Screening of Different Fungi Strains Gongronella sp. WICC F60 and Cordyceps sp. WICC F61 for Degradation of Low-Density Polyethylene

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INTRODUCTION

Plastic waste is a major contributor to numerous issues, including the obstruction of drains, damage to the environment, and adverse effects on human health (Dailin et al., 2022a). Global production of plastic waste reached 348 million metric tonnes in 2017, and projections indicate a four-fold increase in this number by 2050 (Zhai et al., 2023). Reports indicate that the world dumps over 8 million metric tonnes of plastic waste into the ocean annually. This practice is responsible for the extinction of marine life and...
the contamination of food supply for humans (Zhai et al., 2023). Many countries generate a large quantity of plastic waste pyramids, which need to be disposed of in an appropriate manner using effective waste management because their rapid proliferations in wastewater cause major problems (Ghayebzadeh et al., 2020).

Plastic is defined as a substance that is predominantly composed of a high-molecular-weight organic substrate that retains its firmness. Landfills currently house 79% of the world’s total of 6300 million metric tonnes of PW. Only 2% of the plastic packaging material that has been collected over the past 40 years has been recycled (Awoyera & Adesina, 2020).

The types of plastic materials and compounds that are utilized in manufacturing are distinct, each with its own unique set of characteristics (Wiesinger et al., 2021). Polyethylene, also known as PE, is the most prevalent kind of plastic on the globe and comes in a broad variety of densities. Of all these many kinds of plastic, polyethylene is the most common. Different physical qualities are exhibited by the manufactured plastic in accordance with the density of the polyethylene that was used in its production. Because of this, polyethylene can be found in a large number of different products. Low-Density Polyethylene (LDPE), Linear Low-Density Polyethylene (LLDPE), and High-Density Polyethylene (HDPE) are the three densities of polyethylene that are most commonly seen (Mierzwa-Hersztek et al., 2019).

Plastics are primarily composed of binders, fillers, pigments, plasticizers, and other additives (Evode et al., 2021). Four distinct categories can be applied to plastics that break down quickly. These categories are photodegradable bioplastics, bio-based bioplastics, compostable bioplastics, and biodegradable bioplastics. Plastic waste can be broken down through either physiochemical process, which are referred to as “abiotic” processes, or biodegradation processes, which are referred to as “biotic” processes. Usually, the first phase that is often regarded to play a vital role in any degradation processes is the breaking down of the polymeric substance by the physical forces and mechanical nature of nature. This step is typically considered to be the most crucial phase (Awoyera & Adesina, 2020).

Microorganisms play many important roles in biotechnology industries such as agriculture (Islam et al., 2022), feed (Dailin et al., 2019), food (Selvamani et al., 2021), wastewater (Dailin et al., 2022c) and pharmaceutical (Nordin et al., 2020). Microorganisms create enzymes that drive the biodegradation process on the plastics’ surface (Dailin et al., 2022b). Microbes such as bacteria and fungi attach themselves to the plastic film, which then prevents the enzymes from functioning properly. After that, they start to develop on the film by utilising it as a substrate and a source of nutrients in order to do so. This leads to the gradual depolymerization of the polymers, which a process known as mineralization then compiles. This process will result in the production of H₂O (water), CO₂ (carbon dioxide), and CH₄ (methane) as its final products (Srikanth et al., 2022).

Certain fungi utilise both their internal and external enzyme systems in order to break down plastics (Temporiti et al., 2022). This process is known as plastic degradation. The intracellular enzymatic system not only functions as an internal mechanism for detoxification but also plays a significant part in the adaptation process of fungi (Srikanth et al., 2022). Ambient factors such as moisture, pH, temperature, and so on influence the action of fungi on the surface of plastics. All these conditions are present in the surrounding environment. Temperature is another factor that plays a significant part in the biodegradation process. Degrading polymers with a high melting point takes significantly longer than degrading polymers with a low melting point. In order for fungi to become active, there must be an adequate supply of moisture. In order for enzymes to effectively break down plastic polymers, the surrounding environment must have a sufficient pH level. Temperature also plays an essential part in the biodegradation process.

