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Anaerobic Degradation Pathway of Linear Alkylbenzene Sulfonates (LAS) in Sulfate-Reducing Marine Sediments

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Linear alkylbenzene sulfonates (LAS) are among the principal synthetic surfactants used worldwide. Their presence in the environment has been reported in a significant number of studies, and it has been generally assumed that LAS are not biotransformed in the absence of oxygen. However, laboratory experiments performed by our group using anoxic marine sediments have reported LAS degradation percentages that can reach up to 79% in 165 days. Here, we show for the first time the initial reaction metabolites (generated via fumarate addition to the LAS molecules), their biotransformation into sulfophenyl carboxylic acids (SPC), and the progressive degradation of these by successive β-oxidation reactions. Advanced mass spectrometry has been used to carry out the identification of these compounds. This is the first time that an anaerobic degradation pathway for LAS is described, and these results represent a significant advance in understanding the final fate of these and other similar compounds in anoxic environments.

Introduction

The contamination of aquatic environments by organic compounds (e.g., pharmaceuticals, surfactants, endocrine disruptors, polymers) has recently become the focus of increasing regulation and public concern due to their possible, but still mostly unknown, deleterious effects on human health and/or on the wildlife living in these ecosystems (*1*). Under certain conditions, some of these chemicals can also persist in the environment for relatively long times. In the case of surfactants, the most extensively studied compound is nonylphenol (NP), a recalcitrant degradation intermediate of alkylphenol ethoxylates (APEO), which are widely used in the formulation of detergents, cleaners, paints, pesticides, etc. NP exhibits estrogenic properties (*2*), altering the male/female ratio of several species of fish in rivers and lakes and, therefore, jeopardizing their reproduction. As a consequence, the use of APEO in household applications was banned a few years ago in EU.

Our target compound, the anionic surfactant linear alkylbenzene sulfonates (LAS), is one of the most widely used synthetic surfactant on a world scale: its total annual production is around 3 million tons (*3*). LAS are commonly used as a mixture of different homologues and isomers (Figure 1a) in all kind of household detergents, dishwashing products, and multipurpose cleaners. After use, they are efficiently removed (typically >95%) in wastewater treatment plants (WWTPs), like many other surfactants, by degradation and sorption processes (*4*). The remaining fraction of this compound reaches aquatic ecosystems through both treated and untreated wastewater discharges. Aerobic degradation of LAS can take place quickly in WWTPs and in the water column, by the generation of sulfophenyl carboxylic acids (SPC), resulting from the ω-oxidation of the alkyl chain followed by successive α and/or β-oxidations (*5*) (Figure 1b). These metabolites are also biodegradable, but are less toxic than LAS, and do not show any estrogenic activity (*6*). Currently, the main concern is from reports that high concentrations of LAS (of the order of several g/kg) occur in anaerobically digested sludges from WWTPs (*7*), which are later applied to agricultural soils. Values of up to several tens of mg/kg have been also detected in highly contaminated anoxic sediments (*8*). Several laboratory assays have not found any evidence of LAS anaerobic degradation taking place (*9*−*11*), so consequently it has been generally assumed that these compounds accumulate in this kind of environment because they are not biotransformed in absence of oxygen.

Recently, several researchers (*12*−*14*) have reported the disappearance of LAS from continuous stirred tank reactors (CSTR) and from up-flow anaerobic sludge blanket (UASB) reactors, although no metabolites were identified. Evidence of the anaerobic degradation of this surfactant into SPC has been confirmed by our research group in sulfate-reducing marine sediments, where the presence of significant concentrations of these metabolites at anoxic depths ($E_h = -380$ mV) had been previously observed in pore water (*15, 16*). This degradation was confirmed by incubating these sediments in the laboratory under controlled conditions after addition of known amounts of LAS (*17, 18*). Primary degradation percentages of up to 79% were observed after 165 days when 20 ppm or less of LAS were spiked, and SPC were identified as the main degradation metabolites, with concentrations of up to 3 μg mL^{-1} being measured. Results from these assays also showed that there are two factors that

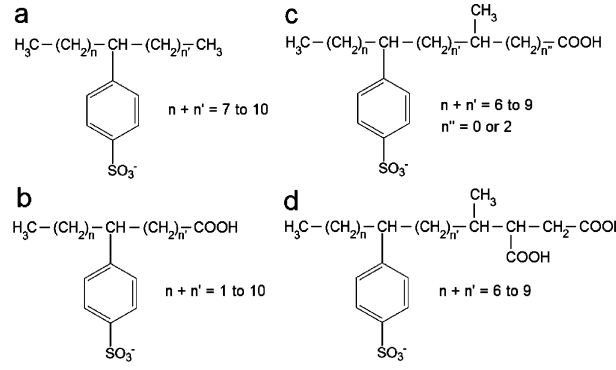

FIGURE 1. Molecular structures of (a) linear alkylbenzene sulfonates (LAS), (b) sulfophenyl carboxylic acids (SPC), (c) methyl sulfophenyl carboxylic acids (Me-SPC), and (d) 4-methyl sulfophenyl dicarboxylic acids (Me-SPdC).

