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Laccase-Catalyzed Degradation of Perfluorooctanoic Acid

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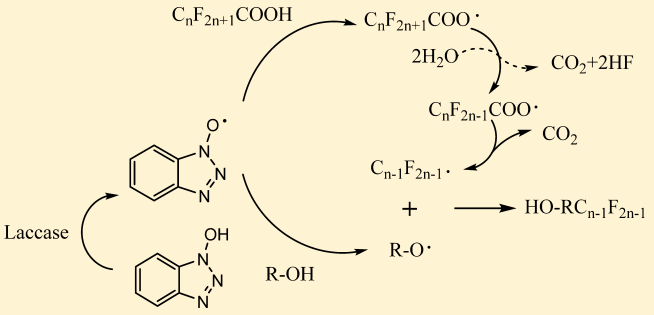
Supporting Information

ABSTRACT: This study examined the decomposition of perfluorooctanoic acid (PFOA) in enzyme-catalyzed oxidative humification reactions (ECOHRs). ECOHRs make up a class of reactions that are ubiquitous in the environment. Approximately 50% of PFOA in a mineral buffer solution decomposed upon addition of laccase and 1-hydroxybenzotriazole after 157 days with a pseudo-first-order rate constant of 0.0044 day^{-1} (r^2 = 0.89). No shorter carbon-chain perfluorocarboxylic acids were detected as degradation products during the experiment. However, partially fluorinated shorter-chain alcohols and aldehydes were identified by high-resolution mass spectrometry. These partially fluorinated compounds were likely products resulting from PFOA degradation via a combination of free radical decarboxylation, rearrangement, and coupling processes. Fluoride was detected in the reaction solution, and the concentration indicated a 28.2% defluorination ratio during the treatment. This finding suggests that PFOA may be transformed during humification, and ECOHRs can potentially be used for the remediation of PFOA.



INTRODUCTION

Perfluorocarboxylic acids (PFCAs) make up a group of compounds that have extreme thermal and chemical stability[1] because of the strong fluorine−carbon bond (~110 kcal/mol).[2,3] Due to their unusual characteristics, PFCAs have been used in nearly every aspect of daily lives.[4,5] Although the excellent chemical inertness is a huge advantage in the application of PFCAs, it also causes considerable environmental concerns because of their ubiquitous presence in the environment and toxicity to animals.[6−8]

High concentrations of PFCAs were frequently detected at sites impacted by aqueous film-forming foam in firefighting practices, with a median concentration of 26 μg/L in groundwater and 21 μg/g in soil samples.[8] The National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control and Prevention (CDC) in 1999 revealed that PFCAs such as perfluorooctanoic acid (PFOA) were present in all the human serum samples in the United States.[9] PFCAs were associated with carcinogenicity, infertility, birth defects, and reduced immune function at parts per million levels.[10−13] PFOA has been included in the Environmental Protection Agency Contaminant Candidate List 3[14] and is a potential candidate of Substance of Very High Concern under the European chemical regulations.[15] Several studies reported using electrochemical, photolytic, or sonochemical oxidation and catalyzed hydrogen peroxide propagation to break down PFCAs.[16−20] However, these approaches require large energy inputs and/or special devices, thus limiting their applications.

For potential remediation applications, it is desirable to identify an approach that can decompose PFCAs under naturally relevant conditions. Enzyme-catalyzed oxidative humification reactions (ECOHRs) could serve such a role but have not been well examined. ECOHRs refer to an important class of reactions that are facilitated by extracellular enzymes such as peroxidases and phenoloxidases to mediate the polymerization of small molecule humic precursors into humic substances in the environment.[21] These enzymes oxidize phenolic or anilinic substrates into radical and quinone intermediates that are further covalently bound with each other via coupling.[22] The active intermediates formed during ECOHRs can also attack other inert compounds such as polychlorinated biphenyls (PCBs)[23] and polycyclic aromatic hydrocarbons (PAHs),[24] thus incorporating them into humification and leading to their decomposition and detoxification.

