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ENVIRONMENTAL CHEMISTRY FOR A SURFACTANT ECOTOXICOLOGY STUDY SUPPORTS RAPID DEGRADATION OF C_{12}-ALKYL SULFATE IN A CONTINUOUS-FLOW STREAM MESOCOSM

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Abstract—Environmental chemistry is a vital part of ecotoxicology studies conducted in mesocosms. Quantification of exposure can be particularly challenging for biodegradable surfactants in once-through stream mesocosms like the Procter & Gamble Experimental Stream Facility (ESF). In the fall of 1991, a study was conducted with the anionic surfactant C_{12}-alkyl sulfate (C_{12}-AS). Analysis of chemical feed tank concentrates indicated that the in-stream concentrations should be very close to the nominal concentrations (26, 78, 233, 700, 2,100 μg/L). However, measured concentrations were lower than expected. The concentrations at the head of the streams were 4 to 23% below nominal concentrations and there was an additional 14 to 33% decline in head to tail C_{12}-AS concentrations. Total residence time in the streams is about 4.3 min. Because analytical losses had been minimized using individual sample recovery factors, it was concluded that these losses were due to rapid C_{12}-AS biodegradation. The results of this analytical program are used to define the in-stream C_{12}-AS concentrations to express subsequent community- and ecosystem-level no-observed-effect concentrations. In addition, the environmental chemistry program described will serve as the model for future programs in support of surfactant ecotoxicology studies conducted at the ESF.

Keywords—Anionic surfactant Artificial stream MBAS Biodegradation Experimental ecosystem

INTRODUCTION

Model stream ecosystems, or mesocosms, have been used extensively to evaluate chemical and biological processes in streams under controlled conditions [1]. Because test chemicals added to stream mesocosms travel realistic transport pathways and create realistic exposure patterns, these studies provide useful information for ecological risk assessments [2]. While lentic mesocosms have often been used to meet regulatory requirements in the evaluation of pesticides in fresh water systems, there has been recognition of a growing need for lotic mesocosm tests to evaluate the hazards of industrial chemicals and effluents in receiving streams [3].

The Procter & Gamble Experimental Stream Facility (ESF) was built to evaluate the potential for ecological effects of high-volume consumer product chemicals. Dose–response ecotoxicology tests have been conducted at the ESF to determine no-observed-effect concentrations (NOEC) for a variety of surfactants [4–9]. Belanger [10] reviewed the fate and effects of surfactants in microcosm, mesocosm, and field tests. Surfactants, or surface-active agents, are a significant component of several consumer products, such as laundry detergents, shampoo, toothpaste, and cosmetics. Surfactants typically are disposed of following consumer use down-the-drain to wastewater treatment plants.

Environmental chemistry is a vital part of every ecotoxicology test conducted in mesocosms, especially for biodegradable surfactants in once-through stream mesocosms like the ESF. Most reviews of mesocosm analytical programs focus on the analysis of the test chemical. In his review, Giddings [2] primarily focused on three reasons for the test chemical analysis during a mesocosm study: (1) to learn the fate of the chemical; (2) to quantify chemical exposure; and (3) to confirm that the test chemical has been accurately applied. Other important aspects of a complete mesocosm analytical program include: test chemical characterization and stability, background water chemistry, and analysis of test chemical concentrates for delivery to the test system.

In the fall of 1991, an ecotoxicology study was conducted at the ESF with the anionic surfactant C_{12}-alkyl sulfate (C_{12}-AS). C_{12}-alkyl sulfate is used worldwide in many consumer products. C_{12}-alkyl sulfate is rapidly degraded by bacteria in sewage treatment plants [11] and is generally at, or below, detection limits of analytical methods for receiving streams [12]. When C_{12}-AS is degraded by bacteria it is rapidly mineralized meaning the principal by-products are carbon dioxide and metabolic water [11]. Because C_{12}-AS is rapidly mineralized, the analytical program designed to support the C_{12}-AS ecotoxicological study was not focused on the ultimate fate of this surfactant in the streams. Instead, our analytical program was designed to meet Giddings' [2] second (quantify exposure) and third (confirm test chemical accurately applied) reasons for test chemical analysis as well as define the background water quality in support of the ecotoxicological evaluation of C_{12}-AS. In addition, this work will describe the role of test chemical characterization and stability and analysis of test chemical concentrates for analytical support to ecotoxicology studies.

MATERIALS AND METHODS

Test material

C_{12}-alkyl sulfate, also known as sodium dodecyl sulfate, 99% active, was purchased from the Sigma Chemical Company, St. Louis, Missouri, USA. Additional characterizations were performed according to Procter & Gamble analytical protocols to verify activity and purity of the test material such as (1) negative

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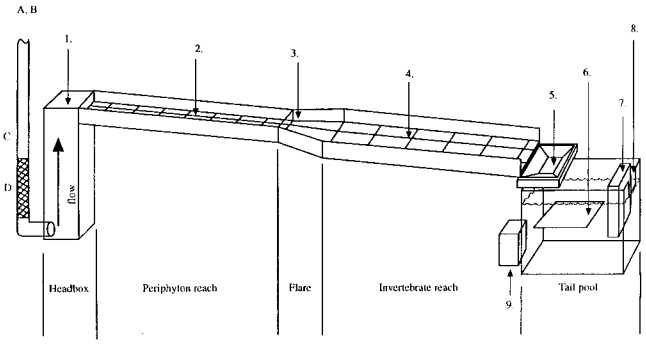

Fig. 1. Diagram of a Procter & Gamble Experimental Stream Facility stream channel indicating sampling locations for all programs. Analytical sampling locations were in the headbox (location 1) and the tail pool (at location 8). Test chemical enters the flow of river water (A) at location C and is immediately dispersed in the in-line mixer (D).

cationic sulfate titration to determine total anionics; (2) infrared spectroscopy to verify identity and to determine if any unreacted intermediates were present; (3) gas chromatography (GC) to ascertain alkyl chain length and identity; (4) fast atom bombardment/mass spectrometry to verify chain length and purity; (5) karl fisher titration to determine moisture content; and (6) a petroleum ether extractables—unsulfonated for unreacted materials. The additional characterizations verified the purity and activity per the manufacturer's literature of minimum 99% activity and purity.

