Study Title

Microbial Metabolism (Biodegradation)
of Perfluorooctane Sulfonate (PFOS)
I. Activated Sludge/Sediment

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1.0 INTRODUCTION

The biodegradation program for perfluorooctane sulfonate (PFOS) was designed to offer a wide range of conditions to maximize the chance for selection and enrichment of microbial populations that could metabolize this chemical as well as other unique fluorochemicals. In addition to enrichment, the program was also designed to optimize for co-metabolism of fluorochemicals. This was done by continual addition of fresh inoculum and complex natural nutrients. The overall goal of these studies, therefore, was to observe loss of parent material and formation of degradation products as quantitatively as possible within the limits of the study designs.

Three aerobic systems were examined: a sewage treatment based system to select for faster growing species (Zymogenous), a soil based system to select for slower growing species (Autochthonous), and a pure culture system for examining specific metabolic capabilities (Cytochrome P_{450} monooxygenase). One anaerobic system was studied: 10% anaerobic digester sludge.

The overall screening program was based on key factors to maximize the chance for enrichment of those organisms capable of metabolizing unique chemicals. Among these factors were:

- testing and enrichment under non-toxic conditions
- the use of natural ecosystems as the basis for enrichment
- the use of natural nutrients from those ecosystems with supplemental trace minerals, co-factors and vitamins
- the continual introduction of new microbes from different natural sources
- the periodic replenishment of natural nutrients without diluting out the species being enriched
- the provision for a realistic time frame for enrichment and acclimation
- the protection of microorganisms from toxic products or metabolites by use of low substrate concentrations, replenishment of nutrients, balanced medium (C:N:P, etc.), proper pH and provision of a protective surface for growth (sandy soil)
- the enrichment in more concentrated (higher biomass and test substance concentration) systems and examination of biodegradation in more dilute systems
- the separation of systems selective for fast growing (zymogenous) and slow growing (autochthonous) species
- use of pure cultures containing the cytochrome P_{450} monooxygenase enzyme system known to metabolize complex molecules.

Throughout the experimental program the principles outlined above for enrichment were incorporated. The results of the studies are summarized in four separate reports and provide a

basis for the future direction of the program to better understand the environmental fate of fluorochemicals. This report summarizes the results of exposure of PFOS to an activated sludge/sediment system.

2.0 TEST SUBSTANCE, INTERNAL STANDARDS, AND SOLUTION PREPARATION

2.1 Test Substance and Internal Standards

The test substance, perfluorooctane sulfonate potassium salt (PFOS, lot no. TN-A-2130), an off-white powder, was received on 25 January 1999 (SLI No. 70-93) from 3M Environmental, St. Paul, Minnesota. Prior to study completion, analytical characterization of the PFOS test substance was not conducted. Therefore, all calculations in the report are based on PFOS purity of 100%. After study completion, a sample of a 1.06 mg/mL PFOS stock solution (SLI No. 70-93A), see Section 2.2.2) was sent to 3M Environmental Laboratory for evaluation of impurities using LC/MS-TOF. Compounds looked for were the C2 to C10 PFOS analogous materials, and many of them were observed in both the 1-µL and 10-µL injections (e.g., masses 249, 299, 349, 399, 449, 499, and 549 were observed as peaks). The C2 to C10 carboxylates related to PFOA were looked for, and some were found. For example, the masses 213, 263, 313, 363, 413, and 463 which correspond to the C4, C5, C6, C7, C8, and C9 perfluorinated carboxylates, respectively, were observed; however, perfluorinated carboxylates were estimated to be present at < 0.2% of the total material. FOSA was looked for but not observed. The perfluorooctane sulfinate was also looked for at mass 483, but not found. The percentage of each component, based on signal intensity, is presented in the following table and is based on an assumption that the signal ratio for each is 1:1 with PFOS.

Perfluorinated Alkyl Sulfonates Observed

Analyte	Peak Response (Area)	Total (%)
Perfluorononane sulfonate (C9) response	0.990	0.400
Perfluorooctane sulfonate (C8) response	223.245	90.23
Perfluoroheptane sulfonate (C7) response	5.786	2.34
Perfluorohexane sulfonate (C6) response	4.262	1.72
Perfluoropentane sulfonate (C5) response	5.326	2.15
Perfluorobutane sulfonate (C4) response	5.190	2.10
Perfluoropropane sulfonate (C3) response	2.622	1.06
Total	**247.421**	**100**

The internal standard, perfluorooctanoic acid (PFOA, lot no. 07216AS), an off-white solid wax, was received on 1 April 1999 (SLI No. 71-94) from Aldrich, Milwaukee, Wisconsin. An additional internal standard, 1,1,2,2-tetrahydroperfluorooctane sulfonate (THPFOS), a brown crystal was received on 18 January 1999 (SLI No. 70-83) from 3M Environmental, St. Paul, Minnesota. Upon receipt at Springborn, the samples of test substance and internal standards were stored in their original containers at room temperature in a dark, ventilated cabinet.

2.2 Preparation of Stock Solutions and Reagents

2.2.1 Internal Standard Stock Solutions. A 25.0 mg/L PFOA stock solution containing 15.0 mg/L THPFOS internal standard was prepared in the following manner. A 1000 µg/mL PFOA solution was prepared by placing 0.1002 g of PFOA in a 100-mL volumetric flask and bringing to volume with methanol. A 1000 µg/mL THPFOS solution was prepared by placing 0.1002 g of THPFOS in a 100-mL volumetric flask and bringing to volume with methanol. A 50-mL aliquot of the PFOA solution and a 30-mL aliquot of the THPFOS solution were placed in a 100-mL volumetric flask and brought to volume with methanol which resulted in a 500 mg/L PFOA solution containing 300 mg/L THPFOS. The 25.0 mg/L PFOA/15.0 mg/L THPFOS internal standard solution was then prepared by placing 2.5 mL of the 500 mg/L PFOA solution containing 300 mg/L THPFOS solution in a 50-mL volumetric flask and bringing to volume with methanol. No visible signs of undissolved substances were observed in any of the methanol solutions.

2.2.2 PFOS Stock Solutions. Two PFOS stock solutions were prepared and used for dosing the activated sludge/sediment, closed vial and toxicity test systems. At the time of test initiation, the actual purity of the PFOS sample had not been determined and was assumed to be 100% for preparation of stock solutions. A 1.06 mg/mL PFOS stock solution (SLI No. 70-93A) was prepared by placing 0.1061 g of PFOS in a 100-mL volumetric flask and bringing to volume with purified reagent water. This stock solution was used to dose the toxicity assay and activated sludge/sediment acclimation flasks. A 1.01 mg/mL PFOS stock solution (SLI No. 70-93E) was prepared by placing 0.1011 g of PFOS in a 100-mL volumetric flask and bringing to volume with purified reagent water. This stock solution was used to dose the closed vial (headspace) aerobic biodegradation test system. These stock solutions were suspensions and were used after vigorous shaking and sonication to deliver homogeneous suspensions to the test systems. Homogeneity was confirmed by the LC/MS analysis of a 1-mL aliquot of an aqueous PFOS stock solution that

resulted in a 100.2% recovery (SLI No. F499-59). Note that the solubility of PFOS in purified reagent grade water is 0.567 mg/mL (SD = 52.8, CV = 9.31%, n = 6) (VanHoven and Nixon, 1999).

A 1.00 mg/mL PFOS primary stock solution (SLI No. 70-93C) was prepared by placing 0.1004 g of PFOS in a 100-mL volumetric flask and bringing to volume with methanol. This stock solution was used in the preparation of quality control samples. A secondary stock solution was prepared by diluting 0.500 mL of this stock to 50.0 mL final volume to produce a 10.0 mg/L PFOS stock.

