

Article

Chemometrics-Assisted Monitoring in Raman Spectroscopy for the Biodegradation Process of an Aqueous Polyfluoroalkyl Ether from a Fire-Fighting Foam in an Environmental Matrix

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Abstract: Surfactants based on polyfluoroalkyl ethers are commonly used in fire-fighting foams on airport platforms, including for training sessions. Because of their persistence into the environment, their toxicity and their bioaccumulation, abnormal amounts can be found in ground and surface water following operations of airport platforms. As many other anthropogenic organic compounds, some concerns raised about their biodegradation. That is why the OECD 301 F protocol was implemented to appreciate the oxygen consumption during the biodegradation of a commercial fire-fighting foam. However, a Raman spectroscopic monitoring of the process was also attached to this experimental procedure to evaluate to what extent a polyfluoroalkyl ether disappeared from the environmental matrix. The relevance of our approach is to use chemometrics, including the Principal Component Analysis (PCA) and the Partial Least Square (PLS), in order to monitor the kinetics of the biodegradation reaction of one fire-fighting foam, Tridol S3B, containing a polyfluoroalkyl ether. This study provided a better appreciation of the partial biodegradation of some polyfluoroalkyl ethers by coupling Raman spectroscopy and chemometrics. This will ultimately facilitates the design of a future purification and remediation devices for the airport platforms.

Keywords: environmental fate; Raman spectroscopy; chemometrics; principal component analysis, biodegradation; kinetics; post-processing; Whittaker filter; partial least square

1. Introduction

The water and/or soils pollution are extensively discussed in the literature, as well as the biodegradation of anthropogenic pollutants and their effects onto the environment. In the specific case of fluorinated aliphatic molecules present as surfactants in many industrial contexts [1], the bibliography is limited for surfactants used as fire-fighting foams [2-9]. These chemical compounds are considered as toxic [10, 11]. This raised concerns about their particular environmental impact [12]. Indeed, these chemicals are known to be persistent into the environment, to present a toxicity to living

organisms and to be bioaccumulated. On airport platforms, fire-fighting fluids are added to water to generate solutions in the form of foams to ease the water spreadability to smother the fire, and its extinguishment, especially for fuel fires, using the low surface tension characteristics of the foam due to the presence of fluorinated molecules. These fire-fighting foams must comply with the specifications related to the equipment to fight fire on/in aircrafts, and their conformity for their use on French airports is certified by the French Civil Aviation Department [13]. Therefore, fire-fighting foams are subject to specific rules [14]. Because of these environmental rising concerns, the French Civil Aviation Department initiated an investigation on methods of detection and quantification of such chemical in aqueous solutions, included at low concentration, their fate in soils and vegetation, and their impact on environment through the determination of their biodegradation and toxicity.

The objective of this article is to focus on the biodegradation process of a polyfluoroalkyl ether present in Tridol S3B, a commercial fire-fighting foam. The product is characterized using conventional OECD 301 F protocol. In parallel, it is characterized by Raman spectroscopy to establish its limit of detection into an aqueous solution. A spectroscopic monitoring of the biodegradation process is then implemented, with a chemometric approach developed for the investigation of the kinetics reactions associated to the biodegradation process for this polyfluoroalkyl ether. Indeed, environmental matrices are complex to analyze in Raman spectroscopy. This technique relies on the presence of strongly polarized chemical bonds from specific spectral signatures e.g. 2H-polyfluorocarboxylates for our surfactants [6]. Nevertheless, undesirable 'effects' could appear on the Raman signal due to the complexity of the analyzed environmental matrices (e.g. spectral overlap). That is why the proposed solution is to use chemometric methods to extract the most relevant information from Raman spectra. Chemometrics has already shown that it is possible to overcome these drawbacks, but also to push back the resolution limits of conventional instrument [15]. To put in a nutshell, we demonstrate a robust approach to monitor the biodegradation of a surfactant by Raman spectroscopy with a chemometrics approach.

2. Materials and Methods

2.1. Presentation of the polyfluoroalkyl ether

Fire-fighting chemicals are aqueous solutions allowing the generation of foam to extinguish fire, in particular fuel ones. Commercial products used on airports platforms are industrially synthesized, and employed at a volume percentage ranging from 3% to 6%. One polyfluoroalkyl ether was tested in this study, and was compliant with specifications emitted by the French Civil Aviation Department, concerning the compliance with the International Civil Aviation Organization (ICAO) requirements. Tested product is an aqueous film forming foam (AFFF), containing fluorinated surfactants, and its general characteristics are summarized in Table 1, according to its safety data sheets. Based on provided information, the presence of polyfluoroalkyl ethers was not obvious. But the biodegradation was presented are extremely compliant with environmental requirements.

Table 1. General characteristics of a commercially-available fire-fighting foam according to its safety data sheet.