**MATERIALS AND METHOD**

**Materials**

LDPE plastic degrading, agar-agar powder, potato dextrose broth (PDB), distilled water and low-density polyethylene (LDPE), 70% ethanol, 2% sodium dodecyl sulphate (SDS), dipotassium hydrogen phosphate (K₂HPO₄), monosodium phosphate (NaH₂PO₄), ammonia sulphate ((NH₄)₂SO₄), magnesium sulphate heptahydrate (MgSO₄.7H₂O), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride hexahydrate (CaCl₂.6H₂O), ferrous sulphate (FeSO₄.7H₂O), zinc chloride (ZnCl₂), copper II sulphate (CuSO₄) and Tween 80.

**Microorganism**

Fungus was obtained from the Wellness Industrial Culture Collection (WICC) at the Institute of Bioproduction Development, Universiti Teknologi Malaysia namely, Gongronella sp. WICC F60 and Cordyceps sp. WICC F61. A sterile inoculating loop was dipped into the fungus suspension strain and streaked over the surface of the PDA agar plates. The petri dish was placed in the incubator at 36 °C for 5 days.

**Preparation of Potato Dextrose Agar**

Upon preparation, a mixture comprising 1000 mL of distilled water, 24 g of PDB, and 27 g of agar agar powder was assembled. Subsequently, the mixture underwent autoclaving at 121 °C for 20 minutes to ensure sterilization. Once sterilized, the combination was allowed to cool to room temperature. After cooling, the liquid was poured into a petri dish and left to settle before being refrigerated until further use.

**Preparation of minimal salt medium (MSM)**

A 20 g K₂HPO₄ and 8 g NaH₂PO₄ were added as buffering agents; 16 g (NH₄)₂SO₄ was added as a nitrogen source; 2 g MgSO₄.7H₂O, 2 g NaCl, 0.6 g KCl, and 0.8 g CaCl₂.6H₂O were added as essential mineral salts. In addition, the medium contained 4 mL mixed trace element solution (0.1 g of each FeSO₄.7H₂O, ZnCl₂, and CuSO₄ in 100 mL distilled water), and 1 mL Tween 80 as a surfactant agent. These ingredients were dissolved in distilled water and diluted to 4 L (pH was fixed at 7.2) and then sterilized in an autoclave (Montazer et al., 2016).

**Pre-treatment of LDPE Plastic Samples**

LDPE sheets with a thickness of 2.3 mm were obtained from Merck. The LDPE sheets were cut into square pieces measuring approximately 1 cm x 1 cm. These cut pieces were then immersed in a 70% ethanol solution for 30 minutes and then dried.
subsequently rinsed with sterile distilled water. Afterward, the LDPE samples were continuously stirred while being boiled in distilled water that had been sterilized for 1 hour. Following this, the LDPE pieces were placed inside a laminar airflow chamber until the surface moisture was removed (Biki et al., 2021).

RESULTS AND DISCUSSION

Determination of Weight Loss of LDPE by the Potential Fungal Isolates

A microbalance was used to measure the weight difference between the samples before and after the deterioration process. This was done in order to assess the extent of the discrepancy. The following table provides a summary of the findings from the experiment on weight loss that was conducted in potato dextrose broth (PDB) over the course of 5, 10, 15, 20, 25, and 30 days. Figure 1 shows the weight of plastic that is being lost in the process of being incubated in Gongronella sp. WICC F60 and Cordyceps sp. WICC F61 which are continuously growing. Based on these findings, it can be deduced that the use of polyethylene as a carbon source caused the weight loss of LDPE that occurred during the incubation time with the various isolates.

**Table 1** Percentage weight loss of LDPE treated with Gongronella sp. WICC F60 and Cordyceps sp. WICC F61

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Weight Loss of Gongronella sp. WICC F60 (%)</th>
<th>Weight Loss of Cordyceps sp. WICC F61 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.38 ± 0.376</td>
<td>3.25 ± 0.04</td>
</tr>
<tr>
<td>10</td>
<td>0.40 ± 0.015</td>
<td>3.17 ± 0.271</td>
</tr>
<tr>
<td>15</td>
<td>0.39 ± 1.290</td>
<td>3.90 ± 0.633</td>
</tr>
<tr>
<td>20</td>
<td>0.67 ± 0.188</td>
<td>3.55 ± 0.340</td>
</tr>
<tr>
<td>25</td>
<td>0.88 ± 1.089</td>
<td>4.62 ± 0.151</td>
</tr>
<tr>
<td>30</td>
<td>1.0703 ± 0.296</td>
<td>5.56 ± 0.546</td>
</tr>
</tbody>
</table>

