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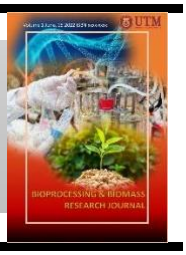

Research Article

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

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ARTICLE INFO

Article History:
Received 29 April 2024
Received in revised form 21 June 2024
Accepted 25 June 2024
Available online 30 June 2024

Keywords:
Biodegradation,
Plastic,
Fungi,
Gongronella sp.,
Cordyceps sp.,
weight loss

ABSTRACT

Over the past decade, polyethylene (PE) products have become increasingly important in several industries. Their biggest concerns are plastic material degradation resistance and environmental longevity. Therefore, this study aims to find fungi that are capable of degrading low-density polyethylene (LDPE) efficiently. This study uses *Gongronella* sp. WICC F60, and *Cordyceps* sp., WICC F61. The fungi were grown on potato dextrose agar for 30 days. Following that, fungal colonies were placed in potato dextrose broth for incubation. The LDPE sample was then cultured with the fungal strain in a minimum salt medium with a 10% inoculum. After 30 days of biodegradation testing, the analysis was done. These studies measured LDPE sample weight loss, pH, and fungal biomass. It was observed that the LDPE sample weight loss increased with time until the end of inclubation. *Gongronella* sp. WICC F60 and *Cordyceps* sp. WICC F61 were able to degrade LDPE plastic. Both fungi show an increase in LDPE weight loss. However, *Cordyceps* sp. WICC F61 shows more efficiency in LDPE degradation compared to *Gongronella* sp. WICC F60. The LDPE weight loss using *Gongronella* sp. WICC F60 was 1.07%, which is lower than *Gongronella* sp. WICC F60's LDPE weight reduction of 5.56%.

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

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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 physicochemical 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 a., 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 H_2O (water), CO_2 (carbon dioxide), and CH_4 (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 (K_2HPO_4), monosodium phosphate (NaH_2SO_4), ammonia sulphate ($(NH_4)_2SO_4$), magnesium sulphate heptahydrade ($MgSO_4.7H_2O$), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride hexahydrate ($CaCL_2.6H_2O$), ferrous sulphate ($FeSO_4.7H_2O$), zinc chloride ($ZnCl_3$), cupper II sulphate ($CuSO_4$) and Tween 80.

Microorganism

Fungus was obtained from the Wellness Industrial Culture Collection (WICC) at the Institute of Bioproduct 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 ^oC 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 ^oC 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 K_2HPO_4 and 8 g NaH_2SO_4 were added as buffering agents; 16 g $(NH_4)_2SO_4$ was added as a nitrogen source; 2 g $MgSO_4.7H_2O$, 2 g NaCl, 0.6 g KCl, and 0.8 g $CaCl_2.6H_2O$ were added as essential mineral salts. In addition, the medium contained 4 mL mixed trace element solution (0.1 g of each $FeSO_4.7H_2O$, $ZnCl_3$, and $CuSO_4$ 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., 2018).

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

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.

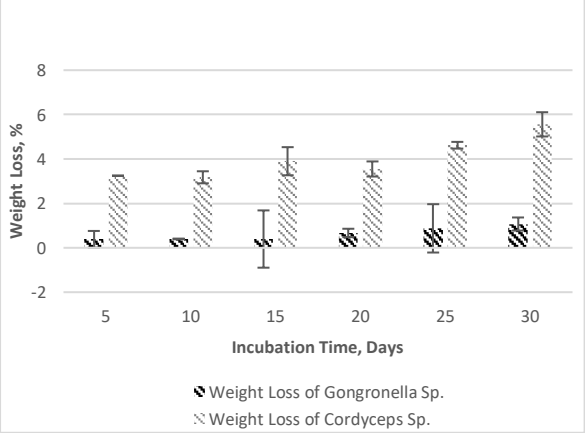

Figure 1 Weight of plastic lost

Table 1 shows the percentage weight loss of LDPE treated with *Gongronella sp. WICC F60* and *Cordyceps* sp. WICC F61. The percentage of weight loss LDPE in *Gongronella sp. WICC F60* was found to be 0.38%, 0.40%, 0.39%, 0.67%, 0.88% and 1.07% respectively. On the other hand, the percentage of weight loss in *Cordyceps sp. WICC F61* was found to be 3.25%, 3.17%, 3.90%, 3.55%, 4.62%, and 5.56%. Based on the findings, it was found that *Gongronella sp. WICC F60* and *Cordyceps sp. WICC F61* have the capability to break down plastic.

