every environment they find themselves. They also have the potential to transform a variety of compounds including plastic polymers. It has been reported that during polymer degradation, the microbes first of all adhere onto the polymer surface which exposes it to microbial colonization. Colonization of the polymer is followed by the secretion of extracellular enzymes which bind to the polymer and causes hydrolytic cleavage. The polymer is then degraded into low weight polymers and mineralized to carbon dioxide ($CO_2$) and water ($H_2O$) to be used by the microbe as energy source [9]. The polymer particles in the organism pass through the cellular membrane where they are broken down within the cells of the organism by cellular enzymes that have the ability to utilize a particular polymer [10]. Using microbes to degrade polypropylene will increase their rate of biodegradation without causing any harm to the environment [11]. Therefore, identifying microbes that can degrade polypropylene is a promising and ecofriendly strategy which could help the natural bioremediation process, favoring the cleaning of natural ecosystems with no adverse impacts.

Mangrove environments are highly productive environments known to harbor a diverse variety of microorganisms [12] which play significant roles in various environmental activities and applications [13]. The high temperature, high levels of salinity, pH, and organic matter, as well as low aeration and moisture improves the substrate conditions thereby, making it conducive for the development of diverse bacteria [14]. Such environmental factors typically enhance the availability of wide genera of microbes with diverse potentials that may include polypropylene degradation. In this paper, we investigated the biodegradation potential of marine bacterial strains isolated from mangrove sediments that can utilize polypropylene as sole carbon source. Previous studies indicate the viability of bacteria isolates from Peninsular Malaysia on the remediation of environmental pollutants including heavy metals [15] oil [16] and polycyclic aromatic hydrocarbons [17].

## 2. Materials and Methods

### 2.1. Materials

Polypropylene granules were obtained from Sigma Aldrich Chemical Co. (Product of USA) with density of 0.9 g/mL at 25 $^{o}$C. The granules were spherical in shape and white in color.

### 2.2. Sample Collection

The sediment samples used in this study were collected bi-monthly through a period of twelve (12) months from Matang mangrove (4$^{o}$50’25.80”N, 100$^{o}$38’9.60”E), Cherating mangrove (4$^{o}$7’36.15”N, 103$^{o}$23’29.46”E), Tanjung Piai (Johor) (1$^{o}$16’5.20”N, 103$^{o}$30’31.36”E), Serkam mangrove in Melaka (1$^{o}$19’37.84”N, 103$^{o}$26’30.61”E), Sedili Basar (Johor) (1$^{o}$55’54.39”N,104$^{o}$7’27.25”E), and Pantai Bisikan Bayu (Kelantan) (5$^{o}$50’0.79”N, 102$^{o}$25’41.07”E), in Peninsular Malaysia. Samples were collected from different depths in the sediment at 1 cm until 5 cm depth using a sterile spatula. Samples were taken from different points with a quadrat of 0.5m x 0.5m locate 2-3m apart from high tide in undisturbed areas [18] filled into sterile plastic bags and transported to the laboratory for further analysis.

### 2.3. Bacterial Isolation and Identification

Bacteria species were isolated by mixing 1 g of sample with 10 ml of normal saline water (0.9 % NaCl) as stock. The mixture was vortexed for 3 h at 180 rpm using Lab-Line 3521 orbit shaker. The resulting suspension was serially diluted and plated [15] on nutrient agar (NA) and incubated at 37 oC for 24 h.

single colonies were further subcultured on freshly prepared NA to obtain individual pure cultures suitable for identification. Isolated bacteria were identified using the Biolog GEN III microplate protocol [19]. An Omnilog reader was used to identify the bacteria species contained in the Biolog's Microbial Identification Systems Software.The authors of the accepted manuscripts will be given a copyright form and the form should accompany your final submission.

## 2.4. Screening for Polypropylene Degradation

Mineral Salt Media (MSM) was used to screen for microplastic degradation as described by [20] with little modifications. The media contain all nutrients except carbon source, necessary for bacterial growth. The bacteria isolated were screened for the ability to use polypropylene as the major carbon source for growth. 0.54g of sterilized polypropylene polymer granules was kept at different locations into sterilized mineral salt medium prior to solidification. The polymer granules became stacked to agar media after solidification. The medium was then seeded with each of the isolated organisms and incubated for a period of 4-8 weeks days at room temperature. Control set was maintained (inoculation on media without polymer) simultaneously. The media was observed for growth.