Test system

The Procter & Gamble ESF is located on the Lower East Fork of the Little Miami River (LEFR) in Milford, Ohio, USA. The ESF is connected to the Clermont County Lower East Fork Waste Water Treatment Plant for treatment of its dosed effluent. The ESF is an indoor stream mesocosm facility that combines aspects of computer control over key parameters such as photoperiod and stream flow rates [8] with elements of the environment. For instance, the ESF is colonized by natural drift of biota from its source river, the LEFR. Because ESF ecotoxicological testing is conducted with once-through flow of natural river water [7], water chemistry changes, including seasonal temperatures, directly influence ESF stream biota.

Seven, 11-m-long experimental streams were utilized in this study. Five streams received C_{12}-AS in a dose–response experimental design. The remaining two streams served as undosed controls. This experimental design is more powerful for ecotoxicology studies than ANOVA-based, replicated designs, given a fixed number of streams [7]. A representative stream channel is shown in Figure 1. Each stream received approximately 164 L/min river water (A on Fig. 1). Dosed streams also received 35 ml/min of concentrated test chemical (C on Fig. 1), which thoroughly mixed with river water in the in-line mixer (D on Fig. 1) prior to entrance into the headbox (1 on Fig. 1). Travel time for river water plus test chemical is approximately 20 s through the biological test area of the stream that includes the periphyton reach (2 on Fig. 1), the flare zone (3 on Fig. 1), and the invertebrate reach (4 on Fig. 1). River water plus test chemical then spills into the tail pool (6 on Fig. 1). When the 0.15-m^3 headbox and 0.5-m^3 tail pool are included, the total residence time for the river water plus test chemical from the in-line mixer to the tail pool drain is approximately 4.3 min. River water plus test chemical is then discharged from the facility to the adjacent wastewater treatment plant.

To ensure that the stream channels have no hydraulic barriers to river water and test chemical flow, flow was visualized with a food-grade dye (FD&C Blue #1) added at the test chemical injection point (C on Fig. 1). Spectrometric analysis of the dye verified that both cross-sectional and longitudinal dispersions of a water-soluble organic test chemical would be homogenous in these streams.

C_{12}-alkyl sulfate dosing system

The test material dosing system design for this facility requires the C_{12}-AS be prepared in a chemical feed tank as a highly concentrated aqueous solution. This concentrate is then pumped into the river water distribution system that passes through an in-line mixing baffle and enters the experimental stream. For the C_{12}-AS experiment, concentrate stock solutions were prepared at nominal concentrations of 124, 371, 1,109, 3,329, and 9,986 mg/L in deionized water to achieve desired in-stream nominal concentrations in the streams of 26, 78, 233, 700, and 2,100 μg/L, respectively. A recharging cycle of 7 d utilizing two chemical feed tanks in a reciprocating manner was used to provide constant delivery of test material over the course of this 8-week study.

The C_{12}-AS concentrates were prepared in 416-L stainless steel chemical feed tanks fitted with variable speed stirrers. The chemical feed tanks were half filled using deionized water and the stirrers started at a very low speed. The C_{12}-AS was slowly added to minimize dust formation and maximize wetting of powder. The C_{12}-AS was allowed to wet totally and stirred prior to further addition of deionized water. The addition of water was achieved during this process via a computerized flow controller. After the C_{12}-AS was totally wetted, deionized water was added to bring the total volume in the chemical feed tank to 416 L. The speed of the stirrers was increased and the C_{12}-AS allowed to mix thoroughly for at least 24 h prior to use or sampling. The C_{12}-AS was then pumped out of the bottom outlet tube into the river water stream flow (location C Fig. 1) via a Pulsa Series model 340 diaphragm pump.

Stability study

Prior to the ecotoxicology study, a stability test was performed to ensure that the C_{12}-AS would remain stable at high concentrations while being delivered (35 ml/min) for a minimum of 7 d. For this study, the lowest concentration (124 mg/L) and highest concentration (9,986 mg/L) of C_{12}-AS concentrates were prepared in carbon-filtered tap water and sampled daily for 8 d. C_{12}-alkyl sulfate was found to be soluble in carbon-filtered tap water at these concentrations. However, when chemical feed tanks were set up for the intermediate concentrations (78, 233, 700 mg/L), C_{12}-AS was insoluble. Deionized water was then substituted for the tap water, and all concentrations of C_{12}-AS were soluble. Deionized water was then used in the remainder of the study. A stability study in deionized water was also conducted during the 8-week ecotoxicological study.

Chemical feed tank sample collection for C_{12}-alkyl sulfate analysis

Samples for the stability study were taken on a daily basis in duplicate using 250-ml glass bottles rinsed with sample prior to final collection. Subsurface grab samples were taken approximately 8 cm below the surface of the continuously mixed chemical feed tanks. The samples were taken to the Procter & Gamble Ivorydale Technical Center Laboratories and retained under refrigeration until analyzed. At these concentrations

(124–9,986 mg C_{12}-AS/L deionized water), samples were stable with refrigeration for at least 12 weeks due to the lack of biological activity in the deionized water. In addition, it is likely that the high C_{12}-AS concentrations in these chemical feed tanks also acted as a preservative. Each chemical feed tank sample was analyzed using the methylene blue active substance (MABS) method described below.