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In an earlier study, PFOA was effectively transformed in ECOHRs mediated by horseradish peroxidase (HRP).[25] Laccase is another enzyme that mediates ECOHRs,[26] and in comparison to HRP, laccase is more suitable for possible in situ remediation applications because it can maintain its activity over a long period of time[27] and uses oxygen instead of hydrogen peroxide as an electron acceptor.

The objective of this study was to investigate the possibility and mechanisms of PFCA degradation by ECOHR. To this end, we have conducted a series of experiments and characterizations using laccase as a model humification enzyme, 1-hydroxybenzotriazole (HBT) as a model mediator, and PFOA as the target chemical. HBT can be oxidized via laccase catalysis to form radicals[28] and has been used as a laccase mediator to degrade PAHs.[24]

MATERIALS AND METHODS

Standards and Reagents. Laccase from *Pleurotus ostreatus* (EC 420-150-4), 1-hydroxybenzotriazole (HBT), and 2,6-dimethoxyphenol (DMP) were purchased from Sigma-Aldrich (St. Louis, MO). The 5-diisopropoxy-phosphoryl-5-methyl-1-pyrroline-*N*-oxide (DIPPMPO) was purchased from Enzo Life Sciences (Farmingdale, NY). PFCAs with total carbon numbers from C4 to C11 and the surrogate standard perfluoro-*n*-[$^{13}C_8$]octanoic acid (M8PFOA) were obtained from Wellington Laboratories (Guelph, ON). The buffer salts, including cupric, magnesium, and manganese sulfates (see Buffer Solution in the Supporting Information for a full list), were ACS grade and from Fisher Scientific (Pittsburgh, PA). High-performance liquid chromatography (HPLC) grade acetonitrile, methanol, and dichloromethane were also from Fisher Scientific.

Experimental Setup. The reactions were conducted in 100 mL of a mineral buffer (Materials and Methods of the Supporting Information) containing 1.0 μM PFOA (414 μg/L). The initial enzyme activity was 1 unit/mL, and the initial HBT concentration was 0, 2, or 20 μM for the different treatments. The laccase solution was freshly prepared and assayed. One unit of laccase activity is defined as the amount of enzyme that causes one unit change in absorbance at 468 nm per minute of a DMP solution at pH 3.8 in a 1 cm light path cuvette[31] (see Materials and Methods and Figure S2 of the Supporting Information). It is noted that the enzyme activity level used in this study (1 unit/mL) fell in a common range (e.g., 0.265−15.9 units/mL) employed in previous laccase bioremediation studies[24,29,30] (see Laccase Activity Assay in the Supporting Information for details).

All reactors were incubated in a shaker at 22 °C, and laccase and HBT were supplemented every 6 days (Experimental Setup in the Supporting Information). Three 0.5 mL aliquots of solution were taken from each reactor for quantification of C4−C11 PFCAs and HBT simultaneously at preselected time intervals. Correction of concentrations was performed to compensate for the variation of solution volume caused by evaporation and supplementation. For product identification purposes, a positive control that contained only PFOA without enzyme or HBT and a negative control that did not contain PFOA but with repeated enzyme and HBT additions were also incubated and processed along with the treatment reactors (Experimental Setup in the Supporting Information). An additional experiment was conducted to compare PFOA degradation in ECOHR in the absence and presence of DIPPMPO, a spin trap that can effectively scavenge HBT free radicals, and details are also provided in the Supporting Information (Experimental Setup).

Chemical Analysis. The samples were spiked with M8PFOA as a surrogate standard before being subjected to solid phase extraction with a HLB cartridge following a procedure reported previously[32,33] with minor modifications. PFCAs and HBT (Table S1 of the Supporting Information) were quantified by a Waters 2690 HPLC system coupled with a Micromass Quattra tandem mass spectrometer (HPLC−MS/MS) (Waters, Milford, MA). Details can be found in the Supporting Information (Materials and Methods).