Product name	Manufacturer	Volume mass (kg/L)	Global composition	Mention about the presence of fluorine	Biodegradation percentage
Tridol S3B	Eau et Feu S.A.	1.01	Ethylene glycol 2-(2-butoxyethoxy)ethanol	Presence indicated without any detail	97 % (number of days not specified)

To both confirm the presence of fluorine and to obtain a more accurate insight on the composition of these fluids, its Raman signature is established as reference. The Raman spectroscopy is a non-destructive and now a cost-effective technique in order to analyze materials and aqueous

solutions [16, 17]. The reference spectrum was obtained using a RXN-2 Raman spectrometer from Kaiser Optics, operating with a 785 nm laser source (red) with a 400 mW output, and a spectral resolution of 1 cm⁻¹. The Raman spectrum (Figure 1) was measured with a sample of 5 mL of the fluid placed in a quartz cell, with an integration time of 30 s. The spectral ranges of interest are comprised between 600 et 1400 cm⁻¹ for the chemical bonds involving fluorine, and in the 1000-1500 cm⁻¹ and the 2800-3000 cm⁻¹ for the ones involving ether covalent bonds [18] (Table 2).

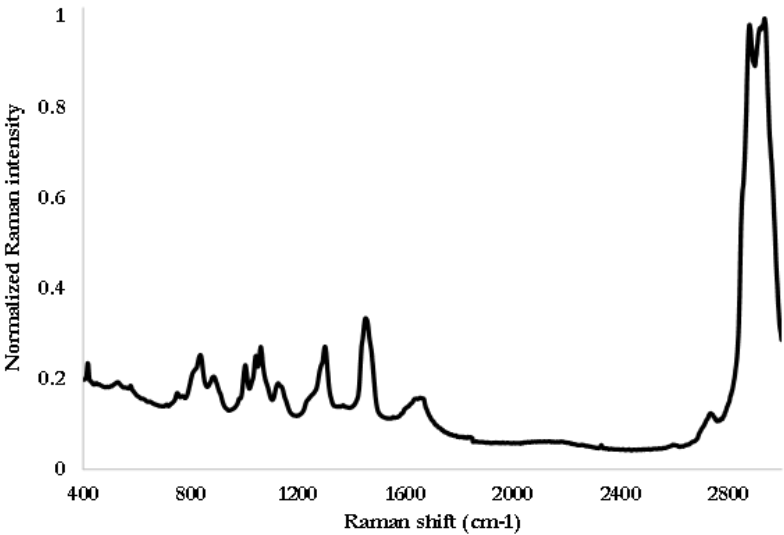


Figure 1. The reference Raman spectrum of the fire-fighting foam Tridol S3B obtained with a Kaiser Optics Raman spectrometer operating with a 785 nm laser (400 mW, 30 s integration time)

Table 2. Raman shift of chemical bonds of a polyfluoroalkyl ether present in fire-fighting fluid.

Raman shift (cm ⁻¹)	Chemical bond of interest with the corresponding vibration type
530	C-F deformation
765	C-F deformation vibration in aliphatic monofluorinated compounds, C-F scissor
830	symmetric stretch C-O-C
990-800	C-F deformation
1110-990	CH2 rocking
1110-1000	C-F stretch in CH2F
1040-1060	aliphatic monofluorinated compound
1250-1050	CH2F with C-F stretch and CH2 rocking
1130	CF stretch on aliphatic difluorinated compound
1300	aliphatic difluorinated compounds C-F stretch
1450	asymmetric stretch C-O-C
	CF stretch
	CF3 attached to an alkyl group
	CF3 attached to an aromatic group
	CH2 vibration of deformation

2.2. Appreciation of the biodegradation based on OECD 301 F protocol

Biodegradability monitoring consists in characterizing the way micro-organisms do degrade a given substance, in an aerobic or in an anaerobic environmental matrix. This monitoring is used to evaluate the persistence of an anthropogenic substance into the environment, in parallel to the collection of chemical and eco-toxicological data.

The determination of the biodegradability can be established through several methods given by a OECD guide [19], and providing six methods to characterize a chemical product as a function of its biodegradability.

The method selected in this study is related to the standard referenced as NF EN ISO 9888, which evaluates the ultimate aerobic biodegradability of organic compounds in aqueous medium and in static conditions (Zahn-Wellens method) [20]. It is similar to the measurement of dissolved organic carbon in a static aerobic environment with constant air flow, temperature, stirring and light, through the appreciation of the chemical oxygen demand instead of the one of dissolved organic carbon. This process describes the degradation of the whole organic carbon present in an environmental matrix.

Therefore, to evaluate the environmental fate of a polyfluoroalkyl ether contained into a fire-fighting foam, a given amount of this foam, only source of dissolved organic carbon besides the inoculum, is introduced into a 1 L-reactor containing a static aerobic media with some nutriment (Figure 2). This inoculum is a sample of micro-organisms collected in an aeration basin for only domestic waste waters.

Dissolved organic carbon is measured with a chemical method during the experiment on homogeneous samples. In parallel to the test with a polyfluoroalkyl ether, a second test was conducted as blank with only the inoculum and the nutriment. The ratio of biodegradation at a date t of the polyfluoroalkyl ether is calculated as follows:

$$\text{biodegradation} = \frac{(DCO_{t_1} - DCO_{blank_{t_1}}) - (DCO_t - DCO_{blank_t})}{(DCO_{t_1} - DCO_{blank_{t_1}})}, \quad (1)$$

DCO_{t_1} and DCO_t respectively are the oxygen chemical demand 3 hours \pm 1/2h after the beginning of the biodegradation test, and at a given time t . Once a 10% is reached, the tested product is considered as to be easily degradable if a 70%-threshold is obtained with the ten following days. The test is considered as finished when the degradation rate remains constant as a function of time. The DCO thus obtained is called the refractory one. The maximum duration of the test is 28 days. It is advised to start with a DCO of 100 mgO₂/L, with a maximum of 1000 mgO₂/L.



