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Research article

# Fungal biodegradation of low-density polyethylene using consortium of *Aspergillus* species under controlled conditions

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ABSTRACT

Low-Density polyethylene is subject to biodegradation using a fungal consortium comprising of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus oryzae* under laboratory conditions. The extent of biodegradation has been compared with the use of potato dextrose broth and czapek dox broth media and also in the presence and absence of Tween 80 additive. Biodegradation was performed replacing the sucrose in czapek dox broth with shredded Low-Density polyethylene as well. The biodegradation was carried out for a period of 55 days. The degree of biodegradation has been analyzed using the loss of weight, FT-IR, and SEM analysis. A maximum weight loss of 26.15% was obtained by using potato dextrose broth over a period of 55 days.

## 1. Introduction

Biodegradation, defined as the decomposition of materials mainly by fungi or bacteria, is a natural process that acts on substances such as leaves, grass, and food scraps. The technique of biodegradation has been extended to break down artificial products, mainly plastics such as aliphatic polyesters, aromatic co-polyesters, and polyethene [1, 2]. Low-Density Polyethylene (LDPE) biodegradation has emerged as an active and attractive area of research in the past 20 years or so, due to the various problems associated with conventional chemical methods, such as the need for high temperature in pyrolysis, highly corrosivity of ozonolysis, and complex by-product treatments in chlorine attacking [3]. Although polyethylene is highly resistant to biodegradation owing to its high hydrophobicity and long chains of carbon [3], many microorganisms have been identified which can degrade LDPE at an appreciable rate [4, 5]. Some of these include bacterial species of the genus *Bacillus* [1, 6], *Streptomyces* [6], and *Pseudomonas* [7], and fungal species of the genus *Aspergillus* [8, 9] and *Penicillium* [10, 11]. In aerobic conditions, the plastics are broken down into monomers and are liberated as carbon dioxide and water, in a moist and warm environment (Equation 1).

$$Organic\ matter + S + O_2 \rightarrow CO_2 + H_2O + NO_2 + SO_2 \quad (1)$$

Under anaerobic conditions, plastics undergo biodegradation and release gases like methane and carbon dioxide (Equation 2) [12].

$$Organic\ matter + H_2O + Nutrients \rightarrow Residual\ matter + CO_2 + CH_4 + NH_3 + H_2S + heat \quad (2)$$

The general process starts with the deterioration of plastics which involves the breakdown of plastics into smaller monomers by various abiotic (ultra-violet (UV) radiation, heating, freezing, or wetting) and biotic (enzymes) processes. Then, the monomers are degraded to smaller monomers so that they can be easily absorbed and metabolized within the cells of the microorganisms in a process called bio-fragmentation [13]. The metabolism process within the microorganisms takes place with the help of enzymes such as laccase which converts the microplastic

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to a mixture of carbon dioxide, nitrogen gas, methane, water, and eventually releases adenosine triphosphate (ATP). In the case of LDPE, biodegradation is associated with a change in physical properties including loss of weight and decrease in tensile strength [14] and chemical properties like surface functional groups and hydrophobicity/hydrophilicity [4].

Fungal species from the genus *Aspergillus*, namely, *Aspergillus niger*, *Aspergillus flavus,* and *Aspergillus oryzae* are usually employed in LDPE biodegradation, due to its ability to freely and abundantly grow in soil and garbage sites, and due to its better incubation time compared to other fungal species [15]. Studies have shown that using a consortium of fungal species gives better results than using individual fungi in a variety of research areas such as degradation of textile dyes [16], production of nanoparticles for targeting breast cancer [17], and treatment of dairy wastewater [18]. This observation has been proved to be consistent in the biodegradation of polyethylene as well, where a consortium of fungi showed superior degradation rates when compared to the use of individual fungi [19]. Tween 80 was employed as a surfactant additive for a set of samples as well, to test for a potential improvement in adherence and thus, an increase in biodegradation.

Finally, the extent of biodegradation can be investigated by the characterization of the degraded samples. Several techniques have been employed in literature to measure degradation across the various stages [20]. Polymer deterioration is measured by Scanning electron microscopy (SEM), weight difference, and decrease in tensile strength [21, 22]. Bio-fragmentation can be determined by analysis of outputs provided through Size exclusion chromatography (SEC), High performance liquid chromatography (HPLC), and Fourier-transform infrared spectroscopy (FT-IR). The assimilation of the plastics by the fungal species can be measured by spectroscopic methods and FT-IR [22].

The relevance of finding a safe and environmentally friendly method of degrading LDPE has increased due to its high demand and widespread use. From packaging to toys, LDPE is used in almost all fields of life due to properties such as durability, cost-effectiveness, lightweight, and energy-efficiency [23, 24, 25, 26]. LDPE, on disposal, accumulates in landfills and natural environments, leading to a variety of problems [27]. An adverse effect of LDPE accumulation can be seen in the marine ecosystem [28]. LDPE enters the oceans either as micro- or nanosized plastics or by gradual wear and tear of larger plastics, which in turn cause harmful effects such as neurotoxicity and increase in cellular oxidative stress in marine wildlife [7, 8]. Hence, there is a growing need for effective treatment of plastic wastes. The paper addresses this issue by employing a consortium of fungal species consisting of *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus oryzae* to enhance efficiency and provide a feasible and safe method for LDPE degradation.