Identification of Reaction Products. Samples (5 mL) were taken after 157 days from selective treatments, and negative and positive controls. Each sample was extracted with 1 mL of dichloromethane while being vigorously shaken for 15 min. The extractants were reconstituted in methanol and analyzed with an Orbitrap Elite high-resolution mass spectrometer (Thermo Scientific, San Jose, CA). The identification of the reaction products was based on element compositions and product ion spectra (MS/MS) (Materials and Methods in the Supporting Information). A Dionex ICS-1000 ion chromatograph was used to quantify fluoride (Materials and Methods in the Supporting Information).

RESULTS AND DISCUSSION

Removal of PFOA in Aqueous Solution. As shown in Figure 1A, no appreciable degradation of PFOA was found in the

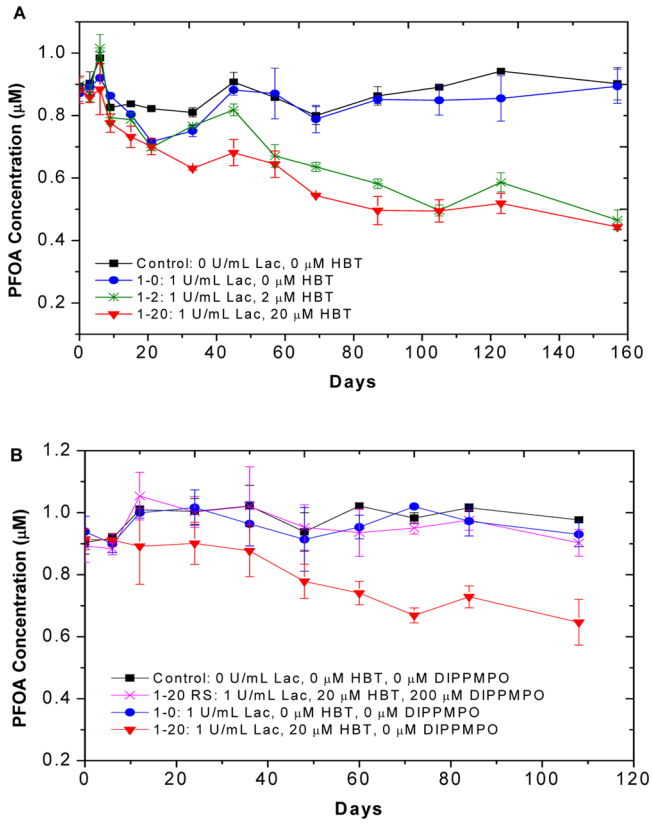

Figure 1. (A) Change in PFOA concentration in ECOHR over time: control, positive control sample to which no laccase or HBT was added; 1-0, 1 unit/mL laccase added every 6 days but no HBT; 1-2, 1 unit/mL laccase and 2 μM HBT added every 6 days; 1-20, 1 unit/mL laccase and 20 μM HBT added every 6 days. (B) Change in PFOA concentration in ECOHR with or without the addition of DIPPMPO as a HBT radical scavenger: 1-20 RS, 1 unit/mL laccase, 20 μM HBT, and 200 μM DIPPMPO added every 6 days.