Figure 2. OECD test for the biodegradation of a fire-fighting foam containing a polyfluoroalkyl ether

2.3 Spectroscopic monitoring and chemometrics to identify the biodegradation process

As discussed above, the evaluation of the environmental fate of a polyfluoroalkyl ether contained into a fire-fighting foam relied on the fact this foam was the only source of dissolved organic carbon besides the inoculum. The Table 1 with the composition of the fire-fighting foam considered in this research article did show the fluid obviously contained other dissolved organic carbon besides the ones related to polyfluoroalkyl ether. Therefore, the sole OECD protocol is not appropriate to identify to what extent the degradation of the polyfluoroalkyl ether is taking also place along with the one of other organic chemicals.

It has been demonstrated Raman spectroscopy was able to specifically identify the presence of C-F chemical bonds despite the fact the amount of polyfluoroalkyl ether present in a fire-fighting foam is low (Table 1). Considering both the volume of reactor and this amount, the question of the

spectroscopic detection limit was raised. Several diluted solutions of the selected commercial fire-fighting foam were then prepared with volumic rates ranging from 4.10^{-3} % to 80 %. Each solution was analyzed by Raman spectroscopy during 8 minutes, with a 30 s integration time, and a 45 s-time interval between each spectrum. Ten spectra for each dilution were then obtained, and a database of over eighty spectra was then generated when including the spectral signature of pure commercial fluid. As expected and as explained in the literature, the greater the dilution the smaller the peaks intensities related to fluorinated chemicals bonds. As a consequence, two chemometric methodologies were applied in order to determine the spectroscopic detection limit of polyfluoroalkyl. The first one is based on the Principal Components Analysis (PCA), and the second one on Partial Least-Square regression (PLS) [21, 22]. These analytical methods are widely used on spectroscopic measurements for data "mining", dimension reduction and/or principal components analysis for characterizing several sets of measured physico-chemical variables.

Once established the limit of detection, a similar experimental setup to the one for the appreciation of the biodegradation based on OECD 301 F protocol was developed. A spectroscopic monitoring was then implemented to specifically appreciate the environmental fate of one fire-fighting foam containing a polyfluoroalkyl ether. Two 1 L-reactors containing a static aerobic media with some nutrients and an inoculum were then prepared. One was used as a reference while 10 mL of pure Triodol S3B were added into the second one. A Raman immersion probe was placed into each reactor (Figure 3a), and was connected to the Kaiser RXN-2 Raman spectrometer to a continuous monitoring over 28 consecutive days. Each probe made a measurement for 30 s, with a 15 minute-interval between each Raman spectrum. Some foam, visible on Figure 2, is naturally generated while stirring the mixture (nutritive fluid, and inoculum) containing a fire-fighting foam. To reduce the risk of foam occurrence, five specific polydimethyl siloxane trimethyl siloxy terminated were added, with molar mass ranging from 770 g/mol to 139000 g/mol, these chemicals making the foam unstable and are widely used in food industry. Normalized Raman spectra of the fluids present in the environmental matrix are provided in Figure 3b. As observed, two peaks of the nutritive fluid, below 800 cm^{-1} , are located near some of the ones observed in Triodol S3B, while siloxane spectral signatures are not interfering with the ones of fire-fighting foams, especially in the $400\text{--}1800\text{ cm}^{-1}$ range. Considering the volume of both fire-fighting foam and of static aerobic media involved, the concentration in fire-fighting foam is of 1 % v/v.

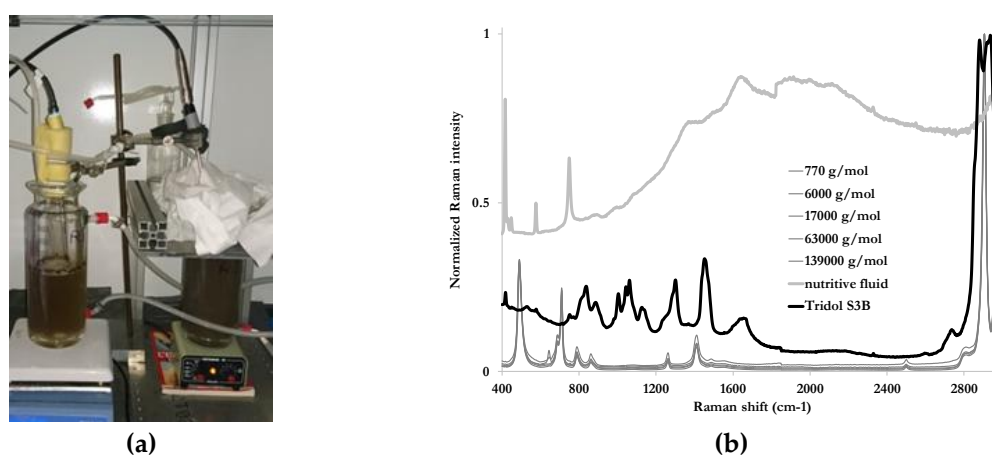


Figure 3. Experimental setup with Raman immersion probes into two identical environmental matrices (one reactor without polyfluoroalkyl ether on the left, and with polyfluoroalkyl ether on the right of the picture) (a), and Raman spectra of each fluid constituting the environmental matrix before adjunction of the inoculum (b)