In-stream sampling

Stream water samples to support the ecotoxicology experiment were taken on a weekly basis into 4-L amber glass bottles with an outer safety coating (VWR). Bottles were rinsed with sample prior to final collection of stream water. The streams were sampled where water from the stream headbox entered the stream channel (Fig. 1, location 1) and the exit of stream water at the tail pool weir (Fig. 1, location 8). Samples were collected using a 600-ml glass beaker and a large glass funnel. The configuration of the headbox and tail pool required small sampling containers to minimize disruption to the streams, especially the dislodging of loosely attached material found on the headbox and tail pool walls. Unlike the chemical feed tank samples discussed above, stream samples require additional preservation measures to inhibit biodegradation of C_{12}-AS during storage and sample processing. Stream samples (3.5 L) were immediately preserved with formaldehyde (final concentration 1%, w/v) to inhibit biological activity. An internal standard, C_{14}-AS, was added to each sample to a final concentration of 250 μg/L. C_{14}-alkyl sulfate has been shown to be below detection limits in river water of this region [12]. The samples were returned to Ivorydale Technical Center laboratories generally within 2 h after sample collection. A subsample was filtered through a Gelman A/E (0.3-μm) glass fiber filter. This filtrate was placed in a new container, relabeled, and refrigerated at 4°C until the final sample preparation could be done. River water samples preserved with 1% formaldehyde, filtered, and stored under refrigeration were shown to be stable in excess of 6 months.

Sample collection for other water quality parameters

Samples were collected from the ESF streams weekly for additional water quality parameters as part of the in-stream ESF analytical program. Weekly grab samples for priority pollutants, pesticides, metals, and PCBs [13] were taken at the weir of the tail pool in approved containers supplied by International Technology Analytical Services (Cincinnati, OH, USA). Sample preservation was as recommended by U.S. Environmental Protection Agency (EPA) methods [13]. Samples were stored on ice and transported to International Technology Analytical Services (Cincinnati, OH, USA) within 1 h after sampling for analysis. Grab samples were also taken from the head of each stream and the river water intake twice a week for the analysis of total and ash-free dry weight of suspended solids [14].

In addition to the weekly analysis of ESF river water, samples were also collected for priority pollutants, pesticides, metals, and PCBs from the LEFR, the source of ESF river water and sediment. These samples were collected once just prior to the start of the ESF colonization. The LEFR water from the ESF water intake point and LEFR sediment from the stream reach where invertebrates were collected [4] were sampled using approved containers supplied by International Technology Analytical Services (Cincinnati, OH, USA). Sample preservation was done according to EPA methods [13] and the samples were transported to International Technology Analytical Services (Cincinnati, OH, USA) within 1 h after sampling.

MBAS analysis of C_{12}-alkyl sulfate in chemical feed tanks

C_{12}-Alkyl sulfate concentrates ranging from 124 mg/L to 9,986 mg/L in deionized water were analyzed using an MBAS method. The MBAS method is a recognized standard colorimetric method [14] for the analysis of anionic materials in aqueous solutions. Methylene blue is a cationic dye, which under acidic conditions will ion-pair with most anionic materials. Following addition of methylene blue to a sample, ion-paired anionics can be extracted into chloroform. The chloroform phase was removed and a direct measurement made at 652 nm on a UV/visible spectrophotometer (Hewlett Packard model 8452 Diode Array, Avondale, PA, USA). Color intensity is directly proportional to anionic surfactant content. C_{12}-alkyl sulfate concentrations were calculated based on standard curves developed with C_{12}-AS. Previous evaluations of the MBAS method have included a cross laboratory study using linear alkyl benzene sulfonate, a widely used anionic surfactant. Distilled, tap, and river water matrices were used to demonstrate 14.8%, 9.9%, and 9.1% RSD (relative standard deviation), respectively, in a survey of 110 laboratories [14]. Although MBAS was an appropriate method for chemical feed tank samples, the selectivity (response to non-C_{12}-AS chemicals in stream water) and sensitivity (requiring large volumes of stream water for analysis) of this method was not sufficient for streamwater samples. For instance, initial evaluations of in-stream samples with MBAS resulted in several false-positive responses in control stream waters, possibly due to large interferences of naturally occurring anionics present in the river water (e.g., humic materials, etc.).

Gas chromatographic analysis of C_{12}-alkyl sulfate in streamwater samples

The GC method used was a modification of the Fendinger [12] method for the determination of AS surfactants in natural waters. Due to time and resource requirements for this method, the more time- and labor-intensive GC method was not used for the chemical feed tank samples where the MBAS method was adequate. The details of sample preparation, including the various solid-phase extractions and BSTFA (*N,O*-bis-[trimethylsilyl]-trifluoroacetamide) derivitization step, are given below. The AS derivatives are quantified using capillary GC (see below). This original paper [12] cites recovery factors of 99 ± 15% for 200 μg/L C_{14}-AS, 88 ± 7 for 200 μg/L C_{12}-AS, and 93 ± 7% for 20 μg/L C_{12}-AS. The method was applied to all stream mesocosm samples.

Formaldehyde preserved streamwater samples, each with C_{14}-AS internal standard, were allowed to equilibrate to room temperature prior to any preparation. Using a vacuum manifold, 100 or 200 ml of stream water were eluted through a C-2 solid-phase extraction cartridge (Varian Sample Prep Products, BOND ELUTE, Harbor City, CA, USA). The C_{12}-AS and C_{14}-AS are retained on the column. This column was stacked onto a strong anion-exchange cartridge (SAX) (Varian, BOND ELUTE). Methanol (10 ml) was used to elute the C_{12}-AS and C_{14}-AS from the C-2 column onto the SAX. The C-2 column was removed and discarded. The SAX was eluted with 2% (v/v) HCl : methanol to remove the C_{12}-AS and C_{14}-AS. The column effluent was collected in a beaker and evaporated to dryness using nitrogen gas. The dried eluent was reconstituted in 5 ml methanol and passed through a strong cation-exchange resin, 5X-80 (BioRad, Richmond, CA, USA). The column effluent was saved and evaporated to dryness. The dried eluent was dissolved with 500 μl of dimethyl formamide. The solution was placed into a septum sealed GC auto sampler vial and capped. The BSTFA

