# FLUORINATED WASTE AND FIREFIGHTING ACTIVITIES: BIODEGRADATION OF HALOGENATED FOAMS FROM PETROCHEMICAL REFINERY SOIL

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**Abstract**

Purpose: Perfluorinated compounds (PFCs) are important constituents in aqueous film forming foams (AFFFs). AFFFs have been used to extinguish fuel fires in the petrochemical industry, in emergency situations and at fire-training sites. Moreover, AFFF contains diethylene glycol butyl ether, also known as DBGE. The interference of DGBE during the biodegradation of aromatic hydrocarbons have not yet been studied and its impact on microcosms must be considered. The objective of this research was to detect the effects of the individual components of full AFFF formulations and characterize shifts in aromatics degradation while measuring byproducts. Methods: The assays were assembled and conducted in 160 ml glass bottles. The biodegradation process in each test containing benzene, toluene and AFFF was monitored by GC-FID. for 20 days. The concentration of aromatics and their biodegradation intermediates were regularly monitored though 100 μL sample injections in a chromatographic column taken from the headspace within the 160 ml bottles. Results: When no DGBE was present, we observed that the biodegradation of each of hydrocarbon components of the mixture occurred much faster in comparison to other assays. A preferential consumption of DGBE over aromatics by the microbial community occurred, whose metabolic pathways prioritize DGBE as substrate, thereby decreasing the degradation of benzene and toluene components. Thus, when there is no DGBE as an agent of competitive inhibition, benzene and toluene consumption is much higher. Conclusions: Our research suggests the discovery of an altered metabolic pathway in aromatic hydrocarbons biodegradation that is directly affected by fluorinated substances. The fluorinated compounds affected the aromatics biodegradation kinetics, as PFCs may contribute to a shift in styrene and catechol concentrations in co-contamination scenarios.

**Keywords**: aqueous film forming foam, bioremediation, chromatography, halogenated waste.

## 1. Introduction

Fires in the petrochemical industry are extinguished using aqueous film forming foams (AFFFs). Perfluorinated compounds (PFCs) are the main constituents of AFFFs, since they are crucial to surface tension reduction. Such property promotes spreading and improves fire suppression. The repeated nature of fire-fighting activities in many places, which may also include PFCs and other AFFF components along with flammable fuels and solvents (e.g. gasoline, diesel and jet fuel) led to continuous release into the water table, causing a complex contamination in both soil and groundwater [1]. In fact, a few Perfluorinated alkylated substances (PFASs) were detected combined with other perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA), as reported by Vecitis et al. [2]. Perfluorinated substances have been detected at many places that are susceptible to the contamination by significant quantities of petroleum hydrocarbons. The usual co-contamination scenarios pose a major challenge for the remediation of contaminated groundwater often accompanied by petroleum industry activity. Therefore, such strategies should be considered within the hydrocarbon co-remediation at PFC-impacted sites.

Among hydrocarbon found in contaminated sites, mixtures of benzene, toluene, ethylbenzene and xylene (BTEX) are very likely to be be found as co-contaminant in petrochemical refinery and storage areas. The compounds are also known for their potential biodegradability

using aerobic microbial communities [3]. Aromatic hydrocarbons compose almost 30% of gasoline, which results in a major exposition to humans in given environments, whether in chronic or accidental releases [4]. By completely understanding the metabolic pathways of hydrocarbon-degrading and PFC-transforming microorganisms it is possible to design effective remediation systems that target both defluorination and hydrocarbon mineralization at AFFF-impacted sites. Fortunately, the wide spectrum of microorganisms capable to aerobically degrade BTEX and other aromatic hydrocarbons has been well documented [5]. Under the appropriate environmental conditions, such compounds can be co-remediated, thus outputting maximum defluorination and hydrocarbon mineralization. Even though it is not yet known if active PFC-adapted microbial populations can affect the biotransformation of aromatic hydrocarbons.

The concentration of PFC substrates may induce different types of enzymes. Different classes of PFC are expected to promote alternate patterns of aromatics biotransformation, since any substrates can induce specific enzyme systems, hence yielding drastic changes in enzyme expression. These alterations correlate to substantial variation in the amounts of transformation products [6]. We hypothesize that the BTEX related oxygenases, which are quite a reliable enzymatic complex, may be affected by co-metabolic PFC substrates. Strictly speaking, BTEX transformation pathways could be driven to alternate pathways by such compounds. The knowledge on AFFF consequences in bioremediation processes, including their environmental fate, would allow better methods to reduce time and resources spent after hydrocarbon fires and chronic scenarios.

