

BIODEGRADATION OF FIREFIGHTING FOAM WASTE IN DIESEL CONTAMINATED SAMPLES

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Abstract

Purpose: Bioremediation allows specific microorganisms to biodegrade petroleum contaminants, yielding carbon dioxide and water as mineralization products. This study applied an adapted microcosm a post-fire refinery soil sample containing diesel and AFFF (aqueous film forming foam). **Methods:** Along with the respirometric assays containing diesel and AFFF, we also determined the toxicity of each contaminated soil to *Lactuca sativa* seeds. Our method was based on the indirect measure of organic compounds biodegradation via CO₂ output through time. The experiment captured CO₂ with an alkaline KOH solution. The CO₂ amount was quantified in the residual KOH, according to conductivity changes. **Results:** A greater respiration rate in soil contaminated with diesel (more than 4.0 ± 1 mS/cm average values compared to other assays) was observed. Higher CO₂ outputs were related to an increased biodegradability. We also observed the growth of a fungus flora on macroscopic scale. The biodegradation activity was near detection limits in AFFF contaminated soils during the entire data collection, justified by toxicity results. The contamination by AFFF significantly affect the soil in tested concentrations, whereas 0% of the seeds germinated in AFFF only and AFFF and diesel mixtures. **Conclusions:** Contamination by firefighting foams was toxic to lettuce seeds, although it is commercially distributed and described as an environmentally safe compound. Our results indicate otherwise, since the fluorinated waste was not biodegradable nor inert. Still, we conclude that the contamination by diesel positively affected CO₂ production in soil as it was successfully degraded by local microflora, whereas AFFF inhibited microbial activity.

Keywords: AFFF, bioremediation, respirometry, toxicity.

1. Introduction

Since the 1970s, economic growth and development in Brazil demanded a major restructuring of the petroleum industry and its entire production chain. The petroleum industries resulting from this expansion are currently one of the main sources of environmental pollution in South America, responsible for serious accidents involving oil and its derivatives. Contaminations may occur at any stage of oil production, including extraction, refining, transport or storage (Kulkamp, 2003).

From the moment fuel reaches the ground, its components are separated into three phases: dissolved, liquid and gaseous. A small fraction of the components of this mixture dissolves in ground water, a second portion is retained in the porous spaces of the soil in its pure liquid form (residual saturation) and the third part evaporates, giving rise to atmospheric contamination (Nadim et al., 1999).

Contamination by petroleum by-products can be hazardous as certain compounds may remain in the environment for long periods. They also contaminate aquifers and springs which are often used for urban and industrial supply (Montagnoli, 2011). Moreover, fires and explosions are as severe as environmental pollution. According to NFPA (1991), both fires and pollution are likely in confined spaces (such as fuel storage tanks at refineries or gas stations) and in wide-open areas (such as wells and spills).

Diesel oil is a widely used hydrocarbon in Brazil, due to the extensive use of roads by transportation and cargo services. According to the ANP (2015), diesel consumption exceeded 56 million m³ this year. Referring to data from 2016, there were at least five major road accidents and refinery fires between July and December. All those accidents lead to the combustion of diesel with the presence of fires. In these cases, the use of water is not always the most appropriate procedure to contain fires, thus extinguishing foams are applied. However, these foams may also remain in the soils mixed with the fired hydrocarbon.

The contamination of hydrocarbons with the extinguishing foam has a considerable impact, since the diesel, is not characterized with high volatilization. Diesel oil also has slower degradation rates compared to other petroleum derivatives such as gasoline. Thus, it is possible that co-contamination may have an even greater permanence in soil and lead to the formation of contamination plumes more easily.

Bioremediation techniques rise as low cost alternatives that can be applied to contamination scenarios, by using biological agents to biodegrade hazardous residues, either *in situ* or *ex situ*. Natural attenuation result in the mineralization of the pollutant, that is, the release of carbon dioxide, water and biomass from indigenous microorganisms (from the site itself, without any interference from other technologies). According to Mariano (2006), this is due to an oxidation-reduction reaction in which the hydrocarbon is oxidized and the electron acceptor is reduced. In aerobic biodegradation, the acceptor would be oxygen (O₂). Three factors are necessary for biodegradation: contact between the microorganism and the substance to be degraded; favorable environmental conditions for the microorganism to perform degradation and; the microorganism must be able to perform the degradation or transformation of the substance (Domingues, 2007).