with 1% TMCS (trimethylchlorosilane, Pierce, Rockford, IL, USA), 500 μl, was injected into the vial and the vial placed on a heating block at 80 to 90°C for 1 h to form the TMS derivative. This derivative was directly injected (1 μl, injector temperature 200°C) onto a 5890 Hewlett Packard GC (Avondale, PA) with flame ionization detection (detector temperature 225°C). The chromatographic conditions used were a Restek, RT_x-1 capillary column (60 m long, film thickness 0.1 μm, 0.25 mm i.d., 100% methylsiloxane) with temperature programming from 50 to 215°C at 10°C/min using a hydrogen carrier gas (41 cm/s).

Other water quality analyses

The following water quality parameters were measured with appropriate U.S. Environmental Protection Agency methods [13] by International Technology Analytical Services (Cincinnati, OH, USA): pH, ammonia nitrogen, residual chlorine, total suspended solids, carbonaceous biological oxygen demand, surfactants determined as anionics, dissolved oxygen, alkalinity, chlorides, total kjeldahl nitrogen, nitrate nitrogen, *ortho*-phosphorous, total phosphorus, dissolved organic carbon (DOC), total organic carbon, sulfate, calcium, magnesium, and sodium. There were 101 components analyzed in river water and sediment samples to determine metals, metalloids, volatile and semivolatile organics, pesticides, and PCBs.

Suspended solids were analyzed twice a week beginning 3 weeks before C_{12}-AS dosing began and continuing through the 8-week exposure. Grab samples were filtered onto a prewashed, prefired, tared glass fiber filter (Gelman A/E). The volume filtered depended on the solids load in the water and was recorded. The solids on the filter were dried to a constant weight (105°C). Organic material was removed by combustion (500°C) to determine ash-free dry weight.

RESULTS

Stability of C_{12}-alkyl sulfate in chemical feed tanks

C_{12}-Alkyl sulfate at concentrations of 124 mg/L and 9,986 mg/L, was stable in the chemical feed tanks for 7 d (Fig. 2) under normal operating conditions. The concentration of C_{12}-AS increased for both concentrations on the eighth day of operation (delivery rate approximately 35 ml/min). During this time the tank volume decreased from 416 L to less than 75 L due to delivery of the test chemical. The results shown in Figure 2 represent averages of two subsurface samples. Because this stability study was conducted in carbon-filtered tap water, rather than deionized water used during the ecotoxicology study, a second stability study was conducted from the week 7 to week 8 sampling day on the 124 and 9,986 mg/L chemical feed tanks. These results verified the stability of C_{12}-AS for the week of use as indicated by recoveries of 106 ± 2% of 124 mg/L nominal and 101 ± 9% of 9,986 mg/L nominal.

Methylene blue active substance analysis of C_{12}-alkyl sulfate in chemical feed tanks during ecotoxicology study

Concentrations of C_{12}-AS were monitored weekly in the chemical feed tanks for all streams. Measured concentrations (8-week average) for all tanks were very close to the nominal, or target, concentrations (Table 1). Chemical feed tank concentrations ranged from 93.2 to 99.7% of the nominal concentration with no pattern associated with total mass of C_{12}-AS in the tank (Table 1). There are 39 sets of duplicate chemical feed tank samples that were analyzed over the 8-week study and averaged to make up the data shown in Table 1. When all data are expressed as percentage of nominal, the average RSD for the MBAS analysis of C_{12}-AS in deionized water was 1.6%.

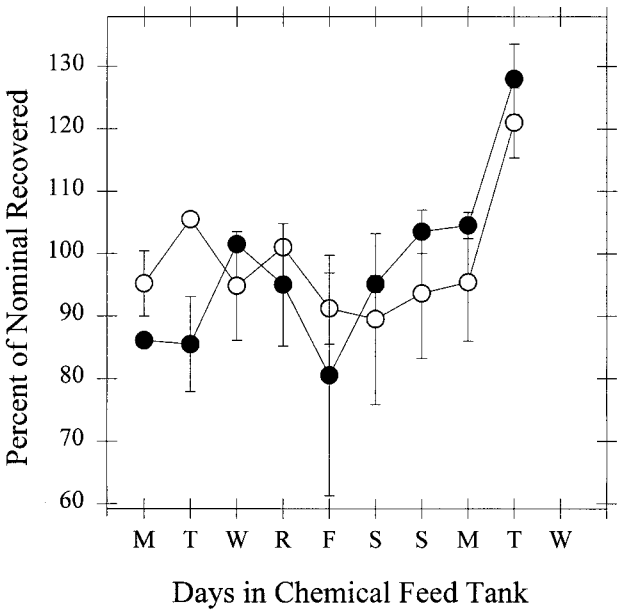

Fig. 2. C_{12}-alkyl sulfate stability in chemical feed tanks during normal delivery operation. The C_{12}-AS was made up in carbon-filtered tap water to test the lowest tank concentrate (124 mg/L, ○) and the highest tank concentrate (9,986 mg/L, ●). The stability study began on a Monday (M) and was sampled daily for 8 additional days. C_{12}-alkyl sulfate was analyzed with the MBAS method. Values plotted are average (±SD) of the two subsurface samples taken per tank, calculated as percentage of nominal, or target, chemical feed tank concentrations of C_{12}-AS.

