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Refining Solid State Fermentation of *Fusarium oxysporum* for Enhanced Polyethylene Terephthalate Biodegradation Efficiency

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Plastic pollution, especially from polyethylene terephthalate (PET), poses a serious environmental threat due to its extensive use and persistence in ecosystems. This research explores the enhancement of PET degradation using the fungus *Fusarium oxysporum* through solid-state fermentation (SSF). *F. oxysporum* was chosen for its known capacity to produce enzymes that can be a marker of complex polymer degradation. The SSF process was refined by adjusting key variables such as moisture content, fermentation weeks, and inoculum size. The effectiveness of degradation was tracked by assessing weight loss and the production of degradation by-products as enzymes. The results indicated that over four weeks, the weight loss of PET increased with higher volumes of *F. oxysporum* inoculum, reaching a peak weight loss of around 2.5% with 15 mL inoculum. Additionally, xylanase activity, a marker of enzymatic degradation, increased over time, with absorbance values nearing 0.45 U/mL at four weeks for both 10 and 15 mL inoculum volumes. These outcomes demonstrate that *F. oxysporum*, under optimized conditions, can substantially improve PET degradation, suggesting its potential in biodegradation efforts to combat plastic pollution. This study lays the groundwork for developing sustainable solutions to the growing issue of plastic waste in the environment.

Keywords: Polyethylene terephthalate; *Fusarium oxysporum*; solid state fermentation; xylanase; plastic waste

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Polyethylene terephthalate (PET) is a common synthetic polymer from the polyester family [1]. It is renowned for its strength and flexibility. Long chains are formed by joining repeating units of ethylene glycol and terephthalic acid together via ester bonds [2]. This ensures PET's resilience and resistance to degradation [3]. It is mostly used in packaging materials such as plastic bottles and food containers, as well as textiles, because of its lightweight and low cost [2]. However, its widespread use has serious environmental consequences. PET polymers take centuries to degrade, filling landfills and harming natural habitats. It degrades over time into microplastics, which contaminate water bodies and soil, resulting in hazardous particle ingestion by marine life, wildlife, and humans. Toxic chemicals seeping from PET can impact the endocrine system in humans and destroy ecosystems [4]. This pervasive environmental hazard highlights the critical need to minimize PET use in order to safeguard both the world and living species from further harm.

There is an intriguing approach to address this issue with microorganisms. Some bacteria can break down plastic, while fungi such as *Fusarium oxysporum* show additional potential [5]. This soil-borne fungus is recognized for its potent enzymes and ability to degrade complex plant components [6]. *F. oxysporum* has been shown to degrade various plastics, including low-density polyethylene (LDPE) and polylactic acid (PLA), through its enzymatic capabilities [7]. However, limited studies have specifically explored its ability to degrade PET, which consists of aromatic compounds and ester bonds similar to those in plant cell walls. By targeting PET's ester linkages, *F. oxysporum* can potentially break the polymer into smaller, more manageable molecules. Enzymes such as xylanase, produced during the degradation process, serve as indicators of PET breakdown [6].

Solid-state fermentation (SSF) presents a promising method for enhancing fungal-mediated PET degradation. SSF is a microbiological process in which fungi grow on solid surfaces with minimal free liquid [8]. This technique mimics the natural environment of fungi, promoting their growth and enzyme production [9]. Furthermore, SSF requires less water than submerged fermentation, rendering it a more sustainable and economical option. Using agricultural waste as the substrate, SSF reduces environmental impact while supporting fungal growth and degradation activity [10]. Optimizing conditions such as inoculum size, incubation time, and liquid-to-solid ratios can significantly enhance the effectiveness of PET

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biodegradation. This study investigates the ability of *F. oxysporum* to degrade PET under SSF conditions. By optimizing SSF parameters and correlating xylanase activity with PET weight loss, this research aims to enlighten *F. oxysporum's* role in PET degradation. The findings could lay the groundwork for developing sustainable strategies to tackle plastic pollution.

EXPERIMENTAL PROCEDURES

Preparation of PET Plastic Strips

PET plastic strips were cut into uniform sizes (approximately 2 cm × 2 cm) to ensure consistent surface area exposure during the biodegradation experiment. The strips were pre-treated by washing with distilled water and ethanol to remove any contaminants before use in the fermentation setup [11].

Subculturing of *F. oxysporum*

F. oxysporum was cultured on potato dextrose agar (PDA) plates for seven days at 28°C to achieve optimal fungal growth. At the end of incubation, actively growing fungal cultures were harvested and used as inoculum for the SSF experiments [12].

