Morphological modification of low-density polyethylene for *Phanerochaete chrysosporium* bioerosion

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ARTICLE HISTORY
Compiled January 5, 2021

Abstract
Disposal of end-of-life low density polyethylene (LDPE) in landfill structures poses an ecological threat through leaching, fragmentation, and additive migration. The present study examines the mycodegradation of morphologically modified low-density polyethylene (LDPE) films by *Phanerochaete chrysosporium*, a lignolytic basidiomycete. Three physicochemical treatments were employed: thermo-oxidation, chemical etching, and synthetic leachate. Surface area imaging over 6 days revealed bioerosion, qualifying *P. chrysosporium* as a suitable agent for LDPE degradation. Analysis of crystallinity indices showed that thermo-oxidation and chemical etching induced conformational changes to the polymer surface, increasing surface area reduction by 19% and 22% respectively. Synthetic leachate (SL) was associated with a 36% increase in surface area reduction. In combination, the three treatments achieved 99% surface area reduction. These trends were corroborated by gaseous evolution in parallel, attributable to the metabolization of fungal isolates. Electrospray Ionization Fourier Transform (FTMS + pESI) profiles observed signs of leachate remediation and organic byproducts. A molecular mechanism for degradation was subsequently proposed based on SL composition.

KEYWORDS
mineralization, secondary metabolism, cross-linking, ligninolysis, bioremediation

1. Introduction

Low density polyethylene (LDPE) is a versatile plastic of immense industrial utility. However, of the 64 million metric tons manufactured every year, only 5.7 percent is recycled [1,2]. Other methods of disposing end-of-life LDPE are often unsafe or counterproductive [3]. As a result, most LDPE accumulates in landfills, where its carbon-to-carbon backbone and branched structure render it recalcitrant and pose an ecological threat through leaching, fragmentation, and additive migration [4,5]. In 1978, Hiroyuki *et al.* proposed two solutions to this polymeric design challenge: engineering biodegradable materials and enhancing microbial degradation [6]. While the former holds long-term potential, this study focuses on biodegradation as a means of addressing LDPE entering existing solid waste structures.

Previous literature has established that fungi exhibit higher enzyme activity than bacteria [7]. Microbial species in genera *Aspergillus*, *Ideonella*, and *Trichoderma* are

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particularly effective at eroding the surface of tertiary sites on polyethylene [8–11]. Studies have also reported that small product degradation is easier than large product degradation, in addition to considering morphological changes to polymer surfaces [7]. However, literature on the impact of physicochemical surface pretreatment on biodegradation is scarce. The objective of this investigation is to enhance LDPE biodegradation by inducing morphological changes to its surface.

1.1. Phanerochaete chrysosporium

Phanerochaete chrysosporium is a white-rot basidiomycete that degrades aromatic hydrocarbons, predominantly lignin, under aerobic conditions [12–14]. Lignin's aliphatic side chains share structural similarities with LDPE, leading to the novel hypothesis that *P. chrysosporium*'s two key enzymes, lignin peroxidase (LiP) and manganese peroxidase (MnP), may possess the ability to mineralize LDPE [15,16]. These enzymes generate H_2O_2 through an oxidation reaction to degrade organic pollutants, viable in pH range 2.5<pH<6 and over a wide range of temperatures, 25 to 60 °C [16,18]. It is capable of growth in both shallow stationary cultures and agitated flask cultures [17].

P. chrysosporium metabolization of various contaminants is shown in Table 1. During secondary metabolism, biofilm formation occurs based on nutrient, oxygen, and trace metal availability [18–22].

Table 1. *P. chrysosporium* Metabolization

Substrate	Mineralization
Organics	CO_2
Nitrogen Compounds	NO_3^{2-}
Hydrogen Compounds	H_2O
Heavy Metals	Ion Adsorption

This study examines the degradation of morphologically modified LDPE using *P. chrysosporium*. The selection of physicochemical pre-treatments is described in the section that follows.

