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# Determination of fluorinated surfactants and their metabolites in sewage sludge samples by liquid chromatography with mass spectrometry and tandem mass spectrometry after pressurised liquid extraction and separation on fluorine-modified reversed-phase sorbents

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**Abstract**

An analytical method was elaborated for simultaneous extraction and determination of fluorinated anionic and non-ionic surfactants in sewage sludge. Surfactant compounds were determined by liquid chromatography–mass spectrometry (LC–MS) after Soxhlet extraction, hot steam extraction and pressurised liquid extraction (PLE) using spiked sludge samples. PLE in a multiple-step procedure consisting of sequential use of ethyl acetate–dimethylformamide and methanol–phosphoric acid resulted in the most efficient extraction procedure. Quantitative analyses of the fluorinated anionic perfluorooctanesulfonate (PFOS) and the partly fluorinated non-ionic alkylpolyglycol ether (FAEO) surfactants were performed by selected ion monitoring LC–MS. Electrospray ionisation or atmospheric pressure chemical ionisation in negative or positive mode was performed. Recoveries between 105 and 120% could be reached. No PFOS and non-ionic FAEO surfactants in concentrations higher than 6 or 10 mg $kg^{-1}$ dry matter were observed in real environmental samples. Therefore aerobic and anaerobic biodegradation was performed to investigate the fate of fluorinated surfactants reaching wastewaters. Biological wastewater treatment in laboratory scale under aerobic or anaerobic conditions led to an elimination by biodegradation.


*Keywords:* Sewage sludge; Environmental analysis; Surfactants, fluorinated; Organofluorine compounds

## 1. Introduction

Among the organic chemicals with the highest production rates surfactants represent one of the major, most multi-purpose groups of organic compounds. Their worldwide production exceeds $9.86\times10^9$ kg per year [1,2]. Fluorine-containing surfactants, available as anionic, non-ionic, cationic and amphoteric surface-active compounds, cover a very small part—estimated total production till 2002 higher than $4\times10^6$ kg per year [3,4]—of this large production volume and the total spectrum of anthropogenic surfactants. Fluorinated surfactants may have the same structures as non-fluorine-containing compounds, yet at least one

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hydrogen atom in the hydrophobic segment of fluorinated surfactants is replaced by fluorine. This partial or total substitution of hydrogen in the hydrophobic segment of the surfactant molecule can lower the water surface tension far below the limit reached by conventional hydrocarbon-type surfactants.

The fluorinated surfactants are known as extremely resistant to chemical attack and therefore "can be used in media where conventional surfactants do not survive" [5], i.e., they are stable to heat, acids, and bases, as well as to reducing and oxidizing agents. This extraordinary stability therefore leads to their special uses, e.g., in fire-fighting foams [6–8] to extinguish fires at high-temperature. In addition, fluorinated surfactants are utilized in pesticides, cosmetics, adhesives, greases and lubricants where they exhibit unique properties, which make fluorinated surfactants irreplaceable in many of these applications. However, these fluorinated surfactants are not only stable against chemical and physical attacks, also persistence against biochemical attack has been reported [9,10].

After their application in aqueous systems, these fluorinated surfactants are reaching the environment either by release into rivers or via wastewater discharge into receiving waters. Predominantly, however, they become adsorbed to sewage sludge. Its use for land treatment or the disposal of sludge on dump sites leads to a remobilization of these recalcitrant compounds. Their polarity and mobility in water and soil allow them to reach the sea or groundwater in unaffected or non-degraded conditions.

Growing concern about the environmental persistence of fluorinated surfactants and their potential for bioaccumulation initiated research concerning the fate of these compounds, nevertheless the published number of papers is quite low. Determination of anionic fluorinated surfactants in aqueous media such as water, groundwater and biota has been favoured [6–14,32–35], whereas reports about non-ionic, cationic and amphoteric fluorinated compounds are missing. Reports regarding the presence of some of these compounds in wastewater [9,10] are rare and in the case of sewage sludge data do not yet exist. All public discussion about the origin of fluorinated surfactants and their main pathways into the environment therefore seems to be based on speculation.

Comprehensive work has been performed to monitor fluorinated surfactants in biota from different compartments of the environment to understand the distribution and the elevated concentrations observable today [11–14]. As a consequence of these results and the concentrations of perfluorooctanesulfonate (PFOS) observed in human blood samples from China, Europe, Japan and the USA [15] PFOS will be phased out in 2003 [16].

Different ecotoxicological or toxic effects arising from fluorinated surfactants have been reported, e.g., to remobilize and to transport other types of contaminants or to reduce biodegradation capacity of the activated sludge process [17,18] as well as to affect the anaerobic sludge digestion process during wastewater treatment [19]. Toxicity to rat liver was observed in in vivo experiments [20]. Additionally tumor promotion was reported [21].

Today the application of liquid chromatography (LC) in combination with mass spectrometry or tandem mass spectrometry (LC–MS or –MS–MS) to fluorinated surfactants permits their substance-specific determination in water, wastewater and biota [9,10,13]. Gas chromatography–mass spectrometry (GC–MS) was also applied to groundwater samples, however, derivatization prior to MS was essential [6]. Previously these compounds were determined by non-specific techniques [22,23]. Later substance-group-specific determination methods performed as surfactant analyses have been applied [24,25], but no reliable results were obtained [26].

As a consequence of the fact that analytical methods are available there arose scientific debates regarding these anthropogenic compounds with ecotoxicological potential. The increasing public concern about fluorinated surfactants in the environment could be observed in the newspapers [27–29]. But statements about the fate and pathways of these compounds into the environment could not yet be reliable. Methods for extraction and determination of fluorinated surfactants from water [6–8], wastewater [9,10] and biota samples [11–14] already exist. Methods to monitor input and output, and to balance the fate of fluorinated surfactants in the different steps of the wastewater treatment process, however, are not available, because quantitative extraction methods for the determination of these compounds from sewage sludge samples are missing.

Our objective therefore was first to develop a robust analytical method—an extraction and quantitative determination procedure providing recoveries above 90%—for anionics and non-ionics fluorinated surfactants. Flow injection analysis bypassing the analytical column in combination with mass spectrometry (FIA-MS) and LC–MS will be applied in order to monitor both surfactants and metabolites. Soft atmospheric pressure ionisation (API) techniques, such as atmospheric pressure chemical ionisation (APCI) and electrospray ionisation (ESI), in combination with MS–MS using collision-induced dissociation (CID), are used. Then such optimised extraction and determination techniques will be applied to real environmental sewage sludge samples collected from treatment plants from North-Rhine-Westphalia (NRW) in order to obtain reliable information about the presence of fluorinated surfactants in sewage sludge.

## 2. Experimental

### 2.1. Chemicals

Ultra-pure water was prepared by a Milli-Q system (Millipore, Milford, MA, USA). Ammonium acetate [$CH_3C(O)NH_4$], sodium sulfate ($Na_2SO_4$, activated at 650 °C prior to use), sodium azide ($NaN_3$), hydrochloric acid (HCl), nitric acid ($HNO_3$), phosphoric acid ($H_3PO_4$), sulfuric acid ($H_2SO_4$), formic acid (HCOOH), acetic acid ($CH_3COOH$), trifluoroacetic acid (TFA, $CF_3COOH$), chloroform ($CHCl_3$), dimethylformamide [DMF, $HC(O)N(CH_3)_2$], 2-propanol [$(CH_3)_2CHOH$], diethyl amine [$(C_2H_5)_2NH$], pyridine ($C_5H_5N$) and tetrahydrofuran (THF, $C_4H_8O$) were of "analytical reagent grade" (Merck, Darmstadt, Germany). Hydrogen peroxide solution 30% ($H_2O_2$) was of "medical extra pure" grade (Merck). Ethyl acetate (EtOAc, $CH_3C(O)OC_2H_5$) and methanol (MeOH, $CH_3OH$) were of "residue analysis" purity grade, while 1,4-dioxane were "nanograde" solvents, all from Promochem (Wesel, Germany). *tert*.-Butyl methyl ether [MTBE, $(CH_3)_3COCH_3$] (Aldrich, Taufkirchen, Germany) was a HPLC grade of 99.999% purity. Nitrogen gas ($N_2$) applied for the evaporation of the organic solvents, drying of solid-phase cartridges and as sheath gas in APCI ionisation was of 5.0 purity (Linde, Germany) and argon used as collision gas was of technical grade (Linde).

### 2.2. Standards

Anionic fluorinated surfactants (cf. Table 1) applied for qualitative and quantitative examinations were industrial blends. Standards used for qualitative applications: perfluorooctanesulfonate (PFOS): Bayowet FT 208, FT 248 and FT 800 (Bayer, Germany), Fluorad FC-93, 95, and 99 (3M, Germany), 9,9,10,10,11,11,12,12,13,13,13-undecylfluorooctane-1-sulfonate: Fluorad FC-98 (3M), perfluorohexanesulfonate (PFHxS): Fluorad FC-100 (3M), perfluorodecanesulfonate (PFDS): Fluorad FC-120 (3M), perfluorooctanoic acid (PFOA): Fluorad FC-143 (3M) and *N*-ethyl-*N*-(heptadecafluorooctane)-sulfonyl-glycinic acid: Fluorad FC-129.

Anionic standards for calibration purposes and for recovery experiments with sludge: PFOS (Fluorad FC-95) and PFOA.

Non-ionic fluorinated surfactants applied for qualitative and quantitative examinations were also industrial blends. Standards used for qualitative applications: perfluorooctane sulfonylamidopolyethoxylate:Fluorad FC-170C (3M), perfluorooctane sulfonylamido polyethoxylate methyl ether: Fluorad FC-171 (3M), partly fluorinated alkylethoxylates (2-perfluoroalkylethanol polyglycolether (FAEO), $C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–H ($n$=6,8,10) Fluowet OTN (Hoechst, Germany). All fluorinated surfactants were gifts from companies cited above.

Fluowet OTN (industrial blend) was used as standard for calibration purposes and for recovery experiments with sludge.

