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Research Article

Biodegradation of Styrofoam by a Genetically Modified Mold Isolated from a 30 Days Vermicompost

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Abstract

Styrofoam, a highly persistent plastic polymer composed primarily of polystyrene, poses a significant global environmental threat due to its recalcitrance to natural degradation and the hazardous byproducts released during conventional disposal. This study explored an eco-friendly alternative by investigating the biodegradation potential of fungal strains isolated from a 30-day vermicompost. The primary goal was to assess the feasibility and efficiency of utilizing both wild-type and genetically modified molds specifically *Aspergillus niger* and *Penicillium chrysogenum* to degrade Styrofoam. Fungal strains were isolated from vermicompost collected in Awka, Nigeria, and identified through morphological and microscopic analysis. Genetic modification was performed via UV mutagenesis for 7 consecutive days. Both wild-type and mutant strains were inoculated into Styrofoam Mineral Salt Vitamins Medium (MSVM) broth containing 5% Styrofoam and incubated in a rotary shaker at 150 rpm for 25 days. Degradation kinetics were monitored at 5-day intervals using spectrophotometric analysis at 680 nm to measure changes in optical density (OD). *Penicillium chrysogenum* and *Aspergillus niger* were successfully isolated and identified. Spectrophotometric data revealed that the genetically modified strain of *P. chrysogenum* exhibited an accelerated degradation process, surpassing the wild-type strain by Day 5 and maintaining higher optical density values throughout the 20-day monitoring period. Conversely, the mutant strain of *A. niger* showed lower degradative potential compared to its wild-type counterpart. The wild-type *A. niger* demonstrated steady degradative efficacy, reaching an OD of 1.247 by Day 20. The findings suggest that indigenous fungi from vermicompost possess the ability to deteriorate Styrofoam, and UV-induced mutation can successfully enhance the degradation efficiency of specific strains like *Penicillium chrysogenum*. These results highlight the potential of using genetically improved microbial agents as a sustainable strategy for managing expanded polystyrene waste.

Keywords: Biodegradation; Styrofoam; UV Mutagenesis; *Aspergillus niger*; *Penicillium chrysogenum*; Vermicompost

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Introduction

Plastic polymers are largely non-biodegradable solid wastes that constitute a major environmental concern globally. The continuous increase in plastic production and disposal has contributed significantly to environmental pollution, with plastics accumulating in terrestrial and aquatic ecosystems [1,2]. These synthetic polymers persist in the environment due to their resistance to microbial degradation, leading to long-term ecological and public health challenges [3,4].

Among commonly used plastics, polystyrene-particularly in the form of expanded polystyrene (EPS), widely known as Styrofoam-is extensively utilized for packaging, insulation, and disposable food containers due to its lightweight nature, durability, and low cost. However, its widespread use has resulted in significant environmental accumulation because of its resistance to natural degradation processes [1]. Improper disposal methods such as open dumping, incineration, and landfilling contribute to environmental contamination and release of hazardous by-products, including toxic gases and persistent organic pollutants [5,6].

The recalcitrant nature of EPS and other plastics is attributed to their high molecular weight and complex chemical structure, which limits their susceptibility to microbial attack. Conventional disposal strategies such as incineration and chemical recycling have been explored; however, these methods often introduce secondary environmental pollution and are not sustainable in the long term [2]. Consequently, there is a growing need for environmentally friendly and sustainable alternatives for plastic waste management.

Biodegradation, which involves the breakdown of complex materials into simpler compounds by microorganisms, has emerged as a promising approach for mitigating plastic pollution. Microorganisms such as bacteria, fungi, algae, and yeast possess enzymatic systems capable of degrading synthetic polymers under suitable environmental conditions [7,8]. These enzymes, including lipases, proteases, and oxidases, facilitate the depolymerization and mineralization of plastics into less harmful substances [9,10]. Biodegradation processes may occur under aerobic or anaerobic conditions depending on environmental factors and microbial composition [11].