## 2.5. Microbial Formulation (Inoculum Preparation)

Bacteria isolated and identified as polypropylene-degrading microorganisms were formulated for biodegradation potential. The isolates were grown on freshly prepared NA to obtain pure cultures at 33 $^{o}$C for 24 hours. The pure cultures were inoculated into nutrient broth and grown to a stationary phase in rotating shaker at 29 $^{o}$C at 150 rpm. Individual suspensions at the same physiological phase were then pooled in equal proportions to set up inoculums for the biodegradability experiment.

## 2.6. Biodegradation Experiment

### 2.6.1. Shake flask experiment

Pure cultures of the plastic degrading bacteria isolated were inoculated into 300 mineral medium broth containing 0.54 g of sterilized polypropylene polymer granules as sole carbon source. Control was maintained with polypropylene polymer granules in a microbe free medium. Triplicates were maintained for the experiment and were left on a shaker (rpm 150) for a period of forty (40) days. The optical density was monitored at every five (5) days for 40 days.

### 2.6.2. Determination of dry weight of residual granule

After 40 days of incubation, the polypropylene polymer granules were collected and the bacterial films colonizing the polypropylene granule was removed by supplementing washed thoroughly using ethanol and distilled water, dried in hot air oven at 50 $^{o}$C overnight and the residual weighed was taken [21], [5], [22]. Residual film weight will be calculated using the formula;

$$\left(\text{Percentage weight loss} = \frac{\text{Initial film weight} - \text{Final film weight}}{\text{Initial film weight}} \times 100\right) \quad (1)$$

### 2.6.3. Determination of the rate of reduction of polymer

The data were further processed to determine the rate constant of polymer granules reduction using the first-order kinetic model as follows:

$$K = -\frac{1}{t}\left(\ln \frac{W}{W_O}\right) \quad (2)$$

where $K$ = first-order rate constant for polymer uptake per day; t = time in days; $W$= weight of residual

polymer (g); $W_0$ = initial weight of polymer (g)

## 3. Results and Discussion

### 3.1. Isolation and Identification of Bacteria

Mangrove forests are very dynamic and highly productive marine ecosystems characterized by high rate of organic matter and biogeochemical cycling. Mangroves are rich in carbon and other nutrients and harbor diverse groups of microbial communities which forms an integral part of the mangrove ecosystem and are able to adapt to extreme conditions [23]. In the present investigation, two mangrove bacteria strains were isolated from mangrove areas in Peninsular Malaysia. Isolates were aerobic bacteria and growth patterns were distinctive enough to enhance identification and differentiation into individual isolates. The bacteria isolated included *Bacillus cereus,* which was a rod-shaped, gram positive bacterium and the other was *Sporosarcina globispora,* a gram positive, round spore-forming bacillus. These bacteria species have been reported to belong to the diversity of bacteria in mangrove soils [13].

### 3.2. Primary Screening of Isolates for Polymer Degradation

The two (2) bacteria isolates were able to grow on carbon free medium containing polypropylene granules as carbon source. The isolates indicated growth signs around the polymer granules within 4 – 5 days after incubation, attaching themselves to the polymer granules which extended around the granule in 10-15 days. No growth was observed on the control samples. The major mechanism involved in biodegradation is the colonization of the surface of plastics which lead to the development of biofilms. Once the organism gets attached to the surface, it utilizes the plastic polymer as carbon source and begins to multiply [9], [24].

### 3.3. *In-vitro* Biodegradation Assay

The biodegradability potential of the bacteria isolates was assessed in a liquid medium containing polypropylene granules as the sole carbon source. The growth of the isolates was monitored throughout the incubation period. The broth culture was subjected to spectrometric analysis at several intervals (days 0, 5, 10, 15, 20, 25, 30, 35, and 40). The growth profile of the bacterial isolates during the biodegradation assay is shown in Fig. 1.

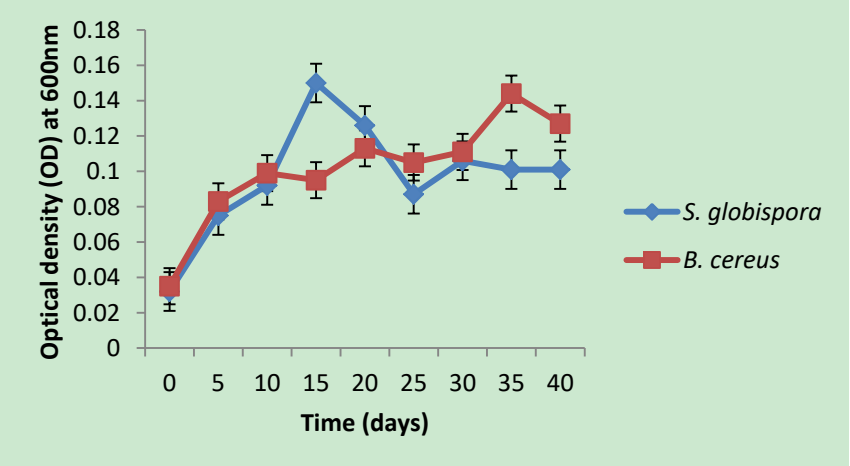


Fig. 1. Growth curve of bacterial isolates during biodegradation studies.