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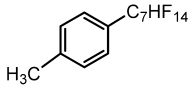
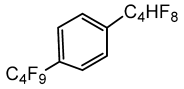
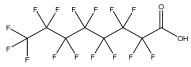
Table 1. Molecular Formulas, Theoretical and Measured Deprotonated Molecular Weights ($[M-H]^-$), Mass Accuracies, Possible Structures, and HRMS Responses of PFOA Degradation Products from ECOHRs

No	Formula	[M-H]$^-$		Mass accuracy (ppm)	Possible structure	Relative intensity[1]
		Theoretical	Experimental			
1	$C_4H_4F_3ON$	138.0167	138.0164	2.0	$CF_3CH{=}CHNHCHO$	0.029%
2	$C_4H_4F_4O$	143.0120	143.0128	0.0	CF_3-CH=CF-CH_2OH	15.4%
3	$C_4H_5F_5O$	163.0182	163.0186	-2.3	$CF_3CF_2CH_2CH_2OH$	212%
4	$C_6H_4F_4O_2$	183.0069	183.0062	3.9	$HCCF{=}CF{-}CF{=}CFCH_2OH$ (O above)	277%
5	$C_8H_{14}F_4O_2$	217.0852	217.0856	-2.0	$CF_3CF(CH_2)_5CH_2OH$ (OH above)	38.8%
6	$C_8H_{11}F_7O_2$	271.0569	271.0558	4.1	$CF_3(CF_2)_2CH(CH_2)_3CH_2OH$ (OH above)	87.5%
7	$C_7H_7F_9O$	277.0275	277.0270	1.8	$C_4F_9C_3H_6OH$	14.0%
8	$C_7HF_{13}O$	346.9742	346.9742	-0.1	$CF_3(CF_2)_5CHO$	1.99%
9	$C_{14}H_8F_{14}$	441.0324	441.0326	-0.4		14.3%
10	$C_{14}H_5F_{17}$	495.0041	495.0040	0.3		14.0%
PFOA	$C_8F_{15}O_2H$	412.9659	412.9648	-2.6		100%

[1]The relative intensity equals the absolute intensity of the product divided by the absolute intensity of PFOA measured by HRMS.

solution with laccase only. However, significant reduction of the PFOA concentration was achieved with periodical addition of HBT (see Figure S1A of the Supporting Information for the results of statistical analysis), indicating the key role of HBT in this process. It is known that laccase converts HBT to its free radicals that can either be quenched via self-coupling or oxidation to benzotriazole[34] or attack other nonsubstrate chemicals,[35,36] such as PFOA in this case. Continuous consumption of HBT was observed in the treatment systems (Figure S3 of the Supporting Information). With periodic additions of 2 and 20 μM HBT (named as 1-2 and 1-20 treatments in Figure 1, respectively), the total quantities of PFOA removed from these two systems were not significantly different (0.0495 and 0.0505 μmol, respectively), even though the total HBT consumption was significantly different between the systems (2.28 and 37.0 μmol, respectively). This is not surprising because it is known that the increase in mediator concentration in an ECOHR system can lead to an increased level of radical quenching that outcompetes the degradation of the target inert chemical.[23] Thus, increasing the HBT concentration does not necessarily enhance PFOA degradation proportionally. The ratios between the removed PFOA quantity and the total HBT consumption were 2.17 and 0.14% in the 1-2 and 1-20 treatments, respectively.

The PFOA degradation time course data in Figure 1A were fitted to the pseudo-first-order rate model (Kinetic Analysis in the Supporting Information), and the results are displayed in Figure S3 of the Supporting Information. The pseudo-first-order rate constant (k) was 0.0042 day^{-1} (r^2 = 0.84) and 0.0044 day^{-1} (r^2 = 0.89) for treatments 1-2 and 1-20, respectively, corresponding to the half-lives of 165 and 157 days, respectively.

Reactions in the Presence of DIPPMPO. An additional experiment was conducted to compare PFOA degradation in ECOHR in the absence and presence of DIPPMPO, a spin trap that can effectively scavenge HBT free radicals[37] (Experimental Setup in the Supporting Information), and the results are presented in Figure 1B. In this additional experimental run, treatments 1-0 and 1-20 without addition of DIPPMPO essentially repeated the trends obtained in the earlier experiment shown in