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play a very important role in explaining the limited degradation of LAS in the absence of oxygen: the relatively low bioavailability of this compound due to its sorption onto the particulate phase, and the inhibition of certain anaerobic microorganisms due to the high concentrations usually employed in other laboratory tests and existing in WWTP anaerobic reactors. No explanation, however, was found of why LAS are biotransformed into the same metabolites (SPC) as those otherwise formed through the aerobic pathway. Initial ω-oxidation of the alkyl chain of LAS is not possible under anaerobic conditions, so the presence of unknown metabolites acting as SPC precursors is expected. Our main objectives in the present work have been (a) to explore in detail the generation of different SPC homologues and isomers as the main anaerobic degradation products of a commercial mixture of LAS; (b) to detect and identify the presence of other metabolites, especially those that could explain the presence of SPC subsequently; and (c) to propose a degradation pathway for the anaerobic degradation of LAS. The methodology used to pursue these objectives is a combination of high performance liquid chromatography−ion-trap−mass spectrometry (HPLC−IT−MS) and ultraperformance liquid chromatography−quadrupole-time-of-flight - mass spectrometry (UPLC−Q-TOF-MS).

Experimental Section

Chemicals. All solvents and reagents used were of chromatography quality and purchased from Scharlau (Barcelona, Spain). Commercial LAS and a $2\Phi C_{16}$LAS standard (99% pure) were supplied by Petroquímica Española S.A (PETRESA, Cadiz, Spain). The proportional composition of the different homologues for LAS was as follows: C_{10} (10.9%), C_{11} (35.3%), C_{12} (30.4%), C_{13} (21.2%), and C_{14} (1.1%). Some of the $2\Phi C_2$ to $13\Phi C_{13}$SPC standards (purity >95%) were kindly supplied by F. Ventura (AGBAR, Spain) and Jennifer A. Field (Oregon State University), and the rest were synthesized by our group.

Sample Collection and Microcosm Establishment. Sediment cores were collected at the Sancti Petri tidal channel (36°28.48′ N, 6°10.71′ W, Cadiz, Spain), where two different depth ranges, 1−10 cm and 10−20 cm, were selected. Sediment slurries were then prepared in an anaerobic chamber (filled with N_2 and CO_2 in a 80:20 v:v proportion) by mixing these sediments with anoxic seawater (flushed with nitrogen) in a 1:3 v:v proportion. 300 mL serum bottles were filled with the mixture, leaving 20 mL of gas space. Commercial LAS was then added in order to reach overall concentrations of 10, 20, and 50 ppm, and bottles without LAS addition were employed as blanks. To account for abiotic degradation, two sets of duplicate bottles spiked with LAS were sterilized with formaldehyde from the beginning and analyzed at the end of the experiment. The microcosms were kept anoxic (based on resazurin indicator) throughout the experiment (165 days) in a temperature-controlled room at 30 °C. Two bottles per LAS concentration and per depth were sacrificed at days 0, 15, 60, and 165 and sterilized with 4% of formaldehyde. Finally, centrifugation at 5000*g* was performed to separate both aqueous and particulate phases, taking 2 aliquots of 100 mL of water from each microcosm for analysis of LAS and degradation products. Further details can be found in ref *17*.

HPLC−IT−MS and UPLC−Q-TOF-MS Analysis of LAS and their Metabolites. Pretreatment of the aqueous samples was carried out using C_{18} solid phase extraction cartridges according to ref *19* and prior to their injection in the HPLC−IT−MS and UPLC−Q-TOF-MS systems. In the first case, the HPLC-MS system consisted of a Spectrasystem liquid chromatograph coupled to a LCQ ion-trap mass spectrometer (Thermo). The chromatographic separation was performed using a reversed-phase C-18 analytical column (LiChrospher 100 RP-18) of 250 × 2 mm and 3 μm particle diameter, from Merck. HPLC solvents were acetonitrile/water in a 80:20 v:v proportion (A) and water with 5 mM of acetic acid and triethylamine (B), flowing through the column in the following gradient (flow = 0.15 mL min^{-1}): 0% of A at t = 0 min, increased linearly to 100% in 40 min and kept isocratic for 10 min. All extracts were analyzed using an atmospheric pressure ionization source with electrospray interface (ESI) operating in full-scan negative ion mode in the m/z range from 80 to 500. Further details can be found in ref *17*.