Many researchers highlight their findings on the biotransformation of perfluorinated compounds, focusing their chemical structure. Recent experimental setups were designed to demonstrate and increase the transformation of the PFC persistent substances [7-9]. Still, fire-fighting foams contain many other substances that help achieving foaming capabilities that allow them to work properly. The complexity of AFFF mixtures is very high and variable according to manufacturer. In retail formulations, the typical components are a solvent (typically a glycol ether), fluorinated surfactants (usually perfluorinated compounds), and hydrocarbon-based surfactants [10, 11]. All components are engineered to provide maximum applicability of foams. Surfactants in fluorine-containing mixtures, for instance, contribute to the AFFF performance as sealants to avoid re-ignition in fuels. Furthermore, diethylene glycol butyl ether, also known as DBGE, is present in retail AFFF. The DGBE compound acts as co-solvent and anti-freezing of AFFFs, and is responsible for 13% to 16% of the total formulation of AFFF. Due to the composition of DBGE, it may also serve as a substrate and carbon source for microbial communities. Therefore, it is expected that DGBE is a competing substrate in co-contaminations where the biodegradation of aromatics takes place. The complex metabolic roadmap involved in AFFF has not yet been fully understood, so the impacts of all components on microcosms must be investigated. Different from PFCs, the DGBE has no fluoride in its structure and it is easily consumed by the microbiota. It is safe to assume that DGBE presence may promote major changes in other substrate uptake ratios that lead to divergences in individual biotransformation steps.

Biodegradation processes are dependent upon many environmental factors, including nutrient availability and substrate interactions. Aromatics and other petroleum hydrocarbons are no exception to this [12]. A confluence of factors may alter conditions to facilitate optimal BTEX biodegradation pathways. Our research approached the AFFF+BTEX co-

contamination to quantify the microbial metabolism of aromatic hydrocarbons via byproducts analysis, which was conducted using gas chromatography. We focused at both DGBE influence and PFCs role in optimizing aromatics mineralization. Our research aimed to put together small pieces to provide insight into a future complete metabolic map of aromatics co-metabolism. Selected individual components of BTEX metabolism were monitored during full AFFF formulations biodegradation at a simulated firefighting soil scenario.

## 2. Methods

The analytical methods applied to AFFF and BTEX compounds in our simulated environmental samples monitored individual metabolites and large scale $CO_2$ production. Both methodologies complimentarily provided new insights towards the behavior of fire-fighting foams when released in a soil matrix. The biodegradation was monitored using simple *headspace* sampling and gas chromatography (GC) with a flame ionization detector (FID). Headspace samples were periodically withdrawn for BTEX biodegradation and $CO_2$ production analysis. We managed to determine and infer how BTEX and DGBE biodegradation presented any relationship with the AFFF co-contamination.

### 2.1. Sample collection

A major release of AFFF occurred at the Replan Petrobras oil refinery in Paulinia, Brazil (22°43'24.2 "S 47°08'00.3" W) on January 8, 1993. It was one of the largest fires to ever strike Brazilian oil refineries, with quantities close to Santos Port fire in 2016. Almost 37,000 L of AFFF was released into the nearby soil when firefighters struggled to extinguish more than 5,000,000 gallons of burning diesel and gasoline [13]. Soil samples were collected from the Replan Petrobras oil refinery in Paulinia where thae fires happened, more than 20 years ago. Soil samples were collected in July 2016 in an area close to the stock tanks refinery fuel. The soil was collected using PFC-free instruments at a 0.8 m depth. All samples were stored in fluoride-free containers at -80°C.

Unfortunately, no records are available on the AFFF brands used at that time. It was most likely Ansul or 3M branded, since the majority of AFFF bought by Brazilian companies were from those brands. We used the Sintex AFFF produced in Brazil in our experiment. The Sintex based in fluortelomer thioamide sulfonated compounds (FtTAoS) formulation replicate the most common AFFF brands.

### 2.2. BTEX biodegradation

#### 2.2.1. Serum bottle assays

Serum bottles were chosen as the recipient of our experiments. The glass bottles had a 160 ml volume. The assays were assembled in sterile environment using the quantities specified in Table 1. The biodegradation of benzene and toluene were evaluated. Ethyl-benzene and xylene, the other components of BTEX mixtures, were not used in our experimental setup. This deliberate experimental design option is based on pilot data containing all BTEX components, where the consumption rates of ethylbenzene and xylene were all proportional.