Although bacterial degradation of plastics-particularly by species of *Pseudomonas*-has been widely studied, recent attention has shifted toward fungal degradation due to the robust enzymatic capabilities of fungi [12,13]. Fungi are key decomposers in natural ecosystems and are capable of colonizing diverse and extreme environments, making them suitable candidates for plastic biodegradation studies [4,14]. Their ability to produce a wide range of extracellular enzymes enhances their capacity to degrade complex and recalcitrant substrates such as synthetic polymers.

Furthermore, microorganisms isolated from contaminated environments such as dump sites, oil-polluted soils, and industrial waste zones often exhibit enhanced degradative abilities due to adaptive evolution [15-17]. These environments serve as reservoirs for microbial strains with potential applications in bioremediation. Studies have also demonstrated that environmental factors such as pH, nutrient availability, and presence of surfactants can significantly influence microbial degradation efficiency [11].

In addition, recent findings have shown that certain biological systems, including insect larvae such as *Tenebrio molitor* and related species, can contribute to the biodegradation of polystyrene materials. However, microbial degradation remains more feasible for large-scale environmental applications due to its scalability and adaptability [1].

Despite these advancements, there remains limited information on the biodegradation of expanded polystyrene by locally isolated fungal species, particularly within the Nigerian context. Most studies have focused on bacterial degradation, with comparatively fewer investigations into fungal-mediated degradation mechanisms and their enzymatic pathways [4,7].

Aim of the Study

This study aims to investigate the biodegradation potential of expanded polystyrene (Styrofoam) using fungal isolates obtained from environmental samples. The study further seeks to identify and characterize these fungal isolates using standard microbiological techniques [18], evaluate their degradation efficiency, and elucidate the underlying biochemical and enzymatic mechanisms involved in the biodegradation process.

The aim of the research topic is to investigate the potential of genetically modified molds, specifically those isolated from a 30-day vermicompost, to biodegrade Styrofoam (polystyrene foam) the hypothesis of this study relates to the belief in the potentials of modified molds in degrading styrofoam compounds.

Materials and Methods

Materials

Styrofoam, 30-day Vermicompost, Nutrient Broth or Agar Plates, Petri Dishes, Sterile Water, Control Molds, Lab Glassware (Flasks, Test Tubes, Pipettes), pH Meter, Incubator, Microscope, Analytical Balances, Spectrophotometer, Gas Chromatograph or Mass Spectrometer, Safety Equipment (Lab coats, gloves, safety goggles), Shakers, Magnetic Stirrers, Autoclave, Inoculation Loop, Pipette Tips, Bunsen Burner, Inoculation Chamber, Colony Counter, pH Buffer Solutions, Water Bath, pipette Calibration Kit.

Collection of vermicompost

Vermicompost was acquired from a waste dump site situated along Ifite-Awka Road. It consisted of a heterogeneous blend of organic waste, including kitchen and yard waste, already undergoing the process of vermicomposting facilitated by *Eisenia fetida* earthworms.

Sample analysis

One gram (1g) of the Vermicompost samples were weighed out aseptically and introduced into 9 ml of sterile distilled water for bacteria, it was properly shaken to homogenize the sample. A 10-fold serial dilution of each of the sample was carried out using distilled water as the diluents. 0.1 ml of appropriate dilutions (10^{-2}) of the sample were pour plated in sterile plates of Sabouraud Dextrose agar plates for the culture of fungi. The culture plates were incubated at 25°C aerobically for 48 - 72 hours (2 - 3 days) for fungi. Developing colonies on SDA were counted to obtain total fungal count. Discrete colonies for the fungi were obtained by sub culturing into SDA plates and were subsequently identified using standard methods.

Total Fungal Count was calculated thus:

$$\text{TFC (CFU|ml)} = \frac{(N) \text{x } 10}{VD}$$

Where:

TFC: Total Fungal Count;

V: Volume plated;

D: Dilution Factor.

Isolation of mold strains

Using a pour plating technique, small, aseptically obtained samples of vermicompost were uniformly distributed into the petri dish then Sabouraud Dextrose Agar (SDA) was poured into it. This pour plating technique was conducted with precision to ensure an even distribution of the sample and maintain the purity of the cultures.

The inoculated SDA plates were carefully sealed to prevent contamination and subsequently incubated at a controlled temperature of 25°C. Incubation lasted for a period of 2 - 3 days.