In the second case, separation of target compounds was performed in a Waters Acquity UPLC system (Waters Corp., Milford, MA) using a 5 cm × 2.1 mm Waters Acquity C18 1.7 μm column. The injection volume was 10 μL and the mobile phase consisted of (A) methanol and (B) water with 5 mM of acetic acid (flow = 0.2 mL min^{-1}). The elution started at 0% A for one min and then was linearly increased to 100% A in 20 min, and kept isocratic for 3 min. The mass spectrometry was performed on a QTof-Micro (Waters Corp., Milford, MA). The capillary and cone voltages were set to 2700 and 45 V, respectively. For MS analysis, the instrument was operated in wide pass quadrupole mode, with the TOF data being collected between m/z 80−800, whereas MS/MS was performed using a collision energy value of 29 eV (previously optimized). An independent reference spray via the LockSpray interference was employed to ensure accuracy and reproducibility, using Val-Tyr-Val as the lock mass (m/z 378.2029) at a flow rate of 10 μL min^{-1}. The accurate mass and composition for the precursor ions and for the fragment ions were calculated using MassLynx software (version 4.0) incorporated in the instrument.

Results and Discussion

Structural Elucidation of SPC Isomers As the Main Degradation Products during Las Anaerobic Degradation. Several microcosms containing a mixture of anoxic marine sediment and seawater were spiked with known amounts of LAS (10, 20, and 50 ppm) and incubated for 165 days. A detailed description of this experiment, as well as the main results for the primary degradation percentages of LAS can be found in ref *17*. Briefly, the appearance of polar metabolites, identified as sulfophenyl carboxylic acids (SPC) by high performance liquid chromatography−ion trap mass spectrometry (HPLC−IT−MS), was associated with a decrease in the LAS concentration during the experiment. It was confirmed that this trend occurred in all microcosms, except those employed as abiotic controls, where no significant change in LAS concentrations nor generation of SPC were observed. The highest concentrations of these degradation products were found in the aqueous phase, due to their high polarity.

For a better understanding, it should be explained that LAS are commercially available as a mixture of different homologues, typically from C_{10} to C_{14}LAS depending on the length of the alkyl chain (Figure 1a). Each homologue contains several isomers that can be considered as external or internal depending on the relative position of the sulfophenyl group link with the alkyl chain. LAS isomers can be identified as $m\Phi C_n$LAS, where n indicates the total length of the alkyl chain and m denotes the C atom of this chain which is bound to the sulfophenyl group. As example, $5\Phi C_{10}$LAS refers to an internal isomer of a short-chain homologue, whereas $2\Phi C_{13}$LAS corresponds to an external isomer of a long-chain homologue. Sulfophenyl carboxylic acids (SPC) are designated in a similar way, but in this case $m = 1$ indicates the C atom belonging to the carboxylic group at the end of the alkyl chain (Figure 1b).

Figure 2 shows several UPLC−Q-TOF-MS chromatograms indicating the presence of SPC in water samples at the end of the experiment (165 days). As expected when using reversed phase (RP) chromatography, their retention times increase