The biochemical degradation products (metabolites) of partly fluorinated alkylethoxylates were obtained by aerobic biochemical degradation of precursor compounds using a wastewater slurry with sewage treatment plant (STP) sludge from Aachen-Soers.

The stock solutions of the non-ionic FAEO and the anionic PFOS or PFOA standards were prepared by dissolution of the liquid compounds (industrial blends) in methanol. From these stock solutions, working solutions with concentrations of 1, 5, 10, 50 and 100 μg/ml for FIA-MS and 1, 2, 3, 5, 10 and 25 μg/ml for LC–MS were prepared by means of serial dilution. Each standard solution was analysed by MS in FIA

Table 1
Determinations of fluorinated surfactants—interfaces, ionisation modes and ions recorded for qualitative and quantitative determinations

| No. | Type of surfactant | General formula/Abbreviation | Systematic name | Trade name | Interface | Ionisation mode (+/−) | Molecular or adduct ion(s) (*m*/*z*) | Recorded ion(s) (*m*/*z*) |
|---|---|---|---|---|---|---|---|---|
| 1 | Anionic | $C_6F_{13}$–$SO_3^-H^+$/PFHxS | Perfluorohexanesulfonate | Fluorad FC-100 | ESI | − | 399 | 399 |
| 2 | Anionic | $C_8F_{17}$–$SO_3^-H^+$/PFOS | Perfluorooctanesulfonate | Fluorad FC-95 | ESI | – | 499 | 499 |
| 3 | Anionic | $C_{10}F_{21}$–$SO_3^-H^+$/PFDS | Perfluorodecanesulfonate | Fluorad FC-120 | ESI | − | 599 | 599 |
| 4 | Anionic | $C_7F_{15}$–$COO^-$ $H^+$/PFOA | Perfluorooctanoic acid | Fluorad FC-143 | ESI | − | 413 | 413 |
| 5 | Anionic | $C_5F_{11}$–$(CH_2)_8$–$SO_3H$ | 9,9,10,10,11,11,12,12,13,13,13-Undecylfluorotredecane-1-sulfonate | Fluorad FC-98 | ESI | − | 461 | 461 |
| 6 | Anionic | $C_8F_{17}$–$SO_2$–$N(C_2H_5)$–$CH_2$–$COO^-$ $H^+$ | *N*-Ethyl-*N*-(heptadecafluoro-octane)-sulfonyl-glycinic acid | Fluorad FC-129 | ESI | − | 584 | 584 |
| 7 | Non-ionic | $C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–OH | 5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Heptadecafluoro-decane sulfonylamido polyethoxylate | Fluorad FC-170C | APCI | + | 677+Δ 44 | 677–941 (Δ 44) |
| 8 | Non-ionic | $C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–$OCH_3$ | 5,5,6,6,7,7,8,8,9,9,10,10,11,11,12,12,12-Heptadecafluoro-decane sulfonylamido polyethoxylate methyl ether | Fluorad FC-171 | APCI | + | 691+Δ 44 | 691–1131 (Δ 44) |
| 9 | Non-ionic | $C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–OH ($n = 6,8,10$)/(FAEO) | Partly fluorinated alkyl-ethoxylates (2-perfluoroalkyl-ethanol polyglycol ether) | Fluowet OTN | APCI | + | 514+Δ 44 | 514–954 (Δ 44) |
| 10 | Surfactant metabolites | $C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–$OCH_2COOH$ | Metabolites of partly fluorinated alkylethoxylates | – | APCI | + | 528+Δ 44 | 528–704 (Δ 44) |

mode or after LC separation on a reversed-phase (RP) $C_{18}$ or on a perfluorinated RP-$C_8$ column.

### 2.3. Sample preparation

#### 2.3.1. Extraction and pre-treatment methods

In order to estimate extraction efficiencies of different extraction methods activated and digested sludge spiked with FAEO, PFOS and PFOA at concentration levels of about 150 μg $g^{-1}$ dry solid matter were submitted to Soxhlet extraction, hot vapour extraction and pressurised liquid extraction (PLE; Dionex trade name ASE, for accelerated solvent extraction). All extracts were brought to dryness under reduced pressure [∼10 Torr (1 Torr=133.322 Pa)] before they were dissolved by a fixed volume of methanol (25 ml). Aliquots of these solutions were analysed by FIA and LC–MS without prior clean-up.

Freeze-dried spiked STP sludge samples (2 g) as described in Section 2.3.3 were extracted using Soxhlet or hot vapour extraction devices and applying solvents or their mixtures which we regarded as effective, e.g., ethyl acetate and methanol partly modified with hydrochloric acid (1% HCl; cf. Table 2). The extracts obtained were concentrated to 10 ml under reduced pressure.

Spiked freeze-dried STP sludge samples (2 g) with known concentrations of FAEO, PFOS and PFOA were extracted by PLE using different solvents or mixtures of these, e.g., dimethylformamide, ethyl acetate, methanol 1,4-dioxane, pyridine, *tert*.-butyl methyl ether and tetrahydrofuran. Temperature was adjusted to 150 °C while pressure applied was varied between 10 714 and 14 285 kPa (cf. Table 2). The recovery examinations applied to the spiked digested sludge samples were optimised prior to their application to real environmental samples.

#### 2.3.2. Preparation of reference materials (spiked extracted samples)

For the development of a quantitative FIA and LC–MS procedure, sewage sludge samples were sequentially extracted with mixtures of ethyl acetate–dimethylformamide (EtOAc–DMF) and methanol–phosphoric acid (MeOH–$H_3PO_4$) using a Dionex ASE 200 system (Dionex, Sunnyvale, CA, USA) in combination with a solvent controller (Dionex). Lyophilised sludge (2 g) was extracted (cf. Table 2, sample 1) and the obtained sludge extracts were brought to a volume of 25.0 ml. FAEO, PFOS and PFOA were added to reach concentration levels of 50 μg $ml^{-1}$. These solutions were subsequently used to develop and optimise FIA and LC–MS procedures.

#### 2.3.3. Preparation of reference materials (blank and spiked sewage sludge samples)

Samples of sewage sludge—activated sludge and anaerobically stabilised sludge—were applied in this study. The sludge samples were known as non-polluted i.e., anionic and non-ionic surfactants were ≤5 μg $g^{-1}$ dry solid matter because of the predominantly municipal discharges contained in the STP inflow. The scheme for preparation of blank and spiked sludge samples was presented in the literature [31] (Fig. 1A–E). Deactivation was performed by adding about 500 mg of sodium azide to the centrifuged sludge. The preparation procedure of blank and spiked sewage sludge samples with known concentrations of anionics and non-ionic fluorinated surfactants was performed as described in the literature [31]. Dry solid matter was determined of lyophilised and ground sludge samples. The absolute total mass concentrations of FAEO, PFOS and PFOA in μg $g^{-1}$ dry solid matter were calculated in each spiked sewage sludge sample. From the quantities of surfactants added and the quantities of sludge obtained after freeze drying the concentration of non-ionic FAEO were calculated with 147 μg $g^{-1}$ dry solid matter while for anionics—PFOS and PFOA—the concentrations were calculated with 148 and 151 μg $g^{-1}$ dry solid matter, respectively.

#### 2.3.4. Oxidative treatment of spiked sludge extracts or sludge samples

Sludge extracts obtained from PLE extraction of unpolluted digested sludge were brought to dryness before they were reconstituted in 5 ml methanol and spiked with fluorinated surfactants (100 μg $ml^{-1}$/surfactant type). The obtained methanolic solution (5 ml) was mixed with oxidation reagents (4 ml) such as hydrogen peroxide–sulfuric acid (1:1, v/v) or hydrochloric acid–nitric acid (3:1, v/v). Mixtures of methanol–water (1:1, v/v, 5 ml) were spiked with the same surfactants (100 μg $ml^{-1}$/surfactant type)

Table 2
Anionic and non-ionic fluorinated surfactant-spiked sludge samples—extraction methods, solvents or solvent mixtures, extraction and separation conditions applied and the obtained recoveries