Using measured chemical feed tank concentrations (Table 1), chemical feed tank delivery rates and river water flow rates (Table 2), expected C_{12}-AS in-stream concentrations were calculated (Table 2). In-stream nominal concentrations had been used to calculate needed chemical feed tank concentrations assuming a chemical feed delivery set point of 35 ml/min and streamwater flows of 166.5 L/min. The data shown in Table 2 incorporate all variables in the expected values averaged over the 8-week study. The delivery of chemical feed was found to match precisely the set point with minimal variance (average RSD = 1.3%). Computer-controlled river water flow rates were also found to be very consistent varying from an 8-week average of 164.4 to 168.0 L/min with an average RSD of 0.2% (Table 2).

An example (high dose, 2,100 μg/L stream) from Table 2 demonstrates how the expected values were derived to obtain

Table 1.Chemical feed tank concentrations of C_{12}-alkyl sulfate with a comparison of nominal, or target, and MBAS-measured concentrations

Nominal C_{12}-AS (mg/L)	Measured C_{12}-As (mg/L)	Measured C_{12}-AS (% of nominal)
124	116 ± 4	93.2 ± 3.3
371	362 ± 24	97.6 ± 6.5
1,109	1,015 ± 49	99.7 ± 4.4
3,329	3,216 ± 119	96.6 ± 3.6
9,986	9,682 ± 347	97.0 ± 3.5

Average ± SD using all measurements collected over 8-week study: two replicates per dose weekly for 8 weeks (n = 16).

Table 2.In-stream concentrations of C_{12}-alkyl sulfate with a comparison of nominal, expected and GC-measured concentrations

Nominal instream C_{12}-AS (μg/L)	Chemical feed tank delivery rate (ml/min)	River water flow rate (L/min)	Expected instream C_{12}-AS (μg/L)	Measured instream (headbox) C_{12}-AS (μg/L)	Measured instream C_{12}-AS (% of expected)
0	none	164.4 ± 0.2	0.0	0.7 ± 1.3	—
26	35.1 ± 0.4	164.6 ± 0.4	24.7	19.8 ± 10.6	−20
78	35.6 ± 0.6	164.6 ± 0.2	78.3	61.0 ± 21.8	−22
233	35.7 ± 0.2	168.0 ± 0.8	234.8	224.3 ± 65.8	−4
700	35.0 ± 0.8	166.2 ± 0.3	677.2	582.0 ± 118.3	−14
2,100	35.2 ± 0.3	165.4 ± 0.3	2,060.5	1,585.8 ± 297.3	−23

Chemical feed tank delivery rates and river water flow rates are provided for the calculation of the expected in-stream C_{12}-AS concentrations based on actual chemical feed tank concentrations found (see Table 1). Average ± SD for chemical feed tank delivery rate based on weekly measurements for the 8-week study. River water flow rate based on data collected and averaged by computer system for 8 h prior to each sampling day. Average ± SD calculated for 8-week study based on these values. Expected C_{12}-AS concentrations = [(measured chemical feed tank concn. from Table 1)∗(chemical feed tank delivery rate)]/river water flow rate. Percentage of expected = [(measured − expected)/(expected)]∗100%.

an expected concentration of 2,060 μg/L. The chemical feed tank for this stream had an 8-week average concentration of 9,682 mg/L (Table 1) and an average chemical feed delivery rate from the tank to the streams of 35.2 ml/min. Using these data, the predicted delivery of C_{12}-AS to the river water can be calculated to be 340.95 mg/min. If the chemical feed system delivers 340.95 mg/min into a stream flowing at 165.4 L/min the expected stream concentration would be 2,060 μg/L.

Gas chromatographic analysis of C_{12}-alkyl sulfate in river water samples

The average recovery for C_{14}-AS internal standard using the GC analysis was 91 ± 29% for all in-stream samples collected (n = 84). C_{12}-alkyl sulfate quantification was done utilizing the specific percentage of internal standard (C_{14}-AS) recovered for each individual sample. This recovery factor was multiplied by the mass of C_{12}-AS measured to normalize the data to a 100% C_{14}-AS recovery.

When the measurement of C_{12}-AS was normalized to the internal standard recovery factor, C_{12}-AS concentrations in the headbox (see location 1 on Fig. 1) of the control streams were essentially at the detection level (approximately 1 μg/L, Table 2). The average of the 8 weekly samples from the dosed streams indicated low variability over the 8 weeks (average RSD of 31%), but with concentrations significantly below the expected values (Table 2). The measured in-stream values (headbox) were 4 to 23% below the expected values (Table 2), which were based on the measured chemical feed tank concentrations (Table 1).

The nominal C_{12}-AS concentrations selected for this study ranged from 0 to 2,100 μg/L with intermediate streams at intervals at a factor of 3 (for instance, 2,100/3 = 700). Although the measured in-stream concentrations were below the nominal and expected C_{12}-AS concentrations, the streams spanned a range of 0 to 1,586 μg/L and maintained an intermediate interval of approximately 3 (Table 2). When the headbox and tail pool C_{12}-AS nominal versus measured concentrations are plotted (Fig. 3), the consistent ratio across the range of concentrations is evident. In addition, the further loss of C_{12}-AS from the headbox to the tail pool is evident in all of the dosed streams (Fig. 3). This ranged from 14 to 33% when averaged over the 8-week study for each stream.

Single samples were taken from each analytical sampling location each week. When these samples are plotted for the tail pool values (Fig. 4), it is evident that the expected C_{12}-AS concentration (horizontal lines in Fig. 4) was achieved in most streams on week 1. There were week-to-week fluctuations of C_{12}-AS in all the streams, but the trend in concentration declined with length of continuous exposure (Fig. 4). The two control streams generally had levels of C_{12}-AS at or below the detection limit (approximately 1 μg/L) except for an increase in both streams on the week 4 sampling, which was just following a large rain event.