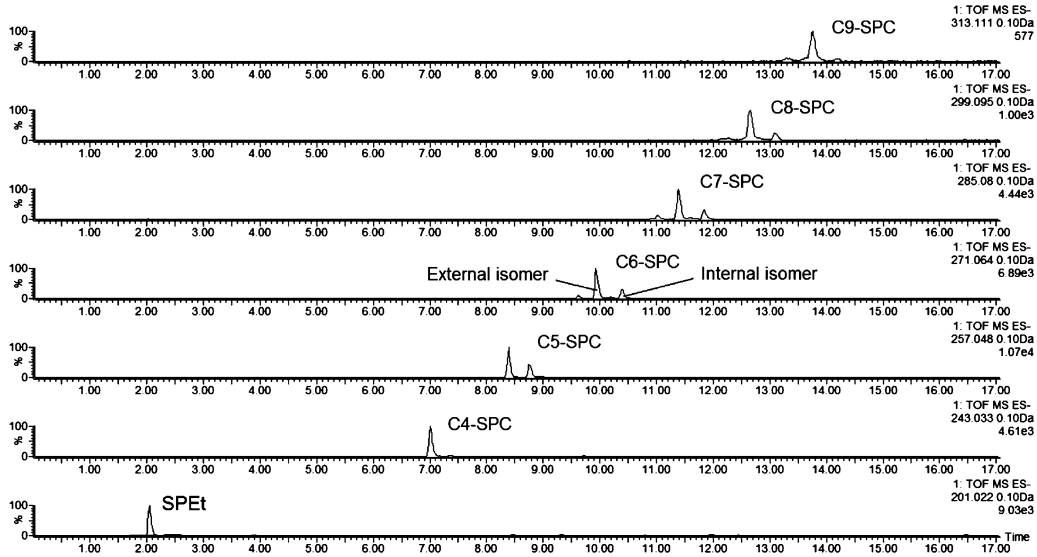

FIGURE 2. UPLC−Q-TOF-MS extracted ion-chromatograms showing the presence of 1-sulfophenyl ethanol (SPEt) and several sulfophenyl carboxylic acid (SPC) homologues and isomers in a water sample taken at the end of the experiment (165 days) from a batch reactor spiked with 10 ppm of LAS.

TABLE 1. UPLC−Q-TOF-MS Retention Times and Accurate Mass Measurements of Molecular Ions of LAS and Their Anaerobic Degradation Metabolites in a Water Sample Taken at the End of the Experiment (165 days) from a Batch Reactor Spiked with 20 ppm of LAS

compound	elemental composition	retention time (min)	theoretical mass (*m*/*z*)	experimental mass (*m*/*z*)	error mDa	error ppm
SPEt	C8H9O4S	1.9	201.0222	201.0221	−0.1	−0.3
C_4SPC	C10H11O5S	6.8	243.0327	243.0328	0.1	0.3
C_5SPC	C11H13O5S	7.9−9.1	257.0484	257.0485	0.1	0.5
C_6SPC	C12H15O5S	9.1−10.7	271.0640	271.0645	0.5	1.8
C_7SPC	C13H17O5S	10.5−12.1	285.0797	285.0790	−0.7	−2.4
C_8SPC	C14H19O5S	11.9−12.9	299.0953	299.0959	0.6	1.9
C_9SPC	C15H21O5S	13.1−14.0	313.1110	313.1115	0.5	1.7
C_{10}SPC	C16H23O5S	14.1−14.5	327.1266	327.1265	−0.1	−0.4
C_{11}SPC	C17H25O5S	15.1	341.1423	341.1429	0.6	1.8
Me-C_{10}SPC	C17H25O5S	15.1	341.1423	341.1429	0.6	1.8
Me-C_{11}SPC	C18H27O5S	15.8	355.1579	355.1575	−0.4	−1.2
Me-C_{12}SPC	C19H29O5S	16.4	369.1736	369.1743	0.7	2.0
Me-C_{13}SPC	C20H31O5S	16.9	383.1892	383.1894	0.2	0.5
Me-C_{14}SPC	C21H33O5S	17.4	397.2049	397.2041	−0.8	−1.9
Me-C_{15}SPC	C22H35O5S	17.9	411.2205	411.2215	1.0	2.4
Me-C_{12}SPdC	C20H29O7S	15.1	413.1634	413.1648	1.4	3.4
Me-C_{13}SPdC	C21H31O7S	15.7	427.1791	427.1797	0.6	1.5
Me-C_{14}SPdC	C22H33O7S	16.3	441.1947	441.1945	−0.2	−0.5
Me-C_{15}SPdC	C23H35O7S	16.8	455.2104	455.2102	−0.2	−0.3
C_{10}LAS	C16H25O3S	16.7−17.8	297.1524	297.1528	0.4	1.2
C_{11}LAS	C17H27O3S	17.1−18.5	311.1681	311.1689	0.8	2.6
C_{12}LAS	C18H29O3S	17.7−18.9	325.1837	325.1828	−0.9	−2.9
C_{13}LAS	C19H31O3S	18.3−19.4	339.1994	339.1991	−0.3	−0.9

as a function of the length of the alkyl chain. Sulfophenyl ethanol (SPEt) and C_4 to C_9SPC homologues (those represented in Figure 2) are the LAS metabolites showing the highest concentration throughout the experiment, although SPC with up to 11 carbon atoms in their alkyl chains have been identified by means of the accurate mass measurement of their molecular ions $[M\text{-}H]^-$ and the use of standards. As can be observed in Table 1, the agreement between the measured and calculated masses is within an error range of <5 ppm. In addition, several peaks corresponding to different isomers can be observed for each SPC homologue (Figure 2). Since the main interaction between these compounds and the RP column takes place preferentially at the end of the alkyl chain opposite to the carboxylic group, those isomers eluting earlier in the chromatogram correspond to external isomers, as their retention times match with those for $4\Phi C_4$SPC to $11\Phi C_{11}$SPC standards; whereas peaks with higher retention times belong to internal isomers. This can be also observed in chromatograms obtained by HPLC−IT−MS (Supporting Information (SI) Figure S1), where the separation between these two types of isomers is sharper due to the different HPLC conditions employed.