## 2. Materials and methods

### 2.1. Fungi

*A. niger, A. oryzae,* and *A. flavus* were associated with the performance of biodegradation studies on polyethylene. Pure cultures of *A. niger* (RV-BT12) and *A. flavus* (RV-BT43) were procured from the Department of Biotechnology at R V College of Engineering, India. *A. oryzae* (RV-BT117) was procured from Microbial Type Culture Collection and Gene Bank (MTCC), India. All the three species were preserved in a suspension medium containing 10% glycerol/10% skimmed milk. Besides *A. oryzae* which has an incubation period of 5 days at temperature 25 °C, the other two species have an incubation period of 7 days at temperature 30 °C.

### 2.2. Preparation of media and consortium

Potato dextrose media and Czapek dox medium were prepared as per the procedure given by HiMedia Pvt. Ltd. [30, 31]. 6 g of potato dextrose broth powder containing dehydrated potato infusion and dextrose was added to distilled water, then dissolved, autoclaved, and cooled. Similarly, Czapek dox media includes 30 g/L of sucrose, 2.0 g/L of NaCl, 1.0 g/L of $K_2HPO_4$, 0.5 g/L of $MgSO_4$, 0.5 g/L of KCl and 0.010 g/L of $FeSO_4$ [30, 31]. The culture of *A. oryzae* was prepared from the freeze-dried form. *A. niger* (RV-BT12) and *A. flavus* (RV-BT43) were inoculated into the cooled potato dextrose broth (PDB) as well as Czapek Dox broth (CDB) media, and then incubated for five days [32]. Post the incubation period, growth of the fungal consortium was observed.

### 2.3. Determination of growth kinetics

30mL of sterilized CDB was transferred to six out of twelve conical flasks. 30mL of sterilized PDB was transferred in the remaining flasks. After inoculation, the flasks were incubated. After every 24 h, two sets of flasks, each consisting of CDB and PDB were removed. The amount of biomass and substrate volumes were recorded post centrifugation. Substrate consumption was quantitatively plotted with reference to the biomass concentration and substrate volume. The specific growth rate for the *Aspergillus* consortium was quantitatively plotted using the slope generated after plotting biomass concentration versus time was generated, the slope of which indicated the specific growth rate for the *Aspergillus* consortium. This information is essential in designing a bioreactor that can facilitate biodegradation studies. The generated data validated the kinetic equations for individual *Aspergillus* species as per several theories.

### 2.4. Treatment of LDPE samples

For the current study, bags made of low-density polyethylene were cut into two sets of square strips measuring 2 cm × 2 cm and 3 cm × 3 cm. 12 polyethylene strips of each set were weighed using a microbalance. To establish the proof of concept as to whether the fungal consortium can solely use LDPE as a carbon source, bags made of LDPE were shredded into small pieces. Both the shredded plastics and polyethylene strips were disinfected and sterilized.

In one set of conical flasks, 0.3 mL of Tween 80 was added as an additive. Tween 80 is utilized as a surfactant to enhance colonization as well as adherence to the polyethylene [33]. In the other set of conical flasks, no additives were added. Subsequently, each of 2 cm × 2 cm and 3 cm × 3 cm LDPE strips were aseptically transferred into the six flasks. The fungal consortium was inoculated into the broth media. Two conical flasks, one each with and without additives were allowed to grow for a period of 20 days, 30 days, and 45 days. The LDPE strips were measured for weight loss and characterized by SEM and FT-IR spectroscopy. The

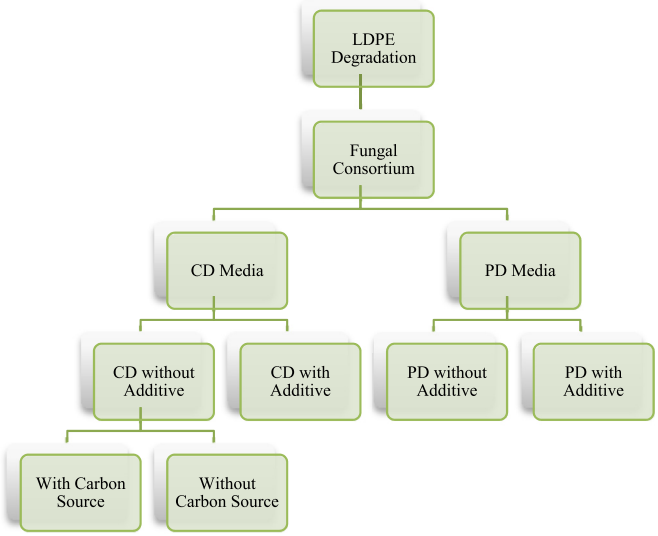


**Figure 1.** A layout of experiments carried out for biodegradation studies.

**Table 1.** Distribution of experimental samples for biodegradation studies.

| Sample ID | Dimensions (cm) | PDB | CDB | Additive | Initial Weight (g) |
|---|---|---|---|---|---|
| A | 2 × 2 | ✓ | | | 0.00524 |
| B | 3 × 3 | ✓ | | | 0.01325 |
| C | 2 × 2 | | ✓ | | 0.00454 |
| D | 3 × 3 | | ✓ | | 0.01169 |
| E | 2 × 2 | ✓ | | ✓ | 0.00547 |
| F | 3 × 3 | ✓ | | ✓ | 0.01089 |
| G | 2 × 2 | | ✓ | ✓ | 0.00503 |
| H | 3 × 3 | | ✓ | ✓ | 0.01269 |

experimental layout has been outlined in Figure 1. The distribution of the polyethylene samples in different media and their initial weights are summarized in Table 1.