On complex samples, such as environmental matrices, the use of chemometric tools could overcome several drawbacks from a classical approach in Raman spectroscopy. First, it is necessary to know a priori all pure components in the sample. If this hypothesis is not true, it could be possible to select a non-selective spectral wavelength and thus, overestimated the concentrations of a

component. Moreover, when there is a strong spectral overlap (e.g. wide bandwidth, fluorescence effect and/or sample complexity), it is impossible to identify a truly selective wavelength. That is why the use of chemometric tools could overcome all these drawbacks in order to give a better representation of the analytic reality.

2.4 Principal components analysis (PCA) and Partial Least-Square (PLS)

Among data analysis techniques, PCA is the most commonly used methodology in order to understand datasets [23, 24]. Indeed, PCA explains the structure of descriptive variables based on data matrix using a small number of variables defined as linear combinations of the original variables (i.e. raw data). This approach decomposes a data matrix D ($m \times n$) into a product of two variables, a matrix of scores denoted T ($m \times k$) and a matrix of loadings denoted P ($k \times n$), which is added to a residual matrix E_{PCA} ($m \times n$):

$$D = T \cdot P^T + E_{PCA}, \quad (2)$$

The dimension of the new space defined by k is determined with the rank of the raw data matrix D . The number of original variables n from m observations are too complex for direct interpretation from D , which explains why it is necessary to "reduce" the space dimension using k Principal Components (PCs) that explain the maximum amount of information. The scores represent the coordinates of the observations (or samples) on the axes of the selected PCs. The loadings denote the contributions of the original variables on the same selected PCs. In other words, since the scores are representations of observations in the space formed by the new axes defined by the PCs, loadings are a representation of the variables on this axis. Geometrically, this change of variables by linear combinations results in a set of new variables called PCs. The direction of each newly created axis describes a part of the global information from the original variables contained in the matrix D . The variance explained by each PCs is sorted in decreasing order. The proportion of variance explained by the first PC, which represents the main part of information, is higher than the second PC, which represents a smaller amount of information, and so on. The same information cannot be shared between two PCs because PCA requires that the PCs are orthogonal to each other.

In our case, the PCA is used directly on the Raman spectra in order to find out when the degradation of the polyfluoroalkyl ethers occurs but especially if there is degradation. One of the drawbacks of this approach is the direct interpretation of the chemical information of the loadings. Indeed, when you analyze spectra, loadings can be negative, while it is not chemically possible to obtain a negative spectrum. As a consequence, some other chemometric methodologies e.g. MCR-ALS [25, 26] or PARAFAC [27] will be needed to reach a more realistic chemical information.

PLS is a supervised statistical method to predict quantitative information or a given state of a system with both spectra and measurements. It consisted in generating a calibration on the basis of a training set of Raman spectra and of corresponding concentrations. It contributes to the elaboration of a statistical forecast model of the concentration with the Raman spectra. Since all data used do contain noise, this calibration generates a calibration matrix P and a minimized error matrix E_{PLS} . It allows here to simultaneously generate models on the variability of concentration in polyfluoroalkyl ether of aqueous solutions and the matrix of their Raman spectra, and so maximize the correlation between latent variables.

$$\text{concentration} = P \cdot (\text{Raman spectra}) + E_{PLS}, \quad (3)$$

Basis vectors are searched for concentrations and another one for Raman spectra, and the understanding the way these sets are related is mandatory. The eigenvectors for each space are calculated to reach the best congruence between each variable factor and its corresponding Raman spectrum in the least-squares sense. Noise being present in both variables and measurements, eigenvectors for both spaces are shifted by different amounts in different directions, due to the independence of noises in corresponding spaces, conducting to an imperfect congruence between variables and data points. PLS will try to restore this optimum congruence, defined as a linear relationship between the scores of the variables and Raman data.

3. Results

3.1. Discrimination of fire-fighting foams and detection threshold

PCA and PLS results for Raman spectra of the fire-fighting foam are respectively presented in Figure 4a and 4b. Results were obtained with Unscrambler X 10.1, using NIPALS algorithm, after a baseline and offset correction on Raman spectra, followed by a normalization with respect to spectra area over the 400–2800 cm^{-1} spectral range. PCA is able to discriminate fire-fighting foam diluted solutions from each other, as illustrated by the spreading of data points on the scores plot (Figure 4a) of the first two principal components. The discrimination and hence the detection limit can be obtained with volume amounts as low as 0.08% of commercial fire-fighting foam in water, corresponding to nearly 10 ppm, based on information provided by the manufacturers (Table 1). A PLS model was also elaborated (Figure 4b) and provided the percentage of fire-fighting foam in an aqueous solution on the sole basis of its Raman spectral response. RMSEC of the PLS model was of at least 0.023%. A linear regression in a log-log scale indicated a R^2 of 0.998, for a slope of 0.9993 and an offset of 0.022% for the prediction of a fire-fighting foam in an aqueous solution. The application of the model to unknown solutions indicated the ability to reach a detection threshold of 140 ppm.