Fragmentation patterns are different for external and internal isomers, as can be observed in Figure 3a and b, where MS/MS mass spectra are shown for two different C_9SPC isomers as an example. In the case of external isomers, the alkyl chain is fragmented by means of a McLafferty rearrangement, which consists of the migration of a hydrogen atom from the carbon atom in the position 4 (or C-4) to the carbonyl oxygen in the carboxylic group (C-1) and the cleavage of the bond between C-2 and C-3. As a result, the molecular ion (m/z 313.1110) is broken and the fragment ion m/z 253.0898 is formed (Figure 3a). This kind of rearrangement has also been reported to take

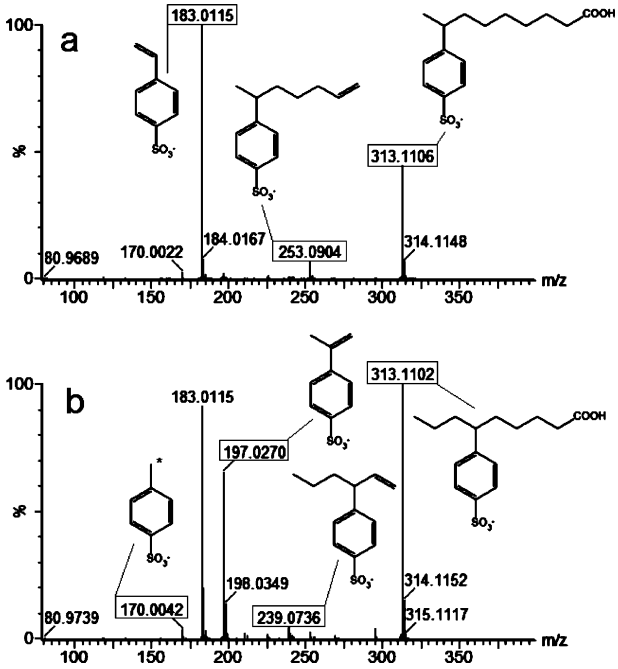

FIGURE 3. UPLC−Q-TOF-MS/MS spectra extracted from the chromatograms corresponding to a water sample taken at the end of the experiment (165 days) from a batch reactor spiked with 20 ppm of LAS. Fragmentation patterns for two different isomers of C_9SPC (a: 8ΦC_9SPC, b: 6ΦC_9SPC) and structures of their fragment ions are shown.

place during MS analysis of alkanes (*20, 21*). Fragments m/z 239.0742 and m/z 197.0272 are generated for C_9SPC internal isomers instead, as can be observed in Figure 3b. The first fragment (m/z 239.0742) is the result of a McLafferty rearrangement again, whereas the latter fragment (m/z 197.0272) is also a characteristic feature in the mass spectra of branched alkylbenzene sulfonates (BAS), used before LAS until the middle of the 1960s (*22*). Both types of SPC isomer share a common fragment, m/z 183.0116 (Figure 3a and b), which has been previously described to be characteristic of LAS and SPC isomers (*19, 23*) and, therefore, confirms the identity of these metabolites as SPC. Although showing a lower intensity, m/z 170.0038 is also detected in both cases prior to the rupture of the benzene ring and generation of HSO_3^- (m/z 80.9646). Abundance of the fragment m/z 170.0038 has been found to be notably higher in the SPC standards (from 2ΦC_2SPC to 13ΦC_{13}SPC) used for identification and quantification, as the linking of the sulfophenyl group to the end of the alkyl chain opposite to the carboxylic group enhances the complete fragmentation of this chain.

