Biodegradation of low-density polyethylene (LDPE) and starch–based plastic (SBP) by thermophiles *Bacillus subtilis* and *Candida tropicalis*

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**Abstract.** This project was carried out to study the biodegradation of low-density polyethylene (LDPE) and starch-based plastics (SBP) by two types of thermophiles microorganisms namely *B. subtilis* and *C. tropicalis* in a lab scale method. A few specific objectives were set to identify the growth curve of both strains on LDPE and SBP films, changes in physical and chemical properties by weight loss, carbon dioxide (CO₂) evolved, topography changes of plastics surfaces and the efficiency on biodegradation rate of LDPE and SBP. The results showed that after 49 days incubation period, the optimum growth of *B. subtilis* for both LDPE and SBP is at week 5 of incubation with 8.9 x 10⁸ CFU/mL and 9.1 x 10⁸ CFU/mL respectively. While for *C. tropicalis* the highest growth was recorded at week 4 of incubation with 9.6 x 10⁸ for both LDPE and SBP. The weight loss reduction percentage of LDPE and SBP by *C. tropicalis* was 3.2% and 22.3% respectively while for *B. subtilis* the results recorded were 4.6% and 12.9% respectively. SEM analysis revealed that there are topography changes for LDPE with bubbling on surface while cracks and holes formed on SBP surface. The strum test used to identify CO₂ evolved in SBP by *C. tropicalis* is 2.7 g/L which was 5-fold higher as compared to LDPE while in SBP by *B. subtilis* the results is 2.5 g/L which 5-fold higher compared to LDPE. Based on this study, it can be concluded that *C. tropicalis* have great potential in degrading SBP as compared to LDPE.

**Keywords:** low-density polyethylene (LDPE), starch-based plastic (SBP), thermophiles, *B. subtilis*, *C. tropicalis*, biodegradation

1. **Introduction**

Plastics or scientifically named as polymers had cover up the needs in human used nowadays. According to [1], the production of plastic has increased twenty fold and this number are expected to double in the next 20 years. Among this amount, it was reported that 12 million tones of plastics leak into the oceans. Polyethylene is the most commonly used plastics among people. Polyethylene can be categories to low-density polyethylene (LDPE), medium-density polythene (MDPE), high-density polyethylene (HDPE) and very low-density polyethylene (VLDPE) [2] Among this, LDPE is commonly used for making grocery bags, food wrapping material, power cable sheathing and laboratory containers, as it is excellently resistant to dilute and concentrated acids, ketones and vegetable oils. The resistant and characterization of LDPE make it harder for degradation and cause a major pollution. [3].
According to [1], there are 25 million tons of plastics accumulate in the coastal and terrestrial environment. Marine ecosystem are more adversely affected by the plastics waste that shares 60-80% of total marine waste. Biodegradable plastics has become the way for new considerations of waste management strategies since these materials are designed to degrade under environmental conditions or in municipal and industrial biological waste treatment facilities [4, 5]. In this study, the use of locally isolated Environmental-Relevant Microorganisms (ERM) is beneficial to treat the plastic pollution. This research focused on the inoculation of thermophiles microorganism’s namely B. subtilis and C. tropicalis to degrade plastic samples for 49 days incubation period. The biodegradation efficiency was done by examined the biofilm cell growth, determination through physical and chemical analysis and spectrometric analysis of plastics surface changes by using scanning electron microscopy (SEM). From this research, the significance findings can be achieved through biodegradation rate by measuring the carbon dioxide evolved and weight loss measurement within 7 weeks period.

2. Materials and methods

2.1. Preparation of medium
There are four medium that being used for this study, which are nutrient broth (NB), medium salt mineral (MSM), Ramsay broth (RB) and nutrient agar (NA). These medium are used as nutrient supplied for microbial growth and further biodegradation studies.

2.2. Preparation of plastic films
Low-density polyethylene (LDPE) plastics bag is obtained from Giant Hypermarket 1 Borneo, Kota Kinabalu, Sabah. This plastic is manufactured by EH Packaging and Plastic Industry that located in Kota Kinabalu, Sabah, while starch-based plastics (SBP) bag is obtained from Maribumi Startech Sdn. Bhd. These plastics samples were cut into equal pieces 5cm × 3cm (l × b) and dried overnight at 60°C. For biodegradation studies, the plastics films were weighed before disinfest with 95% ethanol for 30 minutes. Ethanol was used as disinfectant to remove any impurities attached to its surface [6]. The initial weigh of plastic was recorded as day 0 to see the changes of plastics surfaces during biodegradation process.

2.3. Inoculation of thermophiles strains for biodegradation study
The strains used in this study were Thermophilic microorganisms namely Bacillus subtilis [7] and Candida tropicalis [8]. The strains were obtained from beads at the Animal Physiology Lab, Faculty of Science & Natural Resources, Universiti Malaysia Sabah.