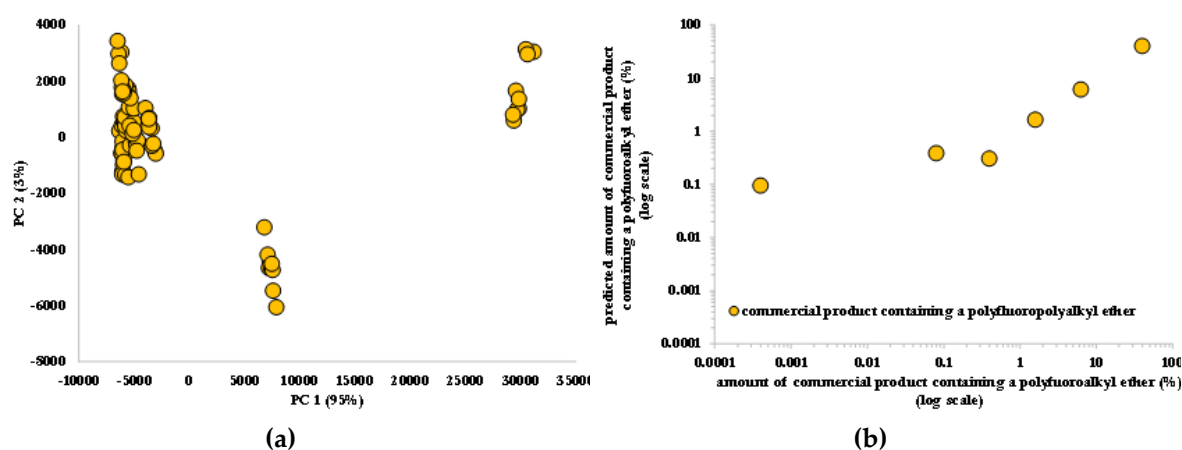


Figure 4. PCA applied to Raman spectra of diluted aqueous solutions of a fire-fighting foam (a) and PLS model to predict the amount of fire-fighting foam Tridol S3B containing a polyfluoroalkyl ether (b)

3.2. Conventional monitoring of the biodegradation process

Biodegradability results are presented in Table 3. According to these test results, the product is easily degradable. Evolutions as a function of time of the degradation of this fire-fighting foam is in Figure 5. No significant difference was observed between it and two other tested fire-fighting foams. They all presented a pause between the 48th and 72th hour depending on the product after de beginning of the degradation process. Nevertheless, and as indicated before, this process describes the degradation of the whole organic carbon present in the environmental matrix. In the case of Tridol S3B, it includes at least ethylene glycol, 2-(2-butoxyethoxy)ethanol, alkyl sulfate, ethanol, polyethylene glycol 400 besides polyfluoroalkyl ethers. Therefore, this test did not indicate to what extent fluorinated organic molecules, considered as persistent into the environment, were really degraded or not.

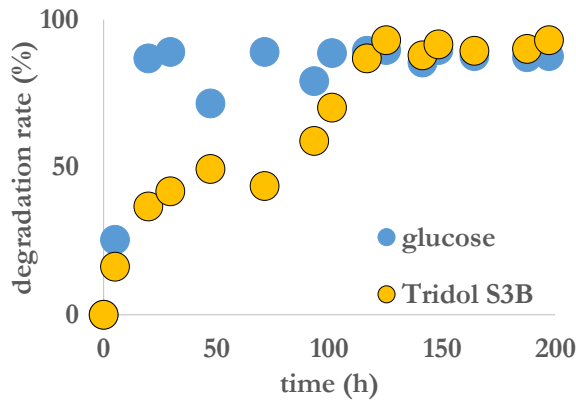


Figure 5. Degradation rate of the fire-fighting foam Tridol S3B as a function of time according to OECD 301 F protocol

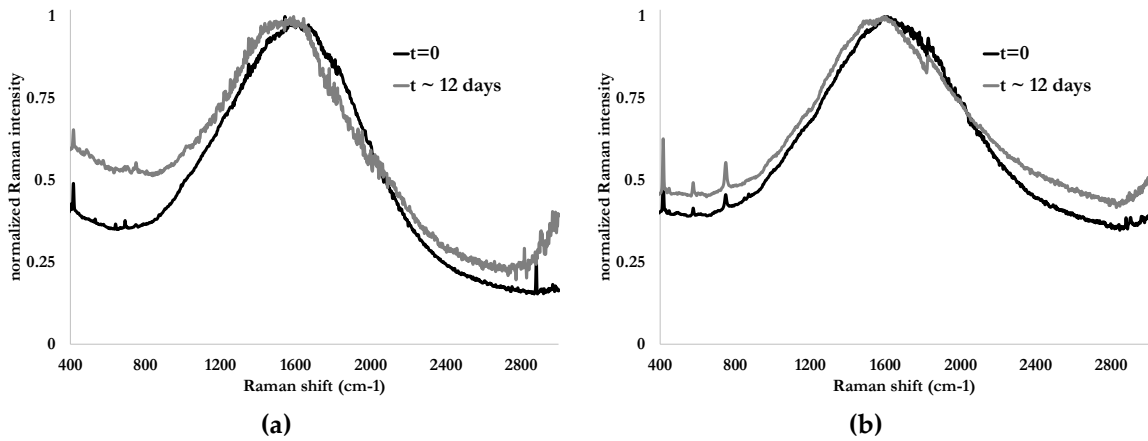
Table 3. Results of aerobic biodegradation tests on the three fire-fighting foams.