Therefore, benzene and toluene were chosen as good indicators towards the whole mixture biodegradability. Due to this, we will refer to the benzene and toluene mixture as BT further in this paper. The experiments also contained AFFF, which were observed for 50 days, with the following components:

- 50 ml of minimum saline media, composed of 80.0 $mg.L^{-1}$ $KH_2PO_4$, 200.0 $mg.L^{-1}$ $K_2HPO_4$, 330.0 $mg.L^{-1}$ $Na_2HPO_4.2H_2O$, 4.8 $mg.L^{-1}$ $NH_4Cl$, 33.4 $mg.L^{-1}$ $CaCl_2.2H_2O$, 22.5 $mg.L^{-1}$ $MgSO_4.7H_2O$ and 0.25 $mg.L^{-1}$ $FeCl_3.6H_2O$. Trace elements were also added to the media as 10.0 $mg.L^{-1}$ $NiSO_4.6H_2O$, 0.75 $mg.L^{-1}$ $(NH_4)_6Mo7O_2.4H_2O$, 100.0 $mg.L^{-1}$ $Na_2SiO_3.5H_2O$, 0.20 $mg.L^{-1}$ $MnSO_4$, 0.15 $mg.L^{-1}$ $SnCl_2.2H_2O$ and 18.1 $mg.L^{-1}$ $Na_2SeO_3$. The buffer $NaHCO_3$ was used at the approximate concentration of 2.7±0.5 $g.L^{-1}$ to achieve pH 7.2.

- 50.0 µL of AFFF. The portion containing perfluorinated substances is known by the brand name Lodyne (composed of a FtTAoS homologues mixture). Along with DGBE, these substances make up most of the Sintex AFFF 6% formulation. It is important to notice that DGBE composes over 80% of the total organic carbon of the mixture. DGBE and Lodyne were each inserted individually to some assays, instead of the full AFFF formulation.

- 9.0 µL BT mixture, with 25.0 $mg.L^{-1}$ of each compound. The final concentration took into account Henry's Law calculations, considering the volatility of the BT compounds. The mixture of BT was stabilized for 24 hours before injection into each vial;

- 5.0 g of soil sample from Petrobras / Replan oil refinery in Paulinia, Brazil.

**Table 1** BT biodegradation assay contents

| Assay ID / Content | $C_M$ (Media control) | $C_N$ (Sterile control) | $C_S$ (Soil control) | A (with AFFF) | B (DGBE only) | C (Lodyne only) |
|---|---|---|---|---|---|---|
| **Media (50 mL)** | X | X | X | X | X | X |
| **Soil (5 g)** | | Sterile | X | X | X | X |
| **DGBE (700 µL)** | | | | | X | |
| **Lodyne (50 µL)** | | | | | | X |
| **AFFF (110 µL)** | | X | | X | | |
| **BT (10 µL)** | X | X | X | X | X | X |

*Autoclaved soil

All vials were sealed using fluoride-free rubber caps to avoid any other halogenated source during manipulation. The caps were checked for their capacity of BTEX volatilization prevention. The materials were verified by pilot assays and controls, showing no adsorption of volatile compounds over time. Moreover, soil-free medium controls ($C_M$) assessed the potential impacts of media components onto fluorinated surfactants. Another set of sterile controls ($C_N$) were prepared by three consecutive soil autoclaving procedures followed by overnight freezing at −20 °C. $C_N$ and full assays (A, B and C) were amended with BT and another source of AFFF related carbon source. The autoclaved control ($C_N$) was supplemented with antibiotic solution to ensure the elimination of microbial activity. These controls were also established to verify any losses of the compounds due to sampling procedures or inadequate sealing. All experiments (Table 1) were run in triplicates in separate microcosms and shaken at 120 rpm in a 28±0.1°C incubator for 51 days. The headspace was

sampled every 6 days with sterile syringes and immediately analyzed. No other co-factors were added to the culture media.

### 2.2.2. Aromatic hydrocarbon analysis

We monitored the biodegradation of BT was monitored by GC-FID. The concentration of BT and its biodegradation intermediates was regularly monitored though 100 µL sample injections in a GS-GasPro chromatographic column (30 m × 0:32 mm; Agilent Technologies, Inc., Santa Clara, USA) taken from the headspace within the 160 ml bottles. Chromatography was performed on Agilent 7890A GC-FID equipment.