One method of monitoring biodegradation is respirometry, which studies an aerobic system from the amount of CO₂ produced and O₂ consumed in contaminated sites. This research used respirometric assays by Bartha and Pramer (1965) adapted to evaluate the indirect CO₂ release of soils contaminated with diesel oil and extinguishing foam. Furthermore, toxicological tests are of great importance in environmental studies and bioremediation processes. Toxicity tests allow researchers to evaluate whether or not biodegradation has successfully reduced the toxic potential of any given contaminant. This work studied, by seed germination tests, the degree of toxicity of contaminants in the soil before and after the treatment by bioremediation. The major milestones when investigating AFFF and diesel oil biodegradation in this research was to relate the biodegradation process from each soil sample with either one of the contaminants or both, thus presenting a mathematical model that best suit the dynamics of biodegradation.

2. Methods

2.1. Soil sampling

Soil samples were taken from the Experimental Garden of the Sao Paulo State University in Rio Claro (22 ° 23'47.4 "S 47 ° 32'40.0" W). The site has a history of contamination with petroleum products for previous biodegradation studies. Thus, we analyzed the activity of autochthonous microorganisms. We analyzed the bioremediation process using such adapted microbiota.

2.2 Respirometry in real climatic conditions and laboratory environment

This work proposed an adapted respirometry test in real climatic conditions replicating those found in major scale contaminations. Our respirometer was used to measure the biodegradation based on alkaline potassium hydroxide solutions (KOH) capacity to capture CO₂ for later chemical analysis. We proposed an outdoors experimental setup to allow natural environment variables to cause weathering. This approach differs from the usual respirometry assays, that are stored in incubators. Plastic containers with lid and disposable cups were used for the insertion of soil and alkaline solutions of KOH (Figure 1).

Our simulated scenarios were similar to the concentrations usually found after fires in the petrochemical industry, specifically the combustion of diesel oil with the use of extinguishing foam (AFFF). Pilot tests were carried out in real weather and controlled laboratory conditions. We monitored the concentrations of both contaminants and CO₂ yields. In order to better evaluate microbial activity in soil, we decided to carry out the duplicates with a full control set. Assays were labeled as follows: control (C) - soil control without contaminants; contaminated control with diesel oil and soil (D); contaminated control with AFFF and soil (F); and co-contaminated assay with diesel and AFFF (B).

2.3. Microcosm setup

The optimized setup was performed as described in Table 1 after a series of pilots and concentration variations to obtain the best balance between representativeness and experimental response.

Table 1 – Respirometric assays.

Assay (ID)	Soil (g)	Diesel oil (mL)	AFFF (mL)
Control (C)	100.00	----	----
AFFF (F)	100.00	----	10.00
Diesel (D)	100.00	5.00	----
Co-contamination (B)	100.00	5.00	10.00
KOH		20.00	

The assays were conducted in triplicate. All 12 assays were performed separately, each in a different flask, using plastic containers with minimum carbon dioxide loss during the process. Figure 1 shows the layout of the assays.

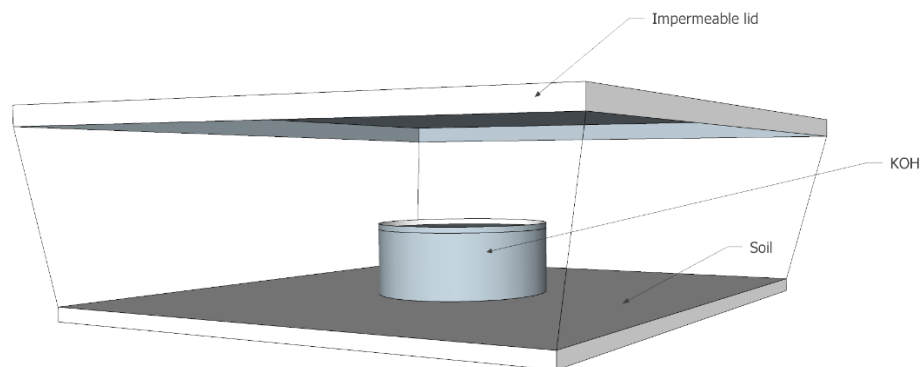


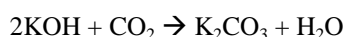
Figure 1 – Experimental setup in plastic containers.