A final PFOS primary stock solution with a concentration of 1.22 mg/mL (SLI No. 70-93D) was prepared by placing 0.1223 g of PFOS in a 100-mL volumetric flask and bringing to volume with methanol. Secondary stock solutions with concentrations of 1.22, 12.2, and 122 mg/L were prepared by placing the appropriate volume of the 1.22 mg/mL primary stock solution in a 50.0-mL volumetric flask and bringing to volume with methanol. The primary and secondary stock solutions were used to prepare calibration standards. Preparation of the calibration standards is detailed in the following table.

Concentration of Stock Solution	Fortification Volume (mL)	Final Volume (mL)	Diluent	Standard Concentration (mg/L)
1.22 mg/mL	0.100	50.0	Methanol	2.44
1.22 mg/mL	0.0500	50.0	Methanol	1.22
1.22 mg/mL	0.0250	50.0	Methanol	0.610
122 mg/L	0.0500	50.0	Methanol	0.122
12.2 mg/L	0.125	50.0	Methanol	0.0305
1.22 mg/L	0.410	50.0	Methanol	0.0100

The calibration standards were stored in amber bottles with Teflon®-lined crimp caps. Aliquots were removed as needed for each LC/MS analysis.

The internal standards were mixed in the same proportions and added (prior to LC/MS analysis) to the calibration standards in the same manner they were added to the test samples (i.e., 25 µL of 25 mg/L PFOA/15 mg/L THPFOS to 2.00 mL of standard).

2.2.3 Tetrabutylammonium Sulfate Stock Solution. Tetrabutylammonium sulfate (TBA-S) solutions (0.5 M) were prepared by placing 35.0 g of TBA-S in a 200-mL volumetric flask and bringing to volume with purified reagent water. This solution was then brought to pH 10 with 10 N NaOH.

2.2.4 Sodium Hydroxide Stock Solution. Sodium hydroxide solutions (10 N) were prepared by placing 400 g of sodium hydroxide in a 1000-mL volumetric flask and bringing to volume with purified reagent water.

2.2.5 Ammonium Acetate Stock Solution. Ammonium acetate solutions (2 mM) were prepared by adding 0.151 g of ammonium acetate to a 1000-mL volumetric flask and bringing to volume with purified reagent water.

3.0 PREPARATION OF SAMPLES AND ANALYTICAL METHODS

The methods used in the analysis of the aqueous and solid samples are summarized in the flow chart presented in Figure 1. For all shake-flask and closed vial test samples, an appropriate volume of test sample (10 mL for the shake-flask samples, and 15 mL for the closed vial samples) was placed into a Nalgene™ centrifuge tube and centrifuged at 1200 x g (rotating radius of 12.5 cm, from RCF Nomagraph, IEC (International Equipment Company), 300 Second Ave., Needham Heights, Massachusetts, 02194) for 30 minutes at room temperature with a Beckman Model 65-6R centrifuge. The procedures used for the resultant aqueous (supernatant) and solid samples are detailed below.

3.1 Preparation of the 20 and 105 mg/L Aqueous Test Samples

A 1.00-mL aliquot of the supernatant was added to a volumetric flask and diluted to volume (25.0 and 100 mL for the 20.8 and 105 mg/L samples, respectively) with methanol. The samples were then filtered through a 0.2-µm filter (Titan, nylon membrane) prior to analysis. A 2.0-mL aliquot was removed from each filtered sample, placed into an autosampler vial, and 25 µL of the 25.0 mg/L PFOA/15.0 mg/L THPFOS internal standard solution was added.

3.2 Preparation of the 0.20 mg/L Aqueous Test Samples

The entire volume (15 mL) of supernatant from each sample was transferred to a separatory funnel. Ten milliliters of the 0.5 M pH 10 TBA-S solution was added to each separatory funnel. The samples were then extracted twice with 300 mL of 50:50 ethyl acetate/hexane. When emulsions formed, an additional 1.00 to 2.00 mL of methanol was added to the separatory funnel and gently mixed until the emulsions vanished. All extracts were reduced to approximately 2.00 mL by rotary evaporation at approximately 35 °C (samples were not allowed to go dry). When water was present in the round-bottom flask, methanol was added and rotary evaporation was continued until the amount of water was minimized. The sample extract was then quantitatively transferred to a centrifuge tube. The sample extract was reduced to a volume of approximately 100-µL under a gentle stream of nitrogen at room temperature, brought to a volume of 10 mL with methanol, vortexed for 30 seconds, and sonicated for 15 minutes. The sample extracts were then filtered through a 0.2-µm filter (Titan, nylon membrane) prior to analysis. A 2.00-mL aliquot was removed from each sample and 15 µL of the 25.0 mg/L PFOA/15.0 mg/L THPFOS internal standard solution was added.

3.3 Preparation of the Solid Samples

Each solid sample was extracted by adding 40 mL of methanol and vortexing for 30 seconds, sonicating for 15 minutes, and shaking on a shaker table at 200 rpm for 30 minutes at room temperature. The samples were then centrifuged at 1200 x g for 30 minutes using a Beckman Model 65-6R centrifuge, and the supernatant was decanted. The supernatant was then filtered through a 0.2-µm filter (Titan, nylon membrane) prior to analysis. If necessary, the sample extract (supernatant) was diluted in methanol. A 2.0-mL aliquot was removed from each sample extract, placed into an autosampler vial, and 25 µL of the 25.0 mg/L PFOA/15.0 mg/L THPFOS internal standard solution was added.

3.4 Preparation of Quality Control Samples

Preparation of the aqueous and solid quality control (QC) samples is summarized in the following table. When necessary and prior to QC fortification, the media were centrifuged at 1200 x g for 30 minutes to separate the solid and aqueous portions. All QC samples were prepared by fortifying the appropriate control matrix (aqueous medium, biomass, etc.) at the concentrations listed below. The QC samples were then treated following the same procedures as the test samples (aqueous samples, Sections 3.1 and 3.2; biomass samples, Section 3.3).

Test ID	QC Sample Type	PFOS Stock Concentration (mg/L)	Volume of Stock Solution Used (mL)	Control Matrix	Volume of Control Matrix (mL)	QC Sample Concentration (mg/L)
Shake-Flask	Aqueous	1000	0.100	1	10.0	10.0
Days 7, 14, 21, 28, and 35		1000	0.100	1	10.0	10.0
		1000	0.200	2	10.0	20.0
	Solids	1000	0.0250	3	10.0	2.50
		1000	0.0250	3	10.0	2.50
		1000	0.0500	3	10.0	5.00
Closed Vial						
Low Conc. Day 0, 16, 30, 61	Aqueous	10.0	0.300	1	15.0	0.200
		10.0	0.300	1	15.0	0.200
		10.0	0.300	2	15.0	0.200
		10.0	0.300	2	15.0	0.200
	Solids	10.0	0.300	3	15.0	0.200
		10.0	0.300	3	15.0	0.200
High Conc. Day 0, 15, 30, 63	Aqueous	1000	0.250	1	10.0	25.0
		1000	0.500	1	10.0	50.0
		1000	1.00	2	10.0	100
	Solids	1000	0.500	3	15.0	33.3
		1000	1.00	3	15.0	66.7
		1000	1.50	3	15.0	100

1 = OECD mineral media
2 = Aqueous portion from centrifuged inoculum blank
3 = Solid portion from centrifuged inoculum blank

3.5 Instrumental Conditions

The following instrumental conditions were used during the analysis of the test samples.

