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Degradation of polystyrene and selected analogues by biological Fenton chemistry approaches: Opportunities and limitations

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HIGHLIGHTS

- Biologically driven Fenton chemistry has only weak effects on polystyrene (PS).
- Small, bioavailable model compounds help to elucidate causes of PS recalcitrance.
- Bioavailability strongly determines PS resistance to Fenton degradation.
- Basic chemical structures inert to Fenton chemistry also restrict PS degradation.

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ABSTRACT

Conventional synthetic polymers typically are highly resistant to microbial degradation, which is beneficial for their intended purpose but highly detrimental when such polymers get lost into the environment. Polystyrene is one of the most widespread of such polymers, but knowledge about its biological degradability is scarce. In this study, we investigated the ability of the polymer-degrading brown-rot fungus *Gloeophyllum trabeum* to attack polystyrene via Fenton chemistry driven by the redox-cycling of quinones. Indications of superficial oxidation were observed, but the overall effects on the polymer were weak. To assess factors constraining biodegradation of polystyrene, the small water-soluble model compounds ethylbenzene and isopropylbenzene (cumene) were also subjected to biodegradation by *G. trabeum*. Likewise, ethylbenzene sulfonate, cumene sulfonate and the dimer 1,3-diphenylbutane sulfonate were used as model compounds for comparison with polystyrene sulfonate, which *G. trabeum* can substantially depolymerise. All model compounds but cumene were degraded by *G. trabeum* and yielded a large variety of oxidised metabolites, suggesting that both the very poor bioavailability of polystyrene and its inert basic structure play important roles constraining biodegradability via biologically driven Fenton chemistry.

1. Introduction

After more than half a century of widespread use, conventional synthetic plastics are increasingly recognized as severe environmental pollutants. About 300 million metric tons of plastics are produced annually (PlasticsEurope, 2015b), of which a sizeable amount of 5–12 million metric tons has been estimated to end up as debris in marine environments (Jambeck et al., 2015). Most literature reports on plastics biodegradation, especially under environmental conditions, show rather little plastics removal by microorganisms (Krueger et al., 2015a).

Two main factors are responsible for the limited degradability of conventional plastics: First, the most widespread plastics (polyethylene - PE, polypropylene - PP, polyvinyl chloride - PVC, polyethylene terephthalate - PET and polystyrene - PS) have highly inert chemical structures and lack functional chemical groups which

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could facilitate chemical attacks. With the exception of PET, they also have backbones consisting exclusively of unreactive carbon-carbon bonds, therefore excluding hydrolysis, on which degradation of most natural polymers depends. Second, the solid state and the macromolecular nature of plastics lead to extremely poor bioavailability, as most plastic particles are too big to pass cellular membranes, while their low surface-volume ratio and the nanoporous three-dimensional structure restrict the access of microorganisms and enzymes to the bulk of the material (Tokiwa et al., 2009). How these two factors interact and determine the biodegradability of plastics is largely unknown.

The aromatic polymer polystyrene accounts for approximately 18 million metric tons annually or 6% of the global plastics market (PlasticsEurope, 2015a). PS and its extended foams have widespread uses in packaging and construction applications, leading to a large potential for release into the environment. Like the even more widespread polyolefins and polyvinyl chloride, PS cannot be attacked via hydrolysis, but requires powerful oxidants to break it down. Reports of PS biodegradation are scarce and typically indicate only little degradation (Sielicki et al., 1978; Mor and Sivan, 2008), with the only notable exception being bacterial strains isolated from mealworm guts (Yang et al., 2015a, 2015b). Although the aromatic side groups of PS have some similarity to the aromatic moieties of the natural macromolecule lignin, that some fungi readily degrade, such organisms have rarely been found implicated in PS biodegradation (Kaplan et al., 1979). Still, fungi could represent promising candidates for PS biodegradation due to their numerous unspecific and powerful extracellular oxidation mechanisms (Guillen et al., 1997; Kerem et al., 1999; Gomez-Toribio et al., 2009; Giardina et al., 2010; Hofrichter et al., 2010).