Fire-fighting foam	Theoretical rate (%) of degradation (according to products SDS)	Biodegradability rate (%) after 5 days	Time (h) necessary to reach a biodegradation rate of 90%
Tridol S3B	97	89	121.5

3.3. Chemometrics-assisted spectroscopic monitoring of the Tridol S3B biodegradation

Raman spectroscopy was implemented on one additional test specifically conducted on Tridol S3B. This choice was comforted by the absence of significant difference between the fire-fighting foams when submitted to conventional OECD protocol as described in the previous paragraph.

Raman spectra from both reactors are illustrated in Figures 6a and 6b for the case of Tridol S3B, at the beginning of the test (dark solid line) and after 288 h (12 days) of biodegradation (grey solid line). The first element to notice is the strong fluorescence due to the light diffusion already present in the nutritive fluid and enhanced by the solid particles in suspension and causing the turbidity of the studied media. The second one is the presence of peaks at 580 cm^{-1} and 750 cm^{-1} in Raman spectra of the fluid containing the fire-fighting foam. These peaks can be attributed to CF_x bonds (x=1, 2 or 3) [18]. The peak near 580 cm^{-1} was barely visible with respect to other one in the case of pristine fire-fighting foam. Thus, the presence of such a peak could be the evidence the fluorinated molecules are still present while the degradation process is supposed to be almost over, as indicated in Figure 5 when evaluating it conventionally. Since these peaks are also present in the nutritive fluid, additional investigations are needed.



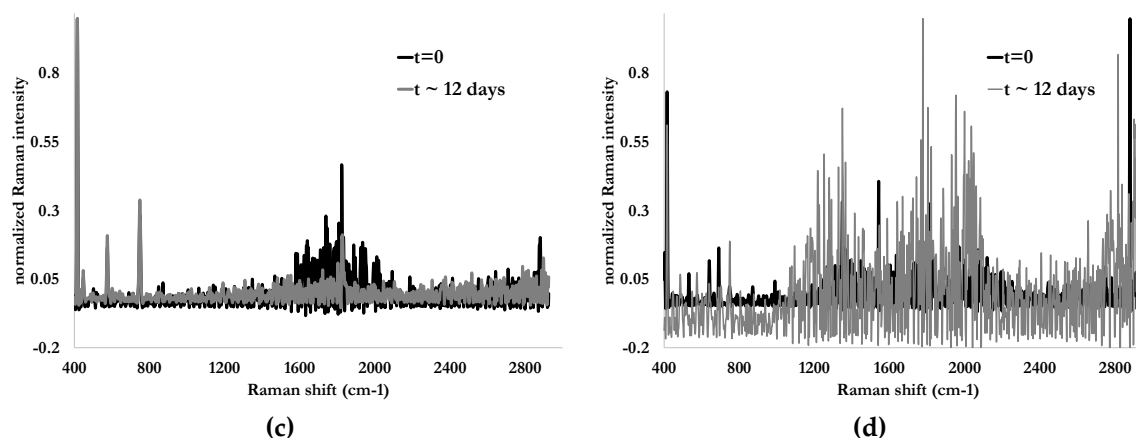


Figure 6. Raman spectra obtained from immersion probes in reactors without fire-fighting foam (a) and with fire-fighting foam Tridol S3B (b) at the beginning of the test and after 12 days in their pristine form, and the same respective spectra after SNV and baseline correction (Whittaker filter) (c, d)

4. Discussion

Raman spectra collected with a probe immersed in each reactor were then first analyzed by means of a PCA using Chemflow, a chemometrics online software [28]. The objective was then to focus on the specific evolution of polyfluoroalkyl ethers. To ease the analysis, some pre-treatments processing were conducted. Indeed, collected Raman spectra have intense and irregularly shaped baselines changing (Figures 6a and 6b). This intense background associated with fluorescence contributions is a common problem in Raman spectroscopy. The raw spectra were then corrected to extract unbiased and effective chemical information. For this purpose, the Weighted Least-Square (WLS), also called Asymmetric Weighted Least-Square (AsLS) removes the fluorescent contributions [29]. Originally, this pre-process algorithm was used to subtract baseline shifts in chromatography [30]. This approach is based on a recursive local fitting of the entire spectrum with a baseline obtaining by using a Whittaker smoother [30] which uses two basic setting parameters λ and P respectively equal to 100 and 0.01. The first one controls the amount of curvature for the baseline. The second one, governs the extent of asymmetry required for the fit and must be in the range 0 to 1, limits excluded. In addition to the baseline correction, the Singular Normal Variate (SNV) was applied for normalization. The SNV calculates the standard deviation of all variables for the given sample [31] which is then normalized with respect to this value. Thus, the sample has a unit standard deviation. Results of these pre-processes are illustrated in Figures 6c and 6d.