The real climatic condition assays were allocated for 5 weeks in the Experimental Garden of the UNESP Campus of Rio Claro to allow biodegradation in local weather. Assays were subsequently exposed to controlled laboratory conditions and monitored for 8 weeks in incubators. Each assay was measured weekly according to the conductivity of residual solutions of KOH in mS (Siemens) after a 5x dilution in CO₂-free water (100 mL).

2.4. Standard curve of conductivity (S) and molarity of CO₂

Lower conductivity data output meant higher the CO₂ values produced by the microbiota within the respirometers. The CO₂ when in contact with the solution reacted with the alkali and formed carbonate ions. Traditionally, the quantification of carbon dioxide is done by acid / base titration following a stoichiometric ratio, however, we applied conductivity to quantify CO₂ saturation levels. Alkaline and carbonates are water soluble, where ions are dispersed and have the ability to conduct electricity. Conductivity is measured by ionic concentration yielded during CO₂ neutralization. Data on

transformation and biodegradation analysis were based on a standard linear standard curve from Siemens to moles based on the chemical equation stoichiometry:



Throughout each weekly respirometric activity, the 0.40 mol / L potassium hydroxide solution (KOH) was converted into a 0.20 mol / L potassium carbonate solution (K₂CO₃) in contact with CO₂. A series of standard solutions were made by varying the volume of the two solutions, where maximum and minimum saturation of CO₂ in KOH had their values calculated. The actual respirometric data from the microbiota was calculated according to the conductivity data from these standard solutions and the calibration curves.

2.5. Toxicity of contaminants in lettuce seeds

The AFFF toxicity was determined in triplicates, within five dilutions as shown in Table 2 using lettuce seeds as test-organisms. The toxicity study of soil contaminants was carried out in three different approaches: evaluation prior to and post biodegradation process and a sole analysis of AFFF toxicity in different dilutions. The toxicity tests using soil used the same setup and contents as the respirometric assays from Table 1.

Table 2 – AFFF dilutions used in toxicity assays.

Assay (ID)	Soil (g)	AFFF (mL)
1:1 (A)	5.00	2.00
1:2 (B)	5.00	1.00
1:5 (C)	5.00	0.40
1:10 (D)	5.00	0.20
1:100 (E)	5.00	0.02

All toxicity tests were performed using lettuce seeds free of any previous chemical treatment with pesticides. In our pre- and post-biodegradation setup, 20 seeds were placed in Petri dishes with filter paper along with a solution of sample leachate supernatant, obtained from stirring at 150 rpm for 24 hours on a shaker table and then subjected to freezing until counting time. The flasks contained 5.0 g of soil and 25.0 ml of deionized water according to Montagnolli et al. (2017). The plates were finally covered by plastic-film so that no moisture loss occurred. They were incubated at 22 ± 1°C for 120 hours. After the incubation time the germinated seeds were counted, measuring the root and hypocotyl of each seedling. A germination threshold of at least 2 mm of radicle growth was considered.

2.6. Mathematical modelling

The respirometric data are adjusted to CO₂ production models, based on Equation 1 proposed by Montagnolli et al. (2015) to determine maximum biodegradation time and expected CO₂ yields from each given substrate.

$$B = B_{max} / (1 + [(B_{max} - B_0) / B_0] e^{-rt}) \quad (1)$$

Where B is CO₂ Produced; B_{max} is the maximum CO₂ production; B₀ is the initial production of CO₂; R is the maximum specific production rate for a particular oil; T is time.

The use of statistical tools and proposition of different models helps to determine which best fits and how biodegradation profiles vary over time. For the data modeling, the SYSTAT SIGMAPLOT 10 platform was used to fit the equations to CO₂ data.

3. Results and Discussion

3.1. CO₂ production yields

The CO₂ production profile was measured through 13 weeks. It is worth mentioning from our pilot assays that a stable CO₂ curve was only observed in PTFE plastic packages, which were not subject to CO₂ leaking throughout the long-term data collection.