Water quality parameters

Water quality parameters and pollutants monitored for this study were found to be very stable with little or no change during the course of the 8-week study. Of the water quality

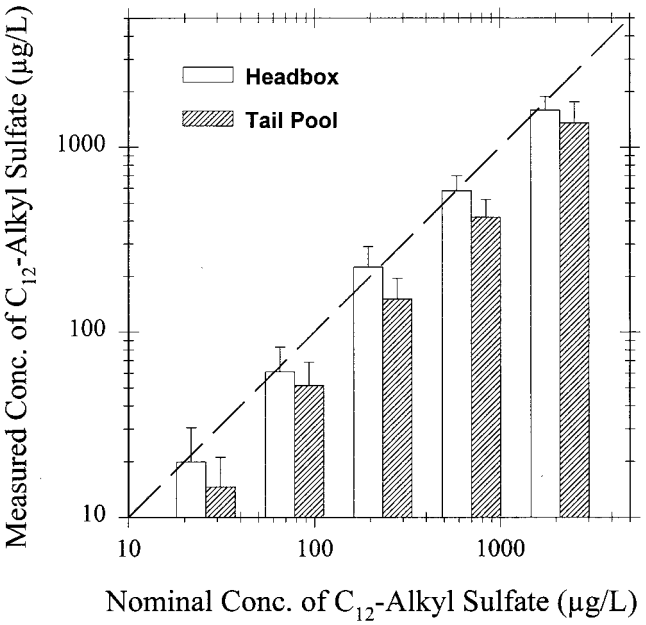

Fig. 3. In-stream C_{12}-alkyl sulfate concentrations of dosed Experimental Stream Facility streams. Average (±SD) of weekly C_{12}-AS concentrations (μg/L) over the 8-week study is shown for the head (□) and tail (▨) of each stream. Dashed line indicates the 1:1 relationship of nominal to measured C_{12}-AS concentrations.

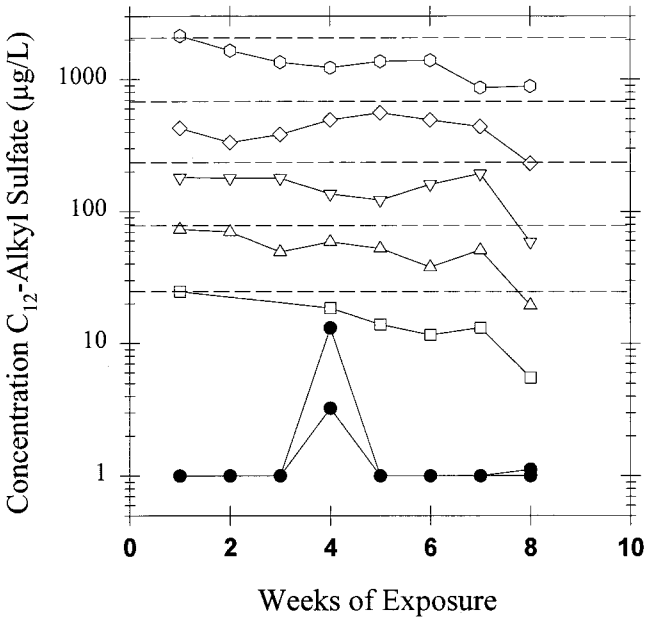

Fig. 4. Tail pool concentrations of C_{12}-alkyl sulfate by week for all Experimental Stream Facility streams. Horizontal dashed lines are expected in-stream concentrations of C_{12}-AS as calculated in Table 2. Control streams (filled circles) were almost always at the detection limit (1 μg/L) except for the week 4 sample. Single samples were taken weekly from the tail pool; missing data points are a result of lost samples.

Table 3.Water quality parameters for Experimental Stream Facility stream water in the control stream monitored weekly[a]

Parameter	Minimum	Maximum	Mean	Standard deviation
pH	7.2	8	7.7	0.22
Ammonia nitrogen	0.25	0.41	0.34	0.18
Residual chlorine	<0.03[b]	0.15	0.04	0.04
Total suspended solids	9.2	36	24.2	9.9
Carbonaceous BOD	<5.0[b]	29	6.6	6.6
Surfactants as anionics	<0.01[b]	0.05	0.04	0.02
Dissolved oxygen	7.3	8.3	7.8	0.31
Alkalinity	97	130	115	9.5
Chlorides	7.8	25	18.8	5.3
Total kjedahl nitrogen	0.43	1.7	1.01	0.37
Nitrate nitrogen	0.57	1.9	1.4	0.39
Orthophosphorus	0.13	0.34	0.27	0.07
Total phosphorus	0.18	0.38	0.29	0.06
Dissolved organic carbon	<8.0[b]	18	9.4	3.3
Total organic carbon	<8.0[b]	9.7	7.9	0.98
Sulfate	21	39	30.8	5
Calcium	34	52	45.2	4.9
Magnesium	9	11	9.9	0.51
Sodium	7.4	24	14.7	4.8
Total suspended solids	7.43	50.37	24.79	14.38

[a] Minimum, maximum, and mean (with standard deviation) values are given over the 8-week study (n = 9). Concentrations are given in mg/L, except for pH.
[b] Specifies minimum detection limit. Where appropriate, detection limit used in calculation of mean.

parameters monitored over the study, all parameters (n = 136 data points) except dissolved organic carbon (n = 8) and total organic carbon (n = 8) demonstrated no higher than 36% RSD for any analyte over this period. The dissolved organic carbon and total organic carbon demonstrated the highest variability with 162% and 206% RSD, respectively (Table 3). These high variances were due to elevated DOC (18 mg/L), and TOC levels (9.7 mg/L), which occurred during the fourth week of the study as a result of a large rain event. The total suspended solids load in the streams had a maximum value about 2.5 weeks after the start of C_{12}-AS exposure (maximum 50.37 mg/L, Table 3). This is the actual time frame of the large rain event, although this rain event is small in comparison to one that occurred 1.5 weeks before C_{12}-AS dosing began in which suspended solids increased to over 380 mg/L. There were no differential organic/inorganic responses as the ash-free dry weight results were parallel to the total suspended solids. The variability of suspended solids from stream-to-stream or at the river water intake was very low. The 8-week average for all streams (24.6 ± 14.2 mg/L) was almost identical to the control stream value (Table 3).