PCA was first conducted on the data set constituted of spectra from both reactors, with a calculation based on 15 principal components on the 400–1200 cm^{-1} spectral range. The first principal component explained nearly 49 % of the variance, and the second close to 9 %, the total explained variance by the 15 principal components being over 99 % (Figure 7a). The scores plot generated by these first two principal components is illustrated in Figure 7b. One can observe the data points from each reactor are separated by the first principal component. Data from reactor 1 only containing the environmental matrix gathered, indicating a system almost unchanged along the selected duration of the test. Data points from reactor 2 (in green in Figure 7b and containing the fire-fighting foam) are globally spread along this first principal component, and converge towards the ones of reactor 1 without reaching it. The first loadings (Figure 7c) was plotted along with Raman spectra of Tridol S3B, siloxanes and nutritive fluid. Peaks related to nutritive fluid at 580 cm^{-1} and 750 cm^{-1} were clearly identified. This component has a loading profile similar to the reference spectrum of the nutritive fluid, which is common to the two reactors (cf. Table 2). In the scores plot, the two data sets are nevertheless separated by this principal component. Therefore, data analysis indicated a difference occurred in the nutritive fluid. A selectivity issue is then raised and these peaks could both be attributed to nutritive fluid and Tridol S3B.

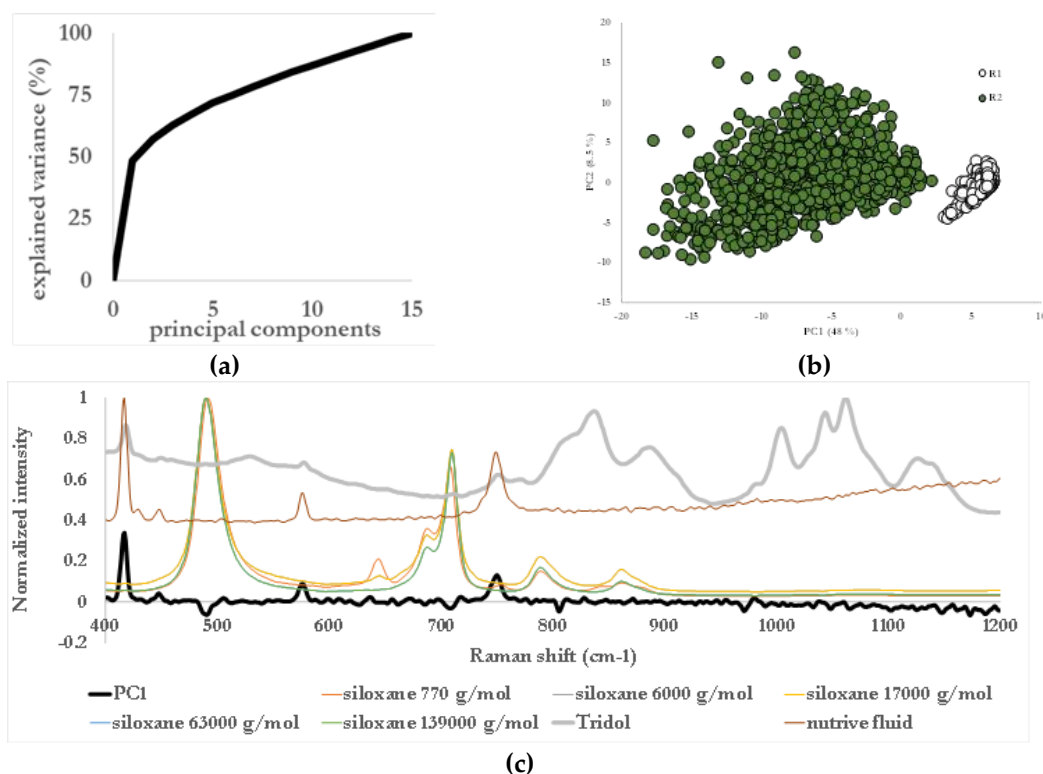


Figure 7. Explained variance (a), scores plot for the first two principal components (a) and loadings (c) for a PCA conducted on Raman spectra (after baseline correction and SNV) obtained from measurements in reactors 1 and 2 during the biodegradation process of Tridol S3B

To overcome this selectivity issue, a new PCA was then conducted on the data set constituted of spectra from both reactors and the ones of a pure fire-fighting foam, with the same data pre-processing and the same spectral range as before for consistency sake. A new scores plot was then generated with the first two principal components (Figure 8) respectively explaining 48% and 8.5% of the variance. Here again the whole data set is organized into two groups, with data points from reactor 1 still gathering, while points related to pure Tridol S3B are mixed to the one of reactor 2. The ones of reactor 2 are still dispersed, and converging towards the ones of reactor 1. Therefore, peaks at 580 cm⁻¹ and 750 cm⁻¹ in the first principal component are related to Tridol S3B, and not exclusively to the nutritive fluid. This indicates the biodegradation of this fire-fighting foam is incomplete.