Real weather assays, susceptible to local climate variations, yielded CO₂ amounts either above (in summer) or below (in winter) our technique measurement window. In other words, respirometry was a very sensible technique to detect variations in CO₂ concentrations within mild weather conditions. Extreme temperatures were beyond the resolution limit of our method. Temperature is a decisive factor in processes that involve microbial activity, and climatic conditions at that time presented high variation and amplitude, this may have affected the biodegradation process. Temperature and pressure directly interfere in the microbial metabolism much more than the contaminants. As a result, all subsequent respirometric assays were conducted in laboratory conditions. Conductivity values were more stable in incubators, thus allowing CO₂ monitoring.

The alkaline solution of KOH standardized allowed the conversion of conductivity to CO₂ moles with the lowest deviation possible. Thus, with the standard solution of KOH and potassium carbonate solution (K₂CO₃), we established a conversion factor (Figure 2).

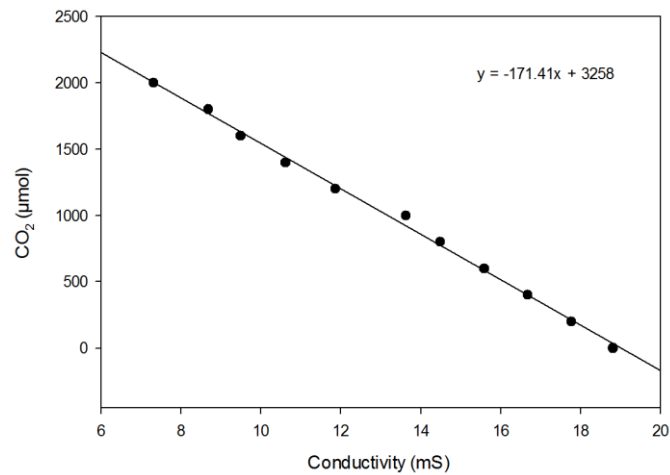


Figure 2 – Standard curve to convert CO₂ concentration from conductivity values in respirometers.

The weekly conductivity data was normalized according to blank KOH solutions. Weekly and accumulated biodegradation results though 90 days are shown in Figures 3 and 4, respectively.

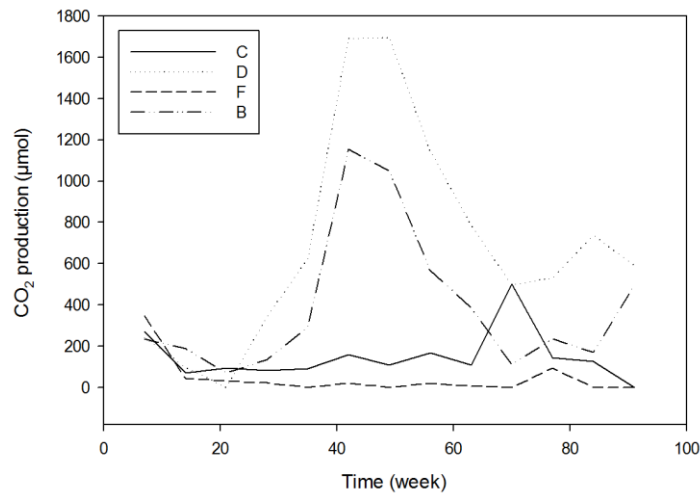


Figure 3 – Weekly CO₂ production.

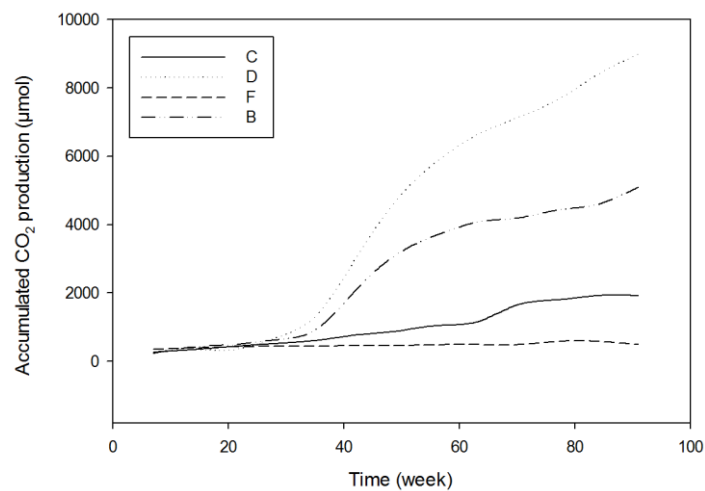


Figure 4 – Accumulated CO₂ production.