1.2. Morphological Treatments

LDPE consists of long aliphatic chains with repeating CH_2— units [4], resulting a closely-packed, branched network. Under normal conditions, this structure interferes with crystallization. However, Drummond *et al.* found a rise in **melting crystallites** in the 110C to 120 °C temperature regime [23,24].

Mijovic also reported that **chemical etching** impacts polymer mechanical properties and surface area roughness, facilitating subsequent fungal anchorage [25].

Landfill leachate is wastewater formed by the percolation of rainwater through a landfill. A high C:N ratio and substrate adsorbability suggest activation of the secondary enzymatic mechanism necessary for LiP and MnP secretion [26,27].

The author also examined 11 other pretreatment methods in preliminary experiments, omitted for brevity.

2. Methods

The aim of this study was to identify physicochemical treatment combinations that maximize LDPE mycodegradation: *P. chrysosporium* inoculation, synthetic leachate, etching, and thermo-oxidation. Table 2 displays the configuration.

Table 2. Treatment Combinations

	Composition				
Group	LDPE	PC	Thermo-oxidation	Etching	Synthetic Leachate
Control[1]	×				
1	×	×			
2	×	×	×		
3	×	×		×	
4	×	×	×	×	
5	×	×			×
6	×	×	×		×
7	×	×		×	×
8	×	×	×	×	×

[1]No morphological or mycoremedial treatment.

2.1. Experimental

LDPE was obtained from commercially-available films of uniform thickness ($\sim 15\mu$m) and cut into strips of surface area 115 mm^2 and mass 3 mg. The films were then sterilized with a bleach-water solution.

2.1.1. Thermo-oxidation

LDPE was heated in a convection oven at 120 °C for 4 minutes. The surface area reduction for each film as a result of this procedure was recorded, averaging 7%.

2.1.2. Chemical Etching

Anhydrous citric acid was used to prepare a 30% citric acid solution, in which LDPE films were immersed for 10 minutes. After removal from solution, the solvent was allowed to evaporate off the polymer surface. In preliminary experiments, LDPE rinsed with distilled water proved less susceptible to biodegradation, so this procedure was eliminated.

2.1.3. Synthetic Leachate

Synthetic leachate (SL) composition was adapted from Azhar *et al.* to simulate landfill wastewater conditions [28] at pH 5.6 and comparable COD:TN:TP ratios. SL was boiled and set with small amounts of potato-dextrose agar (PDA) for 24 hours. The C/N ratio of the SL-PDA medium was approximately 25:1 with a starting pH of 5.7.

2.1.4. P. chrysosporium Inoculation

P. chrysosporium was incubated on a PDA slant at 27 °CC for 24 hours. After sporulation, SL-PDA plates were subsequently inoculated with $2 \cdot 10^7$ fungal spores per gram.

LDPE films were placed in their corresponding media (PDA or SL-PDA) for an observation period of 6 days every 12 days. After the experimental procedure was termi-

nated, LDPE was retrieved using filter paper, sterilized with a bleach-water solution, and analyzed for degradation.

2.2. Surface Area and Crystallinity

Surface area and crystallinity changes were quantified over 6 days using the Java image-processing program, `ImageJ`. Surface area reduction indicates polymer bioerosion, and crystallinity is associated with spherulitic nucleation and roughness in LDPE morphology [29]. Statistical analyses were performed by `StatPlus`.

2.3. CO_2 Evolution

It is well-known that polyethylene degradation yields gaseous byproducts, predominantly CO_2, due to the increase in low molecular weight material and reduction in carbonyl groups. CO_2 evolution was measured by water displacement in a pneumatic trough apparatus.

2.4. Electrospray Ionization Fourier Transform (FTMS + pESI)

Changes in leachate composition in PDA, SL-PDA, and Group 8 samples over 12 days were investigated using an Orbitrap and fourier transform (FTMS + pESI) techniques at 5 μL/min and 60,000 resolution. ESI was performed at 30 V capillary voltage and 60 V tube voltage, low mass conditions.

Compounds were identified by the `ChemBioDraw` software ($\pm$ 0.0001 m/z). NH_4^+, NO_3^{2-}, and NO_2^- data were corroborated by ancillary indicator tests.