The parameters used were modified from the method 113-4332 Agilent Technologies to decrease the running time from 20 min to approximately 7 minutes per sample. The headspace concentrations allowed an improved signal response. The initial temperature was 110°C for 50 seconds, followed by a gradual increase of 110°C to 205°C at 20°C.minute$^{-1}$ and then 205°C to 260°C at 50°C per minute. Finally, temperature was held at 260°C for 2 minutes. The carrier gas helium was set at the flow rate of 41.2 cm.second $^{-1}$ and heated to 115°C at the injection chamber. The injector temperature was set to *split* mode. FID detector temperature was set to 250°C. The BT chromatogram (Figure 1) showed sufficient resolution to quantify the consumption of BT with initial signal intensity greater than 2500 pA.

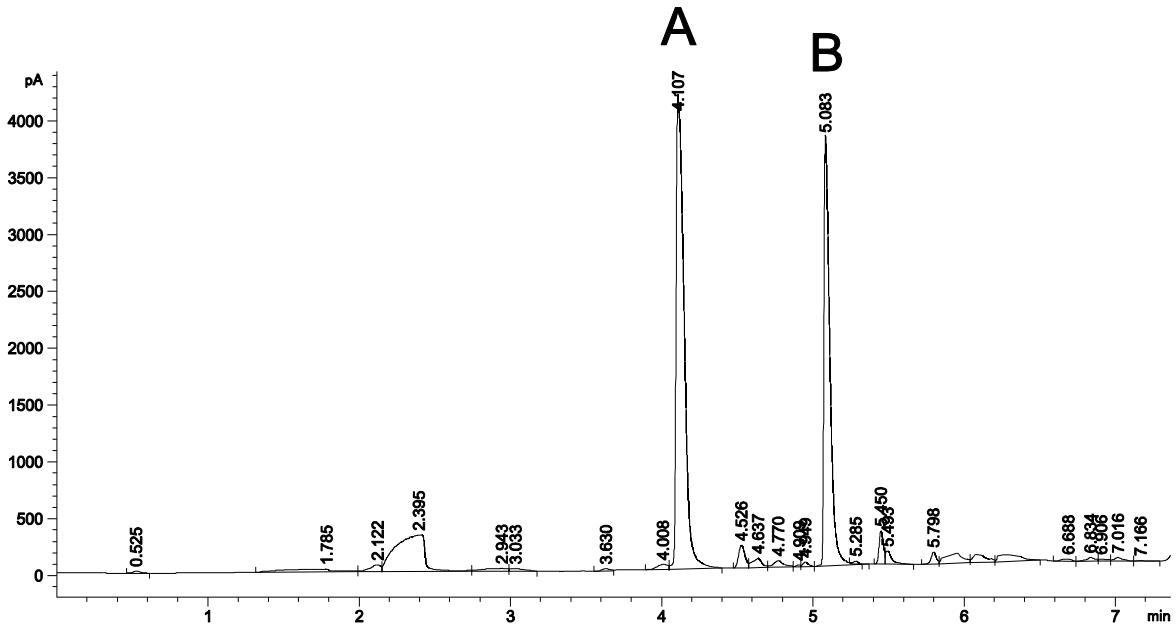


**Fig. 1** Chromatogram from a benzene (peak A) and toluene (peak B) mixture

The gas samples did not undergo any type of pre-preparation. Headspace was injected as is right after removal from serum bottles. The gas withdrawal was performed with a glass gas-tight syringe that perforated directly through the rubber stoppers. Serum bottles were not opened throughout the experiment, to avoid risking any interfere with the biodegradation process. Pressure release was performed every 10 days to re-equilibrate $O_2$ concentration into favorable aerobic conditions. Gas concentration at the micro-atmosphere within bottles was monitored with standard and well established GC-TCD methods for atmospheric gases.

## 3. Results and Discussion

The difference between datasets from A, B and C assays were considered statistically significant compared to controls ($C_M$, $C_N$ and $C_S$) and in relation to each other. Certainly, control assays did not statistically differ when compared to each other, with threshold variations in soil controls ($C_S$). The biodegradation of the individual BT components throughout 51 days is shown in Figure 2.