### 2.5. Addition of plastic as the sole carbon source

To prove that fungal consortia can grow and survive in a media where LDPE is the sole source of carbon, the sucrose in CDB was replaced with shredded LDPE. In this way, the carbon content in the media is solely from the LDPE sample. The *Aspergillus* consortia were inoculated and grown for 40 days. The LDPE shreds were characterized by loss of weight studies and further analyzed by SEM and FT-IR spectroscopy.

### 2.6. Characterization and analysis of the biodegraded polyethylene samples

Degradation efficiency measurements were done for each sample by the residual weight method. The samples were characterized using Scanning Electron Microscope (SEM) and Fourier Transform Infrared Spectroscopy (FT-IR) to analyze the changes brought about by biodegradation on the morphology and structure of polyethylene.

**Table 2.** Loss of weight due to biodegradation.

| Sl. No. | Sample | Initial Weight (g) | Final Weight (g) | Percentage loss of weight |
|---|---|---|---|---|
| a) Period of 20 days | | | | |
| 1. | A | 0.00524 | 0.00493 | 5.916% |
| 2. | B | 0.01325 | 0.01324 | No loss |
| 3. | C | 0.00454 | 0.00454 | No loss |
| 4. | D | 0.01169 | 0.01160 | 0.769% |
| 5. | E | 0.00547 | 0.00546 | No loss |
| 6. | F | 0.01089 | 0.01083 | 0.0551% |
| 7. | G | 0.00503 | 0.00502 | No loss |
| 8. | H | 0.01269 | 0.01268 | No loss |
| b) Period of 30 days | | | | |
| 1. | A | 0.0064 | 0.0054 | 15.625% |
| 2. | B | 0.0140 | 0.0129 | 7.857% |
| 3. | C | 0.0068 | 0.0067 | No loss |
| 4. | D | 0.0142 | 0.0122 | 14.084% |
| 5. | E | 0.0065 | 0.0058 | 10.769% |
| 6. | F | 0.0140 | 0.0101 | 16.78% |
| 7. | G | 0.0064 | 0.0053 | 17.187% |
| 8. | H | 0.0145 | 0.0134 | 7.586% |
| c) Period of 55 days | | | | |
| 1. | A | 0.0065 | 0.0058 | 26.153% |
| 2. | B | 0.0142 | 0.0129 | 2.817% |
| 3. | C | 0.0065 | 0.0048 | 12.307% |
| 4. | D | 0.0142 | 0.0138 | 3.521% |
| 5. | E | 0.0065 | 0.0055 | 15.384% |
| 6. | F | 0.0142 | 0.0116 | 18.309% |
| 7. | G | 0.0065 | 0.0057 | 10.769% |
| 8. | H | 0.0142 | 0.0137 | 9.155% |

#### 2.6.1. Residual weight method

A Sartorius microbalance was utilized for the determination of the initial and final weights of polyethylene samples. The polyethylene strips were placed in the weighing chamber carefully and the high precision readings were recorded. The percentage loss of weight for each sample was calculated using the formula given in Eq. (3). The values obtained were then tabulated and compared.

$$\%Loss\ of\ weight\ = \frac{Initial\ Weight - Final\ Weight}{Initial\ Weight} * 100 \quad (3)$$

#### 2.6.2. Scanning Electron Microscope (SEM) analysis

Prior to carrying out the SEM analysis, the samples were gold plated in a sputter coater as the samples are non-conductive in nature. The samples were then placed in the chamber of the TESCAN VEGA3 LMU SEM assembly. Backscattered electron images were collected to observe the morphology of the polyethylene surfaces. The LDPE surface was captured at a magnification of 1000x, 1500x and 2000x magnification.

#### 2.6.3. Fourier transform infrared spectroscopy

The polyethylene samples were placed under the spectrometer probe of the Alpha FT-IR spectrophotometer. The samples were tested for a range of wavenumbers from 400 to 4000 cm−1. During the FT-IR analysis, the system plots a graph of transmittance (%) vs. wavenumber ($cm^{-1}$). The various peaks in the graphs correspond to functional groups that are present in the sample. The FT-IR analysis was carried out for both, control and degraded sample.

## 3. Results

The current section has been divided into two sections. The results of weight loss analysis among polyethylene samples are discussed in the first section. The second section highlights the results obtained on SEM analysis. In the third section, the FTIR spectrometry results are discussed. The next section highlights the use of shredded polyethylene samples as a sole carbon source for the growth of fungal species. In the final section, growth kinetics are presented which can be used for bioreactor design.

### 3.1. Analysis of loss in weight of the polyethylene sample

The weights of samples before and after degradation were measured using a microbalance. The tabulation of weight loss results for a period of 20, 30 and 55 days in Potato Dextrose broth (PDB), as well as Czapek Dox (CDB) broth, are shown in Table 2, subheadings a, b and c respectively. The comparison of weight loss of LDPE samples measuring 2 cm × 2 cm and 3 cm × 3 cm is illustrated in Figure 2.