| Sample no. | Extraction method | Solvent or solvent mixtures Extraction step | | | | Extraction conditions | | LC separation | Recoveries[a] (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | Temperature $T$ (°C) | Pressure $p$ (kPa) | | Non-ionics | Anionics |
| 1 (Blank)[b] | PLE | EtOAc–DMF (8:2) | MeOH–$H_3PO_4$ (99:1) | MeOH/$H_3PO_4$ (99:1) | MeOH/$H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | n.d.[c] | n.d.[c] |
| 2 (Blank)[b] | PLE | EtOAc–DMF (8:2) | MeOH–$H_3PO_4$ (99:1) | MeOH/$H_3PO_4$ (99:1) | MeOH/$H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | n.d.[c] | n.d.[c] |
| 3[b,e] | Hot vapour | EtOAc | – | – | – | b.p. EtOAc | – | RP-$C_{18}$ | 38 | 19 |
| 4[b,e] | Hot vapour | MeOH | – | – | – | b.p. MeOH | – | RP-$C_{18}$ | 45 | 16 |
| 5[b,e] | Hot vapour | MeOH–HCl (99:1) | – | – | – | b.p. MeOH/HCl | – | RP-$C_{18}$ | 45 | 52 |
| 6[f,g] | Hot vapour | MeOH–HCl (99:1) | – | – | – | b.p. MeOH/HCl | – | RP-$C_{18}$ | <1 | 7 |
| 7[b,e] | Soxhlet | EtOAc | – | – | – | b.p. EtOAc | – | RP-$C_{18}$ | 28 | 13 |
| 8[b,e] | Soxhlet | MeOH | – | – | – | b.p. MeOH | – | RP-$C_{18}$ | 25 | 7 |
| 9[b,e] | Soxhlet | MeOH–HCl (99:1) | – | – | – | b.p. MeOH/HCl | – | RP-$C_{18}$ | 35 | 48 |
| 10[f,g] | Soxhlet | MeOH–HCl (99:1) | – | – | – | b.p. MeOH/HCl | – | RP-$C_{18}$ | 4 | 9 |
| 11[b] | PLE | EtOAc | MeOH–$H_3PO_4$ (99:1) | MeOH/$H_3PO_4$ (99:1) | – | 150 | 14 285 | RP-$C_{18}$ | 98 | 107 |
| 12[b] | PLE | MeOH–$H_3PO_4$ (99:1) | EtOAc | – | – | 150 | 14 285 | RP-$C_{18}$ | 24 | 57 |
| 13[b] | PLE | MeOH–pyridine (95:5) | EtOAc–pyridine (95:5) | – | – | 150 | 14 285 | RP-$C_{18}$ | 40 | 34 |
| 14[b] | PLE | EtOAc–Dioxan (95:5) | – | – | – | 150 | 14 285 | RP-$C_{18}$ | 33 | 8 |
| 15[b] | PLE | EtOAc | – | – | – | 150 | 14 285 | RP-$C_{18}$ | 38 | 7 |
| 16[b] | PLE | MeOH | – | – | – | 150 | 14 285 | RP-$C_{18}$ | 43 | 13 |
| 17[b] | PLE | EtOAc | MeOH | – | – | 150 | 14 285 | RP-$C_{18}$ | 32 | 18 |
| 18[b] | PLE | EtOAc–MTBE (9:1) | – | – | – | 150 | 14 285 | RP-$C_{18}$ | 33 | 25 |
| 19[b] | PLE | EtOAc | MeOH–$H_3PO_4$ (99:1) | MeOH/$H_3PO_4$ (99:1) | – | 150 | 14 285 | RP-$C_{18}$ | 60 | 115 |
| 20[b] | PLE | EtOAc | EtOAc | MeOH/pyridine (95:5) | – | 150 | 14 285 | RP-$C_{18}$ | 50 | 82 |
| 21[b] | PLE | EtOAc | MeOH–pyridine (95:5) | MeOH–pyridine (95:5) | – | 150 | 14 285 | RP-$C_{18}$ | 50 | 82 |
| 22[b] | PLE | EtOAc | EtOAc | MeOH–pyridine (95:5) | – | 150 | 14 285 | RP-$C_{18}$ | 49 | 109 |
| 23[b] | PLE | EtOAc | MeOH–THF (9:1) | MeOH–$H_3PO_4$ (99:1) | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 38 | 41 |
| 24[b] | PLE | EtOAc | MeOH–MTBE (9:1) | MeOH–$H_3PO_4$ (99:1) | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 43 | 39 |
| 25[b] | PLE | EtOAc | MeOH–dioxane (9:1) | MeOH–$H_3PO_4$ (99:1) | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 47 | 41 |
| 26[b] | PLE | EtOAc | MeOH–$H_3PO_4$ (99:1) | MeOH–$H_3PO_4$ (99:1) | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 80 | 222 |
| 27[b] | PLE | EtOAc–DMF (8:2) | MeOH–$H_3PO_4$ (99:1) | MeOH–$H_3PO_4$ (99:1) | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 91 | 254 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 28[b] | PLE | EtOAc | EtOAc | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 67 | 207 |
| 29[b] | PLE | EtOAc | EtOAc | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 34 | 49 |
| 30[b] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | – | 150 | 14 285 | RP-$C_{18}$ | 17 | 47 |
| 31[b] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 10 714 | RP-$C_{18}$ | 77 | 118 |
| 32[b] | PLE | EtOAc–DMF (6:4) | $MeOH–H_3PO_4$ (95:1) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 10 714 | RP-$C_{18}$ | 66 | 232 |
| 33[b] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (95:5) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_{18}$ | 117 | 319 |
| 34[b] | PLE | EtOAc | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | 87 | 122 |
| 35[b] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | 90 | 119 |
| 36[b] | PLE | EtOAc | EtOAc | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | 77 | 106 |
| 37[b] | PLE | EtOAc | EtOAc | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | 45 | 56 |
| 38[b] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | – | 150 | 14 285 | RP-$C_8$[d] | 16 | 52 |
| 39[b] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 10 714 | RP-$C_8$[d] | 77 | 118 |
| 40[b] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (95:5) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | 105 | 119 |
| 41[g] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | 10 | 19 |
| 42[g] | PLE | EtOAc–DMF (8:2) | $MeOH–H_3PO_4$ (95:5) | $MeOH–H_3PO_4$ (99:1) | $MeOH–H_3PO_4$ (99:1) | 150 | 14 285 | RP-$C_8$[d] | 10 | 11 |

[a] Performed as triplicates.

[b] Digested sludge.

[c] n.d., concentration below LOD = 10 mg $kg^{-1}$ dry residue for FAEO and 6 mg $kg^{-1}$ dry residue for PFOS, PFOA.

[d] Column filled with perfluorinated RP materials.

[e] Extraction time: 6 h.

[f] Extraction time: 12 h.

[g] Activated sludge.

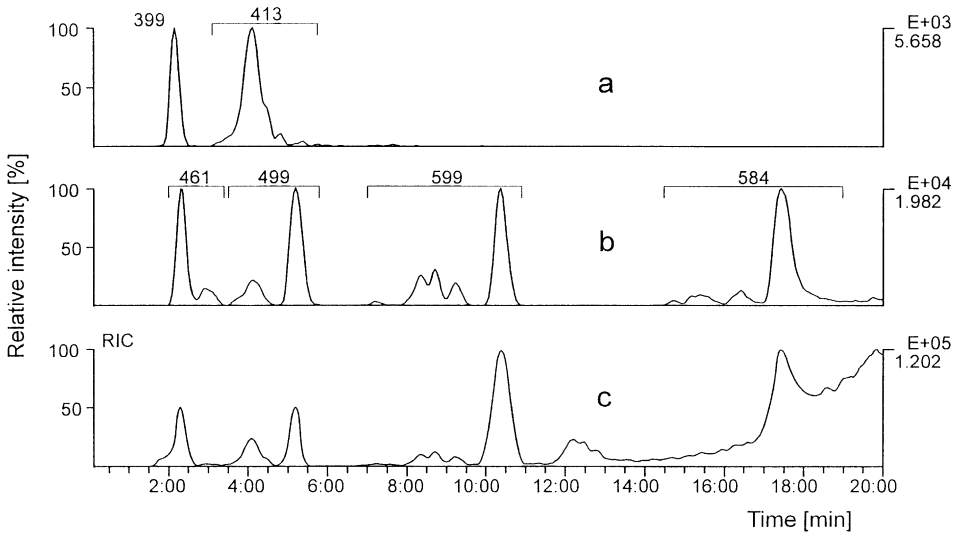


Fig. 1. LC–ESI(−)-MS total-ion current tracing (c) for spiked PLE extract containing the anionic fluorinated surfactants perfluorohexanesulfonate (PFHxS) ($C_6F_{13}$–$SO_3^-H^+$), perfluorooctanesulfonate (PFOS) ($C_8F_{17}$–$SO_3^-H^+$),perfluorodecanesulfonate (PFDS) ($C_{10}F_{21}$–$SO_3^-H^+$), perfluorooctanoic acid (PFOA) ($C_7F_{15}$–$COO^-$ $H^+$) 9,9,10,10,11,11,12,12,13,13,13-undecylfluorotredecane-1-sulfonate [$C_5F_{11}$–$(CH_2)_8$–$SO_3^-H^+$], and *N*-ethyl-*N*-(heptadecafluorooctane)sulfonylglycinic acid [$C_8F_{17}$–$SO_2$–$N(C_2H_5)$–$CH_2$–$COO^-$ $H^+$]; separation by gradient elution performed on perfluorinated RP-$C_8$ column. (a) LC–ESI(−)-MS selected ion current tracings for PFHxS (*m/z* 399) and PFOA (*m/z* 413) from mixture of fluorinated surfactants blends as in (c). (b) LC–ESI(−)-MS selected ion current tracings for $C_5F_{11}$–$(CH_2)_8$–$SO_3H$ (*m/z* 461), PFOS (*m/z* 499), PFDS (*m/z* 599) and $C_8F_{17}$–$SO_2$–$N(C_2H_5)$–$CH_2$–$COO^-$ $H^+$ (*m/z* 584) as in (a). For LC–MS conditions, see Section 2.

before they were subjected to oxidation at different temperatures over a period of 30 min. The reaction mixtures were brought to pH 6 adding aqueous sodium hydroxide before extraction using $C_{18}$-SPE. The cartridges were eluted with 10 ml of methanol and the solution was reduced to a volume of 5 ml in a gentle steam of $N_2$. This solution was applied to a FIA screening and LC–MS analysis (cf. Table 3).

Oxidative destruction of the sludge, bypassing the extraction process was also performed by treating samples (1.5 g) of spiked digested sludge with 6 ml of hydrogen peroxide–sulfuric acid (1:1, v/v) while a mixture of hydrochloric acid–nitric acid (10 ml; 3:1, v/v) was used to oxidise the sludge matrix of spiked STP sludge samples (1 g). Temperatures were adjusted to 70 °C (cf. Table 3). After oxidative digestion of the sludge, the acidic solutions were adjusted to pH 6 adding aqueous sodium hydroxide. The organic compounds were concentrated with $C_{18}$-SPE and eluted with methanol. These eluates were for LC–MS measurements.

### 2.3.5. *Determination of FAEO, PFOS and PFOA in real environmental STP sludge samples*

Lyophilised STP sludge samples (80) obtained from the Ministry for Environment and Nature Conservation, Agriculture and Consumer Protection of the state of NRW were handled as described previously [31]. Portions of 2 g were extracted using the optimised PLE method (cf. Table 2, sample no. 40). The volume-reduced extracts were submitted to FIA and LC–MS determination without prior clean-up.