Degradation rate peaked between the 6th to the 8th weeks in D and B, with the highest microbial activity and CO₂ release values. The D assays were the ones with the highest conductivity output, followed by B, C and F (in this order). There was a decrease in microbial activity and CO₂ release in all respirometers from the 8th to the 13th week. However, the values were never null. The assays C and F were the ones that presented the lowest results of CO₂ production throughout respirometry. We did not observe in C and F typical biodegradation curves, whose profile is usually associated to growth and death of microbial cultures. Instead, constantly low production over time was observed.

The control assays presented basal biodegradation results, with little to no activity. CO₂ release was restricted, since they did not have a large nutrient presence. The only likely substrate were endogenous substrates in the soils matrix.

The respirometry assays D showed the highest microbial respiration and degradation results, and as can be seen in Figure 3. The soil was covered in visible mycelia growth (fungi), confirming viable conditions to the indigenous microbiota. On the other hand, F assays were the ones with the lowest biodegradation rates, even below the control respirometers (C). Such results triggered further analysis in subsequent experiments to verify the toxicity of each contaminant. The toxicity assays are discussed in the next section of this paper.

Assays B also showed signs of biodegradation. A very similar microbial growth was seen on B respirometers compared to the micellar morphology observed in D assays. The visible biomass, however, was less apparent. Growth was very likely allowed due to the presence of diesel in the soil, but the AFFF presence caused a minor disturbance and inhibited full colonial development.

The assays D and B showed degradation peaks of approximately 1700 and 1150 µmoles of CO₂, respectively at 7 and 6 weeks. They both showed a decay of carbon dioxide production. The control assays C presented a constant activity of CO₂ release, not due to degradation and, the F assays had minimum degradation values without significant peaks.

The accumulated CO₂ production (Figure 4) ranks the total organic matter mineralized by microorganisms and provides insights on biodegradability. The D assays contained the most biodegradable substrate, with a cumulative release of CO₂ of approximately 9000 µmol, followed by (B) assays with release of 5000 µmol. F assays ranked last, with a total degradation of 580 CO₂ µmol.

3.2. Toxicological analysis

3.2.1. AFFF toxicity

Due to the observation of AFFF interference in microbial metabolism in respirometric assays, we further expanded our investigation on AFFF toxicity. We verified five different dilutions of the contaminant towards seed growth. The limit and critical point of foam toxicity was found within our five different dilutions.

The vegetable tissue growth is presented in Figure 5. Growth results greater than 1.0 cm have been obtained from the C test, which was also considered the critical toxicity index in our study.

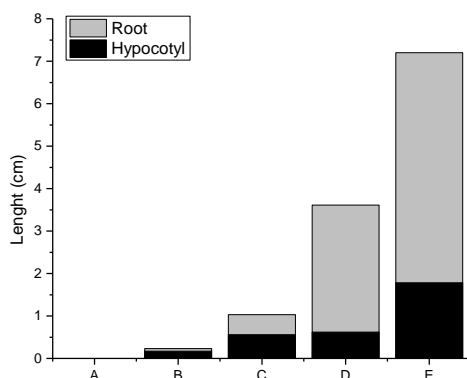


Figure 5 – *Lactuca sativa* tissue growth in AFFF dilutions.

The AFFF contaminant presents a certain level of toxicity, which, in a certain moment, causes the soil to reestablish itself and to allow the germination even in unfavorable environment. According to Barbosa (2000), the contaminant is considered toxic when there is inhibition of growth up to 1 cm, hence AFFF becomes non-toxic only at a concentration of 1: 5 (test C) as previously termed as critical toxicity index.

3.2.2 Toxicity before and after biodegradation

Before biodegradation, only seeds in control C and diesel D germinated, with 100% germination. Assays F and B did not show any growth activity in root and hypocotyl tissues. The qualitative analysis and plotting were averaged from the root and hypocotyl measurements of each quadruplicate assay (Figure 6).