3. Results and Discussion

All results concern the identification and analysis of morphological pre-treatments for enhanced LDPE biodegradation and were statistically significant ($p > 0.05$).

3.1. Crystallinity Index

Figure 1 shows trends in relative percentage crystallinity induced by thermo-oxidation (Group 2) and thermo-oxidation with etching (Group 4).

For both surface modifications, the initial spike occurred on Day 1 after pretreatment. Thermo-oxidation causes a cross-linking reaction where tie chains undergo chain scission and cilia chains recrystallize [30]. The increase in cross-linking is likely responsible for the sharp increase in percentage crystallinity. Elongation of the amorphous region was visually observed, in agreement with the findings of Veitmann *et al.* [31].

Chemical etching performed prior to thermo-oxidation further enhanced crystallization, a result shared by Miyagawa *et al.* who corroborated that chemical etching crystallizes amorphous regions [30].

After initial treatment, the population of crystallites declined due to surface bioerosion as a function of exposure time. Percentage crystallinity remained fairly unchanged by *P. chrysosporium* and SL, and when there were no treatments.

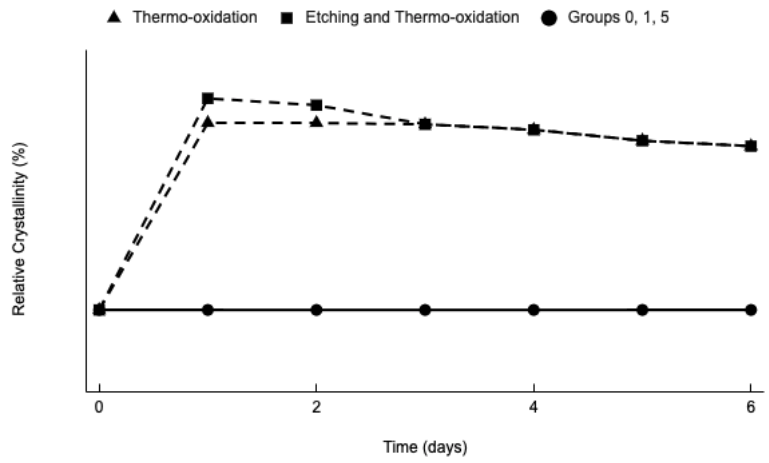

Figure 1. Trends in relative percentage crystallinity induced by thermo-oxidation (Group 2) and thermo-oxidation with etching (Group 4).

3.2. Surface Area Reduction and Gaseous Evolution

Figure 2 tracks the change in average surface area reduction of 3 mg LDPE films after 6 days, with a baseline comparison against Group 1. Prolonged exposure to *P. chrysosporium* (Group 1) yielded a surface area reduction of 5%.

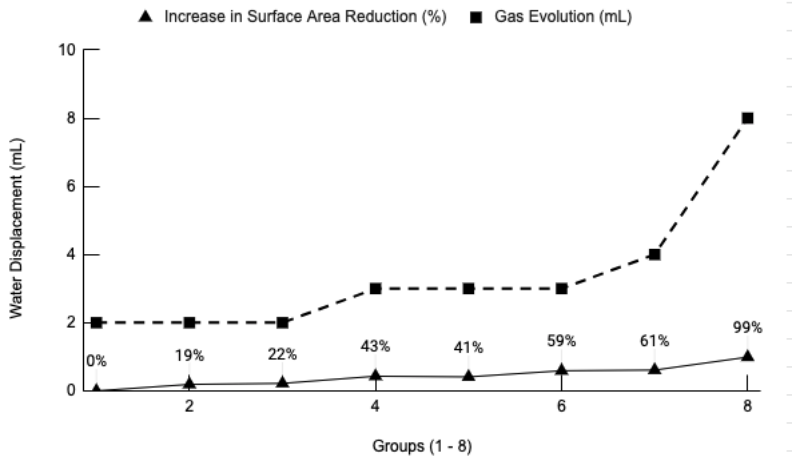

Figure 2. Surface area reduction and corresponding gas production (Groups 1-8).