Instrumental System:	Hewlett-Packard Model 1050 quaternary pump, membrane degasser, autosampler, PE Sciex API 100 LC/MS (single quadrupole), PE Sciex TurbolonSpray® (electrospray)
HPLC Column:	Keystone Betasil C18, 5 µm, 100 Å, 150 x 2 mm column with a Betasil C18 guard column

Mobile phases: A: 2 mM ammonium acetate in purified reagent water
B: 100% methanol

Flow Rate: 0.3 mL/min

Gradient program:

Time (min)	%A	%B
0	60	40
8.5	10	90
11	10	90
13	0	100
17	0	100
20	60	40

Run time: 20 min
Equilibration delay: 10 min
Injection volume: 10 µL

LC/MS parameters

Experiment information:

Scan type: Q1, selected ion monitoring
Scan time: 2.01 sec
Peak Hopping: Disabled
Mass defect: 0 mmu/100 amu
Pause time: 2 msec
Dwell time: 400 msec
Masses scanned (amu)*: 413 (PFOA), 427 (THPFOS), 499 (PFOS), 616, 630 (N-EtFOSE-alcohol)

* Based on 3M analytical method No. ETS-8-11.0

State file information:

Source parameters:

Polarity: Negative
Turbolon spray voltage: -5000 volts
Temperature: 400 °C
Orifice Potential: -20 volts
Nebulizer gas: air (high purity)
Auxiliary gas: nitrogen

4.0 TEST PROCEDURES, RESULTS, AND DISCUSSION

4.1 Toxicity Test

4.1.1 Test Procedures. The potential toxicity of PFOS was investigated concurrently with the activated sludge/sediment acclimation biodegradation study. The assay system consisted of a single stoppered 250-mL Erlenmeyer flask to which 100 mL of an OECD mineral salts medium

(Table 1), containing 20.8 mg/L of PFOS (2.0 mL of SLI No. 70-93A) and 5.00 mg/L [^{14}C]sodium benzoate, was added. The radioactivity was delivered by adding 0.50 mL of a 0.220 mg/L [^{14}C]sodium benzoate stock solution (SLI No. 71-52 RA). The remaining concentration of sodium benzoate was made up by adding 0.080 mL of a non-radiolabeled stock solution containing 6.00 mg/mL of sodium benzoate (SLI No. 61-34V). A 1.0-mL aliquot of activated sludge (approximately 3.0 mg dry weight) collected from the Wareham Wastewater Treatment Plant aeration basin was then added to the flask. A control flask was prepared in the same manner but with the addition of 2 mL of reagent water (no methanol) in place of the PFOS. To the small headspace trap in each flask, 2.0 mL of 1M KOH was added. Flasks were placed on a shaker table set at 150 rpm and incubated at 22 °C. A 100-µL sample was removed from each trap approximately 6 hours after test initiation and again at 24 and 48 hours. The aliquots were analyzed by liquid scintillation counting (LSC). Toxicity was assessed by comparing the rate of $^{14}CO_2$ evolved from the test system flask and control system flask.

4.1.2 Results and Discussion. Table 2 presents the results of the toxicity study with PFOS (20.8 mg/L) and activated sludge. Evolution of $^{14}CO_2$ from the sodium benzoate control flask was not substantially different in the presence of PFOS based on comparison of the rate of $^{14}CO_2$ evolution from both flasks. Thus, at this concentration, PFOS was not anticipated to inhibit the microbial population in this study. However, after the first week, the PFOS concentration in acclimation flasks began to increase due to weekly feeding. Therefore, conclusions about toxicity at concentrations above 20.8 mg/L can not be substantiated, but it could be assumed that PFOS toxicity would not be an issue since all measured concentrations still in the medium (see Table 4) were below 100% of the 20.8 mg/L nominal concentration.

4.2 Activated Sludge/Sediment Biodegradation Study - Acclimation Phase

4.2.1 Test Procedures. The acclimation phase of the biodegradation study in activated sludge/sediment was conducted in 300-mL baffled Erlenmeyer flasks. Inoculum was collected from several sites: the aeration basin from the Wareham, Massachusetts activated sludge plant, the secondary return activated sludge line from the New Bedford, Massachusetts wastewater treatment plant, one disk from a rotating biological contacter (RBC) plant (Bridgewater, Massachusetts), sediment from below the Wareham activated sludge plant's outfall, and a sandy soil collected from a site in Wilson County, North Carolina. The sediment was not characterized, but was a typical dark organic type sediment found below most sewage treatment plant outfalls. The sandy soil was

collected by personnel at American Agricultural Services, Inc., Lucama, North Carolina and was added to provide a surface (protection) for microbial growth and soil microorganisms. The soil was characterized by Agvise Laboratories, Northwood, North Dakota and reported to have the following characteristics: sand, 93%; silt, 4%; clay, 3%; pH 4.4; organic matter, 0.8%; phosphorus, 19 mg/kg; nitrogen, 0.043%; and cation exchange capacity, 3.6 meq/100 g. Aerobic sludge from each wastewater treatment plant was added to the acclimation flasks at a concentration of 1.00 mg/mL (dry weight basis). Sandy soil was added at 10.0 mg/mL medium, and sediment at 1.00 mg/mL medium (dry weight basis) at day 7. Test medium consisted of natural sewage (primary effluent) obtained from the Wareham wastewater treatment plant which had been supplemented with the four OECD mineral salt solutions (at the concentration specified in the OECD ready biodegradation test guidelines, Table 1), trace minerals (Trace Minerals Corp., 0.020 mL/100 mL), yeast extract (Difco, 5.00 mg/100 mL), and activated sludge extract. Activated sludge extract was prepared by adding one part dried RBC sludge to four parts water, autoclaving for 30 minutes, and then centrifuging and filtering through Whatman No.41 paper and Whatman glass fiber filters. The total organic carbon (TOC) of the extract, measured with a Dohrman DC-80 TOC analyzer, was approximately 3500 mg C/L. Sufficient volume (2.0 mL/100 mL of medium) of the extract was added to the natural sewage to bring the TOC level to approximately 100 mg C/L. The extract was refrigerated at 4 °C after preparation and use. The same extract was used throughout the study.

The PFOS test flask contained 100 mL of the medium described above to which 2.0 mL of a sonicated 1.06 mg/mL aqueous PFOS stock solution (SLI No. 70-93A) was added. This resulted in a PFOS concentration of approximately 20.8 mg/L. In addition to the test flask, blank control flasks with no test substance were also established in larger flasks at day 21 (1-L of medium in a 2-L Erlenmeyer flask) for use in preparation of analytical QC samples. Additional flasks, containing only PFOS in OECD salts medium (no biomass, sandy soil, or organic feed) were also established to serve as analytical controls. All flasks were then incubated at 22 ± 2 °C on a rotary shaker (150 rpm) in an environmental chamber.

On a weekly basis, a 10-mL sample of the mixed liquor (10% of the biomass and medium) was removed from the PFOS flasks for analysis of parent material and potential metabolites. The remaining medium in each flask (approximately 90 mL) was centrifuged and 60 mL of the supernatant was removed for analysis. The biomass and medium removed for analysis (70 mL total) were replaced with 70 mL of fresh medium (containing freshly collected natural sewage,

minerals, and carbon nutrients at concentrations originally supplied at day zero), and 1.4 mL of the 1.06 mg/mL PFOS stock solution (SLI No. 70-93A). An additional amount of solids consisting of activated sludge from the Wareham and New Bedford wastewater treatment plants and sediment equivalent to 10% of the original amount supplied at day zero, were also collected each week and added to the test vessels. The Bridgewater RBC wastewater treatment plant sludge was generally collected on a monthly basis and maintained under aeration in the laboratory at ambient temperature. The RBC sludge was not fed during this time period. Flasks were reinoculated from this aerated sample. The blank control flask containing 1.0 L of medium was also treated in the same manner each week and received no PFOS. Analytical control samples were also maintained, sampled, and refortified weekly in the same manner as the activated sludge flasks, but received no biomass or nutrients. Carbon analysis (TOC) was generally conducted on a weekly basis on the supernatants from the flasks after filtration through a 1.0-µm Gelman filter by injection of 1.0-mL samples into a Dohrman DC-80 TOC analyzer. Parameters such as pH, suspended solids, and microbial plate counts were not conducted for these test systems. Weekly OECD medium replacement (70 mL/flask) should have maintained the pH near neutrality.