The loss of weight comparison showed the increasing rate of biodegradation over days for 2 × 2 samples in PD broth media, 2 × 2 sample in CD broth media, 2 × 2 sample in a mixture of PD broth and additive, 3 × 3 samples in CD broth media, and 3 × 3 sample in a mixture

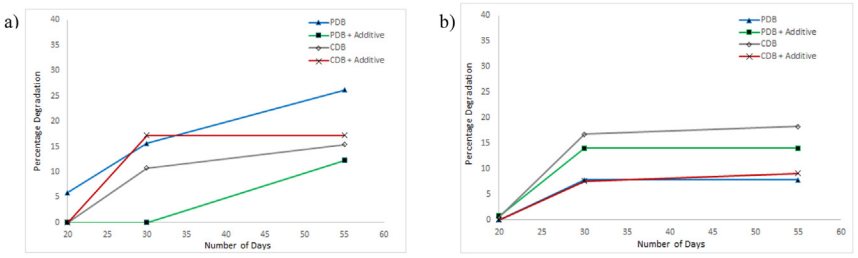


**Figure 2.** Comparison of weight loss in a) 2 × 2 LDPE b) 3 × 3 LDPE samples placed in four different culture media.

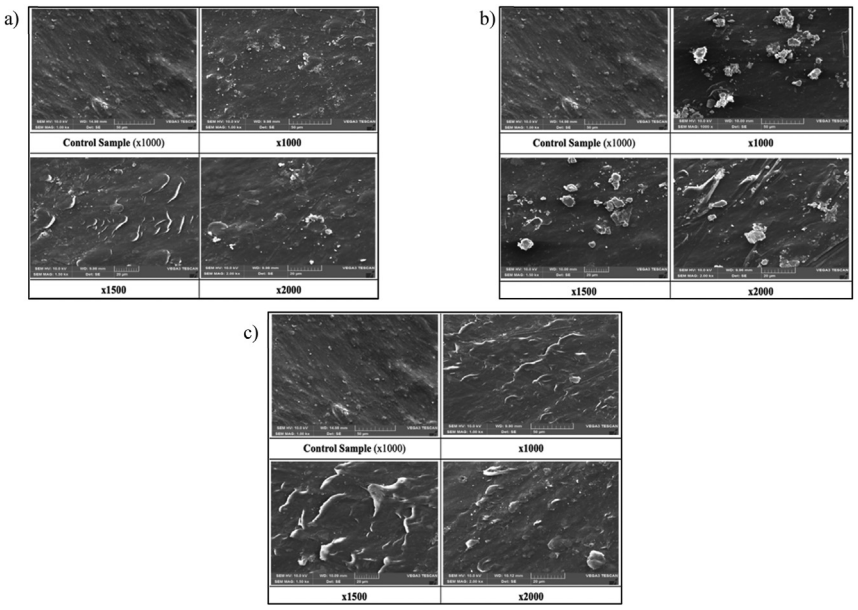


**Figure 3.** Surface morphologies of polyethtylene samples exhibitng highest weight loss percentage: a) Sample A for 20 days magnified at 1000x, 1500x and 2000x; b) Sample G for 30 days magnified at 1000x, 1500x and 2000x; c) Sample A for 55 days magnified at 1000x, 1500x and 2000x.

of PD broth and additive. The highest loss in weight of 26% was exhibited by the LDPE sample placed in PD broth medium after 55 days. The utilization of the Tween 80 surfactant as an additive was insignificant in improving the degradation rates. The weight loss by using a fungal consortium was found to be more than those obtained by using a single species of fungi across a similar time frame [6, 34, 35].

### 3.2. Scanning electron microscopy analysis

The LDPE samples were subjected to 1000x, 1500x and 2000x magnification in a Scanning Electron Microscope to observe the surface morphology before and after biodegradation (Figure 3). The polyethylene strip which was not subjected to fungal degradation displayed a

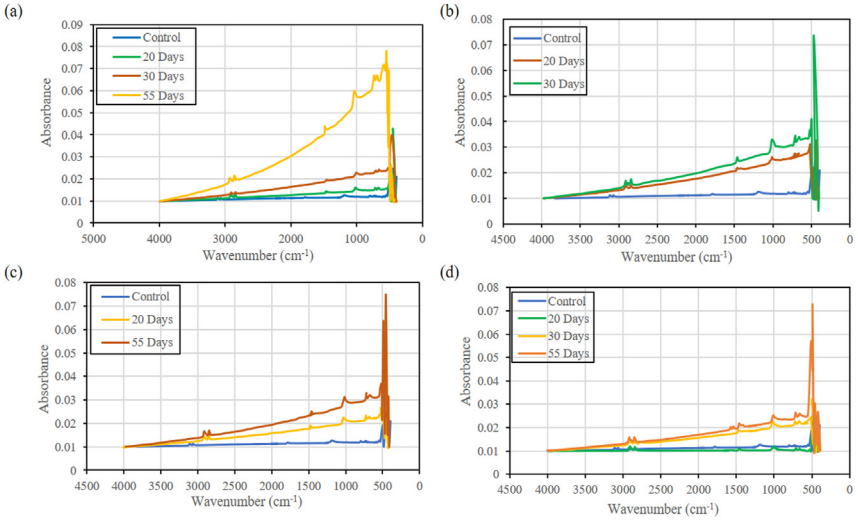


**Figure 4.** Comparison of samples in a) PD media across 0,20,30 and 55 days, b) PD media + additive across 0, 20 and 30 days, c) CD media across 0,20 and 55 days, d) CD media + additive across 0,20,30 and 55 days.