Of the 101 priority and conventional pollutants monitored such as metals, pesticides, and others, most (n = 92) data points reported were nondetectable. Components above detection limits are listed in Table 4. The National Water Quality Criteria [15] limits shown in Table 4 for phenolics, nickel, and zinc show the stream waters to be well below set limits, however, the quantity of mercury at 0.0002 mg/L appears to exceed the criteria of 0.000012 mg/L [15]. The sensitivity of the present approved EPA method utilized by International Technology Analytical Services (Cincinnati, OH, USA) shows only a minimum detection limit of 0.0001 mg/L that is a factor of 10 above suggested limits for mercury using these criteria.

DISCUSSION

C_{12}-alkyl sulfate analysis methods

C_{12}-alkyl sulfate was evaluated using a nonspecific method (MBAS) for chemical feed tanks and a specific GC method for in-stream concentrations. The MBAS method uses a standard curve generated with C_{12}-AS to determine the chemical feed tank concentrations. The precision of our application of this

Table 4.Priority and conventional pollutants detected (above standard EPA method's detection limit, see text for details of each method used) in the Lower East Fork of the Little Miami River water and sediments[a]

Compound	River water at RM 5.5 (mg/L)	River water at RM 3.7 (mg/L)	Sediment at RM 5.5 (μg/g)	Sediment at RM 3.7 (μg/L)	EPA water quality criteria (mg/L) [15]
Arsenic	—	—	1.9	2.2	—
Beryllium	—	—	0.22	0.4	—
Cadmium	—	—	0.53	0.54	—
Chromium	—	—	4.7	6	—
Copper	—	—	4.3	5.5	—
Mercury	0.0002	0.0002	—	—	0.000012[c]
Nickel[b]	0.02	0.021	3	5	0.149
Phenolics	0.5	0.06	—	—	2.56
Zinc[b]	0.008	0.053	17	21	0.523

[a] Samples were collected adjacent to the Experimental Stream Facility intake (river mile, RM 5.5) and at the site of invertebrate tray colonization (RM 3.7), which is downstream of the Lower East Fork WWTP (RM 5.0).
[b] Ni and Zn water quality criteria were calculated at 180 mg/L hardness, an average value for the Lower East Fork basin.
[c] Detection limit for mercury (0.0001 mg/L) is above water quality criteria.

method (RSD = 1.6%) was better than the published values for interlaboratory variability of this method [14].

The GC analysis of in-stream C_{12}-AS concentrations used an internal standard (C_{14}-AS) to normalize for sample-to-sample recovery variability. Over the 8-week study, the recovery of C_{14}-AS (91 ± 29%) was comparable to published values (99 ± 15% in Fendinger et al. [12]). Published recoveries for an equal mass of C_{12}-AS in river water (88 ± 7%) are lower than for C_{14}-AS [12]. Previous investigators have not used any recovery corrections in their analyses for AS concentrations in river water [12] aside from the use of an injection standard when comparing results to an external calibration curve [16]. Due to the importance of accurately defining in-stream concentrations in support of an ecotoxicology test, C_{14}-AS internal standard recoveries were used to adjust C_{12}-AS concentrations for sample-to-sample variability.

Overall, we conclude that both the nonspecific (MBAS) and specific (GC) analysis for C_{12}-AS were accurate and reproducible in the analysis of C_{12}-AS in the chemical feed tanks and in ESF river water.

Dosing of C_{12}-alkyl sulfate to streams

The C_{12}-AS obtained for this study was first analyzed to verify that it met purity and activity specifications provided by the manufacturer. A stability study was then conducted to show that concentrated solutions of C_{12}-AS in the chemical feed tanks were stable during use. Initial studies using carbon-filtered tap water resulted in clear, C_{12}-AS-soluble, solutions at the low (124 mg/L) and high (9,986 mg/L) chemical feed tank concentrates but insoluble precipitates at all concentrations between. Precipitation phase boundary diagrams for C_{12}-AS indicate that for a given concentration of multivalent cations (such as calcium and magnesium found in tap water), there is an intermediate range of C_{12}-AS concentrations that will precipitate [17]. When chemical feed tanks were made up with deionized water, with the cations removed, all concentrations of C_{12}-AS stayed in solution.

Because C_{12}-AS was shown to be stable in the chemical feed tanks for 7 days under normal operating conditions (Fig. 2), weekly samples from these tanks were collected just after the tank was put on-line to deliver C_{12}-AS into the stream. Over the 8-week study, the C_{12}-AS concentrations in the chemical feed tanks were between 93 and 99% of the nominal concentration (Table 1). Because flows of both the chemical feed tank and river water to the streams accurately met the set points (Table 2), and the chemical feed tank concentrations were very close to the nominal values (Table 1), the expected in-stream C_{12}-AS concentrations were very close to the nominal concentrations (Table 2).

In-stream C_{12}-alkyl sulfate concentrations

In-stream C_{12}-AS concentrations were lower than expected. The concentrations in the headboxes were 4 to 23% below nominal (Table 2) while the concentrations in the tail pools were an additional 14 to 33% below these values (Fig. 3). Because the expected values were equivalent to nominal concentrations, and the analyses of in-stream concentrations were individually corrected for recovery, the losses measured are not likely to be due to the analytical method used. Because C_{12}-AS is rapidly biodegraded [11], it appears most likely that these losses are biologically based.