The acclimation procedure was conducted over a 112-day (16 weeks) period. However, samples for analytical measurement of PFOS and potential metabolites were only collected for the first 14 weeks. All samples from this acclimation phase were saved in 100-mL amber Wheaton bottles with Teflon®-lined rubber septa under refrigeration prior to analysis and completion of the PFOS method validation. At week 10 and12, approximately ⅔ of the biomass was removed from the PFOS flask to initiate the sealed vial studies described below. The remaining biomass in the original Erlenmeyer flasks continued to be fed for another four weeks after which time all flasks were transferred to the refrigerator for storage.

4.2.2 Results and Discussion. Table 3 presents the TOC analyses conducted on the centrifuged medium for the blank control, PFOS, and analytical control vessels. The blank control and PFOS vessels were acclimated to the nutrient feed for approximately 27 days prior to dosing. Gradual acclimation to the feed was evidenced by the gradual TOC decrease between days -27 and -1. The PFOS and analytical control vessels were dosed on day 0 and TOC measurements were conducted on a weekly basis for the next ten weeks. The TOC of the analytical control vessel remained in the 0.7 to 1.7 mg/L range throughout the study. The PFOS medium TOC elevated to 34 mg/L on day 14 and, subsequently, settled back to the 15 to 20 mg/L range for the remainder

of the study. TOC values for the blank control ranged from 10 to 26 mg/L from weeks one through ten. Explanations for the TOC fluctuations cannot be provided. Perhaps, the complex nature of the nutrient feeds and their variation at different collection times may have contributed to these results.

Table 4 summarizes the analytical results for days 7, 14, 21, 28 and 35. For these days, analysis was accomplished by combining the biomass and supernatant samples and centrifuging to create a liquid and solid sample. Day 112 was also analyzed, but the biomass sample was not combined with a supernatant sample prior to analysis. Initially, it was assumed that the PFOS might distribute equally between the solids and liquid in the test system. If so, PFOS concentrations in the two matrices should have remained constant throughout the study, if no biodegradation had occurred. However, this was not the case as PFOS was found to initially be distributed primarily in the medium and as the acclimation progressed was found primarily associated with the cells. With the sampling and feeding regime employed, compounds preferentially adsorbing to biomass and not rapidly biodegrading will tend to accumulate in the acclimation test system. However, the accumulation should have been more gradual than that reflected by the mass balance in Table 4. Based on partitioning observed in sealed vessels (Table 8 indicates an average 18% partitioning to biomass and 82% to medium), it can be calculated that even for a compound partitioning 18:82 between solids and medium, an increase from 100% of nominal at day zero to 118% of nominal at five weeks would be expected.

One possible explanation for the mass balances obtained in Table 4 for the activated sludge system may be explained in Table 5 where the results of the analytical control flasks are presented. Mass balance shows good recovery for the first two weeks then a significant decline in recovery from the medium. Concurrent with this decline in mass balance was the observation of a white precipitate forming on the glass at the interface of the medium and headspace of the vessel. A similar precipitate was observed for the flasks containing biomass. While not verified by specific analysis, it appears reasonable that PFOS concentrations were significantly exceeding water solubility and precipitating on the test vessel walls.

4.3 Closed Vial (Headspace) Aerobic Biodegradation Test

4.3.1 Test Procedures. While awaiting validation of the PFOS analytical method, a biodegradation study was initiated to determine if carbon (CO_2) could be evolved from PFOS,

thereby, providing direct evidence for biodegradation. This study was conducted in 20-mL serum vials with a PFOS concentration of 105 mg/L (or 19 mg C/L). Subsequent to the analytical method validation, an additional sealed vessel study was conducted at a much lower PFOS concentration (0.20 mg/L) in 22-mL serum vials.

Both studies employed inoculum from the acclimation flasks after approximately 12 weeks into the acclimation. Inoculum concentration selected for these studies was based on a study in which varying concentrations of activated sludge were added to OECD salts medium containing 50 mg/L yeast extract and resazurin, an oxidation/reduction indicator in sealed 20-mL serum vials. Headspace in the vials was flushed with pure oxygen prior to sealing. A one to ten dilution of this medium was also examined and biomass concentrations ranged from 10 to 6666 mg/L (dry wt. basis). It was found that all of the vessels containing the full strength medium went anaerobic within 2 weeks (Table 6). Biomass concentrations exceeding 2000 mg/L also caused the 1/10 strength medium to go anaerobic within 2 weeks and a biomass concentration of 666 mg/L gave variable results. Concentrations of 10, 30 and 100 mg/L biomass in the 1/10 strength medium remained aerobic for greater than 2 weeks. Based on these data, an activated sludge concentration of 200 mg/L was selected for the sealed vessel studies.

For the low concentration (0.20 mg/L), 64 vials (22-mL) were prepared as follows; 12 blank vials received 15 mL of mineral medium, 12 inoculum control vials received 14 mL of mineral media and 1 mL of inoculum, 20 analytical control vials received 14 mL of mineral media and 1 mL of a 3 µg/mL PFOS stock solution, and 20 PFOS biodegradation vials received 13 mL of mineral media, 1 mL of inoculum, and 1 mL of a 3 µg/mL PFOS stock solution. The PFOS aerobic sludge acclimation system served as the source of inoculum (final concentration of solids = 200 mg/L).

For the high concentration (105 mg/L), 48 vials (with a measured volume of 20-mL) were prepared (16 blank inoculum control vials, 16 analytical control vials, and 16 PFOS biodegradation (fortified inoculum) vials. The blank inoculum control vials contained a mixture prepared by adding 0.5278 g of wet solids to 250 mL of mineral media (200 mg/L solids on a dry weight basis). The analytical control vials contained a mixture prepared by adding 26 mL of the 1.01 mg/mL PFOS stock solution to 224 mL of mineral media. The biodegradation (fortified inoculum) vials contained a mixture prepared by mixing 0.5346 g of wet solids with 224 mL of mineral media and 26 mL of the 1 mg/mL

PFOS stock solution. Each vial contained 13.6 mL of the appropriate medium and the inoculum control and biodegradation vials contained 200 ppm of dry weight biomass.

Analytical samples were removed from selected blank inoculum control vials, analytical control vials, and PFOS biodegradation vials on Days 0, 16, 30, and 61 for the low concentration, and on Days 0, 15, 30, and 63 for the high concentration. For the high PFOS concentration study, half of the samples were analyzed for CO_2 production in the headspace of the vials (after acidification and shaking for at least 1 hour) and the other half of the vials were refrigerated and kept for specific chemical analysis. Analysis for headspace CO_2 was performed on duplicate samples using a ThermoGlass 1200™ carbon analyzer by injection of 200 µL of headspace gas.

4.3.2 Results and Discussion. Tables 8 and 9 summarize analytical data from the PFOS closed vial systems. For the system containing 0.20 mg/L PFOS (Table 8), an average of 0.362 mg/L PFOS was observed in the inoculum blanks throughout the 61-day study. This was due to the fact that both the fortified and blank test vessels received inoculum from the PFOS aerobic sludge acclimation vessel. The fact that this concentration of PFOS remained relatively constant during the course of the study points to the recalcitrance of this molecule. The PFOS concentration in the fortified analytical control (no inoculum) appeared to steadily decrease from 0.232 mg/L at Day 0 to approximately 0.15 mg/L at Day 61. Average PFOS concentrations in test vessels fortified with PFOS and inoculum also showed a slight decline in PFOS concentration from Day 0 (0.333 mg/L) to Day 61 (0.214 mg/L). Since vials and stoppers were extracted with methanol, PFOS was apparently not adsorbing to the test vessel surfaces.

Recoveries or mass balances from the test system containing 105 mg/L PFOS (19 mg C/L) and no inoculum (analytical controls) ranged from 95% to 123% (Table 9). Recoveries from the test vessels fortified with PFOS and inocula were higher and ranged from 121% to 145% over the 63-day test period. Apparent PFOS concentrations decreased from 145 mg/L on day 0 to 132, 132, and 121 mg/L on days 15, 30, and 63, respectively. This represents an approximate 20% decline in PFOS concentration over the course of the study. Whether this represents biodegradation or some sort of matrix effect is not certain. Samples have been retained for mass spectral analysis.