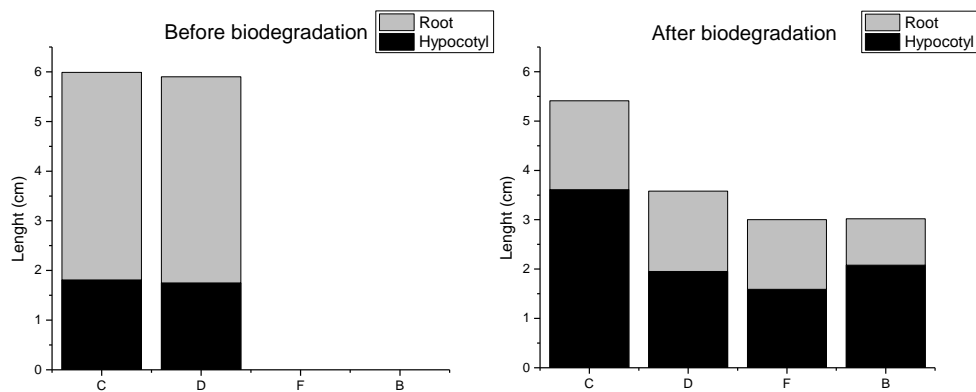


Figure 6 - *Lactuca sativa* tissue growth before and after biodegradation.

According to Figure 6 (left), the growth and germination of lettuce seeds occurred only in assays C and D, both growing 6 cm of vegetable tissue before biodegradation. From this result, we concluded that even prior to the bioremediation process by respirometry, the diesel was not toxic to the soil biota at the proposed concentrations and still had fairly comparative results to the control soil, free of recent contaminations.

The AFFF containing assays F and B showed a high degree of toxicity in the contaminating foam, with 0% of germination when in soil alone, and also with the presence of diesel, which in this situation played a more secondary role. When the diesel was placed separately in D, the development of plant tissue was still observed.

Post-degradation analyzes (Figure 6, right) were also conducted to investigate how bioremediation would affect toxicity. For this test, we checked whether the AFFF showed diminished signs of biodegradation, even if not significant, relative to its pre-degradation toxicity. In dilution tests, high germination rates were obtained in relation to AFFF. Thus, we also decided to conduct undiluted tests to verify if original concentrations, even with little degradation, would decrease the toxicity of AFFF. Thus, as in the dilution tests, duplicate assays of each triplicate experiment were performed, each of them being individual, since the degradation process was isolated, with a total of 24 toxicity experiments.

On the F assays, the toxicity of original concentrations becomes minimal, having practically 100% of germination in all the referring tests. Thus, it could be concluded that considering the 1:1 concentrations, the bioremediation process became effective in reducing contaminant toxicity, but not representative of significant degradation. According to the B assays, the germination was low in both respirometers. In other words, when not combined, contaminants (either diesel or AFFF) presented a favorable environment (soil) for the growth of a microbiota. The germination B assays was approximately 75% on average, non-toxic to the environment, but with lower growth activities. Therefore, the previously toxic contents in respirometric assays F and B had a comparable growth rate to non-toxic contaminants in soil (over 60%) and an approximate tissue growth of 3 cm after the biodegradation process.

3.3. Biodegradation modelling based on microbial growth kinetics

The distribution patterns of CO₂ production within 90 days allowed the analysis and fitting of mathematical models related to the biodegradation process (Figure 7). The adjusted kinetic parameters of this process predicted the expected kinetic behavior of each respirometric assay in their respective accumulated CO₂ production rates during the biodegradation process.