The growth curve reflected a synodical pattern which was characterized by significant growth that indicates increase in microbial cell load. The first 10 days showed a log phase pattern before optimizing on

the 15th day. Such development might imply increase in metabolic activities that enhance double generation of the microbial cells. Reference [25] stated that an increase in the bacterial load has correlation with the degradation of the polymer. However, a slight disparity occurred between the microbes as *B. cereus* recorded optimal log phase at the 35th day (0.14 ABS at 600nm) unlike *S. globispora* which was already at stationery phase (0.1 ABS at 600nm). This variation may be attributed to differences in some cytoplasmic activities peculiar to each bacterial species. At the end of the experiment, the color of the white polypropylene granules changed to yellow indicating bacteria colonization.

### 3.4. Determination of Dry Weight and Reduction Rate of Polypropylene by Bacterial Isolates

The percentage of weight reduction was estimated after forty (40) days of incubation and the degrading ability of the two isolates showed variability as shown in Table 1. Polypropylene granules incubated with *S.* globispora and *B. cereus* showed a weight loss of 11 % and 12 %, respectively with *Bacillus cereus* having the highest percentage removal. This indicated that the weight loss was actually due to a biological process occurring in the media and not due to the chemicals in the media. From the results, it can be concluded that both bacteria had the potential to utilize polypropylene as carbon source but *B. cereus* possess greater potential to degrade polypropylene polymer when compared to *S. globispora.* It is clear that natural polymers can be degraded to some extent by microbes. Degradation of other plastic polymers such as polyethylene by *B. cereus* has been reported [26]. Reference [5] reported the degradation of polyethylene terephthalate (PET) by *Ideonella sakaiensis*.

Furthermore, the potentials of the isolates to significantly impact the weight of the polymer after 40 days was compared from the calculated rate of reduction constant, *K*. Table 2 shows the rate of reduction of polypropylene granules by *B. cereus* and *S. globispora* after 40 days of incubation. The *K* value obtained from treatment with *S. globispora* was 0.002 g $day^{-1}$ as against 0.003 g $day^{-1}$ recorded with *B. cereus.* The result presented a correlation in the rate of reduction, though higher correlation ($R^2$ = 0.8) was found with the *B. cereus*. It therefore implied that the rate of daily reduction was more prevalent in *B. cereus* than *S. globispora*. Hence, it supports the total weight reduction found at the end of the experiment.

Table 1. Mass Reduction Efficiency (%) of Polymer Granules after 40 Days of Incubation with Bacterial Isolates

| Bacterial isolates | Initial mass of granule (g) | Final mass of granule (g) | % reduction |
|---|---|---|---|
| Control | 0.54 | 0.54 | 0 |
| *Bacillus cereus* | 0.54 | 0.47 | 12 |
| *Sporosarcina globispora* | 0.54 | 0.48 | 11 |

Each data point represents the mean of three replicates

Table 2. Rate of Reduction of Polypropylene Granules by *B. Cereus* and *S. Globispora*

| Bacterial isolates | Removal rate $day^{-1}$ (g) | F- value | $R^2$ |
|---|---|---|---|
| *Bacillus cereus* | 0.003 | 0.217 | 0.8311 |
| *Sporosarcina globispora* | 0.002 | | 0.6209 |

## 4. Conclusion

The longevity of synthetic polymers has led to the accumulation of microplastics in marine waters, shores, and sediments for a long period of time. The resistance to degradation and deterioration is creating serious environmental concern. In the present study, two marine bacterial strains were successfully isolated and identified from mangrove sediments. Degradation of polypropylene granules was carried out with *Bacillus*

*cereus* and *Sporosarcina globispora*. Both isolates had the ability to grow on media containing polypropylene granules as sole carbon source. The efficiency of *Bacillus cereus* to degrade polypropylene was higher than that of *S. globispora*. The two isolates demonstrated the potential for the degradation of polypropylene and can therefore be used to reduce the quantity of microplastic waste in the environment.

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