Table 7 presents data for CO_2 analyses of the headspace gas from these systems. Very little difference in headspace CO_2 production was noted between the blank and PFOS containing test vessels. This is also indicative of PFOS recalcitrance. On day 63, CO_2 in the headspace of one PFOS vial was somewhat higher (33.8%) than the inoculum control flasks; however, without more replication of test vessels, conclusions as to the extent of biodegradation can not be made. The hydrolysis (analytical) control showed much lower CO_2 evolution than the blank and PFOS inoculated vessels over the 63-day test period.

5.0 CONCLUSIONS

Overall, PFOS was found to be very recalcitrant in the activated sludge/sediment system. PFOS removal from acclimation medium occurred via partitioning to biomass in the inoculated test systems or to the glass walls of the vessels in analytical control flasks. From the current data, it cannot be concluded whether apparent decline of PFOS concentration was due to biodegradation or some type of matrix effect. Analysis of the QC samples with each set of test system samples resulted in measured concentrations which were consistent with the recovery range determined during the method validation study. Based on these results, it was established that the appropriate quality control was maintained during the analyses of the test samples.

6.0 FUTURE STUDIES

Acclimation to PFOS degradation, if it occurs, may take substantial time. It has been noted that anoxic settling ponds at the 3M plant in Decatur, Alabama contain materials tentatively identified as perfluorooctane sulfinate (reduced PFOS). Thus, it may be beneficial to sample both aerobic and anaerobic sites known to have been exposed to PFOS for a prolonged time period. Microbes in such a system may be able to use PFOS as an electron acceptor or possibly a source of sulfur. Time decay studies with these inocula may provide the best chance to demonstrate PFOS metabolism.

REFERENCES

VanHoven, Raymond L. and Willard B. Nixon. 1999. Determination of the Water Solubility of PFOS by The Shake Flask Method, Wildlife International LTD. Project Number 454C-107, OECD Guideline for the Testing of Chemicals, 105 Water Solubility, May 3, 1999.

Table 1. Composition of OECD inorganic salts medium.[a]

Solution	Reagents[b]	Concentration, g/L
A[c]	KH_2PO_4	8.50
	K_2HPO_4	21.75
	$Na_2HPO_4 \cdot 2H_2O$	33.40
	NH_4Cl	0.50
B	$CaCl_2$	27.50
C	$MgSO_4 \cdot 7H_2O$	22.50
D	$FeCl_3 \cdot 6H_2O$	0.25
	HCl	0.05

[a] The mineral media was prepared by mixing 10 mL of Solution A with 800 mL of water, then adding 1 mL of Solutions B, C, and D, and then bringing to a volume of 1 L with water.
[b] Proportionate amounts of salts with different waters of hydration may be substituted.
[c] The pH of this solution should be 7.4.

Table 2. Toxicity assay of PFOS in activated sludge.

		[^{14}C]Radioactivity in CO_2 Trap	
Test Vessel	**Time (hours)**	**DPM[a]**	**Percent Applied Dose**
Blank Control[b]	6	<LOQ[c]	0
	24	34100	31.0
	48	46700	42.4
PFOS[d]	6	<LOQ	0
	24	33400	30.4
	48	47000	42.7

[a] DPM = dissociations per minute
[b] Blank Control vessels were comprised of 100 mL of mineral salts medium which contained 5 mg/L [^{14}C]sodium benzoate
[c] <LOQ = less than the Limit of Quantitation (60 dpm)
[d] PFOS vessels were comprised of 100 mL of mineral salts medium which contained 20 mg/L of PFOS and 5 mg/L [^{14}C]sodium benzoate

Table 3. Total organic carbon (TOC) analyses during the pre- and post dosing phases of the activated sludge/sediment acclimation study.[a]

	TOC (mg/L)		
Test Day	Blank Control	PFOS	Analytical Control
Pre Dose			
-27	NA[b]	NA	NA
-20	42.35	45.75	NA
-16	35.67	38.16	NA
-13	24.45	24.76	NA
-8	21.67	17.48	NA
-1	11.98	11.39	NA
Post Dose			
0	NA	---[c]	NA
7	10.07	10.23	1.699
14	14.93	33.85	1.467
21	11.17	17.44	1.604
28	15.54	14.86	1.293
35	21.22	14.94[d]	0.999
42	26.04	15.78	0.876
49	16.62	16.91	0.703
56	16.31	18.56	1.297
63	14.10	19.61	1.224
70	12.06	18.25	0.899

[a] Samples were filtered through a 1.0-µm Gelman filter and duplicate 1.0 mL samples were analyzed on the Dohrman DC-80 carbon analyzer.

[b] Not analyzed.

[c] Initial TOC measurement at the start of the post-dose phase (T_0). Remainder of analyses indicated after 7 days of incubation prior to feeding.

[d] One sample was an outlier and that data point was not used.

Table 4. PFOS concentration measurements from the activated sludge/sediment acclimation flasks.

Sample No./Type[a]	Nominal (mg/L)	PFOS Concentration					
		Measured (mg/L)			Measured (%)		
		Biomass	Medium	Total	Biomass	Medium	Total (Mass Balance)
Test Samples							
AS399-01-1/Day 7	23.5	10.0	16.5	26.5	42.6	70.0	113
AS399-01-2/Day 14	24.4	11.5	14.3	25.8	47.1	58.6	106
AS399-01-3/Day 21	24.8	19.6	9.43	29.0	78.9	38.0	117
AS399-01-4/Day 28	25.0	33.3	11.6	44.8	133	46.2	179
AS399-01-5/Day 35	25.0	21.2	5.47	26.7	84.8	21.9	107
AS399-01/Day 112	22.0	24.7	3.64	28.4	112	16.6	129
Blank Samples							
AS399-06-01/Day 7	0.00	< 0.0100[b]	< 0.0100	< 0.0100	NA[c]	NA	NA
AS399-06-02/Day 14	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
AS399-06-03/Day 21	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
AS399-06-04/Day 28	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
AS399-06-05/Day 35	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
QC Medium Samples[d]							
C599-19/Days 7-28[e]	10.0	NA	11.3	NA	NA	113	NA
C599-20/Days 7-28[e]	20.0	NA	22.6	NA	NA	113	NA
C599-21/Days 7-28[e]	20.0	NA	22.1	NA	NA	110	NA
C599-59/Day 35[e]	10.0	NA	10.5	NA	NA	105	NA
C599-60/Day 35[e]	20.0	NA	22.7	NA	NA	114	NA
C599-61/Day 35[e]	20.0	NA	20.9	NA	NA	105	NA
C899-14/Day 112[e]	5.00	NA	5.02	NA	NA	100	NA
C899-15/Day 112[e]	10.0	NA	10.3	NA	NA	103	NA
C899-16/Day 112[e]	20.0	NA	22.5	NA	NA	112	NA
QC Biomass Samples							
C599-95/Days 7-35[f]	2.50	2.56	NA	NA	102	NA	NA
C599-96/Days 7-35[f]	2.50	2.58	NA	NA	103	NA	NA
C599-97/Days 7-35[f]	5.00	5.33	NA	NA	107	NA	NA
C899-11/Day 112[f]	2.50	2.61	NA	NA	104	NA	NA
C899-12/Day 112[f]	2.50	2.60	NA	NA	104	NA	NA
C899-13/Day 112[f]	5.00	5.76	NA	NA	115	NA	NA

[a] Day 0 samples were not taken.
[b] Values expressed as < 0.0100 were below the limit of quantitation
[c] NA = not applicable
[d] QC = quality control sample.
[e] QC sample fortified in control medium.
[f] QC sample fortified in blank biomass.