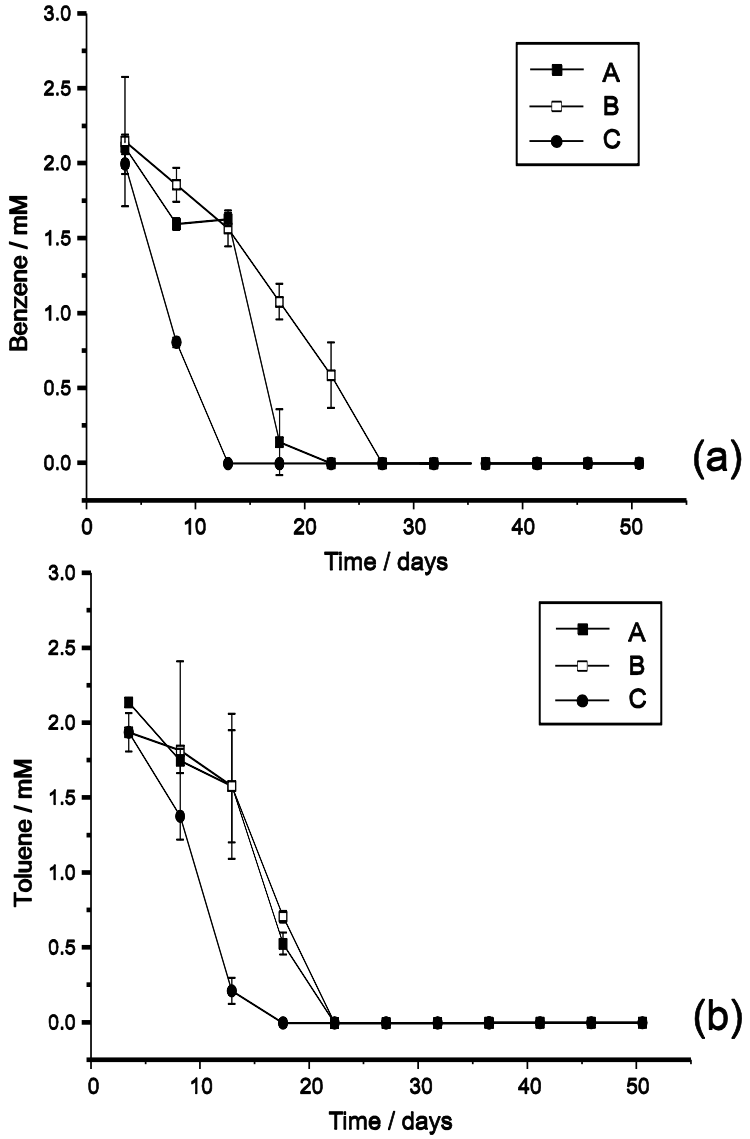


**Fig. 2** Benzene (a) and toluene (b) concentration in the biodegradation assays A (AFFF), B (DGBE only) and C (Lodyne only)

Without DGBE, either pure or contained in the full AFFF mixture, the biodegradation of each BT components was seen to occur in a much higher rate than other assays. This is highly noticeable in Figure 2a. In average, A and C consumption ceased 9.0 days earlier than the B assays. We believe that this is due to a preferential consumption of DGBE over BT by the indigenous microbes, whose metabolic pathways will always prioritize DGBE as substrate, thereby decreasing the degradation of BT components. Consequently, when BT is available

as the sole carbon source, specific metabolic pathways are activated. On the other hand, no DGBE also means no competitive inhibition, as BT consumption is then much higher.

As expected, the soilless assays ($C_M$) had minimal to null microbial activity that yielded detectable aromatics biodegradation. Thus, the output of these controls showed that no major leaks or adsorption of volatiles occurred throughout the experiment.

Benzene benefited the most by the addition of perfluorinated substances in its culture medium, especially in C assays (Figure 2a). There was a significant ($p > 0.05$) increase in benzene and toluene biodegradation whenever fluorinated compounds were added (A and C assays). Such differential biodegradation profile between assays prompted us to perform a detailed analysis of the chromatogram to search for intermediates of aromatic hydrocarbon biodegradation (Figure 3), cross referencing peaks with 20 other common known BT degradation intermediates.