Identification of mold

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the modified slide culture technique using lactophenol cotton blue stain for the microscopic evaluation under X10 and X40 magnification of the microscope [18]; with reference to the Manual of Fungal Atlases [19].

Genetic modification of mold strains

The mold strains underwent genetic modification using the UV mutagenesis technique. For random mutation induction, a 24-hour subculture of the mold was exposed to 2 hours of UV light daily within an enclosed chamber. This UV exposure regimen was maintained for 7 consecutive days, facilitating the tentative induction of random mutations in the mold strains. Subsequent to the UV exposure, the mold cultures were sub-cultured to promote the proliferation of potentially mutated strains

Styrofoam mineral salt vitamins medium agar composition (gL^{-1})

Styrofoam, 5.0g; $(NH_4)_2SO_4$, 1.0g; KH_2PO_4, 1.0g; K_2HPO_4, 8.0g; $MgSO_4.7H_2O$, 0.2g; NaCl, 0.1g; $CaCl_2.2H_2O$, 0.02g; $FeSO_4$, 0.01g; $Na_2MoO_4.2H_2O$, 0.5 mg; $MnSO_4$, 0.5 mg; Inositol, 0.2 mg; p-amino benzoic acid, 0.2 mg; pyridoxine, 0.4 mg; thiamine, 2.0 μg; biotine, 2.0 μg; vitamin B, 120.5 μg; DW, 1000 ml; pH 7. The medium used will be the same one that was used previously by Suzuki. A solid medium was prepared by adding 20g of agar powder to 1000 ml of the MSV medium before autoclaving.

Degradation by monocultures

2 fungal plugs of the isolates were inoculated into separate 50 ml of MSVM broth containing varying concentrations (5%) of

Styrofoam in 100 ml flasks. They were then incubated at 150 rpm in a rotary shaker for 25 days at 30°C. The level of degradation was assessed through spectrophotometric analysis at 680 nm, all of which were considered at 5-day intervals.

Styrofoam degradation process

The mold strains were aseptically inoculated into separate 50 ml volumes of MSVM broth. Each flask containing shredded Styrofoam samples. The inoculated flasks were incubated at 150 rpm in a rotary shaker for a duration of 25 days, maintaining a constant temperature of 30°C. The degradation process was monitored at 5-day intervals. The assessment of degradation levels involved spectrophotometric analysis at 680 nm, providing quantitative data on the degradation rate as well as microbial cell growth. Additionally, the entire degradation process was carried out at room temperature to simulate conditions relevant to potential real-world applications.

The extent of degradation of styrofoam by the isolates was studied using mass spectrophotometry (MS) analysis of the Styrofoam residue from the degradation at 5-day intervals, with a wavelength of 680 nm.

Results

Samples	**TFC (Cfuml^{-1}) No. of fungi colonies on plate**	**Organism Distribution**
	TFTC	
Sample A	TFTC	*Penicillium chrysogenum* and *Aspergillus niger*

Table 1: Total fungal count (Cfuml^{-1}) and fungal distribution.

Key: TFTC= Too few to count; Cfuml^{-1}= Colony forming unit per ml.

Isolates	**Colony morphology**	**Microscopy**	**Identity**
1	Colonies were compact with white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidial heads are large (up to 3 mm by 15 to 20 μm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5 μm in diameter), dark brown to black and rough-walled	*Aspergillus niger*
2	Cultures on SDA are fluffy, bright yellowish green with bluish green tint, funiculose with bundles of hyphae, reverse yellowish pink with reddish purple tint. Rather good in growth.	Conidiophores hyaline, erect, developed from aerial hyphae, branched penicillately at the apexes with primary and secondary metula, verticillate phialides and catenulate conidia in each phialide, forming rather open-spaced yellowish green conidial heads: phialides lanceolate or abruptly sharpened. Conidia phialosporous, pale green, dark in mass, globose to subglobose, 1-celled, minutely echinulate on the surface.	*Penicillium chrysogenum*

Table 2: Colonial morphology and microscopy of fungi isolates.

	Normal	Mutant
Day 0	0.130	0.040
Day 5	0.098	0.125
Day 10	1.570	1.545
Day 15	1.538	1.600
Day 20	1.425	1.662

Table 3: Optical density value for styrofoam degradation by *Penicillium chrysogenum*.