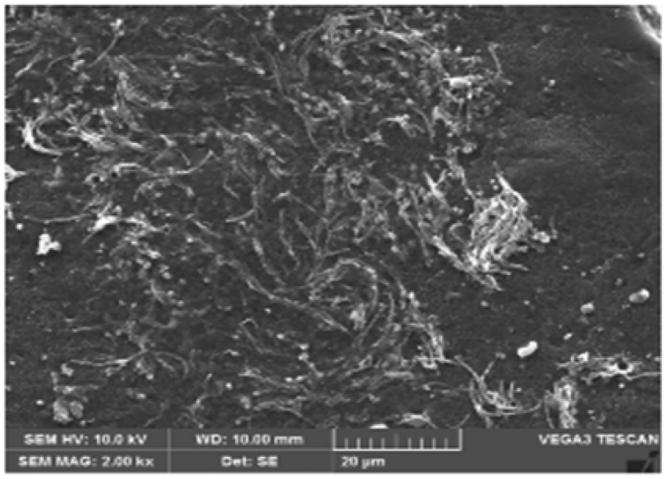


**Figure 5.** SEM analysis of shredded LDPE sample subjected to biodegradation.

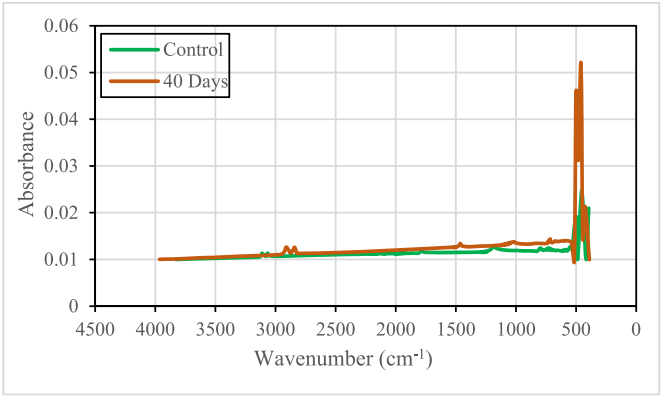


**Figure 6.** Comparison of samples with polyethylene as sole carbon source for the control sample and biodegraded sample after a period of 40 days.

smooth surface view with no defects in all levels of magnification. The LDPE sample inoculated in PD media with additives did not show appreciable degradation. Hence, it was not considered for SEM analysis. Sample A showed the highest percentage loss of weight in 20 days, Sample G in 30 days, and Sample A in 55 days. The resulting SEM images of control and biodegraded samples based on the highest percentage loss of weight are shown.

SEM analysis showed that the polyethylene strips treated with *Aspergillus* consortium showed appreciable surface erosion, folding, and pitting in the form of cracks, holes, scions, and cavities. This observation is consistent with previous studies of polyethylene degradation by single *Aspergillus sp* [36, 37, 38] and also by consortium [19].

### 3.3. Fourier transform infrared analysis

Control and bio-degraded samples of LDPE were subjected to FT-IR analysis. FT-IR analysis confirmed the degradation of the polyethylene sample by illustrating the structural changes between degraded and control samples. The absorbance versus wavenumber curves obtained from FT-IR results of control and biodegraded samples kept in different media are shown in Figure 4. The peaks present at 2915 $cm^{-1}$ and 2845 $cm^{-1}$ for the control sample indicated C–H bond stretching. Additionally, the peak obtained at 1464 $cm^{-1}$ was confirmed to be $CH_2$ bend bonds and the peak at 717 $cm^{-1}$ was determined to be due to $CH_2$ rock vibrational mode. Both these results are fairly consistent in accordance to observations made by Asensio et al. [39] and Noda et al. [40]. In the degraded samples over 20, 30 and 55 days, a clear decrease was evident in the intensity of the peaks at wavenumbers corresponding to 2915 $cm^{-1}$, 2845 $cm^{-1}$, 1464 $cm^{-1}$, and 717 $cm^{-1}$. In accordance to the work carried out by Chatterjee et al. [41], such a decrease indicated clear signs of biodegradation. The FT-IR curves after 55 days indicated that these peaks had significantly reduced in intensity such that they are almost vanishing and only just narrowly identifiable. Correspondingly, a plot of absorbance versus wavenumber would indicate an increase in peak intensity of the peaks at the above-mentioned wavenumbers.

### 3.4. Biodegradation analysis of the shredded polyethylene sample

Shredded LDPE samples were subjected to biodegradation in the Czapek Dox medium in the absence of any other source of carbon. The shredded polyethylene sample was solely the source of carbon for the growth of the *Aspergillus* fungal consortium. Characterization tests like loss of weight, SEM, and FT-IR analysis were done on the shredded Polyethylene samples to establish biodegradation.

#### 3.4.1. Loss of weight analysis

The loss of weight obtained when polyethylene is the sole carbon source (14.656%) is lesser than that obtained when other carbon sources are provided for the same media. The loss of weight in the shredded polyethylene sample in the absence of sucrose suggests that the fungal consortium can utilize it as the sole carbon source for its growth and metabolism.

#### 3.4.2. SEM analysis

The LDPE samples that showed the highest loss of weight were subjected to 1000x, 1500x and 2000x magnification in a Scanning Electron Microscope to observe the surface morphology before and after biodegradation. The resulting SEM images of control and the biodegraded sample is shown in Figure 5.

The SEM images of the biodegraded shredded LDPE sample exhibited the presence of fiber-like structures. Clear signs of surface erosion, folding, and pitting are evident in the SEM images which are consistent with previous studies [19]. In Figure 5, the breaking of fiber-like structures is evident at numerous sites, which suggests that the carbon chain of polyethylene is broken down by fungal activity.