## 2.4. *Gas chromatographic analysis*

GC–electron-capture detection (ECD) analyses were performed on a Varian (Darmstadt, Germany) Model 3400 GC system equipped with a fused-silica capillary column. The conditions were as follows: carrier gas, nitrogen; linear gas velocity, 42 cm $s^{-1}$; injector temperature, 280 °C; detector temperature, 280 °C; column, DB-XLB (J&W Scientific, Folsom, CA, USA), film thickness 0.5 μm (30 m×0.32 mm I.D.). For analysis 50 μl splitless injections were

Table 3
Oxidative treatment of sludge extracts (PLE) and sludge samples spiked with fluorinated surfactants

| Sample no. | Extraction method | PLE sludge extracts obtained by application of | | | Treatment[a] | | Concentrated from aqueous phase by | LC separation | Recoveries[a] (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Solvents for extraction | Temperature $T$ (°C) | Pressure $p$ (kPa) | Oxidation reagents | Temperature $T$ (°C) | | | Non-ionics | An-ionics |
| 1 (Blank) | PLE | 1×EtOAc–DMF (8:2) 3×MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | – | – | – | RP-$C_{18}$ | n.d.[b] | n.d.[b] |
| 2 (Blank) | PLE | 1×EtOAc–DMF (8:2) 3×MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | – | – | – | RP-$C_8$[c] | n.d.[b] | n.d.[b] |
| 3 | PLE | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 79 | 82 |
| 4 | PLE | EtOAc | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 75 | 81 |
| 5 | PLE | EtOAc–DMF (8:2) | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 72 | 91 |
| 6 | PLE | EtOAc–DMF (8:2) | 150 | 14 285 | HCl–$HNO_3$ (3:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 70 | 71 |
| 7 | PLE | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 3 | 43 |
| 8 | PLE | EtOAc–HCOOH (9:1) | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 50 | n.d.[b] |
| 9 | PLE | EtOAc | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 11 | 17 |
| 10 | PLE | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 29 | 15 |
| 11 | PLE | MeOH–$H_3PO_4$ (99:1) | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 28 | 145 |
| 12 | PLE | EtOAc | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 79 | 7 |
| 13 | PLE | Dioxan | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 40 | 6 |
| 14 | PLE | MeOH–pyridine (95:5) | 150 | 14 285 | $H_2O_2$–$H_2SO_4$ (1:1) | 50 | $C_{18}$-SPE | RP-$C_{18}$ | 23 | 9 |
| 15[d] | – | – | – | – | $H_2O_2$–$H_2SO_4$ (1:1) | 70 | $C_{18}$-SPE | RP-$C_{18}$ | 91[e] | 87[e] |
| 16[d] | – | – | – | – | $H_2O_2$–$H_2SO_4$ (1:1) | 70 | $C_{18}$-SPE | RP-$C_8$[c] | 101[e] | 97[e] |
| 17[d] | – | – | – | – | HCl–$HNO_3$ (3:1) | 70 | $C_{18}$-SPE | RP-$C_{18}$ | 90[e] | 85[e] |
| 18[d] | – | – | – | – | HCl–$HNO_3$ (3:1) | 70 | $C_{18}$-SPE | RP-$C_8$[c] | 97[e] | 98[e] |
| 19 | Destruction[f] | – | – | – | $H_2O_2$–$H_2SO_4$ (1:1) | 70 | $C_{18}$-SPE | RP-$C_{18}$ | 24[e] | 57[e] |
| 20 | Destruction[f] | – | – | – | HCl–$HNO_3$ (3:1) | 70 | $C_{18}$-SPE | RP-$C_{18}$ | n.d.[b,e] | n.d.[b,e] |

[a] Performed as triplicates.
[b] n.d., concentration below LOD = 0.5 μg $ml^{-1}$ for FAEO and 0.48 μg $ml^{-1}$ for PFOS, PFOA.
[c] Column filled with perfluorinated RP materials.
[d] Surfactant-spiked ultra-pure water–methanol.
[e] Performed as duplicates.
[f] Oxidative destruction of sludge matrix.

made. GC conditions were programmed as follows: initial oven temperature 50 °C, kept for 3 min, increased to 150 °C at 5 °C $min^{-1}$ within 10 min.

### 2.5. Flow injection analysis

For coupling of FIA and MS, APCI or ESI both from Finnigan (San Jose, CA, USA) were applied for the determination of the non-ionic or anionic fluorinated surfactants.

The conditions in FIA–MS and –MS–MS on a TSQ 700 bypassing the analytical column were as follows: injection volume, 10 μl; mobile phase methanol–water (30:70) containing 0,05 *M* ammonium acetate. The overall flow-rate was 0.6 ml $min^{-1}$ (Waters Model 510 pump). FIA–MS analysis was performed by scanning at 1 s from 200 to 1200 u.

### 2.6. Liquid chromatographic conditions

LC separations of fluorinated surfactants were carried out with a Multospher 100 RP 5-5 column ($C_8$, 5 μm, spherical; 250×4.6 mm I.D.) from CS Chromatographie Service (Langerwehe, Germany) or using a PF-$C_8$ column (150×4.6 mm I.D.) filled with spherical perfluorinated RP-$C_8$ material (5 μm) from Fluor Technologies (FTI), which was bought from ICT (Bad Homburg, Germany). For elution of non-ionic, anionic and amphoteric fluorinated compounds methanol (A) in combination with a mixture of methanol–Milli-Q-purified water (20:80; v:v) (B) was applied. The gradient for separation of non-ionics on both the RP-$C_{18}$ and perfluorinated RP-$C_8$ columns was programmed as follows: starting with A–B (60:40) the concentration was changed linearly to A–B (90:10) within 12 min. Up to 30 min the composition was kept constant. The overall flow-rate was adjusted to 0.8 ml $min^{-1}$.

The gradient for separation of anionic and amphoteric surfactants on both the RP-$C_{18}$ and perfluorinated RP-$C_8$ column was programmed as follows: starting with 20% A the concentration was changed linearly to 90% A within 12 min. Up to 30 min the composition was kept constant. Anionic surfactants were separated on perfluorinated RP-$C_8$ column using a concentration of 2 m*M* diethyl ammonium acetate for ion-pairing purpose. The overall flow-rate was adjusted to 0.8 ml $min^{-1}$.

LC separations were achieved with a SpectraSystem P4000 pump [Thermo Separation Products (TSP), San Jose, CA, USA]. A Waters Model 510 pump was used for post-column addition of 0.1 *M* ammonium acetate solution in the APCI mode. A Waters 996 photodiode array detection (DAD) system in combination with a Millenium 2010 data system (Millipore) was connected in-line with the APCI or ESI interface.

Applying APCI, 0.2 ml $min^{-1}$ of 0.1 *M* ammonium acetate was added after passing the DAD system, resulting in an overall flow-rate of 1.0 ml $min^{-1}$. In ESI mode 0.2 ml $min^{-1}$ of eluent was added after passing the column prior to DAD. The flow split ratio then was adjusted to 1:2 in favour of the MS in the ESI mode compared to waste.

Applying LC–MS, 10 μl of standard solutions or extracts of sludge were injected onto the column.

For column cleaning purposes, the RP-$C_{18}$ and perfluorinated RP-$C_8$ column were cleaned with a mixture of equivalent amounts of chloroform, methanol, tetrahydrofuran and 2-propanol modified with 0.5% of TFA to minimise retention time shifting.

### 2.7. MS and MS–MS systems

A TSQ 700 mass spectrometer combined with a DEC 5000/33 data station was used for research work and the following conditions for APCI ionisation using ammonium acetate were chosen: vaporizer temperature, 400 °C; capillary temperature, 180 °C. Corona voltage was operated at 5 kV. The potential of capillary, tube lens and API octapole were chosen as 50, 50 or −3 V, respectively. Sheath gas pressure was operated at $2.81\times10^5$ Pa. Under the above-mentioned conditions, the ion source pressure was 0.3 Torr, and the pressure in the vacuum system of the mass spectrometer was $2\times10^{-5}$ Torr.

The electron multiplier operated at 1200 V and the conversion dynode at 15 kV. In the MS–MS mode the ion source pressure was 0.5 Torr. Under CID conditions the pressure in quadrupole 2 (collision cell) was, unless otherwise specified in the captions to the figures, 1.3 mTorr. The collision energy was adjusted from −10 to −50 eV. The electron multiplier voltage in quadrupole 3 varied between 1200 and 1700 V with a conversion dynode voltage at 15 kV.

Low-resolution FIA and LC analyses on the TSQ 700 were performed, recording APCI or ESI mass

spectra scanning from 100 to 1200 u at 1 or 3 s, respectively. FIA bypassing the analytical column with MS or MS–MS (daughter- and parent ion-mode) detection was performed accumulating 50 scans. The mass spectrum averaging the total ion current from the beginning of the signal up to the end in FIA–MS mode was termed "overview spectrum". APCI and ESI ionisation on the TSQ 700 were first checked in positive and negative MS or MS–MS mode followed by determinations adjusting the conditions with highest sensitivity and efficiency (cf. Table 1).

### 2.8. Quantification by MS

Quantification was performed by means of calibration curves and reconstructed by results obtained in selected ion monitoring (SIM) detection mode [selected ions (cf. Table 1) in APCI(+): FAEO (OTN): *m/z* 514–954 ($\Delta m/z$ 44); metabolites of OTN: *m/z* 528–748 ($\Delta m/z$ 44); selected ions in ESI(−): PFOS: *m/z* 499 and PFOA: *m/z* 413].

Recoveries were determined by summation of areas of the fluorinated surfactants in the SIM traces of the confirmation ions in FIA–MS or LC–MS mode.

Retention times ($T_R$) observed with calibration standards were used for additional confirmation in the LC–MS mode.