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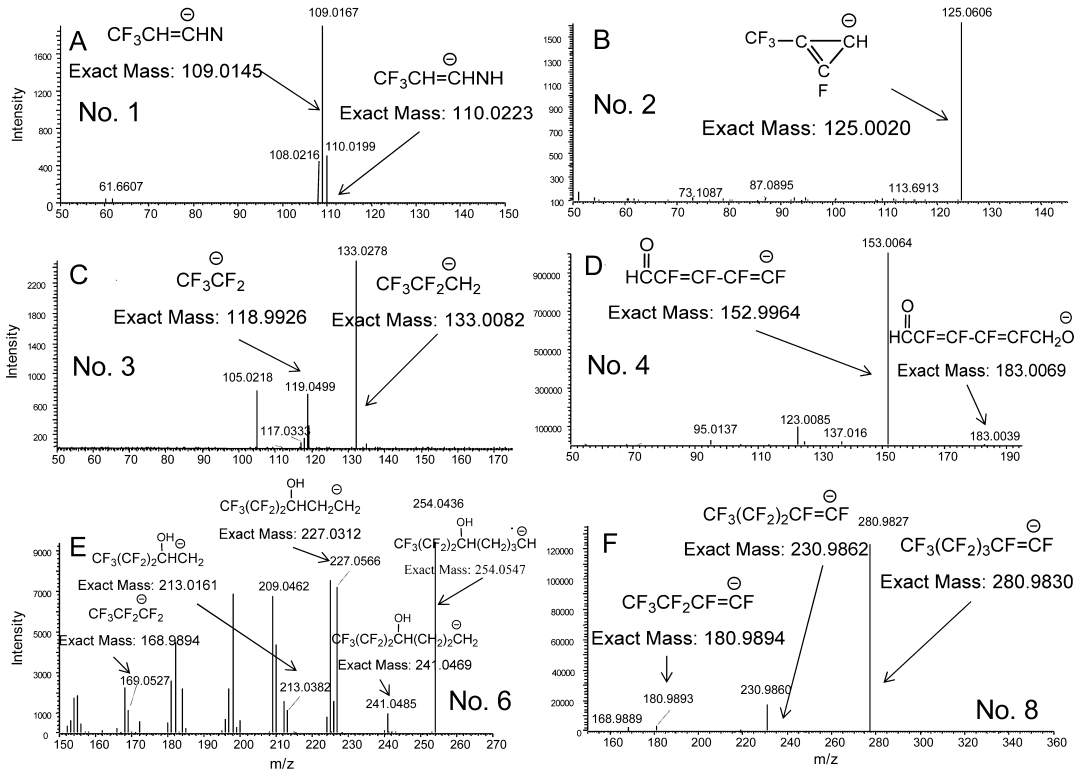

Figure 2. MS/MS spectra of PFOA degradation products given by high-resolution mass spectrometry and their possible transition ions. (A) Product 1: $C_4H_4F_3ON$, measured m/z 138.0164. (B) Product 2: $C_4H_4F_4O$, measured m/z 143.0128. (C) Product 3: $C_4H_5F_5O$, measured m/z 163.0186. (D) Product 4: $C_6H_4F_4O_2$, measured m/z 183.0062. (E) Product 6: $C_8H_{14}F_4O_2$, measured m/z 271.0558. (F) Product 8: $C_7HF_{13}O$, measured m/z 346.9742.

Figure 1A. The remaining PFOA levels were 93 ± 4.5 and 66 ± 7.6% for treatments 1-0 and 1-20, respectively, after 108 days (Figure 1B), while those were 95 ± 4.6 and 56 ± 4.0%, respectively, in the earlier run at 105 days (Figure 1A). PFOA degradation was, however, significantly suppressed in the 1-20 treatment with addition of DIPPMPO (see Figure S1B of the Supporting Information for the results of statistical analysis). This is strong evidence that laccase-produced HBT radicals played a key role in degrading PFOA.