**Table 3.** Growth kinetics data for Potato Dextrose broth media and Czapek Dox broth.

| Time, *t* (h) | Initial Weight (g) | | Final Weight (g) | | Weight Difference (g) | | Substrate Volume (mL) | | Biomass Concentration, *x* (g/L) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Media | PDB | CDB | PDB | CDB | PDB | CDB | PDB | CDB | PDB | CDB |
| 24 | 0.933 | 0.933 | 1.063 | 1.000 | 0.130 | 0.067 | 28 | 25 | 4.643 | 2.680 |
| 48 | 0.880 | 1.041 | 1.044 | 1.120 | 0.164 | 0.079 | 27 | 27 | 6.074 | 2.926 |
| 72 | 1.043 | 0.872 | 1.115 | 1.193 | 0.072 | 0.321 | 26 | 26 | 2.769 | 12.346 |
| 144 | 0.852 | 1.240 | 0.956 | 1.584 | 0.104 | 0.344 | 25 | 21 | 4.160 | 16.381 |
| 192 | 0.915 | 0.734 | 1.427 | 1.595 | 0.512 | 0.861 | 23 | 23 | 22.261 | 37.435 |
| 336 | 1.119 | 1.024 | 1.200 | 1.082 | 0.081 | 0.058 | 20 | 24 | 4.050 | 2.417 |

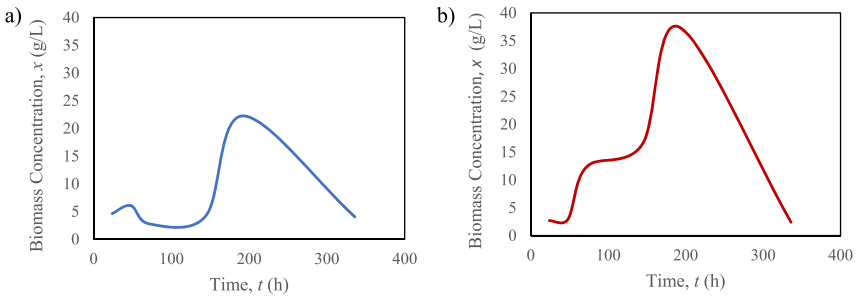


**Figure 7.** Growth curves of *Aspergillus* consortium for a) PD media and b) CD media.

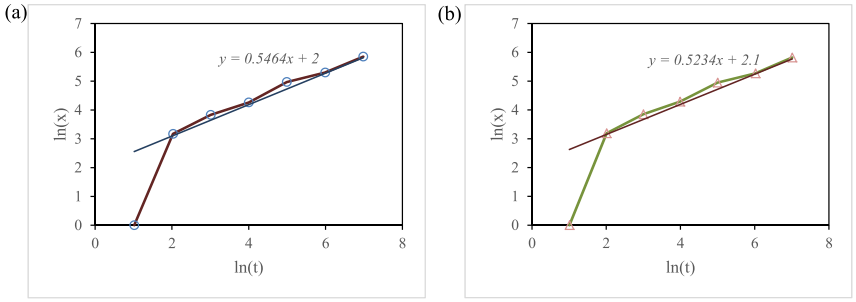


**Figure 8.** Specific growth rate curves of *Aspergillus* consortium for a) PD media and b) CD media.

### 3.4.3. FT-IR analysis

Control and degraded samples of shredded Polyethylene were subjected to FT-IR analysis. The absorbance versus wavenumber curves obtained from FT-IR results of control and biodegraded samples kept in different media are shown in Figure 6.

The intensity of peaks at wavenumbers 2915 $cm^{-1}$, 2845 $cm^{-1}$, 1464 $cm^{-1}$ and 716 $cm^{-1}$ have considerably increased when compared to the control sample, which indicates that the population of C–H bonds have decreased [41]. The depletion of C–H bonds indicates that polyethylene has undergone biodegradation in the presence of the fungal consortium, similar to the degradation of the samples in media. The loss of weight in the shredded polyethylene sample in the absence of sucrose suggests that the fungal consortium can utilize it as the sole carbon source for its growth and metabolism.

### 3.5. Analysis of growth kinetics

The measured weight difference of the fungal consortium and its corresponding time periods are shown, along with the calculated biomass concentration, in Table 3 for Potato Dextrose broth and Czapek Dox broth. The growth rate curve was then plotted between biomass concentration and time as shown in Figure 7.

The specific growth rate was determined by plotting graphs natural logarithm of biomass concentration versus the natural logarithm of time of growth as shown in Figure 8. The linear curve so obtained is illustrated in Eq. (4).

$$ln(x) = \mu^* ln(t) + ln(i) \quad (4)$$

where, $x$ = biomass concentration, g/L; $t$ = time of growth, h; $\mu$ = specific growth rate, $h^{-1}$; $ln(i)$ = intercept.

The specific growth rate thus obtained is approximately 0.5442 $h^{-1}$ for potato dextrose broth and 0.5242 $h^{-1}$ for czapek dox broth. This value of specific growth rate is explicit only to the *Aspergillus* consortium used in the experiments. The batch time of the bioreactor can be determined using this predetermined specific growth rate.