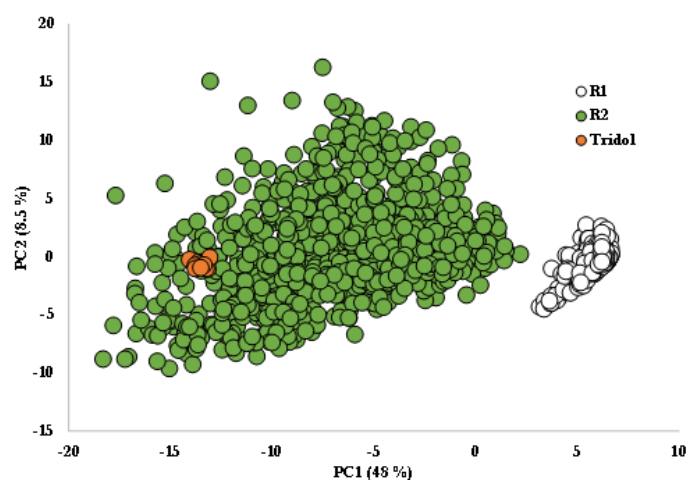


Figure 8. Scores plot for the first two principal components for a PCA conducted on Raman spectra (after baseline correction and SNV) obtained from measurements in reactors 1 and 2 during the biodegradation process of Tridol S3B, and Raman spectra of pure Tridol S3B

5. Conclusions

Fire-fighting foams are used on airports platforms to avoid airplanes fires to spread when they unfortunately occur. Some of these foams do contain surfactants based on polyfluoroalkyl ethers and are therefore considered as anthropogenic pollutants. As so, some abnormal amounts were measured both in ground and surface water, raising concerns on their persistence into the environment, their toxicity, their bioaccumulation, and hence about their environmental fate. To evaluate their biodegradation, a fire-fighting foam was selected and submitted to an OECD method from a guide dedicated to biodegradability, and related to protocol dedicated to the estimation of its biodegradability in compliance with NF EN ISO 9888 for the ultimate aerobic biodegradability of organic compounds in aqueous medium and in static conditions. In parallel, a Raman spectroscopy monitoring of the biodegradation process was implemented, coupled with a data analysis based on principal components analysis (PCA) to first identify a structure in the data, if any, along with the identification of chemical species related to polyfluoroalkyl ethers chemical bonds, easily detected because of the high polarizability of C-F bonds.

Results from OECD 301 F protocol indicated that over 97 % of the organic charge in the environmental matrix was consumed roughly after 12 days, justifying to put an end to the test. This process describes the degradation of the whole organic carbon present in the environmental matrix, without any distinction between anthropogenic chemicals compounds such as ethylene glycol, 2-(2-butoxyethoxy)ethanol, alkyl sulfate, ethanol, polyethylene glycol 400 also present besides polyfluoroalkyl ethers. The analysis of Raman spectra obtained from the spectroscopic monitoring did indicate a different environmental fate, in particular in the case of fluorinated molecules. The environmental matrix containing fluorinated molecules did have a significant different behavior than the one without them. And although it evolved towards an uncontaminated system, it never reached it, including after the 12 days of the OECD tests which indicated an almost total degradation. Investigations on eigenvectors issued from PCA calculations did reveal the presence of specific peaks (530 cm^{-1} , 765 cm^{-1} , 890 cm^{-1} , 980 cm^{-1}) consistent with chemical bonds in fluorinated molecules, indicated their persistence in the environmental matrix. The OECD protocol can be considered as adapted to globally identify the biodegradation of an organic compound, but as not properly adapted to precisely identify to what extent some chemical are persistent in the environment. Raman spectroscopy could then be considered as a relevant complement to the specific implemented protocol.

As a perspective, some additional biodegradation tests will be implemented including a spectroscopic monitoring. The tests will include fire-fighting foams and other chemicals used on airports. Besides the identification of any other partial biodegradation with respect to results obtained with a conventional OECD 301 F protocol, the objective will be to describe the kinetics of the biodegradation process along with the spectroscopic signatures and the concentration profiles of the chemicals present during the process. Such an analysis will also rely on chemometrics methods, including principal components analysis (PCA) and multivariate curve resolution (MCR), where each spectrum can be described as a linear combination of the signal of the chemical compound or a part of a sample (i.e. mixture) with a proper spectral signature.

Author Contributions:

	conceptualization	methodology	software	validation	formal analysis	investigation	resources	data curation	writing - original draft	writing - review & editing	visualization	supervision	project administration
Marchetti M	X	X	X		X	X		X	X	X	X	X	X
Offroy M	X	X	X	X	X				X	X	X	X	
Abdat F.	X	X	X		X	X	X		X	X	X	X	
Branchu P.		X		X						X			
Bourson P.				X						X			
Jobard C.						X	X			X			
Durmont J.-F.		X				X	X	X		X			
Casteran G.										X			X

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Conflicts of Interest: The authors declare no conflict of interest.

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