Identification of Degradation Products. The degradation products of PFOA in ECOHR systems were analyzed via high-resolution mass spectrometry (HRMS) (mass accuracy of <5 ppm), which allowed accurate determination of element compositions. Possible PFOA degradation products (Table 1) were first identified by comparing the mass spectrum of the 1-20 treatment sample after incubation for 157 days with those of corresponding positive (PFOA only) and negative (no PFOA but with laccase and HBT) controls. No products were confirmed with authentic standards because no authentic standards were available. The products identified in this study are the ones having relatively high MS responses (Table 1). Products 3 (212%) and 4 (277%) have very high relative intensities compared to that of PFOA (100%), followed by product 6 (87.5%). It should be noted, however, that the peak intensities of different chemicals are strongly dependent on their molecular structures and do not quantitatively reflect the concentrations of the chemicals in the absence of standards. Structures of selected products were further deduced according to their product ion spectra without standards (MS/MS) (Figure 2). For those compounds (products 5, 7, 9, and 10) not showing distinct product ions, speculated structures were given on the basis of possible reaction pathways and element compositions. As shown in Table 1, most of the identified products were partially fluorinated alcohols and/or aldehydes and fluoroalkyl-substituted aromatic compounds. The aromatic ring in products 9 and 10 was attributed to the reactions with an amino acid that entered the reaction system as an impurity in the laccase solution. This amino acid was determined to be phenylalanine (Figure S5 of the Supporting Information). Similarly, the amine in product 1 was probably from the amino acid residues in the enzyme solution. The double bond in products 2 and 4 may be formed from free radical rearrangement as reported previously.[25] Release of fluoride was considered to be an important indicator of PFOA degradation. The fluoride concentration in the sample after incubation for 157 days was 35.5 ± 2.18 μg/L, while its levels were below the detection limit (1.5 μg/L) in both controls. The fluoride concentration would be 125.7 μg/L if complete mineralization of PFOA occurred. Therefore, 28.2% of PFOA defluorination was achieved in this study by a calculation method reported previously.[16]

Mechanisms of PFOA Degradation. On the basis of the results discussed above, the mechanism of PFOA degradation in ECOHR can be proposed (Figure 3). The reaction was initiated by transferring an electron from the carboxyl headgroup of PFOA to an HBT radical followed by Kolbe decarboxylation to form a perfluoroheptyl (C_7F_{15}) radical. The C_7F_{15} radical can go through hydrolysis with concurrent elimination of an HF molecule and a fluoride to form $C_6F_{13}COOH$. This Kolbe decarboxylation cycle was well recognized as the mechanism of eliminating the CF_2 unit from PFCAs in electrochemical and persulfate oxidation processes,[16,38] leading to the formation of a perfluoroalkyl acid with one fewer CF_2 unit. Each process involves the generation of a corresponding perfluoroalkyl radical. Alternatively, shorter-chain perfluoroalkyl radical can also be