In FIA–MS mode the calibration curves for non-ionic FAEO and their metabolites were linear over a concentration range of 5–100 μg ml$^{-1}$ [FIA–MS: $r^2$=0.9877 (FAEO) and $r^2$=0.9810 (metabolites of FAEO); $r^2$=0.9777 (PFOS) and $r^2$=0.9883 (PFOA)]. Calibration curves for FAEO, PFOS and PFOA obtained after separation on RP-$C_{18}$ or perfluorinated RP-$C_8$ phase prior to LC–MS were also linear over a concentration range of 2–25 μg ml$^{-1}$. [RP-$C_{18}$: $r^2$=0.9977 (FAEO) and $r^2$=0.9968 (metabolites of FAEO); $r^2$=0.9959 (PFOS) and $r^2$=0.9961 (PFOA); perfluorinated RP-$C_8$: $r^2$=0.9974 (FAEO) and $r^2$=0.9988 (metabolites of FAEO); $r^2$=0.9989 (PFOS) and $r^2$=0.9937 (PFOA)]. The detection limits (LODs) in the LC–MS mode were calculated by a signal-to-noise ratio of 3 (*S/N* 3:1) taking into account the amount of sample extracted, the volume of the extract analysed and the absolute spiking quantities. LODs of the compounds dissolved in the spiked extracts or in the extracts of spiked sludge samples were estimated to 0.5 μg ml$^{-1}$ for FAEO and 0.48 μg ml$^{-1}$ for PFOS, PFOA or partly fluorinated sulfonates [limit of quantification (LOQ): 1.0 μg ml$^{-1}$]. The LODs of the three compounds, FAEO, PFOS and PFOA in spiked sewage sludge, determined by LC–MS, were calculated to 10 mg kg$^{-1}$ dry residue (LOQ: 20 mg kg$^{-1}$) for non-ionic or 6 mg kg$^{-1}$ dry residue (LOQ: 10 mg kg$^{-1}$) for anionic surfactant compounds, respectively. The metabolites of partly FAEO compounds (Fluowet OTN) were quantified by using a $C_{18}$-SPE concentrated mixture as standard obtained after biodegradation of precursor blend.

Each concentration of FAEO, PFOS and PFOA standards applied to establish calibration curves had been checked by a 10- or 3-fold determination in the FIA–MS or LC–MS SIM mode, respectively. Then the entire procedure of extraction and determination of these compounds was validated by 5-fold FIA or 3-fold LC–MS determinations. The analyses in the Soxhlet, hot vapour or PLE screening examinations using spiked sludge samples and the analyses of real environmental sludge samples all were performed in triplicate.

### 2.9. Sampling areas

Wastewater from sewage treatment plant (STP) effluent mixed with effluent wastewater of the pre-settling tank of Aachen-Soers municipal STP of the city of Aachen, Germany, was taken in order to cultivate the immobilized aerobic biocoenosis. Effluent of the sludge stabilization tank of the same STP were used for anaerobic degradation experiments. Glass foam beads (SIRAN Carrier No. 023/02/300) produced by Schott Engineering (Mainz, Germany) were used for immobilization of both, aerobic and anaerobic biocoenoses. All parts of the reactors were made of glass and the pipes were made of PTFE [30].

Two types of non-polluted sewage sludge—activated sludge and anaerobically stabilised sludge—were taken from the aeration tanks or anaerobic sludge stabilisation tank of Aachen-Soers STP.

Lyophilised anaerobically stabilised sludge samples taken from different municipal wastewater treatment plants of NRW were obtained from the Ministry for Environment and Nature Conservation, Agriculture and Consumer Protection of NRW. Samples of activated sewage sludge and anaerobically stabilised

sludge, not contaminated with fluorinated surfactants, were taken from Aachen-Soers STP.

### 2.10. Biodegradation experiments

The laboratory scale reactors for aerobic and anaerobic treatment, spiking methods and how to obtain wastewater samples were already described [30]. The different types of fluorinated surfactants selected for biodegradation were dissolved in 1 ml of methanol or water–methanol before they were added into the reactors. The absolute quantities of surfactants spiked into the wastewater contained in the biodegradation devices were chosen in order to reach an initial concentration of 10 or 5 mg $l^{-1}$ of FAEO compounds and their methylated derivatives, PFOS, PFOA and partly fluorinated sulfonates.

After spiking the wastewaters contained in the aerobic or anaerobic biodegradation units the reaction media were mixed by stirring before the first samples, representing the initial concentration, were taken. A standardised sampling period of 24 h was chosen and was modified in accordance with the different degrees of biodegradability observed. In order to monitor FAEO, PFOS, PFOA and partly fluorinated sulfonates, 10 ml wastewater samples were taken from the biodegradation reactors. Commercially available solid-phase extraction (SPE) cartridges filled with $C_{18}$ material from Mallinkrodt Baker (Deventer, The Netherlands) were used for concentration of the surfactants and their metabolites. SPE cartridges filled with 100 mg of $C_{18}$ material were conditioned as prescribed by the manufacturer (5 void volumes of methanol followed by 5 void volumes of ultra-pure water) before they were applied for extraction and concentration of 7 mg of organic carbon per cartridge. The glass-fibre filters used for the pre-treatment of the wastewater samples were obtained from Schleicher & Schüll (Dassel, Germany). Before use, the glass-fibre filters were heated to 400 °C.

The samples were frozen and stored at −80 °C before SPE extraction, if not extracted immediately after collection. After the extraction procedure the cartridges were rinsed with two column volumes of purified water and dried in a gentle stream of nitrogen. 2 ml of methanol in three portions were applied for eluting analytes. Without further pretreatment these eluates could be used for FIA– and LC–MS measurements.

To ensure anaerobic conditions a redox potential below −380 mV was adjusted by addition of aqueous sodium acetate added in a concentration of 50 mg $l^{-1}$. The anaerobic conditions were controlled by electrode measurement.

For aerobic treatment diffused aeration using compressed air adjusted to 2 l $min^{-1}$ was administered into the aerobic lab-scale reactor by plate diffusers.

All reactors were stirred by means of magnetic stirrers. The biodegradation experiments were performed in a dark room at 20 °C. Under aerobic and anaerobic conditions the quantities of wastewater which were pumped through the closed-loop systems equipped with columns filled with glass foam beads were adjusted to about 1 l $h^{-1}$.

The generation of digester gas was monitored by volume measurement and analysis of volatile fluorinated compounds in the digester gas was performed using GC–ECD.

The variations in the concentrations of fluoride ions in the reaction mixtures during aerobic and anaerobic treatment were monitored by use of a fluoride ion selective electrode (Ingold, Steinbach, Germany).

## 3. Results and discussion

When we started with our research work there was a lack of information on the presence of fluorinated surfactants in sewage sludge because the matrices wastewater and sewage sludge can be termed as “difficult matrices”. The determination of these amphiphilic compounds was being hampered by their reduced extractability and co-extracted matrix. Orientative results proved that methods for determination of fluorinated surfactants in sewage sludge extracts without clean up turned out to be quite complicated. Balances of these compounds in wastewater treatment processes as the most important source of fluorinated surfactants in the environment could not yet be performed.

When we applied different extraction techniques (Soxhlet, hot vapour or PLE) to artificially contaminated sludge for screening purposes PLE proved to be the most promising method, whereas the extraction efficiency of Soxhlet and hot vapour extraction was relatively low.

To reach our objectives the elaboration of an analytical method for fluorinated surfactants was divided

into three main steps: (1) elaboration of an analytical determination technique which is essential for reliable quantification and its adaptation to the complex matrices co-extracted from sewage sludge samples, (2) generation of artificially contaminated sewage sludge containing fluorinated surfactants and (3) application to exhaustive simultaneous extractions with the aim that both types of surfactants, anionics and non-ionics, should be extracted with satisfactory recovery rates near to 100%. The results and the path by which this aim can be gained are described as follows: (1) we started with the elaboration of an analytical quantification technique for fluorinated surfactant-spiked sludge extracts heavily loaded with matrix. Checking the two quantification procedures, FIA–MS and LC–MS, using extracts with known concentrations of fluorinated surfactants was essential to judge both quantitative methods. While FIA–MS bypassing the analytical column is known to provide quick results, LC–MS is the more time-consuming method, while it reduces interferences with matrix compounds.

In parallel with FIA–MS and LC–MS optimisation using spiked extracts without any clean-up, sewage sludge samples were spiked with known concentrations (2) followed by lyophilisation before these samples (3) were extracted exhaustively to find out the most promising extraction technique.

### *3.1. FIA and LC–MS determinations of anionic and non-ionic fluorinated surfactants*

The quantitative determinations of anionic and non-ionic fluorinated surfactants were optimised with the application of a matrix stock solution which had been prepared by spiking PLE extracts. Because of the extraction efficiency of the solvents methanol, ethyl acetate and DMF matrix components were extracted from digested sludge to a large extent while the pollution of the extracted sludge samples by fluorinated surfactants was, however, estimated quite low. MS quantification of anionic and non-ionic fluorinated surfactants was performed in SIM (selected ion monitoring) mode.

Application of the FIA–MS quantification approach [36] performed with spiked PLE sewage sludge extracts failed because of the high load of co-extracted matrix compounds, which led to quite unstable selected ion current traces. Even application of the more selective product or parent ion scans in FIA–MS–MS mode was unselective owing to isomeric or isobaric compounds co-extracted by PLE.

Results of FIA proved that an LC separation prior to MS determination was necessary to get rid of matrix compounds. Reversed-phase LC separations of the spiked matrix extracts performed on a RP-$C_{18}$ column without prior clean-up step were not quite promising. Calculations by means of peak areas after ESI(−) or APCI(+) followed by MS detection for anionics and non-ionics, respectively, gave evidence of comparable or even surplus recoveries as observed for LC–MS performed with standard solutions. Extraordinary high recoveries observed with spiked sludge samples under gradient RP-$C_{18}$ separation conditions consequently must be induced by as yet unidentified matrix compounds. These compounds are present in the PLE extracts, but are not recognisable in spectra and are not separated from fluorinated surfactants.

In order to dispense with these matrix compounds, investigations using the high stability of fluorinated surfactants were performed by applying oxidation reagents such as hydrogen peroxide–sulfuric acid or hydrochloric acid–nitric acid to the spiked sludge extracts. After oxidation reactions had been performed adjusting different temperatures, recoveries of fluorinated surfactants varying between “not detectable” and 144% could be observed (cf. Table 3) in eluates of $C_{18}$-SPE extracts.