Identification of the Initial Degradation Products: 4-Methyl-Sulfophenyl Carboxylic and 4-Methyl-Sulfophenyl Dicarboxylic Acids As Precursors of SPC. Laboratory assays performed with river water (*24*) and seawater (*5*) have reported that SPC showing the same alkyl chain length as the parent compound are the initial aerobic degradation metabolites of LAS. These intermediates are generated by ω-oxidation, so, for example, the long-chain C_{12}SPC would be the first degradation product of C_{12}LAS. However, this mechanism cannot take place in anoxic sediments or sludges since it requires the presence of molecular oxygen. Analysis of water samples from our anaerobic degradation experiments by HPLC−IT−MS (SI Figure S1) reveals the presence of a homologue series (Δ $m/z \pm 14$) showing higher masses (from m/z 369 to m/z 411) than those known for the SPC having the longest alkyl chains. The characteristic fragment m/z 183 can be also observed in their mass spectra, so their molecular structures should be related to those for LAS and SPC. These compounds have not been detected in abiotic controls and standards. Since the maximum length of the alkyl chain in the commercial mixture of LAS employed in this experiment is 13 carbon atoms (C_{14}LAS accounts for only 1% of the total), the occurrence of these new compounds suggests the addition of an organic molecule to the original structure of the surfactant, probably at the beginning of the degradation process. On this point, several authors (*20, 21, 25, 26*) have reported that increasing the number of carbon units of an alkyl chain is a newly identified mechanism for the anaerobic degradation of alkanes in the marine environment. Previous studies (*25, 27*) have shown that several sulfate-reducing bacterial strains, such as AK-01 or CV2803, are able to oxidize hexadecane or pentadecane into 4-methyl-18:0 and 4-methyl-17:0 fatty acids, respectively. In the same way, we postulate here that the alkyl chain of LAS homologues would be degraded following a similar pathway, so the previously mentioned homologue series should correspond to 4-methylated sulfophenyl carboxylic acids (or Me-SPC) (Figure 1c): from Me-C_{12}SPC (m/z 369.1736) to Me-C_{15}SPC (m/z 411.2205) in the cases of C_{10} to C_{13}LAS degradation, respectively. Using UPLC−Q-TOF-MS we have confirmed the presence of these candidates as LAS metabolites (Figure 4a) and, by accurate mass measurement, have obtained their elemental composition (Table 1). Table 1 also shows that the only difference in mass between these compounds (Me-SPC) and the previously characterized SPC is the addition of CH_2 groups, presumably to the alkyl chain. Me-C_{10} and Me-C_{11}SPC can be also detected resulting from the degradation of longer Me-SPC by β-oxidation, which will be discussed in more detail in the following section. Separation between external and internal Me-SPC isomers has also been achieved by HPLC (SI Figure S1), showing slightly lower retention times than those corresponding to SPC isomers due to the branching (corresponding to the presence of a methyl group) in their alkyl chains.

In addition, Kropp et al. (*21*) demonstrated that the anaerobic oxidation mechanism that generates these 4-methylated compounds from alkanes involves the initial addition of fumarate to form a substituted alkylsuccinate. This biochemical reaction starts with the abstraction of a hydrogen atom from the subterminal carbon of the alkyl chain by a glycyl radical enzyme, resulting in the formation of a radical intermediate, which would add across the double bond of fumarate. Rabus et al. (*26*) found the same reaction occurring during the initial attack of a denitrifying bacterium (strain HxN1) on *n*-hexane, forming (1-methylpentyl) succinate as a metabolite. Degradation of these succinate-compounds results in the formation of the previously mentioned 4-methylated metabolites. In our case, we have been able to identify the presence of the precursors of Me-SPC in water samples from the experiment. Although these compounds show lower intensities than SPC and Me-SPC, probably due to their higher reactivity and faster degradation, Figure 4b shows the presence of 4-methyl sulfophenyl dicarboxylic acids (hereafter Me-SPdC) corresponding to the initial attack of the anaerobic marine bacteria community on the LAS homologues by the addition of fumarate. As expected, their retention time is lower compared to Me-SPC due to the presence of a second carboxylic group (Figure 1d); and only four homologues (from Me-C_{12}SPdC to Me-C_{15}SPdC, m/z 413.1634−455.2104, respectively) have been found (Table 1), each deriving from the degradation of a different LAS homologue. Finally, confirmation of the identities of both Me-SPC and Me-SPdC homologues has been also obtained using MS/MS. As an example, the MS/MS mass