## 4. Discussions

Over the years, several studies have focused on the biodegradation of LDPE using microorganisms. Although several studies have employed bacterial cultures in biodegradation of LDPE in natural environments, minimal work has been carried out using fungal species in controlled environments. In the current work, a fungal consortium comprising of *Aspergillus niger*, *Aspergillus flavus,* and *Aspergillus oryzae* has been used for biodegradation of LDPE under laboratory conditions. Residual weight, SEM, and FT-IR methods were performed on the biodegraded samples to calculate the degradation efficiency for a period of 20, 30, and 55 days. The loss of weight in the polyethylene samples can be attributed to the breakdown of the carbon backbone by fungal enzymes. The resultant monomers and oligomers are used directly by the fungal species as a carbon source [8]. The loss of weight can be attributed to the formation of biofilms over the LDPE samples, which decreased the hydrophobicity and contact surface between the fungi and LDPE samples. SEM analysis was able to effectively capture the level of scission before and after subjecting the polyethylene sample to the microbial attack. As reported by [42], the polyethylene sample subjected to biodegradation for a minimal duration (20 days) exhibited minimal signs of exfoliation and had a homogenous film structure as compared to the 30 and 55 days sample. The FT-IR spectrometry can analyze the micro destruction of the chemical structure of polyethylene brought about by biodegradation. Concurrent to the studies made by Asensio et al. [39], Noda et al. [40] and other studies, the formation, and disappearance of C–H peaks and carbonyl peaks in the polyethylene samples, were associated with biodegradation. whose results were commensurate to those obtained from the literature.

## Declarations

### Author contribution statement

Glen Cletus DSouza: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ryna Shireen Sheriff, Varun Ullanat, Aniruddh Shrikrishna: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Anupama V. Joshi, Lingayya Hiremath, Keshamma Entoori: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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### *Data availability statement*

Data included in article/supplementary material/referenced in article.

### *Declaration of interests statement*

The authors declare no conflict of interest.

### *Additional information*

No additional information is available for this paper.

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## References

[1] H. V Sowmya, M.K. Ramalingappa, B. Thippeswamy, Biodegradation of polyethylene by Bacillus cereus, Adv. Polym. Sci. Technol. An Int. J. 4 (2) (2014) 28–32.

[2] K.M. Abd El-Rahman, S.F. Abdellah Ali, A.I. Khalil, S. Kandil, Influence of poly(butylene succinate) and calcium carbonate nanoparticles on the biodegradability of high density-polyethylene nanocomposites, J. Polym. Res. 27 (8) (2020) 231.

[3] L. Contat-Rodrigo, A. Ribes Greus, Biodegradation studies of LDPE filled with biodegradable additives: morphological changes. I,, J. Appl. Polym. Sci. 83 (8) (2002) 1683–1691.

[4] S.K. Sen, S. Raut, Microbial degradation of low density polyethylene (LDPE): a review, J. Environ. Chem. Eng. 3 (1) (2015) 462–473.

[5] N. Verma, S. Gupta, Management of polythene waste through biodegradation by using few species of *Aspergillus*: an environment friendly approach, in: *Biotechnology and Biological Sciences: Proceedings of the 3rd International Conference of Biotechnology and Biological Sciences* (BIOSPECTRUM 2019), August 8-10, 2019, Kolkata, India, 2019, p. 325.

[6] B. Nowak, J. Paj\kak, M. Drozd-Bratkowicz, G. Rymarz, Microorganisms participating in the biodegradation of modified polyethylene films in different soils under laboratory conditions, Int. Biodeterior. Biodegrad. 65 (6) (2011) 757–767.

[7] S. Nanda, S. Sahu, J. Abraham, Studies on the biodegradation of natural and synthetic polyethylene by Pseudomonas spp,, J. Appl. Sci. Environ. Manag. 14 (2) (2010).

[8] K. Kathiresan, Polythene and plastics-degrading microbes from the mangrove soil, Rev. Biol. Trop. 51 (3–4) (2003) 629–633.

[9] N. Priyanka, T. Archana, Biodegradability of polythene and plastic by the help of microorganism: a way for brighter future, J. Environ. Anal. Toxicol. 1 (4) (2011) 1000111.

[10] T. Volke-Sepúlveda, G. Saucedo-Castañeda, M. Gutiérrez-Rojas, A. Manzur, E. Favela-Torres, Thermally treated low density polyethylene biodegradation by Penicillium pinophilum and Aspergillus Niger, J. Appl. Polym. Sci. 83 (2) (2002) 305–314.

[11] V. Mahalakshmi, S.N. Andrew, Assessment of Physicochemically treated plastic by fungi, Ann. Biol. Res. 3 (9) (2012) 4374–4381.

[12] R. Mohee, G.D. Unmar, A. Mudhoo, P. Khadoo, Biodegradability of biodegradable/degradable plastic materials under aerobic and anaerobic conditions, Waste Manag. 28 (9) (2008) 1624–1629.

[13] A.A. Shah, F. Hasan, A. Hameed, S. Ahmed, Biological degradation of plastics: a comprehensive review, Biotechnol. Adv. 26 (3) (2008) 246–265.

[14] B.M. Kyaw, R. Champakalakshmi, M.K. Sakharkar, C.S. Lim, K.R. Sakharkar, Biodegradation of low density polythene (LDPE) by Pseudomonas species, Indian J. Microbiol. 52 (3) (2012) 411–419.

[15] P.P. Bosshard, Incubation of fungal cultures: how long is long enough? Mycoses 54 (5) (2011) e539–e545.

[16] S. Saroj, S. Dubey, P. Agarwal, R. Prasad, R.P. Singh, Evaluation of the efficacy of a fungal consortium for degradation of azo dyes and simulated textile dye effluents, Sustain. Water Resour. Manag. 1 (3) (2015) 233–243.

[17] P. Rajeswari, et al., Green synthesis of silver nanoparticles by Aspergillus consortium and evaluating its anticancer activity against Human breast adenocarcinoma cell line (MCF7), Pharm. Biol. Eval. 4 (2017) 28–36.