An alternative bypassing the extraction process was also performed. Here, determination of fluorinated surfactants followed oxidative destruction of the sludge using hydrogen peroxide–sulfuric acid or hydrochloric acid–nitric acid at 70 °C. Despite the high stability of fluorinated surfactants against heat and oxidation reagents, the method was not found to be applicable, because besides sludge matrix the fluorinated surfactants were destroyed.

A solution to the problems with matrix disturbing quantitative determination could be accomplished by application of a new fluorinated reversed-phase material applied for LC separations in the course of our examinations. This analytical phase based on perfluorinated RP-$C_8$ material which was successfully used for separation of all PLE extracts without any previous clean-up. False-positive results we had obtained with the use of RP-$C_{18}$ in LC separations of spiked extracts and PLE extracts of spiked sludge became

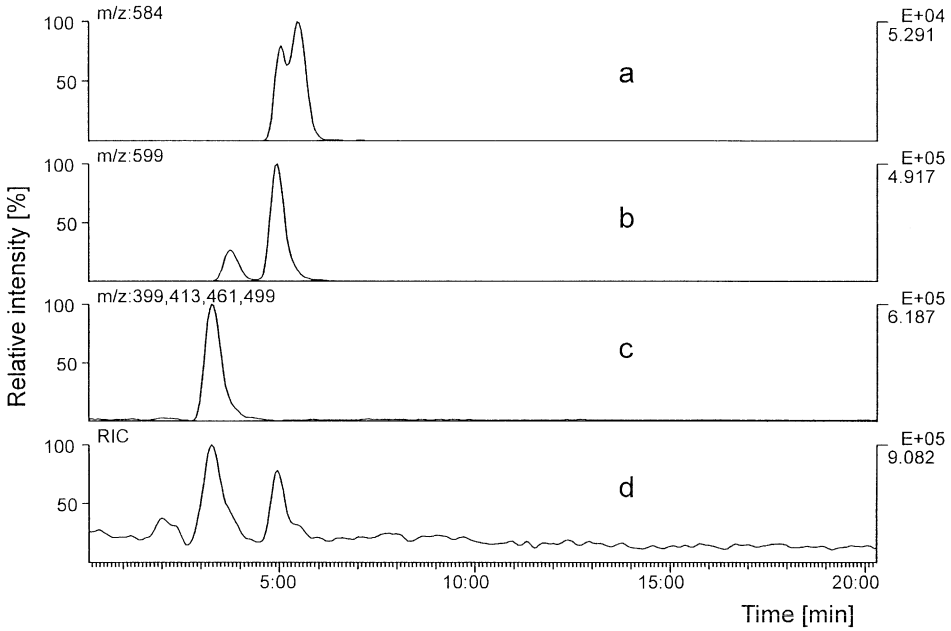


Fig. 2. LC–ESI(−)-MS total-ion current tracing (d) for fluorinated surfactant spiked PLE extract as in Fig. 1c. LC separation performed by gradient elution on RP-$C_{18}$ column. (a) LC–ESI(−)MS selected ion current tracing for $C_8F_{17}$–$SO_2$–N($C_2H_5$)–$CH_2$–$COO^-$ $H^+$ (*m*/*z* 584) from mixture of fluorinated surfactants blends as in (d). (b) LC–ESI(−)-MS selected ion current tracing for PFDS (*m*/*z* 599) as in (a). (c) LC–ESI(−)-MS selected ion current tracings for PFHxS (*m*/*z* 399), PFOA (*m*/*z* 413), $C_5F_{11}$–$(CH_2)_8$–$SO_3H$ (*m*/*z* 461), and PFOS (*m*/*z* 499) as in (a). For LC–MS conditions, see Section 2.

more reliable applying this material for separation. Results of LC separations of anionic surfactants spiked into PLE extracts were recorded with the perfluorinated RP-$C_8$ column (Fig. 1) which recently became commercially available. Selected mass traces and total ion mass trace are presented for comparison with RP-$C_{18}$ chromatograms (Fig. 2).

### *3.2. Comparison of different extraction methods using spiked sludge samples*

The most conventional extraction methods such as Soxhlet or hot vapour extraction applied to the extraction of spiked sewage sludge samples may provide high recovery rates up 90% and more. This was also found in literature for the extraction of nonylphenols and bisphenol A [31], but the reason why these results were obtained is that no well-adapted unrealistic spiking techniques had been performed prior to extraction. Surfactants are bipolar compounds with a hydrophilic and a lipophilic segment. Particularly in fluorinated surfactants the lipophilic moiety exhibits a great potential to interfere with the lipophilic matrix such as sewage sludge. Therefore the described spiking technique [31] was also applied in this examination to elaborate reliable extraction methods for fluorinated surfactants although the procedure cannot simulate the adsorptive effects which happen during wastewater treatment. Nevertheless, a strong adsorption of these compounds onto the sludge matrices can be observed and, therefore, it represents the most realistic pathway to judge and to improve extraction techniques already existing today. Activated and digested sewage sludge samples spiked in this way were used to select the most effective extraction method from the extraction techniques, Soxhlet extraction, hot vapour extraction and PLE. Aliquots of these extracts applied to FIA–MS and LC–MS analyses proved that an evaluation of the results recorded by FIA–MS was impossible because of unstable ion current signals in MS detection. The application of FIA–MS–MS did not improve results, so all quanti-

tative determinations were elaborated by LC–MS (cf. Tables 2 and 3).

### 3.2.1. Soxhlet extraction and hot vapour extraction of spiked sludge samples

The results obtained by LC–MS applying RP-$C_{18}$-separation to Soxhlet or hot vapour extracts of digested sludge samples are presented in Table 2 (cf. sample nos. 3–5 or 7–9). Ethyl acetate and methanol, either single or in combination with hydrochloric acid were applied during extraction periods of 6 h. The recoveries from spiked digested sludge were not quite convincing, since none of the methods examined reached recoveries of more than 52 or 48% in the simultaneous extraction for both anionic and non-ionic compounds. The application of these extraction methods to spiked activated sludge were disappointing. Extraction failed completely, because all recoveries observed were below 10% despite extraction periods were expanded to 12 h (cf. Table 2, cf. samples 6 or 10).

### 3.2.2. Pressurised liquid extraction (PLE)

In the combination with PLE, DMF, 1,4-dioxane, ethyl acetate, methanol, pyridine, MTBE or mixtures of these, sometimes modified by phosphoric acid were used for extraction with different pressures (10 714 and 14 285 kPa). Results after RP-$C_{18}$-LC–MS are presented in Table 2 (cf. samples 11–33).

For spiked sludge samples without clean-up step partly extraordinary high recoveries sometimes larger than 300% were observed under RP-$C_{18}$ separation conditions. LC separations were repeated with an analytical column filled with perfluorinated RP-$C_8$ material. The obtained results became more reliable with recoveries varying between 77 and 122% (cf. Table 2, samples 34–39).

It became obvious that only the multi-step procedures with acidified methanol developed such high efficiencies in extraction, that both fluorinated surfactant types, anionics and non-ionics could be extracted completely.

The application of the optimized PLE procedure in combination with perfluorinated RP-$C_8$ separation to activated sewage sludge resulted, however, in quite low recoveries (cf. Table 2, samples 41, 42). Extraction efficiencies were comparable with those observed under Soxhlet or hot vapour extraction conditions (cf. samples 5 and 9). The reason for this failure is the high adsorptive potential of the activated sewage sludge matrix, which is different to sludge matrix observed with digested STP sludge.

### 3.3. Determination of fluorinated surfactants in real environmental STP sludge samples

The most efficient PLE method was obtained by the application of EtOAc–DMF–MeOH–$H_3PO_4$ (cf. Table 2, no. 40) providing recoveries which proved to be stable, permanently reproducible and gave evidence of an extraction percentage of about 100% (conc. range: ∼150 μg $g^{-1}$; cf. Section 2.3.3). This PLE method was applied to real environmental STP sludge samples but the results we obtained by LC–MS using perfluorinated RP-$C_8$ material and ESI(−) or APCI(+) were disappointing. Neither anionic nor non-ionic surfactants were present at concentrations higher than 10 or 6 mg/kg dry residue for non-ionics and anionics, respectively.

Therefore, the questions arose, whether fluorinated surfactants had been present in the sewage from the very beginning or not present at all, what had happened to these surfactants during aerobic wastewater treatment or anaerobic sludge treatment process?

### 3.4. Behaviour of fluorinated surfactants under biochemical degradation

Extraordinary stability of anionic and non-ionic surfactants against physicochemical or chemical attack is known [9,37]. Biodegradability of non-ionic FAEO compounds under aerobic conditions, however, has been observed [10]. To elucidate and perhaps explain the results obtained during STP sludge investigations we selected different types of anionic [$C_7F_{15}$–COOH (PFOA), $C_8F_{17}$–$SO_3H$ (PFOS)] and non-ionic fluorinated surfactants [$C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–H ($n$=6,8,10) (partly FAEO), $C_8F_{17}$–$(CH_2)_2$–$SO_2$–N$(C_2H_5)$–$(OCH_2CH_2)_x$–OH (perfluorooctanesulfonylamidopolyethoxylate), $C_8F_{17}$–$(CH_2)_2$–$SO_2$–N$(C_2H_5)$–$(OCH_2CH_2)_x$–$OCH_3$ (perfluorooctanesulfonylamidopolyethoxylate methyl ether)]. These compounds then were submitted to aerobic and anaerobic wastewater treatment to obtain knowledge about their stability in biochemical processes.