Table 5. PFOS concentration measurements from the analytical control flasks.

Sample No./Type[a]	Nominal (mg/L)	PFOS Concentration					
		Measured (mg/L)			Measured (%)		
		Biomass	Medium	Total	Biomass	Medium	Total (Mass Balance)
Test Samples							
AS399-07-01/Day 7	21.2	NA[b]	21.1	21.1	NA	99.6	100
AS399-07-02/Day 14	21.2	NA	19.8	19.8	NA	93.2	93.2
AS399-07-03/Day 21	21.2	NA	11.1	11.1	NA	52.4	52.4
AS399-07-04/Day 28	21.2	NA	17.8	17.8	NA	83.9	83.9
AS399-07-05/Day 35	21.2	NA	10.2	10.2	NA	48.0	48.0
AS399-07/Day 112	21.2	NA	5.74	5.74	NA	27.1	27.1
Blank Samples							
AS399-09-01/Day 7	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
AS399-09-02/Day 14	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
AS399-09-03/Day 21	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
AS399-09-04/Day 28	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
AS399-09-05/Day 35	0.00	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
QC Samples[d]							
C599-19/Days 7-28	10.0	NA	11.3	NA	NA	113	NA
C599-20/Days 7-28	20.0	NA	22.6	NA	NA	113	NA
C599-21/Days 7-28	20.0	NA	22.1	NA	NA	110	NA
C599-59/Day 35	10.0	NA	10.5	NA	NA	105	NA
C599-60/Day 35	20.0	NA	22.7	NA	NA	114	NA
C599-61/Day 35	20.0	NA	20.9	NA	NA	105	NA
C899-14/Day 112	5.00	NA	5.02	NA	NA	100	NA
C899-15/Day 112	10.0	NA	10.3	NA	NA	103	NA
C899-16/Day 112	20.0	NA	22.5	NA	NA	112	NA

[a] Day 0 samples were not taken.
[b] Values expressed as < 0.0100 were below the limit of quantitation
[c] NA = not applicable
[d] QC = quality control sample.
[e] QC sample fortified in control medium.
[f] QC sample fortified in blank biomass.

Table 6. Selection of test conditions for the sealed PFOS CO_2 evolution study.[a]

	Solids Concentration, mg/L (dry weight basis)[b]											
	Full Strength Medium						$^1/_{10}$ Strength Medium					
Day	10	30	100	666	2000	6666	10	30	100	666	2000	6666
0	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
4	+++	+++	+++	--+	---	--+	+++	+++	+++	+++	+++/-	---
5	+++	+++	+++	--+	---	—	+++	+++	+++	+/-+/-+/-	++/--	---
6	+/-+/-+	+++	+++	--+	---	---	+++	+++	+++	+/-+/-+/-	+/---	---
7	-+/-+/-	+++	+/-+/--	--+/-	---	---	+++	+++	+++	+/-+/-+/-	---	---
9	--+/-	+/-+/-+/-	+/---	---	--	---	+++	+++	+++	+/-+/-+/-	---	---
10	--+/-	+/---	+/---	---	---	---	+++	+++	+++	+/-+/-+/-	---	---
12	---	---	---	---	---	---	+++	+++	+++	+/-+/-+/-	---	---
14	---	---	---	---	---	---	+++	+++	+++	+/-+/-+/-	---	---
16	---	---	---	---	---	---	+++	+++	+++	--+/-	---	---
17	—	---	---	---	---	---	+++	+++	+++	--+/-	---	---
21	---	---	---	---	---	---	+++	+++	+++	---	---	---

[a] Test system = 2-mL serum vials, 15 mL medium consisting of OECD salts, Wareham raw sewage, 100 mg C/L sludge extract, resazurin and activated sludge. For $^1/_{10}$ strength medium, raw sewage and sludge extract were diluted 1 to 10 in the test system.

[b] Aerobicity was judged by observing medium color; + = pink color and presence of O_2; - = colorless and no O_2; +/- = faint pink color.

Table 7. CO_2 analysis of the headspace in sample vials during the closed vial (headspace) aerobic biodegradation test with PFOS.[a]

Test Vessel	Replicate	Headspace C, mg/L				% Theoretical			
		Day				Day			
		0	15	30	63	0	15	30	63
Inoculum Control[b]	A	NA[e]	7.61	9.22	11.54	NA	NA	NA	NA
	B	NA	7.55	9.25	10.49	NA	NA	NA	NA
Analytical Control[c]	A	NA	2.32	3.14	3.27	NA	NA	NA	NA
	B	NA	2.68	2.78	3.58	NA	NA	NA	NA
PFOS Fortified[d]	A	NA	6.53	8.10	9.99	NA	-7.70	-8.33	-7.52
	B	NA	7.19	8.39	15.62	NA	-2.86	-6.20	33.78

[a] Averaged for duplicate test vessels.
[b] Contains "acclimated' activated sludge in mineral salts medium.
[c] Contains 79.3 mg/L (20 mg C/L) PFOS in mineral salts medium.
[d] Contains "acclimated' activated sludge in mineral salts medium and 79.3 mg/L PFOS.
[e] NA = not applicable

Table 8. PFOS concentration measurements for the 0.200 mg/L test samples from the sealed vessels (headspace).

Sample No./ Sample Day	Nominal (mg/L)	PFOS Concentration					
		Measured (mg/L)			Measured (%)		
		Biomass	Medium	Total	Biomass	Medium	Total (Mass Balance)
Inoculum Control							
HS0699-10/0	0.000	0.0400	0.300	0.340	NA[a]	NA	NA
HS799-03/16	0.000	0.023	0.365	0.388	NA	NA	NA
HS799-04 /16	0.000	0.020	0.373	0.393	NA	NA	NA
HS799-121/30	0.000	0.005	0.341	0.346	NA	NA	NA
HS799-122/30	0.000	0.019	0.316	0.335	NA	NA	NA
HS899-69/61	0.000	0.0309	0.352	0.395	NA	NA	NA
HS899-70/61	0.000	0.0222	0.310	0.340	NA	NA	NA
Avg.	0.000	0.0229	0.337	0.362	NA	NA	NA
PFOS + Inoculum [b]							
HS0699-15/0	0.212	0.0841	0.249	0.333	39.7	117	157
HS799-08/16	0.212	0.0321	0.175	0.207	15.1	82.5	97.7
HS799-09/16	0.212	0.0131	0.207	0.220	6.18	97.6	104
HS799-10/16	0.212	0.0351	0.271	0.306	16.6	128	144
HS799-126/30	0.212	0.0521	0.188	0.240	24.6	88.7	113
HS799-127/30	0.212	0.0251	0.108	0.133	11.8	50.9	62.8
HS799-128/30	0.212	0.0471	0.202	0.249	22.2	95.3	118
HS899-74/61	0.212	0.0066	0.101	0.108	3.11	47.6	50.8
HS899-75/61	0.212	0.0748	0.220	0.295	35.3	104	139
HS899-76/61	0.212	0.0273	0.100	0.127	12.9	47.2	60.0
Avg	0.212	0.0397	0.180	0.219	79.9 [c]	85.9 [c]	105
Blank Analytical Control							
HS0699-08	0.000	NA	<0.0100[d]	NA	NA	<0.0100	NA
HS799-01/16	0.000	NA	<0.0100	NA	NA	<0.0100	NA
HS799-02/16	0.000	NA	<0.0100	NA	NA	<0.0100	NA
HS799-119/30	0.000	NA	<0.0100	NA	NA	<0.0100	NA
HS799-120/30	0.000	NA	<0.0100	NA	NA	<0.0100	NA
HS899-67/61	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
HS899-68/61	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
Analytical Control							
HS0699-12/0	0.212	NA	0.232	0.232	NA	109	109
HS799-05/16	0.212	NA	0.197	0.197	NA	92.9	92.9
HS799-06/16	0.212	NA	0.198	0.198	NA	93.4	93.4
HS799-07/16	0.212	NA	0.196	0.196	NA	92.5	92.5
HS799-123/30	0.212	NA	0.179	0.179	NA	84.4	84.4
HS799-124/30	0.212	NA	0.187	0.187	NA	88.2	88.2
HS799-125/30	0.212	NA	0.188	0.188	NA	88.7	88.7
HS899-71/61	0.212	NA	0.159	0.163	NA	75.0	75.0
HS899-72/61	0.212	NA	0.142	0.144	NA	67.0	67.0
HS899-73/61	0.212	NA	0.152	0.154	NA	71.7	71.7

[a] NA = not applicable
[b] Net values after subtraction of inoculum control average values.
[c] Ratio equivalent to 18% PFOS on solids and 82% in medium.
[d] Values presented as less than were below the limit of quantitation

Table 8. Continued. PFOS concentration measurements for the 0.200 mg/L test samples from the sealed vessels (headspace).