**Figure 1** Weight of plastic lost

As shown in Table 2, further observation of WICC F61 spanned from the fifth to the thirty-first day. During the lag phase, wherein fungi adapt to their new surroundings, biomass levels were initially low on the fifth day. Gongronella sp. WICC F60 demonstrated the highest biomass production, approximately 0.06%, by the 10th day. Conversely, Cordyceps sp. WICC F61 exhibited its peak on day 15 with only 0.03%. However, beyond the 15th day mark, the biomass started declining due to diminishing nutrients and other factors. The ensuing phase, termed the death phase, denotes the eventual decomposition of mycelium triggered by various stimuli, including exposure to external stress conditions.

**Table 2** Observation of WICC F61 spanned from the fifth to the thirty-first day

<table>
<thead>
<tr>
<th>Time (Days)</th>
<th>Biomass of Gongronella sp. WICC F60 (%)</th>
<th>Biomass of Cordyceps sp. WICC F61 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.01 ± 0.004</td>
<td>0.01 ± 0.005</td>
</tr>
<tr>
<td>10</td>
<td>0.06 ± 0.038</td>
<td>0.02 ± 0.017</td>
</tr>
<tr>
<td>15</td>
<td>0.05 ± 0.038</td>
<td>0.03 ± 0.019</td>
</tr>
<tr>
<td>20</td>
<td>0.02 ± 0.014</td>
<td>0.02 ± 0.014</td>
</tr>
<tr>
<td>25</td>
<td>0.01 ± 0.005</td>
<td>0.01 ± 0.002</td>
</tr>
<tr>
<td>30</td>
<td>0.02 ± 0.011</td>
<td>0.01 ± 0.005</td>
</tr>
</tbody>
</table>

**Figure 2** Organisms’ biomass is composed of Gongronella sp. WICC F60 and Cordyceps sp. WICC F61

Changes in pH during LDPE Degradation

Microorganism growth is influenced by the pH profile of the culture medium. Every living thing has a pH level that is ideal for its growth and development. If this pH value is altered, growth will not be optimal.
Following a 30-day incubation period, the pH of the liquid medium containing LDPE was assessed using a pH meter and compared to the initial pH of the experimental medium. It was observed that for both substrates, the pH of the LDPE-containing medium gradually decreased over the 30 days, dropping from 6.66 to 6.63 for Gongronella sp. WICC F60 and from 6.8 to 6.62 for Cordyceps sp. WICC F61 as illustrated in Figure 3. The reduction in pH in environments where fungi degrade plastic is attributed to the production of enzymes that degrade plastic, leading to subsequent chemical changes. Fungi secrete enzymes such as cutinase, lipase, proteases, and lignocellulolytic enzymes that act on plastic, causing oxidation or hydrolysis as well as the formation of functional groups that increase the plastic’s hydrophilicity.

CONCLUSION

Gongronella sp. WICC F60 and Cordyceps sp. WICC F61 were able to degrade LDPE plastic. Both fungi exhibit a proportional increase in LDPE weight loss. However, Cordyceps sp. WICC F61 shows more LDPE weight loss compared to Gongronella sp. WICC F60. The weight loss LDPE in Gongronella sp. WICC F60 was 0.38%, 0.40%, 0.39%, 0.67%, 0.88%, and 1.07%. For Cordyceps sp. WICC F61, weight reduction was 3.25%, 3.17%, 3.90%, 3.55%, 4.62%, and 5.56%. Gongronella sp. WICC F60 and Cordyceps sp. WICC F60 both show a decrease in pH over time. Both fungi show an increase in biomass and a decrease at day 20. After 30 days of Gongronella sp. WICC F60 incubation, plastic degrades by 5.56%. During the 10th day of incubation, Gongronella sp. WICC F60 displayed the highest biomass production, approximately 0.06%. In contrast, Cordyceps sp. WICC F61 reached its biomass peak on the 15th day, with only 0.03% biomass production.

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References