Because of its feasibility and in parallel large amount of information obtainable, biodegradation in

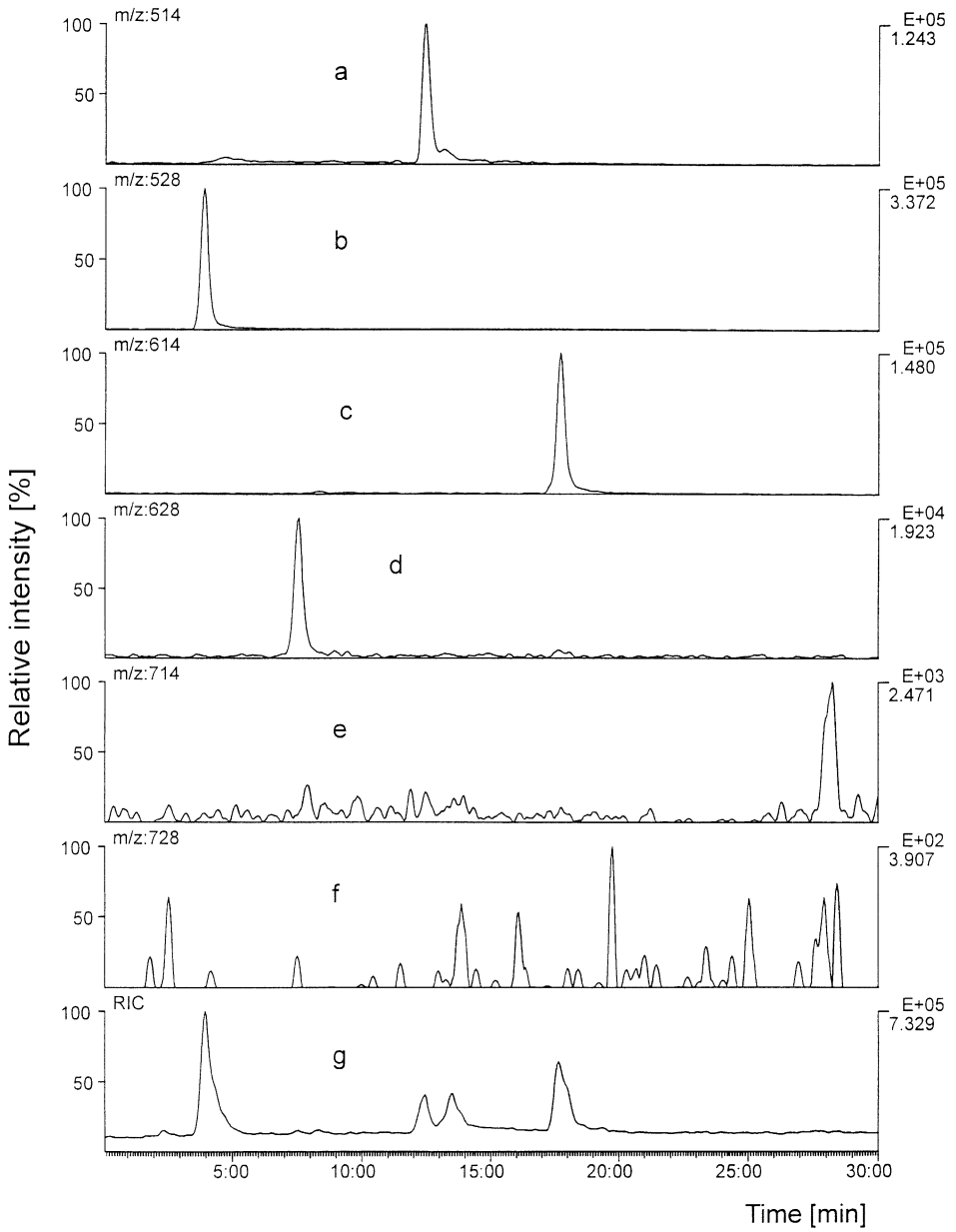


Fig. 3. LC–APCI(+)-MS total-ion current tracing (g) for wastewater sample spiked with FAEO blend [$C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–OH ($n$=6,8,10)] prior to aerobic biodegradation; Concentrated by $C_{18}$-SPE, eluted by methanol and separated on perfluorinated RP-$C_8$ column using gradient elution. (a,c,e) Selected ion current tracings for non-ionic fluorinated compounds as in (g) according to $n$=6 ($m/z$ 514), $n$=8 ($m/z$ 614), $n$=10 ($m/z$ 714). (b,d,f) Selected ion current tracings for aerobic metabolites [$C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–$OCH_2COOH$] of non-ionic fluorinated compounds as in (g) according to $n$=6 ($m/z$ 528), $n$=8 ($m/z$ 628), $n$=10 ($m/z$ 728). For LC–MS conditions, see Section 2.

closed-loop systems with a minimum of activated or anaerobic fixed-bed biocoenosis located on glass foam beads was perform [30]. In order to monitor the decrease of precursor compounds and the generation of metabolites, degradation was first monitored by FIA–MS after concentration and determination of spiked compounds by off-line $C_{18}$-SPE and methanol elution. This screening allowed a rapid recognition and follow-up of the biodegradation processes. To confirm results, LC–MS in ESI(−) or APCI(+) mode

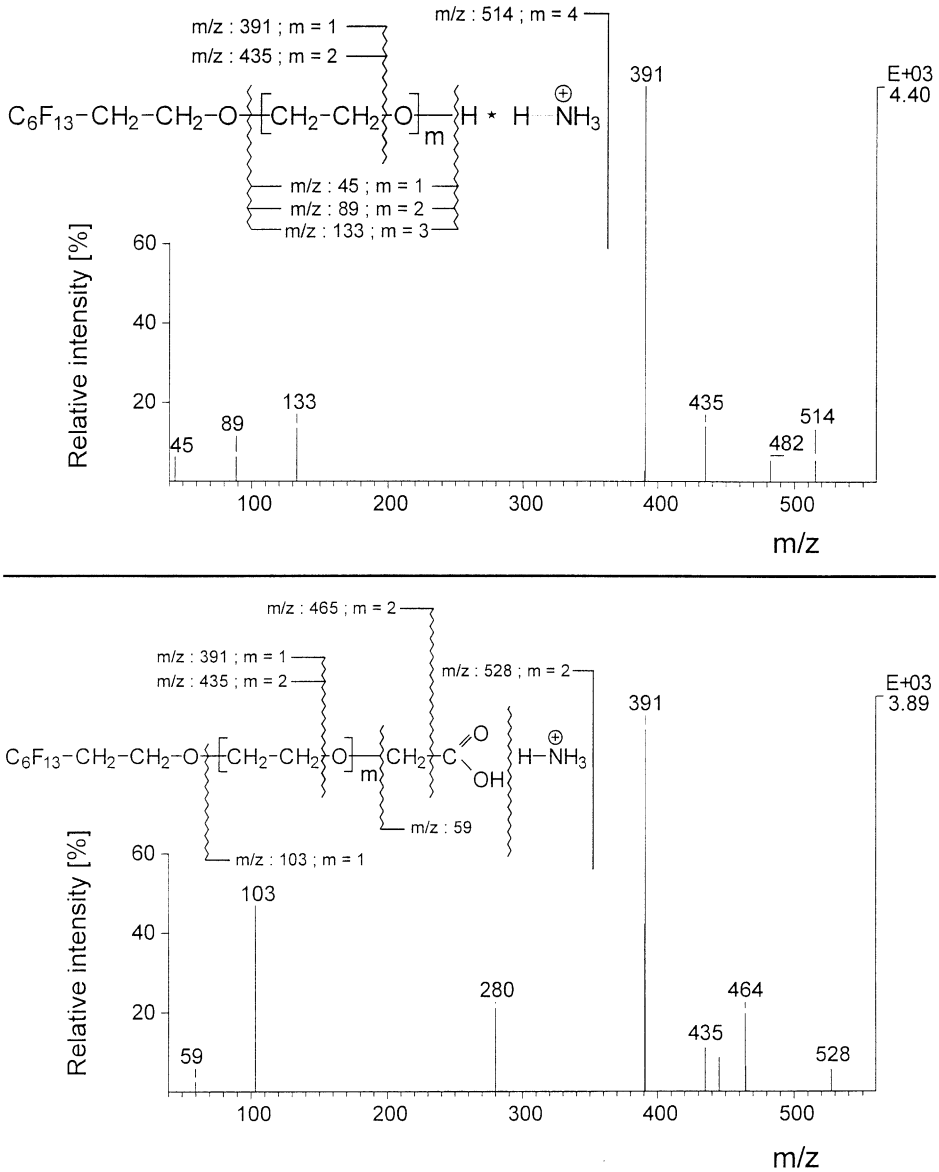


Fig. 4. LC–APCI(+)-MS–MS product-ion mass spectrum and proposed fragmentation scheme for (top) selected parent ion at *m/z* 514 of precursor FAEO blend; (bottom) product-ion mass spectrum and fragmentation scheme for metabolite ion at *m/z* 528 obtained by biodegradation of non-ionic FAEO mixture. LC conditions as in Fig. 3, for MS–MS conditions, see Section 2.