Sample No./ Sample Day	Nominal (mg/L)	PFOS Concentration					
		Measured (mg/L)			Measured (%)		
		Biomass	Medium	Total	Biomass	Medium	Total (Mass Balance)
QC[a] Medium							
C699-266/0	0.200	NA	0.195	NA	NA	97.5	NA
C699-267/0	0.200	NA	0.186	NA	NA	93.0	NA
C799-40/16	0.200	NA[b]	0.195	NA	NA	106	NA
C799-41/16	0.200	NA	0.186	NA	NA	111	NA
C799-42/16	0.200	NA	0.195	NA	NA	105	NA
C799-43/16	0.200	NA	0.186	NA	NA	98.5	NA
C799-145/30	0.200	NA	0.162	NA	NA	81.0	NA
C799-146/30	0.200	NA	0.176	NA	NA	88.0	NA
C799-147/30	0.200	NA	0.170	NA	NA	85.0	NA
C799-148/30	0.200	NA	0.163	NA	NA	81.5	NA
C899-94/61	0.200	NA	0.221	NA	NA	111	NA
C899-95/61	0.200	NA	0.207	NA	NA	104	NA
C899-96/61	0.200	NA	0.229	NA	NA	114	NA
C899-97/61	0.200	NA	0.218	NA	NA	109	NA
QC solids							
C699-262/0	0.200	0.219	NA	NA	110	NA	NA
C699-263/0	0.200	0.213	NA	NA	107	NA	NA
C799-38/16	0.200	0.219	NA	NA	115	NA	NA
C799-39/16	0.200	0.213	NA	NA	110	NA	NA
C799-149/30	0.200	0.211	NA	NA	106	NA	NA
C799-150/30	0.200	0.210	NA	NA	105	NA	NA
C899-98/61	0.200	0.211	NA	NA	106	NA	NA
C899-99/61	0.200	0.205	NA	NA	103	NA	NA

[a] QC = quality control sample
[b] NA = not applicable

Table 9. PFOS concentration measurements for the 105 mg/L test samples from the sealed vessels (headspace).

Sample No./ Sample Day	Nominal (mg/L)	PFOS Concentration Measured (mg/L)			Measured (%)		
		Biomass	Medium	Total	Biomass	Medium	Total (Mass Balance)
Blank Inoculum							
HS799-41/0	0.000	< 0.0100[a]	< 0.0100	< 0.0100	NA[b]	NA	NA
HS799-42/0	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
HS799-135/15	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
HS799-136/15	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
HS899-25/30	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
HS899-26/30	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
HS999-25/63	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
HS999-26/63	0.000	< 0.0100	< 0.0100	< 0.0100	NA	NA	NA
PFOS + Inoculum							
HS799-45/0	105	1.31	151	152	1.25	144	145
HS799-46/0	105	2.86	134	136	2.72	127	130
HS799-139/15	105	0.912	138	139	0.869	131	132
HS799-140/15	105	1.10	145	146	1.05	138	139
HS899-29/30	105	1.25	142	143	1.19	135	136
HS899-30/30	105	2.50	136	139	2.34	130	132
HS999-29/63	105	1.42	125	127	1.35	119	121
HS999-30/63	105	2.27	130	132	2.17	124	126
Analytical Control							
HS799-43/0	105	NA	120	120	NA[a]	114	114
HS99-44/0	105	NA	115	115	NA	109	109
HS799-137/15	105	NA	129	129	NA	123	123
HS799-138/15	105	NA	109	109	NA	103	103
HS899-27/30	105	NA	123	123	NA	117	117
HS899-28/30	105	NA	124	124	NA	118	118
HS999-27/63	105	NA	108	108	NA	103	103
HS999-28/63	105	NA	99.5	99.5	NA	94.7	94.7
QC[c] Medium							
C799-131/0	25.0	NA	25.3	NA	NA	101	NA
C799-132/0	50.0	NA	53.7	NA	NA	107	NA
C799-133/0	100	NA	104	NA	NA	104	NA
C799-165/15	25.0	NA	29.5	NA	NA	118	NA
C799-166/15	50.0	NA	59.1	NA	NA	118	NA
C799-167/15	100	NA	115	NA	NA	116	NA
C899-02/30	25.0	NA	27.5	NA	NA	110	NA
C899-03/30	50.0	NA	52.6	NA	NA	105	NA
C899-04/30	100	NA	106	NA	NA	106	NA
C999-103/63	25.0	NA	26.8	NA	NA	107	NA
C999-104/63	50.0	NA	53.6	NA	NA	107	NA
C999-105/63	100	NA	110	NA	NA	110	NA

[a] Values presented as less than were below the limit of quantitation.
[b] NA = not applicable
[c] QC = quality control sample

Table 9. Continued. PFOS concentration measurements for the 105 mg/L test samples from the sealed vessels (headspace).

Sample No./ Sample Day	Nominal (mg/L)	PFOS Concentration					
		Measured (mg/L)			Measured (%)		
		Biomass	Medium	Total	Biomass	Medium	Total (Mass Balance)
QC[a] Solids							
C799-134/0	33.3	35.7	NA[b]	NA	107	NA	NA
C799-135/0	66.7	69.9	NA	NA	105	NA	NA
C799-136/0	100	99.0	NA	NA	99.0	NA	NA
C799-168/15	33.3	37.1	NA	NA	111	NA	NA
C799-169/15	66.7	68.9	NA	NA	103	NA	NA
C799-170/15	100	107	NA	NA	107	NA	NA
C899-05/30	33.3	37.7	NA	NA	113	NA	NA
C899-06/30	66.7	75.4	NA	NA	113	NA	NA
C899-07/30	100	102	NA	NA	102	NA	NA
C999-106/63	66.7	68.1	NA	NA	102	NA	NA
C999-107/63	66.7	72.3	NA	NA	108	NA	NA
C999-108/63	100	103	NA	NA	103	NA	NA

[a] QC = quality control sample
[b] NA = not applicable

Figure 1. Flow chart of extraction procedures.