We recently reported that the fungus *Gloeophyllum trabeum*, which causes brown-rot decay of wood, is capable of causing substantial biodegradation of the water-soluble PS analogue polystyrene sulfonate (PSS) (Krueger et al., 2015b). Driving an extracellular Fenton mechanism by the redox cycling of hydroquinones, *G. trabeum* caused up to 80% reduction in the number-average molecular mass (M_n) within 20 days. The aims of the current study were to (i) elucidate whether and to what extent its Fenton chemistry-based biodegradation potential enables *G. trabeum* to attack and degrade solid PS, and (ii) identify factors influencing PS degradation in comparison to the previously reported degradation of PSS. For this purpose, we used low molecular weight model compounds, which are readily water-soluble and hence sufficiently bioavailable, to assess compound structure-related mechanistic constraints to biodegradability in more detail. Ethylbenzene and isopropylbenzene (cumene) served as model compounds possessing structural elements of PS, while their sulfonated analogues and sulfonated 1,3-diphenylbutane (DPB) represented PSS structures (Fig. 1). The use of these model compounds allowed us to differentiate between limitations caused by restricted bioavailability, and those related to inert chemical structures.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade and obtained from various commercial sources (Sigma-Aldrich, Munich, Germany; ABCR, Karlsruhe, Germany; Merck, Schwalbach, Germany; Carl Roth, Karlsruhe, Germany; Th. Geyer, Renningen, Germany). Cumene sulfonate was purchased from Chemos (Regenstauf, Germany). PSS (M_w ~ 70,000; Sigma-Aldrich, Munich, Germany) was ultrafiltrated before use as described before (Krueger et al., 2015b). PS films were produced by Versalis (Milan, Italy). DPB and its corresponding disulfonate (DPB sulfonate; both > 90% purity) were customly synthesised by Chempur (Karlsruhe, Germany).

2.2. Cultivations

Gloeophyllum trabeum DSM 1398 was obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and maintained on 1.5% agar plates of Wetzstein mineral medium (Wetzstein et al., 1997) with an addition of 10 g L^{-1} milled wheat straw (6 mm particle size) as the carbon source.

Prior to experiments, *G. trabeum* was grown on 1% malt extract agar plates for one week. Three plugs (1 cm diameter) of mycelium were used to inoculate preculture flasks containing 30 mL of 5 g L^{-1} Oxoid CM57 malt broth (Oxoid, Vienna, Austria). After one week, liquid cultures were filtered over a sterile sieve, washed with 50 mL Wetzstein mineral medium and transfered to bottles containing 25 mL Wetzstein mineral medium devoid of a source of carbon and energy (250 mL total volume). Polystyrene films (ca. 5 cm^2 size, about 80–100 μm thickness) were degreased and sterilised in 70% ethanol prior to use, dried and added to cultures at one film per flask. The respective model compounds were added from sterile stock solutions to a concentration of 400 μM in the liquid phase. For ethylbenzene and cumene, Henry coefficients of $H^{cc} = 3.22$ and $H^{cc} = 2.14$, respectively, were assumed for the distribution between water and air phases inside the bottles (Sander, 2015), as these literature values appeared reasonably precise in preliminary experiments (data not shown).

Afterwards, degradation bottles were sealed gas-tight and left to equilibrate in the dark at room temperature for 60 min before initial sampling of 2.2 mL. Bottles were then incubated at 28 °C without shaking. Further samples were taken at 1, 5, 10, 15 and 20 days. All experiments were conducted in triplicates.

2.3. Abiotic Fenton conversions

Polystyrene films were degreased and sterilised in 70% ethanol, and subjected to pure Fenton's reagent for 48 h at 28 °C in Erlenmeyer flasks filled with 30 mL Wetzstein medium. Varying concentrations of H_2O_2 (0–0.5%) were applied. One film was used per flask.

To investigate the effect of Fenton's reagent on model compounds, reactions were run for 48 h in 10 mL Wetzstein mineral medium in 120 mL closed bottles with varying concentrations of H_2O_2 (0%; 0.01%; 0.1%; 0.5%). Model compounds were used at 400 μM in the liquid phase. Two ml of an H_2O_2 stock solution or distilled water were added with a cannula through the closed cap to start the reactions. For optimal mixing, all samples were shaken at 120 rpm and 28 °C. Harvested samples were directly filled into chromatography vials and analysed. Controls contained all components, but no model compounds. All experiments were conducted in triplicates.

2.4. Sample preparation

For contact angle (CA) and X-ray photoelectron spectroscopy (XPS) analyses, PS films were cleaned following a protocol modified from Gilan et al. (2004). After harvest, films were stored in distilled water at 4 °C over night. Afterwards, they were subjected to three incubations of 2 h in 2% SDS solution at 50 °C with the last 15 min under additional ultrasonication followed by rinsing in distilled water, 5 min rinsing in 70% ethanol, further 15 min incubation in 70% ethanol at 120 rpm shaking and a final washing step with distilled water. The films were left to dry at room temperature for 48 h.

For Ultra Performance Liquid Chromatography (UPLC) and