An increased surface area loss of 14% was observed in samples pretreated by thermo-oxidation (Group 3) at 120 °C. The procedure causes cross-linking, which breaks carbon-hydrogen bonds and gives rise to carbon-carbon single bonds. These tertiary sites have lower bonding energies and are therefore vulnerable points in LDPE morphology to extracellular enzymes [31].

Etching (Group 3) increased surface area reduction by 17% over a 6 day observation period. This result accompanies previous findings that on a molecular level, chemical-etching cleaves bonds in amorphous polymeric regions. The presence of citric acid may also be responsible for enhanced microbial activity by increasing surface bonding sites for hyphal extension and adding various polar groups [31].

An increase of 36% in surface area reduction was noted in leachate samples (Group 5). While the mechanism for this remains unclear, it is well-known that ligninolytic activity catalyzed by *P. chrysosporium* generally occurs in nitrogen-starved environments [32]. The SL-PDA growth media had a high C:N ratio, was sulfur-limited and phosphorus abundant, and contained Mg^{2+} and Ca^{2+}, trace metals known to catalyze secondary metabolic responses in *P. chrysosporium*. Carbohydrate levels were also low. SL did contain abundant NH^{4+}, but Jeffries *et al.* previously reported that it does not significantly suppress ligninolytic activity [28,33]. This leads to the following proposed mechanism: the synthetic composition of leachate met ligninolytic conditions, facilitating high levels of LDPE mineralization.

In conjunction, the three morphological treatments (Group 8) increased surface area reduction by a maximum of 99%.

This data was corroborated by an analogous trend in gas evolution associated with fungal respiration and degradation products, quantified by water displacement in a pneumatic trough. Displacement in a sample with no fungal isolates was 0.7 mL. Group 8 showed an 8 mL change in water level, and Ideal Gas Law calculations based on LDPE and SL composition approximately confirmed the surface area reduction results [34,35].

3.3. Electrospray Ionization Fourier Transform

Figures 3, 4, and 5 show the FTMS + pESI profiles of SL, SL exposed to *P. chrysosporium* for 12 days, and SL with LDPE film exposed to *P. chrysosporium* for 12 days respectively.

Significant decreases were observed in the CO_3^{2-} peak at 121.0278 *m/z* and NH_4^+ peak at 180.0859 *m/z* after 12 days of exposure. Heavy metal solubility appeared to rise. For example, Mn^{2+} at 350.9300 *m/z* can be observed in three unique peaks on Day 12. A number of organics were also observed in samples containing degraded LDPE products. ESI results suggested some signs of SL remediation, later confirmed by indicator tests for NH_4^+, NO_3^{2-}, and $NO2^-$. This aligns with conclusions drawn by previous research on *P. chrysosporium* metabolism and biodegradative potential for leachate [36–38].

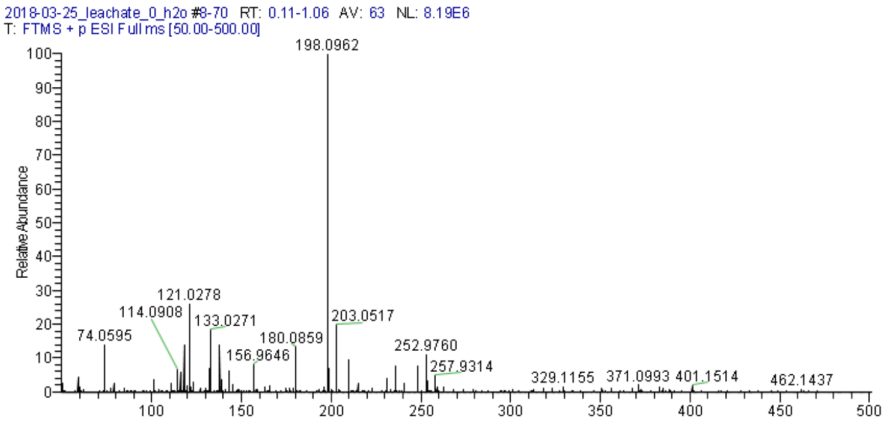

Figure 3. FTMS + pESI profile of Synthetic Leachate on Day 0.