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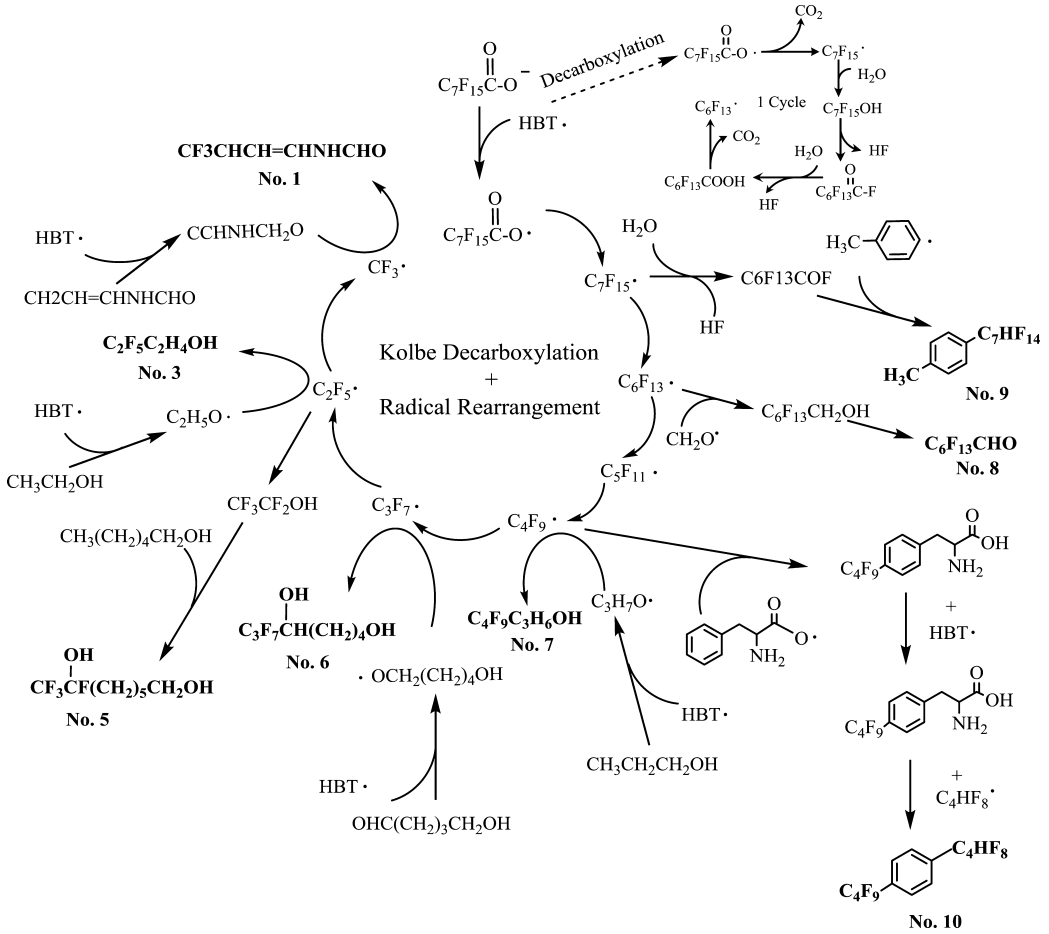

Figure 3. Proposed PFOA degradation pathways during ECOHR treatment with laccase and 1-hydroxybenzotriazole.

formed directly from longer-chain radical via free radical rearrangement.[25] The HBT radicals not only initiated PFOA decarboxylation but also converted other nonfluorinated organic chemicals in the solution to free radicals that cross-coupled with the perfluoroalkyl radicals to form the products (Table 1) that are either partially fluorinated or contain a perfluoroalkyl moiety. Such free radical coupling is common to laccase-mediated reactions.[39,40] These nonfluorinated chemicals were most likely the impurities such as amino acids or fermentation byproduct alcohols (e.g., 1-propanol, 1-hexanol, and 1,5-pentanediol) in the laccase solution that was confirmed by HRMS analysis (Figures S5 and S6 of the Supporting Information).

In summary, this study indicates that ECOHR can effectively transform PFOA to shorter-chain, partially fluorinated products in the presence of HBT. Because these products are seemingly analogues of PFCA precursors, it is possible that some of them may be further transformed to their corresponding shorter-chain PFCAs under extreme oxidative conditions, but these partially fluorinated products, as well as shorter-chain PFCAs, are believed to be more environmentally benign.[41] It should be noted that laccase substrates with phenolic functional groups are abundant in natural organic matter, which may serve as mediators for ECOHRs.[42] Such natural processes may effect only very slow PFOA degradation given the low PFOA concentrations and variable humification enzyme activities in the natural environment, but it is possible to enhance the humification reactions through an engineering approach for remediation purposes.

■ ASSOCIATED CONTENT

Supporting Information

Additional experimental details, figures, and a table. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.5b00119.

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Notes

The authors declare no competing financial interest.

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