[18] H. Djelal, A. Amrane, Biodegradation by bioaugmentation of dairy wastewater by fungal consortium on a bioreactor lab-scale and on a pilot-scale, J. Environ. Sci. 25 (9) (2013) 1906–1912.

[19] H. V Sowmya, B. Ramalingappa, G. Nayanashree, B. Thippeswamy, M. Krishnappa, Polyethylene degradation by fungal consortium, Int. J. Environ. Res. 9 (3) (2015) 823–830.

[20] N. Lucas, C. Bienaime, C. Belloy, M. Queneudec, F. Silvestre, J.-E. Nava-Saucedo, Polymer biodegradation: mechanisms and estimation techniques–A review, Chemosphere 73 (4) (2008) 429–442.

[21] S. Zahra, S.S. Abbas, M.-T. Mahsa, N. Mohsen, Biodegradation of low-density polyethylene (LDPE) by isolated fungi in solid waste medium, Waste Manag. 30 (3) (2010) 396–401.

[22] M.K. Sangale, M. Shahnawaz, A.B. Ade, Potential of fungi isolated from the dumping sites mangrove rhizosphere soil to degrade polythene, Sci. Rep. 9 (1) (2019) 1–11.

[23] C. Rivard, L. Moens, K. Roberts, J. Brigham, S. Kelley, Starch esters as biodegradable plastics: effects of ester group chain length and degree of substitution on anaerobic biodegradation, Enzym. Microb. Technol. 17 (9) (1995) 848–852.

[24] M.A. Begum, B. Varalakshmi, K. Umamagheswari, Biodegradation of polythene bag using bacteria isolated from soil, Int. J. Curr. Microbiol. App. Sci. 4 (11) (2015) 674–680.

[25] U. Witt, T. Einig, M. Yamamoto, I. Kleeberg, W.-D. Deckwer, R.-J. Müller, Biodegradation of aliphatic–aromatic copolyesters: evaluation of the final biodegradability and ecotoxicological impact of degradation intermediates, Chemosphere 44 (2) (2001) 289–299.

[26] D. Burd, Plastic Not Fantastic, *Proj. Reports Canada Wide Sci. Fair*, 2008, pp. 1–5.

[27] P.K. Roy, S. Titus, P. Surekha, E. Tulsi, C. Deshmukh, C. Rajagopal, Degradation of abiotically aged LDPE films containing pro-oxidant by bacterial consortium, Polym. Degrad. Stabil. 93 (10) (2008) 1917–1922.

[28] D.K.A. Barnes, F. Galgani, R.C. Thompson, M. Barlaz, Accumulation and fragmentation of plastic debris in global environments, Philos. Trans. R. Soc. B Biol. Sci. 364 (1526) (2009) 1985–1998.

[30] Potato Dextrose Broth, Himedialabs.com, 2015. Retrieved from, https://www.himedialabs.com/TD/M403.pdf.

[31] Czapek Dox Broth, Himedialabs.com, 2018. Retrieved from, https://himedialabs.com/TD/M076.pdf.

[32] C. Thom, K.B. Raper, in: A Manual of the Aspergilli, 60, LWW, 1945 no. 4.

[33] M. Boley, K. Langenbach, and B. Beck, Rapid Identification of Cell Lines Sensitive to Clostridium perfringens Epsilon Toxin, American Type Culture Collection, Manassas, VA 20110.

[34] N. Ojha, et al., Evaluation of HDPE and LDPE degradation by fungus, implemented by statistical optimization, Sci. Rep. 7 (1) (2017) 1–13.

[35] I. Brunner, M. Fischer, J. Rüthi, B. Stierli, B. Frey, Ability of fungi isolated from plastic debris floating in the shoreline of a lake to degrade plastics, PloS One 13 (8) (2018), e0202047.

[36] R. Pramila, K.V. Ramesh, Biodegradation of low density polyethylene (LDPE) by fungi isolated from marine water a SEM analysis, Afr. J. Microbiol. Res. 5 (28) (2011) 5013–5018.

[37] V. Mahalakshmi, A. Siddiq, S.N. Andrew, Analysis of polyethylene degrading potentials of microorganisms isolated from compost soil, Int. J. Pharm. Biol. Arch. 3 (5) (2012) 1190–1196.

[38] N. Raaman, N. Rajitha, A. Jayshree, R. Jegadeesh, Biodegradation of plastic by Aspergillus spp. isolated from polythene polluted sites around Chennai, J. Acad. Indus. Res. 1 (6) (2012) 313–316.

[39] R.C. Asensio, M.S.A. Moya, J.M. de la Roja, M. Gómez, Analytical characterization of polymers used in conservation and restoration by ATR-FTIR spectroscopy, Anal. Bioanal. Chem. 395 (7) (2009) 2081–2096.

[40] I. Noda, A.E. Dowrey, J.L. Haynes, C. Marcott, Group frequency assignments for major infrared bands observed in common synthetic polymers, in: Physical Properties of Polymers Handbook, Springer, 2007, pp. 395–406.

[41] S. Chatterjee, B. Roy, D. Roy, R. Banerjee, Enzyme-mediated biodegradation of heat treated commercial polyethylene by Staphylococcal species, Polym. Degrad. Stabil. 95 (2) (2010) 195–200.

[42] T. Mumtaz, M.R. Khan, M.A. Hassan, Study of environmental biodegradation of LDPE films in soil using optical and scanning electron microscopy, Micron 41 (5) (2010) 430–438.