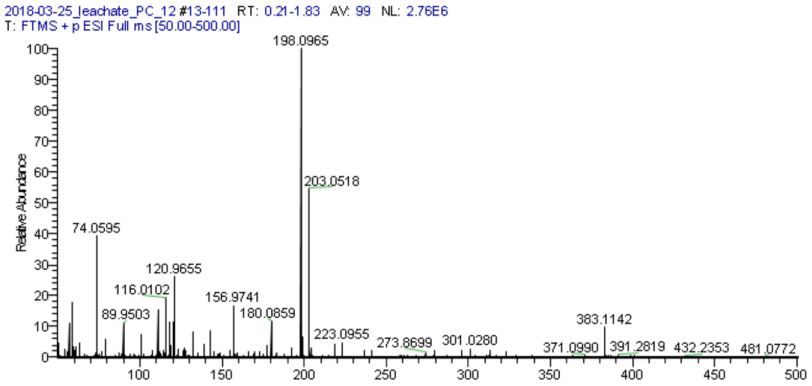

Figure 4. FTMS + pESI profile of Synthetic Leachate after 12 days of exposure to *P. chrysosporium*.

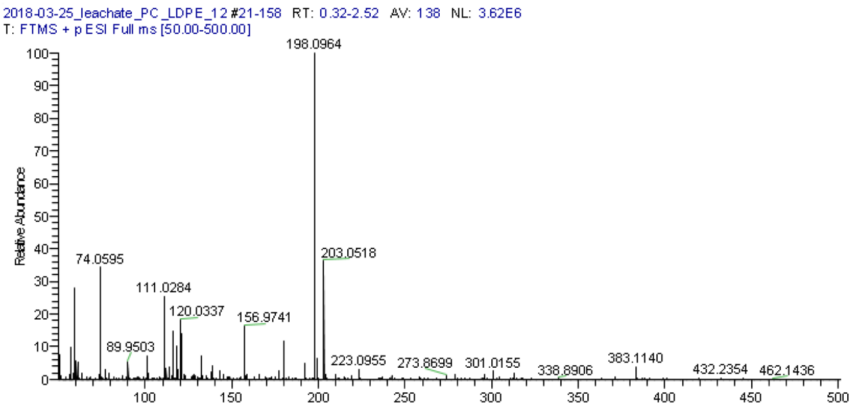

Figure 5. FTMS + pESI profile of Synthetic Leachate and degraded LDPE product after 12 days of exposure to *P. chrysosporium*.

4. Conclusions

This study expands upon the limited body of research amalgamating physicochemical surface treatments and biodegradative approaches for effective mineralization. Surface area imaging revealed bioerosion, qualifying *Phanerochaete chrysosporium* as a suitable candidate for LDPE degradation. Trends in the crystallinity index over 6 days show that thermo-oxidation and chemical etching induced conformational changes to polymer surface morphology, and that crystallinity values decreased as a function of mycoremedial exposure. Thermo-oxidation was associated with a 19% increase in surface area reduction, etching showed a 22% increase, and synthetic leachate media showed a 36% increase. Together, they achieved a 99% increase in surface area reduction. These results were accompanied by a rise in gaseous evolution associated with CO_2 and the respiration of fungal isolates. Therefore, the following molecular mechanism was proposed. Cross-linking and amorphous crystallization increased surface vulnerability to ligninolytic enzymatic activity, and *P. chrysosporium* secondary metabolites were produced in response to appropriate chemical conditions in leachate. FTMS + pESI profiles of leachate after 12 days of exposure to *P. chrysosporium* suggested signs of leachate mycoremediation: reductions in CO_3^{2-} and NH_4^+ levels, improved heavy metal solubility, and the presence trace organics were observed. This approach holds promise for attenuating challenges in LDPE disposal, especially in landfill structures.

Acknowledgements

The author thanks Drs. K. Walker and T. Hughes for technical guidance, along with the Department of Chemistry at Stanford University for ESI instrumentation.

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