Sorption of C_{12}-AS to ESF sediment was considered as a possible alternative route of C_{12}-AS loss from the water column because the in-stream C_{12}-AS concentrations (Table 2, Figs. 3 and 4) are water column concentrations, with removal of suspended solids above 0.3 μm during sample preparation. Suspended solid concentrations ranged from 50.37 mg/L on week 2.5, during the large rain event, to a low of 7.43 mg/L (Table 3). There appeared to be no week-to-week in-stream C_{12}-AS changes corresponding to these changes in suspended solids, except for an increase in C_{12}-AS on the first sampling point after the rain event (week 4) in the control streams (Fig. 4).

A study was conducted to evaluate the potential for C_{12}-AS to adsorb to ESF sediments. The ESF sediments were slurried with ESF river water and $^{14}C_{12}$-AS was added. This mixture was allowed to equilibrate for 3 d before the phases were separated and the amount of ^{14}C was quantified. No detectable ^{14}C was found following combustion of the sediments and up to 95% of the added ^{14}C was measured in the river water (D.E. Whittington, unpublished data).

The week-by-week decline in tail pool C_{12}-AS concentrations (Fig. 4) is consistent with microbial biodegradation results for C_{12}-AS reported in Guckert [9]. Biodegradation studies conducted during the C_{12}-AS ecotoxicology study indicated that time required to mineralize the entire amount of added C_{12}-AS (approximately 70 μg/L $^{14}C_{12}$-AS in these assays) to $^{14}CO_2$ dropped below 2 h in the highest-dose stream (1,586 μg/L) after 8 weeks of exposure to C_{12}-AS [9]. This extremely rapid rate of C_{12}-AS biodegradation influenced not only the microbial community but the entire ESF stream ecosystem [4,9] and is consistent with the analytical results presented here.

Historical ESF data [8] of river water quality parameters indicate that many of the parameters measured in the C_{12}-AS study (Tables 3 and 4) are similar in comparison to previous ESF studies. The major exception for the C_{12}-AS study was the elevated DOC value that occurred during the fourth week of the study as the result of a large rain event (Table 3). The average values for DOC for 1989 and 1990 ESF studies were 4.4 to 4.9 and 5.8 to 6.5 mg/L [8], respectively, versus the average of the 1991 C_{12}-AS experiment of 3 to 5 mg/L if the high of 18 mg/L were excluded. The comparison demonstrates the river appears to be fairly stable, except when natural events alter its composition, The importance of the fourth week DOC value is that during this period many biological effects were observed [4,9,18]. In addition, in-stream analytical results suggest that C_{12}-AS concentrations increased in the incoming river water during week 4 (Fig. 4). The water quality data generated will be used to help interpret the biological and ecological changes and effects. Pollutant analyses showed the river water and sediments to be free of many components that might endanger the health of the ecosystem (Table 4). The components that were present above detection limits of the methods used were believed not to be of greater quantity than normally occurring in environmental matrices. In addition, the LEFR has been shown to have a robust and diverse macroinvertebrate community [4]. Changes in water quality and solids' quality or quantity are also statistically blocked across all streams during the study.

Environmental chemistry in support of ecotoxicology studies

In this study, an environmental chemistry program that supports an ecotoxicology study is described. The analytical needs for an ecotoxicology program are different than programs used to quantify in situ environmental concentrations. Environmental concentrations of many test chemicals, especially biodegradable surfactants, tend to be very low and are many times at the levels of detection [12,16]. For a stream mesocosm study, the analytical method may need to quantify several orders of magnitude

for the added test chemical. In addition, extra care must be exercised to minimize analytical losses. For many environmental monitoring programs, analytical recoveries above 80% are considered to be sufficient [12,16]. However, a 20% loss of test chemical due solely to the analytical method may result in a mesocosm NOEC that inappropriately underestimates the actual ecosystem-level NOEC.

In this study, test chemical stability was evaluated to ensure weekly evaluations of the C_{12}-AS concentrate were sufficient. The C_{12}-AS concentrate (e.g., the chemical feed tank) was evaluated to determine what in-stream concentrations should be expected. Computer-controlled pumps are used to accurately deliver test chemical and river water to the streams. In-stream concentrations are individually corrected for an analytical recovery factor. All of these steps are taken to minimize analytical losses. With these steps, we feel confident that the measured concentrations of C_{12}-AS accurately reflect the water column concentrations in our streams. The losses we noted (expected to headbox, headbox to tailpool) are, therefore, losses due to in-stream biological processes, such as microbial biodegradation.

Giddings [2] indicated that analysis of the concentrated test chemical solution was an appropriate indirect measure of test chemical application, if direct analysis was not possible. The C_{12}-AS results indicate that for a biodegradable surfactant under continuous exposure, this strategy would overestimate the actual in-stream concentrations. We feel, however, that a combination of both the test chemical concentrate and the in-stream concentrations provides the most accurate measure of the test chemical while also providing additional understanding about the fate of this material in the mesocosm.

Although the measured concentrations of C_{12}-AS were below expected values, the streams spanned a range of 0 to 1,586 μg/L and maintained an intermediate interval of approximately 3. The range and separation of C_{12}-AS concentrations provides sufficient discrimination power to develop NOEC values for the biological endpoints measured. The analytical program described in this paper has defined the in-stream concentrations that will be used in all other analyses of the ecotoxicological evaluation of C_{12}-AS. The periphyton [9,18] and macroinvertebrate studies [4] will use the 8-week average headbox concentration to develop NOECs. The protozoa (P. McCormick, unpublished data) and single-species [19] studies, both of which were conducted in the stream's tail pools, will use the average tail pool concentrations for the development of NOECs. Finally, the surprising head to tail loss of C_{12}-AS in these fast-flowing stream mesocosms will also be used in the evaluation of the C_{12}-AS biodegradation program conducted during the ecotoxicology study [9].

This environmental chemistry program will serve as the model for future programs in support of surfactant ecotoxicological studies conducted at the Procter & Gamble Experimental Stream Facility.

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