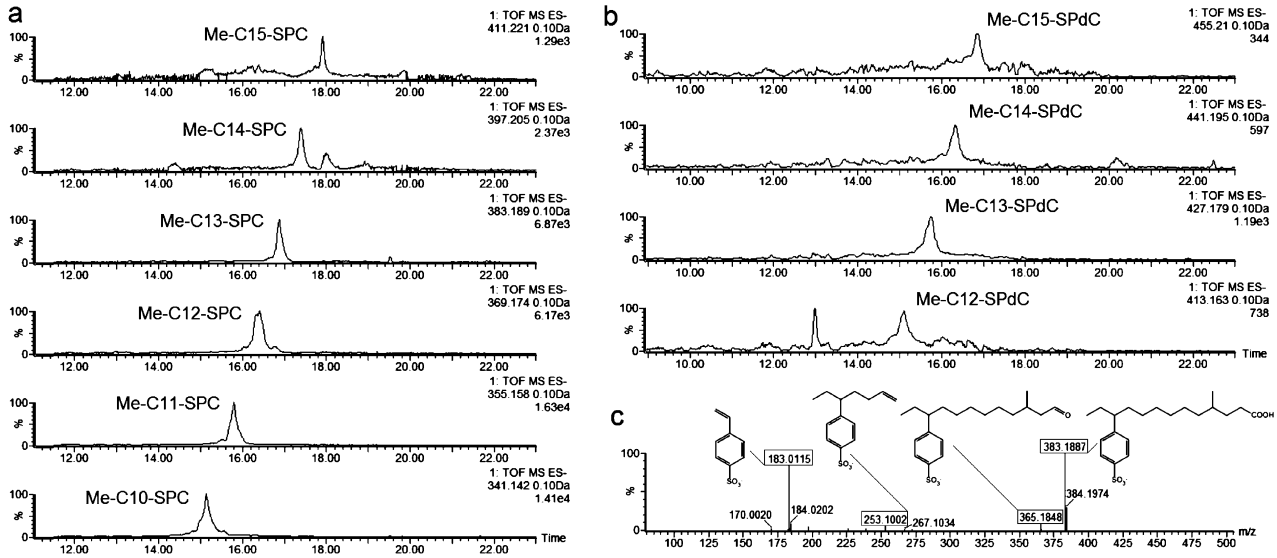

FIGURE 4. UPLC−Q-TOF-MS extracted ion-chromatograms for a water sample taken at the end of the experiment (165 days) from a batch reactor spiked with 20 ppm of LAS showing the presence of (a) methyl-SPC (from Me-C_{10} to Me-C_{15}SPC), and (b) 4-methyl-SPdC (from Me-C_{12} to Me-C_{15}SPdC). A MS/MS negative ion-mode spectrum for 4-methyl-C_{13}SPC and structures of their fragment ions are shown as example (c).

spectrum of Me-C_{13}SPC is displayed in Figure 4c, where, among other identified fragments, the characteristic fragment ion m/z 183.0116 can be clearly observed, as it shows the highest intensity after the molecular ion $[M\text{-}H]^-$.

Proposed Pathway for the Anaerobic Degradation of LAS. In this study, we present for the first time evidence supporting a mechanism of fumarate addition to the alkyl chain of LAS homologues in order to explain the generation of sulfophenyl carboxylic acids (SPC) as the major degradation products of this surfactant under anoxic conditions. In addition, and using the data obtained by HPLC−IT−MS and UPLC−Q-TOF-MS reported in the preceding section, we have been able to propose the following scheme for the anaerobic degradation pathway of LAS in marine sediments (Figure 5).

First, dicarboxylic acids (Me-SPdC) are formed by the addition of fumarate to the subterminal carbon atom (C-2) of the alkyl chain of LAS (Figure 5A and B). This kind of initial attack was described for the first time for the anaerobic degradation of toluene (*28*) and other aromatic hydrocarbons such as 2-methylnapthalene (*29*), but for those it takes place on the terminal carbon (C-1) instead. Fumarate addition to C-2 is preferred not only in the case of LAS, but also for ethylbenzene for sulfate-reducing microorganisms (*28, 30*) and *n*-alkanes (*20, 21, 25, 26*). As Rabus et al. (*26*) reported for *n*-hexane degradation, these activation steps are not energetically equivalent. First, the cleavage of the C−H bond in C-2 in any *n*-alkane (or the alkyl chain of LAS) requires more energy than in the methyl group (C-1) of toluene (401 versus 368 kJ/mol). However, the cleavage of the C−H bond in C-1 in *n*-alkanes requires even more energy (419 kJ/mol). Therefore, the reaction of LAS with fumarate takes place preferentially on C-2 rather than C-1. This reaction is also thermodynamically favored ($\Delta G^\circ = -34.4$ kJ/mol using the estimation method proposed by Mavrovouniotis (*31*)), which would explain the formation of the Me-SPdC as the initial reaction metabolites in the anaerobic degradation of LAS.

The next stage in the degradation pathway represented in Figure 5 results in the appearance of Me-SPC (Figure 5C). We propose that there is a C-skeleton rearrangement similar to that described in refs *20, 32, and 33* for the degradation of alkanes. Briefly, it consists on the migration of the carboxyl group from C-3 to C-2 and a subsequent decarboxylation due to the release of the carboxyl group in C-1. Without additional study, however, it is difficult to discern if this mechanism generates Me-SPC or they are just a result from direct decarboxylation of Me-SPdC, a mechanism that is also proposed in ref *20*. Later, the resulting products undergo β-oxidation, a process that can take place in absence of oxygen and generates 2-methylated SPC (from Me-C_{10} to Me-C_{13}SPC) (Figure 5D). The following degradation steps comprise further successive β-oxidations of the alkyl chains to form different SPC homologues. During this sequence, a C_3 unit is released (Figure 5D and E), which may be used for the regeneration of fumarate and later activation of other LAS molecules, according to Wilkes et al. (*32*) and Davidova et al. (*33*). By following the proposed pathway, the degradation of LAS homologues which have even numbers of carbon atoms (C-even, such C_{10} and C_{12}LAS) should result in the generation of Me-SPC (Figure 5C and D) which predominantly have odd numbers (C-odd) of carbon atoms, while SPC generated later would be C-even again (Figure 5E and F). On the other hand, C-even Me-SPC and C-odd SPC are expected to occur during the biotransformation of C_{11} and C_{13}LAS. This is in agreement with results from previous anaerobic degradation assays performed with *n*-alkanes (*25, 27*), where C-odd or C-even fatty acids are formed depending on the number of carbon atoms of the parent compound.