Solid State Fermentation (SSF)

The SSF experiments were conducted using three different liquid-to-solid ratios: 70% liquid:30% solid, 60% liquid:40% solid, and 80% liquid:20% solid, representing varying moisture levels. Distilled water with inoculum was used as the liquid component in the SSF setup. Three inoculum volumes (5, 10, and 15 mL) of *F. oxysporum* culture were used for each condition. The PET strips with fungal inoculum in a flask were placed in a sterile condition in an incubator, where moisture content and temperature (maintained at 28°C) were controlled to sustain fungal growth over the 10-week experimental period [13].

Xylanase Assay

Xylanase activity is defined as the amount of enzyme required to release 1 µmol of D-xylose per minute under specific conditions. The enzyme activity was expressed in U/mL units [14].

Weight Loss Determination

Weight loss was determined by weighing the PET strips at the start of the experiment and after every two weeks [15]. The percentage of weight loss was calculated as in Equation (1):

$$\text{Weight Loss Percentage} = (\text{Initial Weight} - \text{Final Weight}/\text{Initial Weight}) \times 100 \quad (1)$$

RESULTS AND DISCUSSION

Weight Loss and Enzyme Activity Across Inoculum Volumes and Liquid-to-Solid Ratios

Figures 1, 2, and **3** represent the weight loss percentage and enzyme activity (U/mL) over 10 weeks for three different liquid-to-solid ratios (70% liquid: 30% solid, 60% liquid: 40% solid, and 80% liquid: 20% solid) across 5, 10, and 15 mL inoculum volumes.

Inoculum of 5 mL

Figure 1 shows that at week 6, weight loss peaked under the 60% liquid: 40% solid condition, reaching 1.8%. This coincided with the highest xylanase activity of 0.30 U/mL. The higher solid content likely provided more accessible nutrients, e.g., nitrogen (yeast) and carbon (sucrose), for *F. oxysporum* to metabolize, facilitating initial degradation. Solid substrates in SSF enhance fungal growth by providing structural support and concentrated nutrients, as reported by Mahgoub et al. (2022) [8]. However, insufficient moisture in this condition may have slowed enzyme diffusion, slightly limiting overall efficiency [8].

Inoculum of 10 mL

Figure 2 depicts the peaked weight loss and enzyme activity at week 4 under the 70% liquid:30% solid condition, achieving 2.3% weight loss and 0.42 U/mL enzyme activity. The increased inoculum size supported higher fungal colonization, while the balanced moisture levels promoted enzymatic activity. Balanced moisture ensures substrate accessibility and prevents oxygen diffusion issues, as observed by Ibarruri et al. (2021) [9]. Excess liquid in the 80% liquid:20% solid condition likely inhibited fungal growth by diluting essential nutrients and creating anaerobic conditions [9].

Inoculum of 15 mL

Figure 3 portrays the most efficient PET degradation, with weight loss reaching 2.6% and enzyme activity peaking at 0.47 U/mL under the 70% liquid:30% solid condition at week 4. Larger inoculum volumes accelerated fungal colonization and enzyme production, but overpopulation may have caused competition for nutrients in later weeks, leading to diminished efficiency [7]. High inoculum densities are known to cause resource competition, reducing microbial activity over time [7].