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

Time	Weight Loss of *Gongronella sp. WICC F60*, %	Weight Loss of *Cordyceps sp. WICC F61*, %
5	0.38 ± 0.376	3.25 ± 0.04
10	0.40 ± 0.015	3.17 ± 0.271
15	0.39 ± 1.290	3.90 ± 0.633
20	0.67 ± 0.188	3.55 ± 0.340
25	0.88 ± 1.089	4.62 ± 0.151
30	1.0703 ± 0.296	5.56 ± 0.546

Determination of Dry Mycelium Weight of the Fungal Isolates

The length of time that the plastic was exposed to sunlight and its incubation by fungi both played a role in the amount of dry mass that was lost. In comparison to the other cultures, growth was detected in the flasks that had been infected with fungal isolates known as WICC F60 and WICC F61 after 30 days of incubation. **Figure 2** reveals that the organisms' biomass is composed of *Gongronella sp. WICC F60* and *Cordyceps sp. WICC F61*.

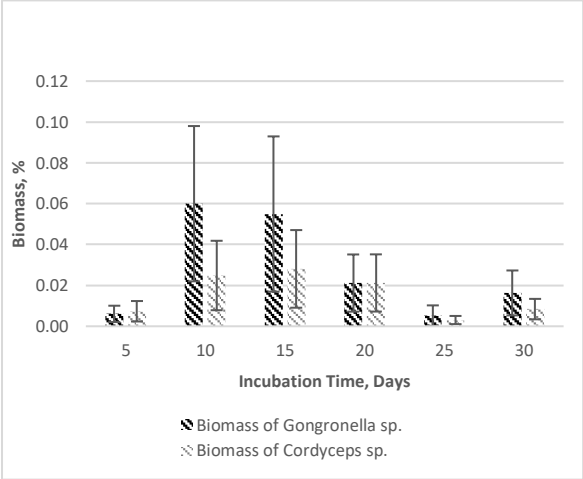

Figure 2 Organisms' biomass is composed of *Gongronella sp. WICC F60* and *Cordyceps sp. WICC F61*

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

Time	Biomass of *Gongronella sp. WICC F60*, %	Biomass of *Cordyceps sp. WICC F61*, %
5	0.01 ± 0.004	0.01 ± 0.005
10	0.06 ± 0.038	0.02 ± 0.017
15	0.05 ± 0.038	0.03 ± 0.019
20	0.02 ± 0.014	0.02 ± 0.014
25	0.01 ± 0.005	0.01 ± 0.002
30	0.02 ± 0.011	0.01 ± 0.005

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.

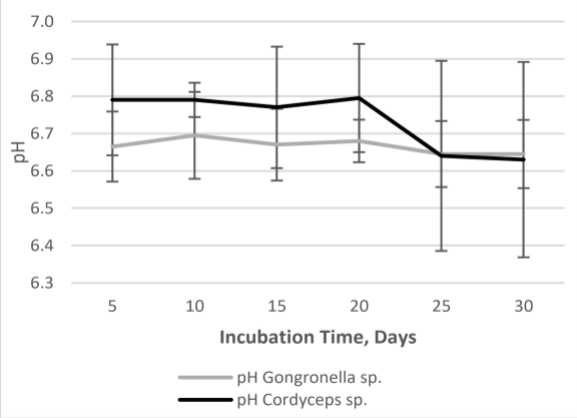

Figure 3 pH vs incubation time of the liquid medium containing LDPE

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.

Acknowledgement

The authors would like to thank the Ministry of Higher Education, Malaysia through FRGS grant No. (FRGS/1/2020/TK0/UTM/02/16).

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