![Figure 1](image)

Figure 1. The selected strains on basal medium (a) B. subtilis with LDPE (b) B. subtilis with SBP (c) C. tropicalis with LDPE and (d) C. tropicalis with SBP.
The experiment was performed by adding LDPE and SBP films in the two media, which are mineral salt medium (MSM) for B. subtilis and Ramsay broth (RB) for C. tropicalis, in accordance with the Standard test method BS EN ISO 846:1997 (with some modification). For biodegradation study, a total of 10% of bacterial colony B. subtilis were inoculated into each MSM flasks and C. tropicalis in RB flasks (Figure 1). Three strips of each type of plastic films are added to 2 different flasks that contain mineral salt medium (MSM) and Ramsay broth (RB) and then 2 flasks of LDPE and starch-based plastics (SBP) film are inoculate with thermophilic B. subtilis and C. tropicalis while the other 2 are leave and maintain as control. The experiment were conducted with B. subtilis were left in rotary shaker (180 rev min⁻¹) at 32°C for 49 days and C. tropicalis are left for 30°C at 200 rev min⁻¹. Within this period, a few observations was made to study the biodegradation process of plastics by both strains.

2.4. Biofilm cell growth study
An aliquot for each of incubated flasks were taken at regular intervals for first 30 days of incubation for quantification of microbial cells by serial dilution technique. This is followed by plating on Nutrient Agar for viable colony count. For this study, biofilm layer formed on the plastics films is remove by immerse the plastics strips in 2mL of 0.9% NaCl for 2 hours and vortex for 5 to 10 minutes [9]. Aliquot from this suspension is taken and serially diluted and plated on Nutrient agar plates for colony counting. The colony forming units from biofilm cells is calculate according to equation below.

\[
\log \left( \frac{\text{CFU}}{\text{cm}^2} \right) = \log \left( \frac{\text{Average CFU}}{\text{Drop volume}} \times \text{dilution counted} \right)
\]  

(1)

2.5. Carbon dioxide (CO₂) evolution
The degradation of plastics can be measured by determine the carbon dioxide evolve according to OECD Guidelines 1992 Method 301 B. After incubation, all the flasks including control flask is calculate gravimetrically as a result of degradation of polymeric chain that trap in absorption flask contains KOH (1.0 M). Barium chloride solution (0.1 M) was added into the flask containing KOH which resulted in barium chloride precipitation (using CO₂ released from the breakdown of polymer). CO₂ produced were calculated gravimetrically by measuring the amount of CO₂ evolved by addition of BaCl₂. The changes of CO₂ evolved was recorded.

2.6. Determination of weight loss in LDPE and SBP films
The weight loss of LDPE and SBP films were calculated in form of percentage (%) biodegradation. For weight loss measurement, the plastic strips were sampling for every 7 days of interval. The residual of LDPE and SBP were washed in 70% of ethanol and rinsed through distilled water to remove any kind of impurity and organic matter films. Then the samples were dried overnight at 60°C to record the final reading. The weight loss was calculated by using the equation below.

\[
\text{Biodegradation (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100
\]  

(2)

2.7. Spectrometric analysis of SEM
The changes of the surface topography for treated and untreated LDPE and starch-based plastic (SBP) films were examined by Scanning electron microscopy (SEM). The treated samples after 49 days of incubation were washed with 70% ethanol and distilled water for a few minutes to remove the cell. Then the samples were coated with gold for 40 second and were observed under high-resolution scanning electron microscope (EVO LS15; Carl Zeiss, Germany).
3. Results and discussion

3.1. Microbial cell growth on plastics

The results showed that the growth curves of *C. tropicalis* on both LDPE and SBP were slightly increase from week 1 to 7 along the biodegradation study. For *B. subtilis* the growth was increase from week 1 to 5 and slowly decrease along the incubation period. The highest growth curve of *B. subtilis* can be observed in SBP at week 5 with $9.1 \times 10^8$ CFU/ml. While for *C. tropicalis*, the highest growth rate can be shown at week 4 in SBP with $9.6 \times 10^8$ CFU/ml. The formation of biofilms by both plastics within 7 days had reduced the hydrophobicity of both polymers and yet it improved the degradation rate. This can be observed after a week of incubation whereas the microbial growth of both strains were slightly increased. This indicates that the biodegradation were rapidly progressed which means it has make an easier movement for microbial attack to degrade the plastic. In addition, the presence of plastics as sole source of carbon may favor the growing of both strains, especially for petroleum-based microbes. In this study, there were no other nutrients supplied to the cultures such as glucose [10] and the changes of microbial growth can be seen through the presence of LDPE and SBP.

![Graph of microbial growth on plastics](image)

**Figure 2.** The microbial growth of (a) *B. subtilis* and (b) *C. tropicalis* on LDPE and SBP during incubation periods.
The graph also showed that both strains that supplied with SBP was rapidly developed as compared to LDPE. This is due to the composition of SBP which made by starch and contain high nutrient content as compared to LDPE. The results also showed that *C. tropicalis* was more efficient in degrading LDPE and SBP as compared to *B. subtilis*. According to [11], microorganisms effectively degrade the plastics when it attach to the surface. As SBP surface is hydrophilic, *C. tropicalis* was found to degrade this compound more effective as compared to *B. subtilis*.