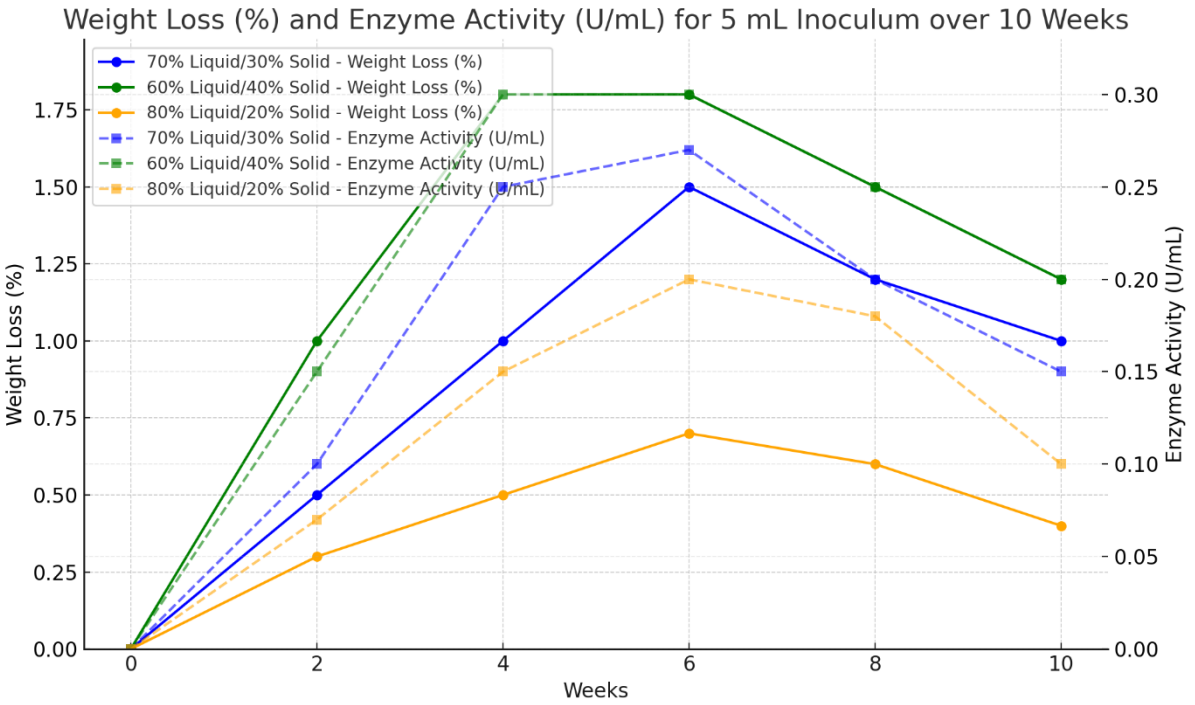

Figure 1. The percentage of weight loss and enzyme activity (U/mL) over 10 weeks for three different liquid-to-solid ratios (70% Liquid/30% Solid, 60% Liquid/40% Solid, and 80% Liquid/20% Solid) with a 5-mL inoculum.

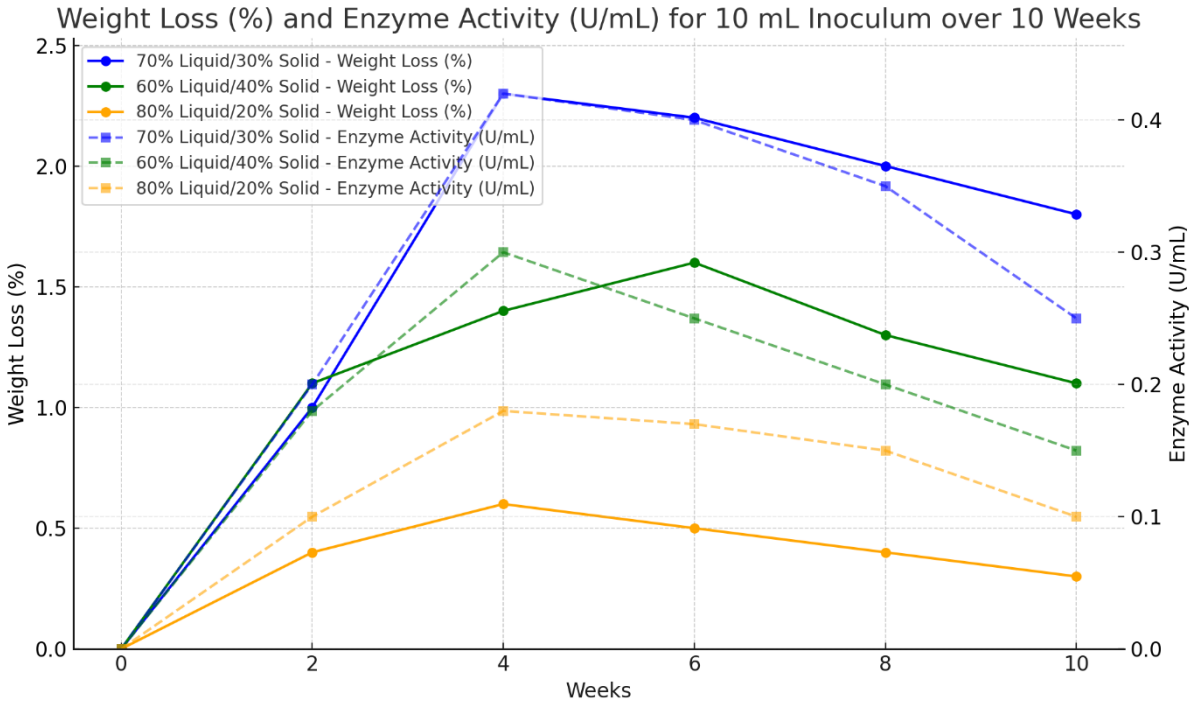

Figure 2. Weight loss percentages and enzyme activity (U/mL) over 10 weeks for the three liquid-to-solid ratios using a 10-mL inoculum.