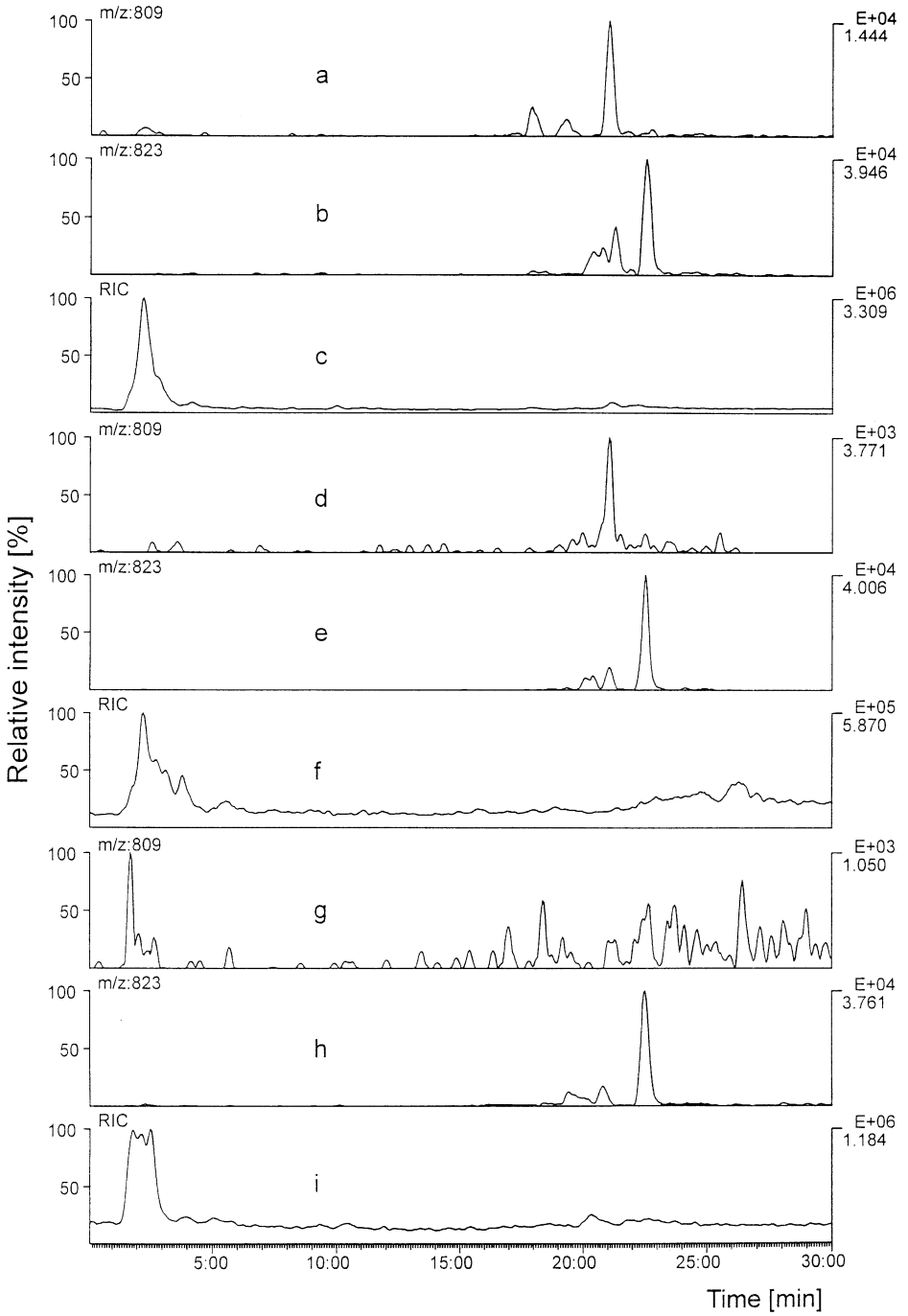


Fig. 5. LC–APCI(+)-MS total-ion current tracings (c,f,i) for wastewater samples during anaerobic biodegradation containing partly fluorinated decanesulfonylamidopolyethoxylates (I) [$C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–OH] and partly fluorinated decanesulfonylamidopolyethoxylate methyl ethers (II) [$C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–$OCH_3$]; (a,d,g) selected ion current tracings for non-ionic fluorinated compounds (I) as in (a) proving their elimination by anaerobic biodegradability; (b,e,h) selected ion current tracings for non-ionic fluorinated compounds (II) as in (b) proving their persistence against anaerobic biodegradation; (a,b,c) $t = 0$, (d,e,f) $t = 4$ d and (g,h,i) $t = 10$ d. Concentration, elution and separation as in Fig. 3. For LC–MS conditions, see Section 2.

was performed. Short intervals between the sampling dates during aerobic and anaerobic degradation ensured that even arising intermediates with short half-life could be recognised during the monitoring processes provided that the metabolites are concentrated by SPE and ionised by API.

Results proved that under aerobic conditions with the exception of the partly FAEO surfactant mixture [10] none of the surfactants could be minimised by metabolic (primary degradation) or mineralisation processes. The results of aerobic degradation of the non-ionic fluorinated surfactant blend $C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–H ($n$=6,8,10) are shown in Fig. 3 after LC–MS analysis of SPE extracts. Besides precursor surfactant compounds in mass traces (cf. Fig. 3b,d,f) signals of metabolites could be separated (cf. Fig. 3c,e), while metabolites of homologue compounds with $n$=10 could not be observed (Fig. 3g).

LC–APCI-MS–MS spectra (Fig. 4) recorded from the most prominent ions ($m/z$=514 and 528) of the mixture of precursor compound and carboxylic metabolites [$C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_{x-y}$–$CH_2COOH$] were comparable with spectra obtained by APCI [34] and thermospray ionisation (TSP) [10]. Fragmentation behaviour under CID conditions are presented in the insets (Fig. 4). These product ion spectra proved that precursor compounds had oxidised and been converted to carboxylic compounds.

The anaerobic treatment (redox potential below −300 mV) of all anionic fluorinated surfactants PFOA and PFOS, however, resulted in a decrease of concentration of these compounds in turbid water. While PFOS disappeared quite rapidly, within 2 days, PFOA remained stable during breakdown of PFOS. After the disappearance of PFOS, the degradation was continued with the result that PFOA could no longer be detected after 25 days.

The application of these anaerobic conditions to the non-ionic fluorinated surfactants $C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–H ($n$=6,8,10), $C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–OH and $C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–$(OCH_3)$ only resulted in an elimination of $C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–OH while the non-ionic FAEO compounds and the methylether were not reduced in concentration. The results of degradation of both sulfonylamido compounds [$C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–OH=(I; $m/z$ 809) and $C_8F_{17}$–$(CH_2)_2$–$SO_2$–$N(C_2H_5)$–$(OCH_2CH_2)_x$–$OCH_3$) = (II; $m/z$ 823)] after 4 (Fig. 5d,e,f) and 10 days (Fig. 5g,h,i), is recognisable in the selected ion mass traces.

This degradation behaviour for compound (II) was expected because it was also observed during anaerobic treatment of not fluorinated methylated polyether surfactants [30]. Stability of partly fluorinated compound $C_nF_{2n+1}$–$(CH_2)_2$–O–$(CH_2CH_2O)_x$–H against anaerobic biodegradation was, however, quite astonishing.

Our examinations performed by LC–MS proved that either the aerobic or anaerobic biological treatment of fluorinated surfactants led to remarkable reductions in concentration or even to a complete elimination of these compounds within some days. With the exception of the metabolites generated from FAEO compounds, polar metabolites extractable from the wastewater phase using $C_{18}$-SPE could be detected neither under aerobic nor anaerobic conditions. Non-polar, volatile fluorinated compounds in the digester gas could also not be detected by GC–ECD.

The mineralisation of fluorinated surfactants should lead to increased concentrations of fluoride ions in the aerobic and anaerobic reactor systems. However, elevated concentrations of fluoride ions could not be observed.

## 4. Conclusions

First, a robust method for simultaneous reliable quantitative determination of anionic and non-ionic fluorinated surfactants in digested sewage sludge had to be elaborated. Unpolluted digested sludge as real environmental matrices for the generation of fluorinated surfactant-spiked samples was applied. The generation of spiked sludge samples within a concentration range of about 150 μg $g^{-1}$ dry solid matter allowed realistic adsorption and absorption process of fluorinated surfactants onto the sludge. For their extractability adsorption proved to be determinative. The sequential application of mixtures of EtOAc–DMF and methanol–phosphoric acid in combination with PLE was found to be essential for exhaustive extraction. Because of the high adsorptive potential of activated STP sludge the extraction of fluorinated surfactants failed.

For the FIA–MS approach in many cases of surfactant quantification the quickest determination technique could not be performed successfully with PLE extracts without prior clean-up. Even gradient RP-$C_{18}$ LC was complicated because of matrix compounds co-extracted from STP sludge and not yet eliminable by column clean-up.

The application of FIA or LC–MS–MS as a powerful alternative determination techniques [38,39] failed and in parallel proved the lack of fluorinated surfactant-characteristic ions or neutrals. As solution to the matrix problems observed with RP-$C_{18}$ LC–MS or MS–MS separations of PLE extracts applying perfluorinated RP-$C_8$ LC became the method of choice. Its application led to a shift in $T_R$ resulting in clear separations for compounds with fluorinated alkyl chain length of >7 carbon atoms. Stabilisation of eluent pH and long lasting overnight column rinsing were found to be essential for stable $T_R$ values.

The examination of more than 80 real STP sludge PLE samples indicated that fluorinated surfactants were not present in concentrations higher than the LODs. As this finding was very astonishing because of the ubiquitous dispersion of PFOS [11–15] in the environment and in biota we decided to check biodegradability of fluorinated surfactants. With the results obtained from biochemical degradation experiments under aerobic and anaerobic conditions our findings became more explainable and reliable. LC–MS analyses of SPE extracts from lab-scale closed-loop biodegradation reactors proved that in the aerobic biological wastewater treatment process only the FAEO compounds were metabolised, whereas anaerobic treatment conditions were effective for elimination of anionic PFOS and PFOA surfactants. Under these conditions the non-ionic perfluoralkylsulfonylamidopolyethoxylates were also found to be degradable, whereas their methylated ether compounds were neither aerobically nor anaerobically reduced in concentration.

An oxidative destruction step by application of $H_2O_2$–$H_2SO_4$ or HCl–$HNO_3$ (aqua regia) for mineralisation of extracted sludge matrix or sewage sludge led to an unexpected destruction of the fluorinated surfactants. Fluorinated surfactants contained in spiked ultra-pure water samples, however, were found to be stable against chemical oxidation. The reason we believe was the absence of catalytic heavy metal ions. All degradation processes were monitored by LC–MS and MS–MS. It could be confirmed that with the exception of the arising metabolites of FAEO compounds only an increase of polyethylene glycol concentrations could be observed during anaerobic degradation of perfluoralkylsulfonylamidopolyethoxylates.

As no increasing concentration of fluoride ions could be found, degradation of the hydrophobic fluorine-containing moiety, which is responsible for the ecotoxicological behaviour of these compounds in the environment could be excluded. The lack of knowledge concerning the whereabouts of the fluorinated segment in this type of surfactants remained, which is why an elucidation of the fate of the fluorinated lipophilic part of fluorinated surfactant molecules is now under way.

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