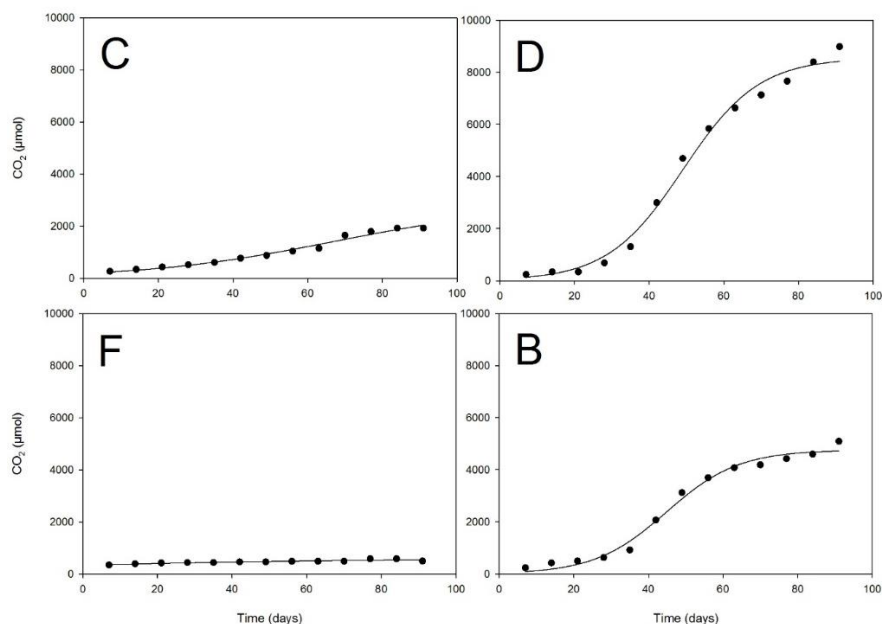


Figure 7 – Fitted CO₂ production curve.

The most significant parameter extracted from the modelling is presented as Bmax, which corresponds to maximum CO₂ produced from the substrate. Biodegradation ceases beyond 90 days after initial inoculation. The Bmax value for B assays is 4757.38 µmol, whereas C assay is expected to produce 2832.86 µmol. The lowest expected CO₂ yield occurs in F assays, with 588.90 µmol. The kinetic analysis confirms that the foam is a biodegradation inhibitor.

Higher CO₂ values produced during biodegradation were expected when both contaminants (diesel oil and AFFF) were combined, due to AFFF surfactant properties. The foam promotes contact surfaces between diesel and aqueous soil medium, allowing an interaction zone containing the hydrophobic medium and microorganisms present in hydrophilic medium. Higher values of diesel oil degradation are to be expected, since its hydrocarbon structure could be broken down more easily than AFFF. Fluorinated compounds are extremely rigid, due to CF bonds in their hydrophobic part, which in turn decreases their biodegradability.

However, our research reached an unexpected result, in which diesel oil was the contaminant that had the highest rates of biodegradation, whereas firefighting foam halved significantly most microbial activities. This data was reaffirmed by seed testing, verifying that the foam could not perform its role as a surfactant. It was also a low biodegradable contaminant, with extremely high toxicity before the microbial attenuation process. The toxicity of the foam however, decreased after 90 days, when and a high germination could be finally observed.

Conclusions

Biodegradation viability studies offer alternatives towards the treatment of contaminants with minimum resources. Our research provided new insights on diesel contaminants and extinguishing foam. The simulated post-fire scenario within our respirometers allowed the development on how the environment would react to the entry of these contaminants and how the toxicity would be reestablished after contamination.

The results of the study under real climatic conditions were satisfactory, however, it was observed that, during the winter and summer seasons, large differences in temperature broaden the CO₂ production value outside of respirometers resolution limits. Still, we can conclude that temperature is a crucial factor in the AFFF + diesel biodegradation process. In other words, the proposed respirometry technique was found not suitable for degradation studies with large climatic variations. The high sensitivity of the method, combined with high variability in respiration rates, caused contaminations in colder places to be inhibitory or extremely high when seasonality factors are not isolated.

With seed testing prior to the respirometry process, we found that there is a high degree of toxicity in the AFFF extinguishing foams, contrary to what is presented by most manufacturers and suppliers. However, after the bioremediation treatment from respirometric assays, germination in F and B could be verified. Thus, long-term biodegradation is expected to occur, even if not significantly. The diesel oil, however, had a high degradation, showing increased microbial activity during the 90 days of study. It is also worth mentioning that the soil already had a history of contamination with petroleum products, thus natural selection towards the most adapted microorganisms may have happened. Still, the combination of respirometry and phytotoxicity methods can be applied to different types of contaminated soils, where laboratory tests are essential to predict the kinetics of on-site degradation and toxicity parameters.

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