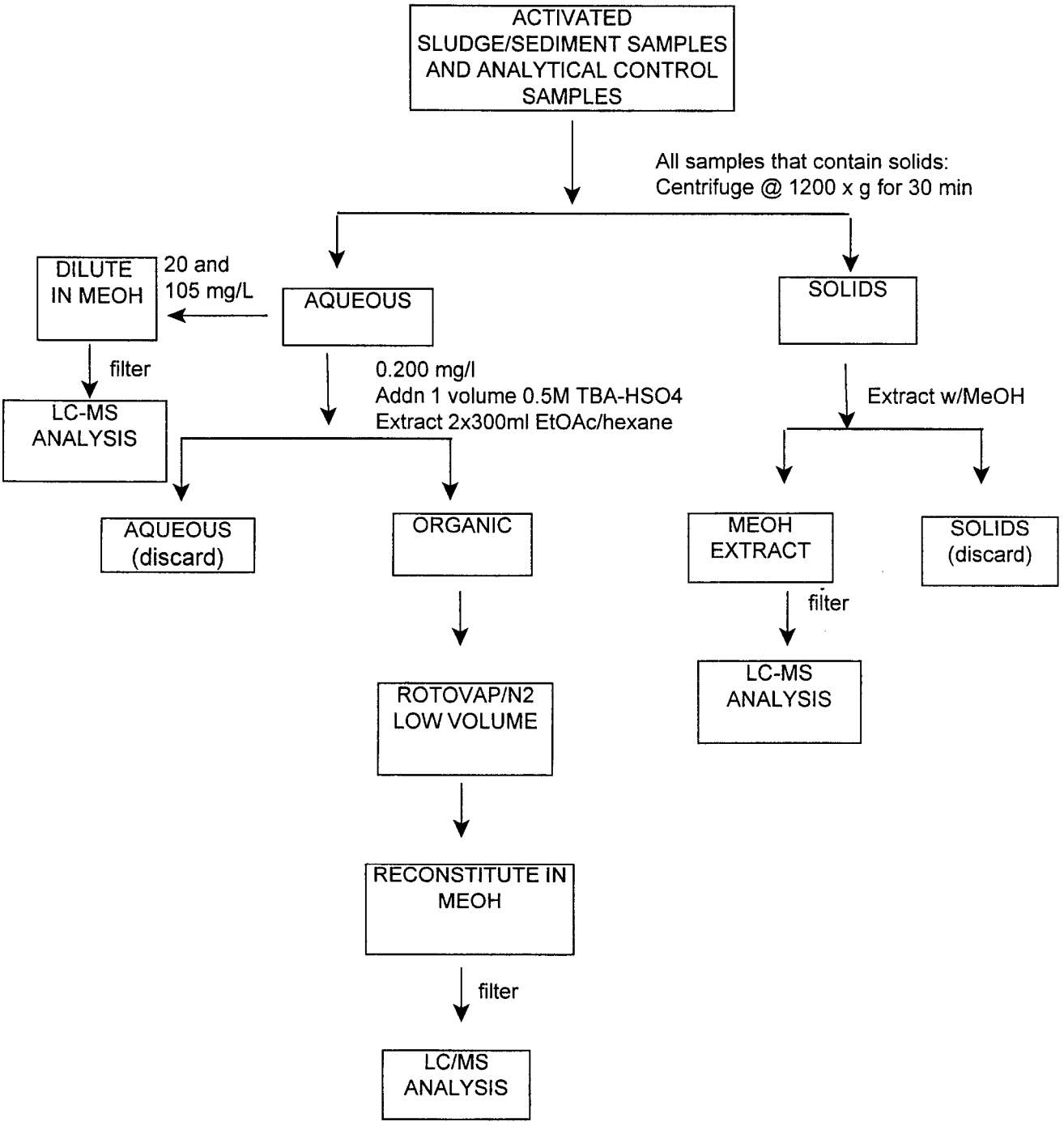
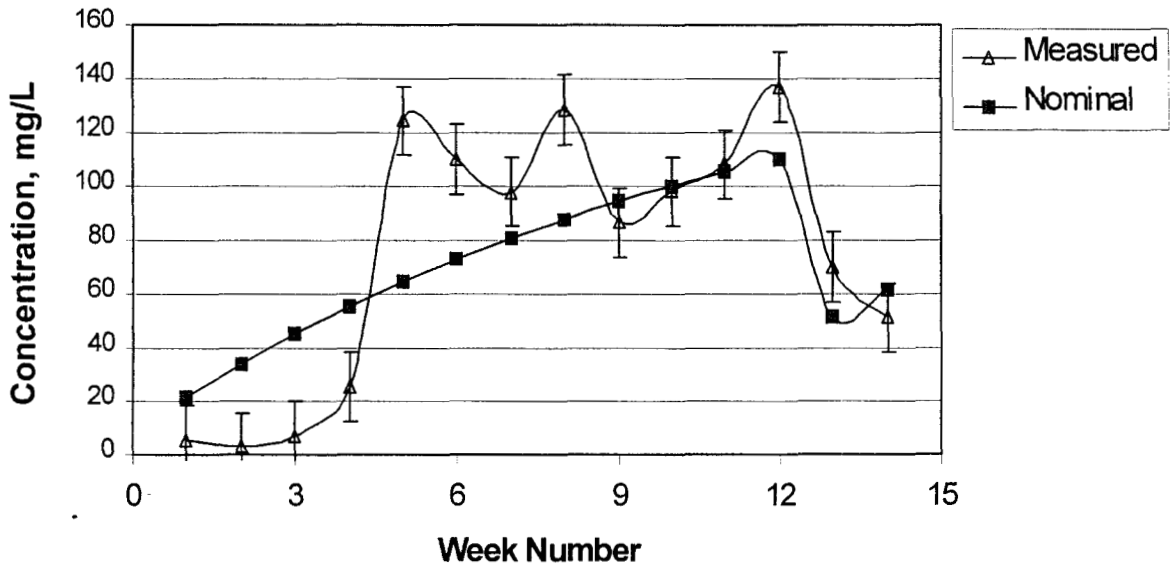


Figure 2. Total measured PFOS concentration during acclimation in activated sludge/sediment.[a]



[a] Nominal values were calculated using the following assumptions: a) 10% of the biomass and 70% of the medium was removed and replaced each week with fresh biomass and 14.84 mg/L PFOS (70% of nominal) and b) 92.0% of PFOS partitions to biomass in the system, 0.3% remains in the medium, and 1.2% binds to glass.

Figure 3. PFOS concentration in the analytical control flask.

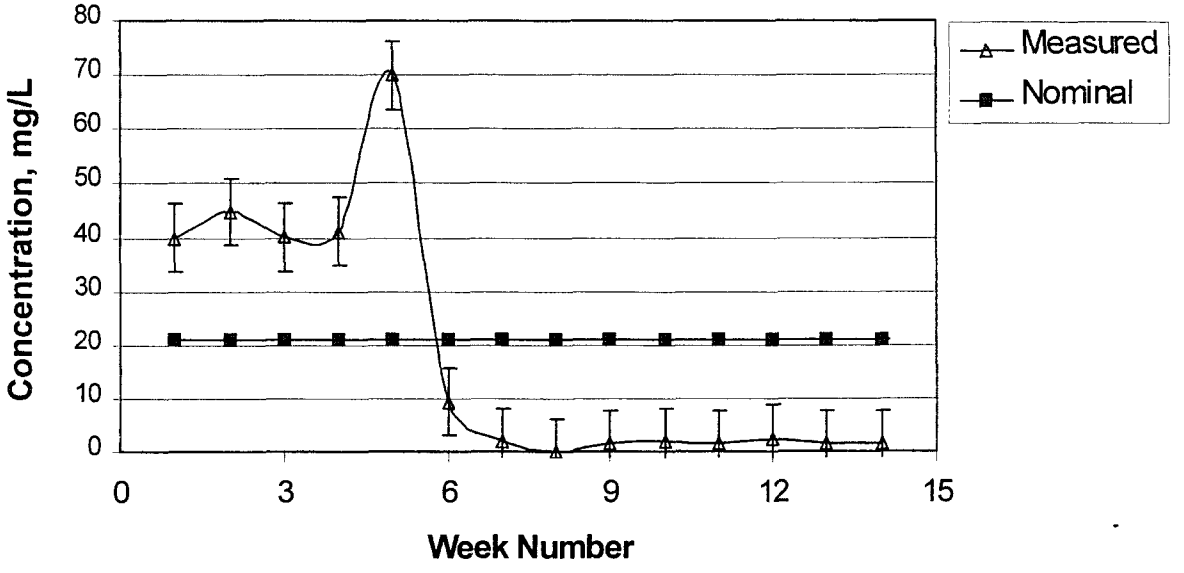


Figure 4. Concentration of PFOS versus time during the closed vial (headspace) aerobic biodegradation test.