3.2. Carbon dioxide (CO$_2$) evolution

Figure 3 showed the amount of CO$_2$ produced from the breakdown of LDPE and SBP after the biodegradation study. It was found that the CO$_2$ evolved by SBP is higher than LDPE for both cultured in *B. subtilis* and *C. tropicalis*. The data indicates that microbes utilized the oxidation products in the pre-oxidised films as carbon sole source. As SBP is made by starch, it becomes the sole of nutrients for microbial growth in turns increase the mineralization profile of plastics sample. The CO$_2$ evolved by *C. tropicalis* and *B. subtilis* in SBP was the highest with 2.69 g/L and 2.5 g/L respectively. The supplied nutrient by starch increase the favorable conditions for microbes. This finding showed that SBP degradation is more efficient than in previous study by [12]. He stated that the degradation of LDPE by *Aspergillus clavatus* produced only 2.32 g/L of CO$_2$. However, the degradation of LDPE in this study by both strains produced only 2.15 g/L and 2.19 g/L of CO$_2$ which is 1 – fold lower than the previous study.

![Figure 3](image.png)

**Figure 3.** The amount of CO$_2$ produced after degradation of plastic at room temperature.

3.3. Biodegradation of plastic

The biodegradation of plastic was quantitatively assessed by measuring the percentage reduction of LDPE and SBP in shake flask experiment under control conditions. Figure 4 showed the percentage of weight loss in LDPE and SBP by *B. subtilis* and *C. tropicalis*. The data obtained indicates that the percentage of weight loss was increase as the incubation period. The highest percentage reduction was found in LDPE with 3.2% while SBP recorded 22.3% by *C. tropicalis*. For *B. subtilis*, the highest reduction was observed with 4.6% for LDPE and 12.9% for SBP respectively. This finding suggested that the presence of both strains *B. subtilis* and *C. tropicalis* as degrader had increase the process of plastic degradation. Microbes that grow utilizing the polymers lead to an increase in weight due to the adherence of microbes. The percentage weight reduction of LDPE and SBP films can be attributed to the breakdown of carbon backbone due to enzymatic degradation by both strains studies.
Figure 4. The percentage of weight loss (%) of LDPE and SBP within 7 weeks incubation periods by B. subtilis and (b) C. tropicalis.

3.4. Topography changes on polymeric surface
The extent of biodegradation was examined by observing the changes in surface morphology through SEM analysis. Figure 5 showed the physical changes of both polymeric surfaces before and after 49 days of incubation periods. Analysis on SEM showed that the topography of LDPE surfaces before inoculated with C. tropicalis was flatted and smooth which indicates that there was no reaction of biodegradation by C. tropicalis. However after being degraded, the surface was observed distinct in which a weakening surface of LDPE had been eroded with greasy surface. LDPE structure showed cracks and formed a micro bubbles in polymer matrix. This is due to scissoring of long chain polymer and evolution volatile products from LDPE surface [13]. On the other hand, the structure of SBP after biodegradation study was observed to be crumpled on the surface. It was found that there are a few holes formed on the surface with ragged edges and narrow gaps with smooth edges extending lengthwise along the film’s surface. The destruction of surface was suggested due to the microbial attack of C. tropicalis that had weakened up the polymeric structure and penetrate through SBP matrix during the degradation process. According to [14], SBP films was easily disintegrated under mild pressure during
the degradation process. The element starch in SBP film has increased the surface roughness compared to LDPE films. An increase in surface roughness could provide a plenty of anchoring places for microorganisms which is less possible in case of LDPE films [15]. The adherence of cells to SBP surface may due to biofilm formation [16], indicating strong adhering capabilities of *C. tropicalis* on SBP film until it caused the surface destroyed with a few holes.

![Bubbles formation](image1)

![Cracks](image2)

![Holes](image3)

**Figure 5.** SEM images under 1.5 kV magnification of (a) LDPE surface before incubation (b) LDPE surfaces after incubated with *C. tropicalis* (c) SBP before incubation (d) SBP after incubated with *C. tropicalis*.

4. **Conclusion**

The present work indicates that both strains namely *B. subtilis* and *C. tropicalis* is capable in degrading low density polyethylene (LDPE) and starch- based plastic (SBP). Generally, *C. tropicalis* have a higher degrading effectiveness as compared to *B. subtilis*. The weight loss reduction (22.9%) in SBP by *C. tropicalis* proved that biodegradation of plastic had occur after 49 days of incubation. This showed that the relatively significant degradation was forced the strains to consume SBP as the only carbon source. Thus this condition had leads to development of biofilm and high growth rate on SBP surfaces.
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