Finally, 1-sulfophenyl-ethanol (SPEt) has been detected as the ultimate degradation product in the proposed pathway (Figure 5G) once the alkyl chain has been completely metabolized. Desulfonation and/or ring cleavage are expected to occur then. Several types of sulfate-reducing bacteria (*34, 35*) such as *Desulfitobacterium spp.* or strain RZLAS have been reported as capable of carrying out these last two processes. The mechanism that produces SPEt, however, is still unclear. C_4SPC is the shorter SPC homologue detected in the samples, and it may undergo β-oxidation, which would produce SPEt as two carbon atoms are removed. However, this reaction produces a hidroxyacyl intermediate instead of an alcohol in the case of alkanes (SI Figure S2a). Consequently, another carboxylic acid, having two carbon atoms less, would be generated. In the case of C_4SPC, the formation of a ketone in C-3 is not possible (SI Figure S2b), and only the alcohol may be formed. We hypothesize that, after this, two carbon atoms would be retrieved, resulting in the formation of 1-sulfophenyl-ethanol. This is stated with

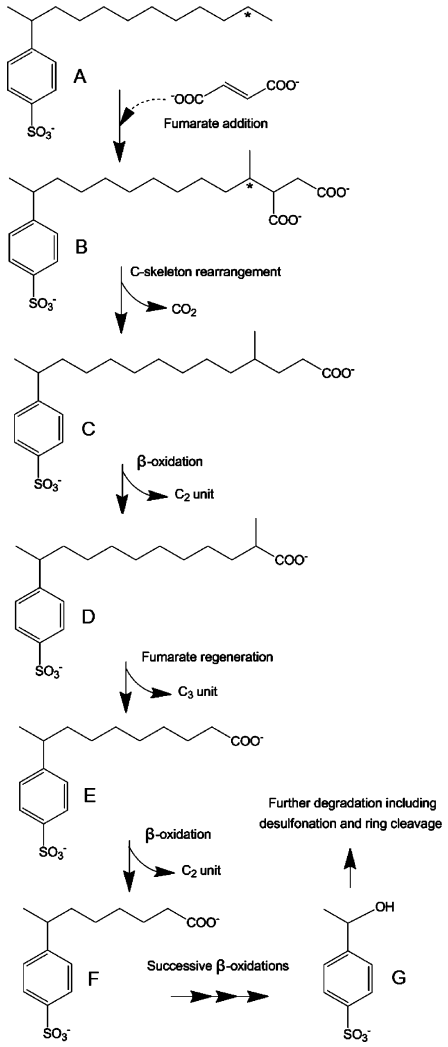

FIGURE 5. Proposed pathway for the anaerobic degradation of LAS in sulfate-reducing marine sediments (2ΦC$_{12}$LAS is shown as example). The following compounds have been identified by UPLC−Q-TOF-MS: A. C$_{12}$LAS, B. 4-methyl-C$_{14}$SPdC, C. 4-methyl-C$_{14}$SPC, D. 2-methyl-C$_{12}$SPC, E. C$_{10}$SPC, F. C$_8$SPC, and G. 1-sulfophenyl-ethanol. The place where the fumarate addition takes place (C-2) is marked with an asterisk.

some reservation, as this mechanism has not been previously described in the literature. However, a similar intermediate was detected during the anaerobic degradation of phytols in marine sediments (*36*). In addition, 1- sulfophenyl-ethanol has not been detected in abiotic controls, but it has shown a high intensity peak in the rest of microcosms, and the absence of SPC having shorter alkyl chains than C_4SPC suggests that SPEt may be derived from this homologue.

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Supporting Information Available

HPLC−IT−MS chromatograms showing the presence of SPC and Me-SPC in water samples from the experiment (Figure S1) and proposed pathway for the formation of 1-sulfophenyl-ethanol (Figure S2). This information is available free of charge via the Internet at http://pubs.acs.org/.

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