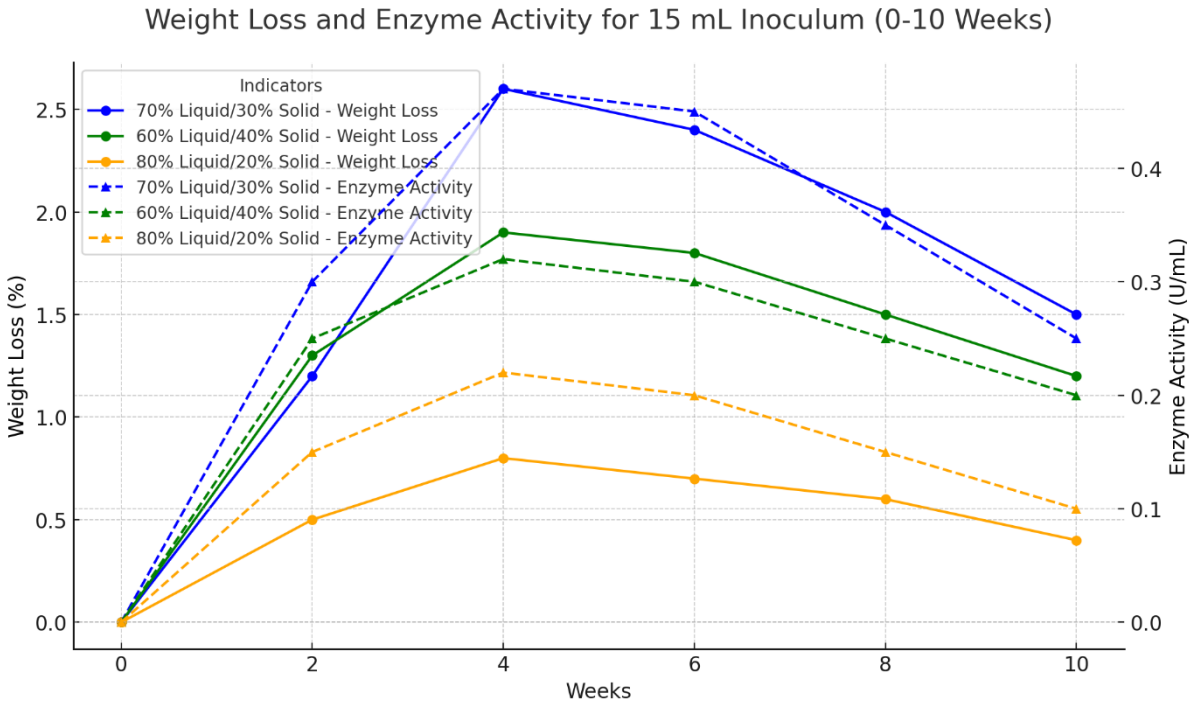

Figure 3. Weight loss (%) and enzyme activity (U/mL) over 10 weeks for the three liquid-to-solid ratios using a 15-mL inoculum.

Moisture and Nutrient Dynamics

The ratio of 70% liquid:30% solid condition consistently proved optimal across all inoculum volumes. This ratio balances moisture availability with sufficient solid substrate for fungal attachment and enzyme secretion. Excess moisture in the 80% liquid and 20% solid condition may have diluted essential nutrients or created anaerobic zones, inhibiting fungal activity [10]. In contrast, the ratio of 60% liquid and 40% solid conditions likely restricted fungal access due to lower moisture availability, reducing enzymatic efficiency [8].

Observations on Degradation Patterns

Weight loss peaked early in the fermentation process (week 4 or 6) before stabilizing. This pattern aligns with microbial degradation studies, where readily degradable substrates are consumed first, followed by slower degradation of more recalcitrant components [16]. Similarly, enzyme activity peaked during periods of high substrate availability and declined as nutrient levels decreased. This observation is consistent with [17], who reported that enzyme activity in SSF systems decreases as substrates are consumed and metabolic byproducts accumulate.

CONCLUSION

This study demonstrates the potential of *F. oxysporum* for the biodegradation of PET plastic through solid-state fermentation. The optimal conditions for PET degradation were identified as a 15 mL inoculum size and a 70%:30% liquid-to-solid ratio, yielding a 2.5% weight loss and peak xylanase activity of 0.47 U/mL by week 4. Although the degradation rate is modest, this research provides a promising foundation for future studies aimed at optimizing biodegradation efficiency. Future work should focus on enhancing the activity of xylanase and other enzymes to improve the overall degradation rates. This approach has promising implications for the bioremediation of plastic waste, particularly in industrial settings.

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