	Normal	Mutant
Day 0	0.616	0.357
Day 5	0.697	0.390
Day 10	0.786	0.492
Day 15	0.898	0.265
Day 20	1.247	0.165

Table 4: Optical density value for Styrofoam degradation by *Aspergillus niger*.

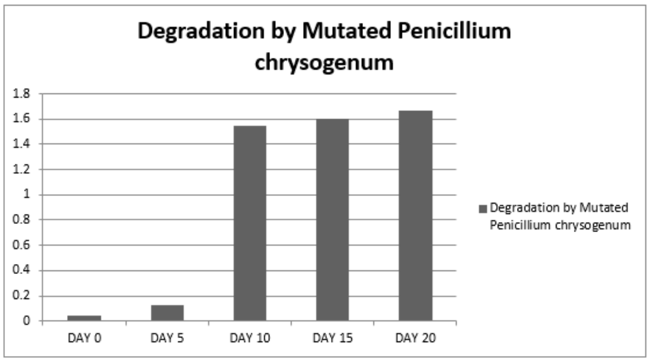

Figure 1: Chart showing the rate of degradation of mutated *Penicillium spp*.

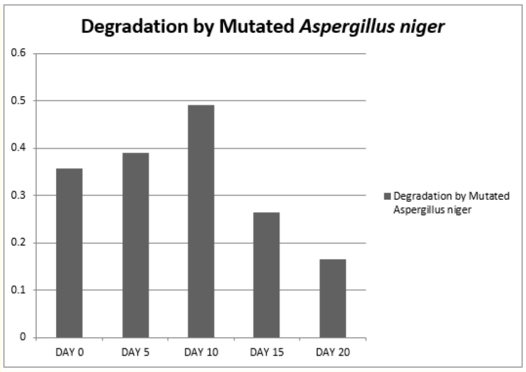

Figure 2: Chart showing the rate of degradation of mutated *Aspergillus niger*.

Discussion

The widespread environmental impact of Styrofoam, a highly persistent polymer, has prompted an urgent search for sustainable solutions. Conventional disposal methods significantly contribute to pollution, necessitating the exploration of innovative approaches. A novel method capitalizes on the use of microbial enzymatic activity, providing a promising avenue for environmentally friendly waste management Our study investigates the biodegradation of Styrofoam using genetically modified molds isolated from a 30-day vermicompost.

Table 1 shows data for the fungi isolates identified from the vermicompost, the study reports the fungal density in the vermicompost sample as 'Too few to count' which suggest a limited fungal count in sample A. However, Despite the challenges associated with low fungal counts study identified: *Penicillium chrysogenum* and *Aspergillus niger.* This is consistent with the study of [20] which isolated *Aspergillus niger* from compost and vermicompost in Italy, [21] identified both organism from vermicompost collected in India

Results for Styrofoam degradation for both *Penicillium chrysogenum* and *Aspergillus niger* is presented in table 3 and 4. Table 3 compares the optical density values measuring the degradation of Styrofoam sample over a 20-day period (Day 0, day 5, day 10, day 15 and day 20) for normal and the genetically modified (mutant) *Penicillium chrysogenum*. The results suggest that both the normal strain and genetically modified strain of *Penicillium chrysogenum* has a discernible impact on Styrofoam degradation efficiency as observed by the change in the optical density values indicating degradation kinetics and potential of *Penicillium chrysogenum*. This finding is consistent with the study of [22] which reported efficacy of *Penicillium chrysogenum* in the degradation of different plastics including polystyrene which is the parent precursor of Styrofoam.

However, the mutant strain continues to have a slightly higher optical density over the normal strain over the 20-day period On day 1, the normal strain exhibited a higher optical density (0.130) compared to the mutant strain (0.040), suggesting a potentially faster initial response. By day 5, the mutant strain surpassed the normal strain, indicating an accelerated degradation process while for the normal strain indicates lagging phase (Normal: 0.098, Mutant: 0.125).