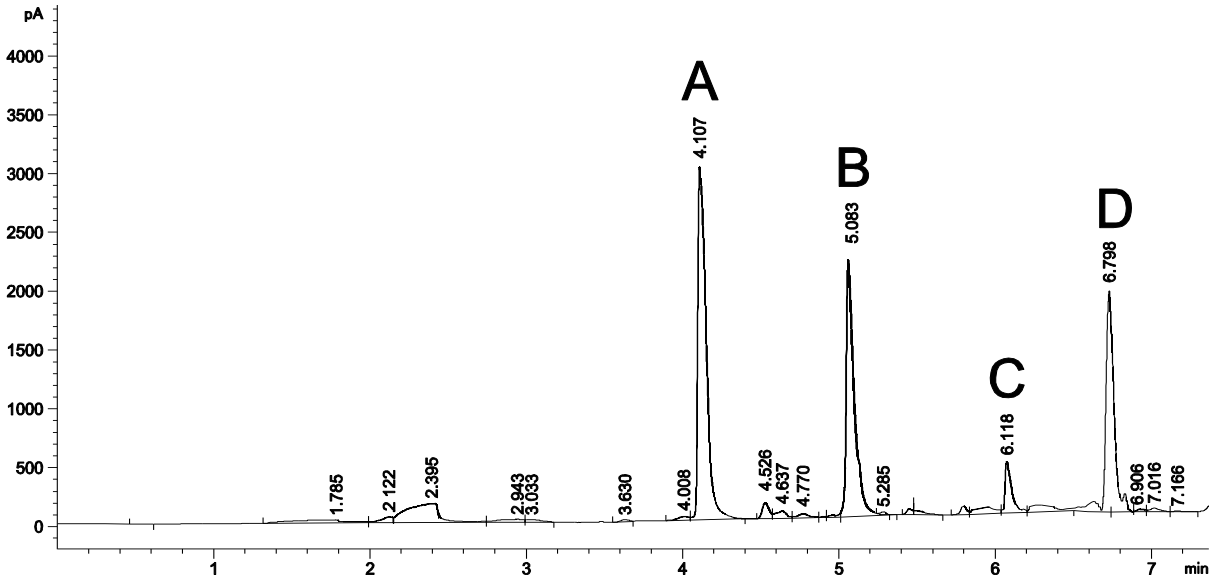


**Fig. 3** Chromatogram after biodegradation in a benzene (peak A) and toluene (peak B) mixture, showing possible cathecol (peak C) and styrene (peak D) byproducts formation

The determination of enzymatic mechanisms is beyond the scope of this research; however, our results suggest the formation of styrene and cathecol. The retention times for peaks C and D (Figure 3) were the same as peaks measured from pure standard solutions of each compound. This confirms previous reports on aromatics bioprocesses that changes, even though slightly, their biodegradation patterns when exposed to PFCs. The microbial adaptation to aromatics (especially benzene and toluene) was broadly studied, as perspicaciously complied by El-Naas et al. [14] in their review. Recent studies on aromatics suggests that the adaptation of microbial communities affects the usually reported biodegradation pathways, since co-metabolites may result in higher efficiency of aromatics removal from a contaminated site. It has been long demonstrated (2 decades ago) by Yeom et al. (1997) that even microbes adapted to only one of aromatic compounds such as *Alcaligenes xylosoxidans* may switch their enzymatic mechanisms towards other compounds, acquiring the ability to degrade toluene, m-xylene and ethyl benzene faster than control assays though unknown pathways. This study is part of a collective effort to bring up the pieces to the whole

metabolical map of aromatics in bioremediation context. In summary, we found that PFCs can trigger a new metabolic pathway that causes a previously unreported biodegradation profile in aromatics co-contamination scenarios with organo-halogenated compounds.

## 4. Conclusions

The biodegradation process varied significantly in C assays for aromatic hydrocarbons, in which an alternative production rate of other byproducts could be detected. Thus, we propose that transient components of the benzene and toluene degradation may be differentially formed, causing the BT+AFFF scenario to go through earlier metabolic stages under the presence of PFCs in a contamination scenario. The AFFF and DGBE contribute to the development of different concentrations of byproducts, although no production/consumption spike occurred in A and B assays. While the experimental approach cannot confirm a novel metabolic pathway during aromatic hydrocarbons biodegradation, there is a divergent result among assays triggered by fluorinated substances. Our data points toward to a high sensitivity to PFCs in the AFFF formulation from soil microbiota, that responded with a shift in their benzene and toluene degradation routes in terms of intermediate products concentration. Still, we encourage other research groups to evaluate the differential production of other possible BTEX intermediates and accurately report the effects of selected PFCs during the biodegradation aromatic hydrocarbons.

## 5. References


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