Meanwhile, on the day 10, the normal strain achieved to its stationary phase at (1.570) while the mutant strain keep increasing spontaneously indicating that the genetically mutated strain of *Penicillium chrysogenum* exhibited more degradation on the Styrofoam compared to the normal strain of *Penicillium chrysogenum.*

Similarly, *Aspergillus niger* demonstrated degradative potential against the Styrofoam samples as optical density value changes was observed for both normal and mutant strains. This is consistent with the findings of [23] which observed the efficacy of *Aspergillus niger* in the degradation of an array of plastics which includes: polyethylene, polystyrene and polyurethane their study reported *Aspergillus niger* as the organism with highest degradation percentage amongst the five fungi tested for. Unlike the *Penicillium spp*, the mutant strain of *Aspergillus niger* was observed in this study to impact less degradative potential than the normal strain as observed in the trend and changes in the optical density values.

The more the concentration the more the optical density.

These findings underscore the promising potential of genetically modified molds in enhancing Styrofoam degradation efficiency. The consistent performance superiority of the mutant strains suggests their applicability in sustainable waste management practices.

Conclusion and Recommendation

In light of our investigation into the biodegradation of Styrofoam using genetically modified molds isolated from a 30-day vermicompost, our findings underscore the tremendous potential of exploring genetically modified fungi for the effective management of Styrofoam pollution.

Further genetic exploration: Conduct additional genetic analyses to understand the specific modifications responsible for the enhanced degradation efficiency observed in the mutant strains. This knowledge could aid in optimizing genetic engineering approaches for improved environmental remediation.

Long-term degradation studies: Extend the duration of the degradation study to assess the long-t erm performance and sustainability of genetically modified molds. This would provide valuable insights into the durability and reliability of these strains in real-world waste management scenarios.

Environmental impact assessment: Undertake a comprehensive environmental impact assessment to evaluate the potential consequences of releasing genetically modified molds into natural ecosystems. Ethical considerations and potential ecological ramifications should be thoroughly examined.

Scaling up for practical applications: Explore the scalability of the genetically modified molds for practical applications in waste management on a larger scale. Assess the feasibility of deploying these strains in real-world settings to address Styrofoam pollution.

Collaborative research and validation: Collaborate with other research institutions and experts to validate and replicate the findings. Independent verification would strengthen the credibility of the study and contribute to the broader scientific understanding of Styrofoam degradation.

Appendix

Figure 3: Mutated *Penicillium* on SDA.

Figure 4: Mutated *Aspergillus* on SDA.

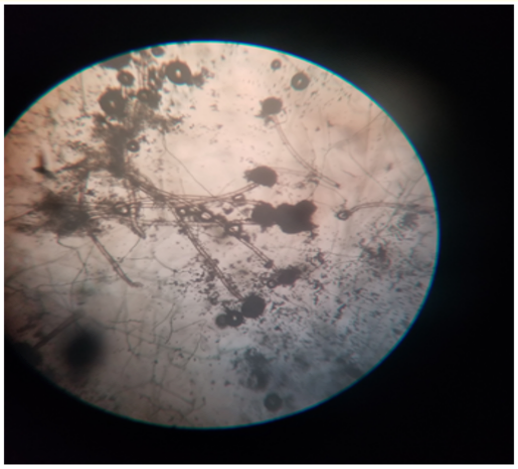

Figure 5: Microscopic view of mutated *Aspergillus niger*.

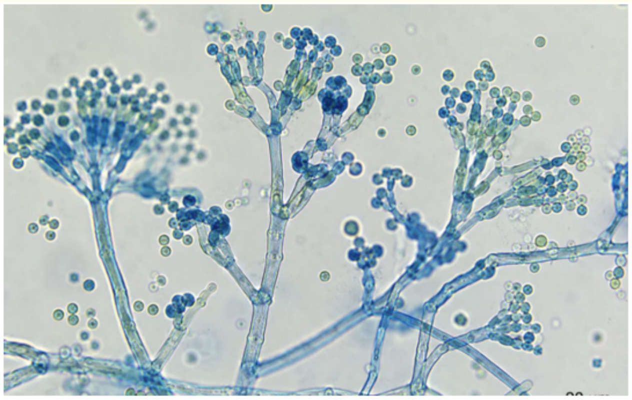

Figure 6: Microscopic view of mutated *Penicillium spp*.

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