Environ. Sci. Technol. **2007**, *41,* 3573−3579

Anaerobic Degradation of Linear Alkylbenzene Sulfonates in Coastal Marine Sediments

PABLO A. LARA-MARTíN,† ABELARDO GÓMEZ-PARRA,† THORSTEN KÖCHLING,‡ JOSÉ LUIS SANZ,‡ RICARDO AMILS,‡ AND EDUARDO GONZÁLEZ-MAZO*,†

Departamento de Química Física, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Campus Río San Pedro s/n, 11510 Puerto Real, Cádiz, Spain, and Unidad de Microbiología Aplicada, Centro de Biología Molecular, Universidad Autónoma de Madrid, Crta. de Colmenar km 15, 28049 Madrid, Spain

This research shows for the first time the degradation of linear alkylbenzene sulfonates (LAS) under anaerobic conditions, together with the presence of metabolites and the identification of microorganisms involved in this process. This compound is the most widely used surfactant and its main environmental concern is related to its persistence in the absence of oxygen as LAS accumulates in anaerobic sediments and sewage sludges. Laboratory experiments performed with anoxic marine sediments spiked with 10−50 ppm of LAS demonstrated, however, that its degradation reached 79% in 165 days via the generation of sulfophenyl carboxylic acids (SPCs). Almost all of the added LAS (>99%) was found to be attached to the sediment while the less hydrophobic SPCs were predominant in solution, as their concentration increased progressively up to 3 ppm during the full course of the experiment. Average half-life for LAS has been estimated to be 90 days, although higher values should be expected when the LAS concentration exceeds 20 ppm, due to inhibition of the microbial community. Sulfate-reducing and methanogenic activities proved to be intense during the experiment. Several sulfate-reducing bacteria and firmicutes/clostridia have been identified as possible candidates for effecting this degradation. Our results imply that the persistence of LAS in anoxic compartments, such as marine sediments, should be reconsidered when evaluating its environmental risk.

Introduction

Linear alkylbenzene sulfonates (LAS) are anionic surfactants that were marketed for the first time in the mid-1960s as replacements for the poorly degradable tetrapropyl benzenesulfonates (TPS). Today LAS is still the most widely used synthetic surfactant, with an annual worldwide production of around 2.5 million tons (*1*). More than 80% of the consumption takes the form of household detergents, as key components of washing powders and liquids, dishwashing products, and multipurpose cleaners. After use, LAS are ultimately discharged into aquatic ecosystems through both treated and untreated wastewater discharges, and are deposited into agricultural soils as part of sludges from wastewater treatment plants (WWTPs). Therefore, significant levels of LAS have been reported in both terrestrial and aquatic environments (*2*−*4*).

The removal of LAS in aerobic WWTPs is very efficient (*5*), typically more than 99%, and aerobic degradation in water (*6*) and soils (*7*) takes place in a few days by the generation of sulfophenyl carboxylic acids (SPCs) resulting from the ω-oxidation of the alkyl chain and then consecutive α- and β-oxidations. However, a considerable fraction of the total LAS discharged ends up in river and coastal sediments because this surfactant has a high adsorption capacity as a consequence of its affinity for the organic carbon in the sediment (*8*). These sediments tend to present anaerobic conditions below a topmost layer a few millimeters thick in the case of aquatic areas subjected to pollution. It is generally assumed that this compound is not biotransformed in these kinds of anoxic environments because of the high concentrations (of the order of several g/kg) found in anaerobic sewage sludge; nor have various laboratory assays found evidence of this occurring (*9*−*11*). Apart from the absence of oxygen, some authors have attributed this to inhibition of the anaerobic digestion due to the high concentrations usually employed in laboratory tests and found also in WWTP anaerobic reactors (*12*, *13*), and to the low bioavailability of LAS due to adsorption to the particulate phase (*14*); these have been considered as key factors for explaining this recalcitrance.

On the other hand, previous studies undertaken in recent years have shown that the degradation of LAS in absence of oxygen may be possible, because the moieties of the LAS molecule (sulfonate, benzene, and the linear alkyl chain) have all been described as acting as sources of carbon and energy for microorganisms under anaerobic conditions; several bacterial species that carry out these reactions have also been identified (*15*−*19*). Furthermore, several researchers (*14*, *20*, *21*) have recently reported the disappearance of LAS from continuous stirred tank reactors (CSTR) and from up-flow anaerobic sludge blanket (UASB) reactors. However, this disappearance does not imply unequivocally that an anaerobic degradation process is taking place because no LAS metabolites have been identified or quantified (*14*).

Results from some field studies (*3*, *22*, *23*) have suggested the possibility of the disappearance of linear alkylbenzenes, precursors in LAS synthesis, and of LAS itself, in anoxic sediments. Specifically, sediment cores that our research group collected at several points in a tidal channel subjected to untreated domestic wastewater discharges (*3*) have shown the presence of significant concentrations of SPCs at anoxic depths ($E_h = -380$ mV). This presence was associated with a sharp decrease in LAS values with depth in the sediment column. Bioturbation was discounted as a mechanism responsible for this trend due to the regularity shown by the porosity profiles and the absence of meio- and macrobenthos from the zone due to the heavy pollution. Anaerobic degradation was suggested but not proved, so this is the main reason why the present experiment was designed. This experiment includes not only the monitoring of the levels of LAS and SPCs in microcosms filled with anoxic marine sediments, but also a descriptive survey of the microbial community present in this environment in order to pinpoint possible candidates for effecting the biodegradation process. Marine sediments are only recently becoming the focus of

* Corresponding author phone: +34 956 016159; fax: +34 956 016040; e-mail: eduardo.gonzalez@uca.es.
† Universidad de Cádiz.
‡ Universidad Autónoma de Madrid.

10.1021/es062372z CCC: $37.00 © 2007 American Chemical Society
Published on Web 04/18/2007

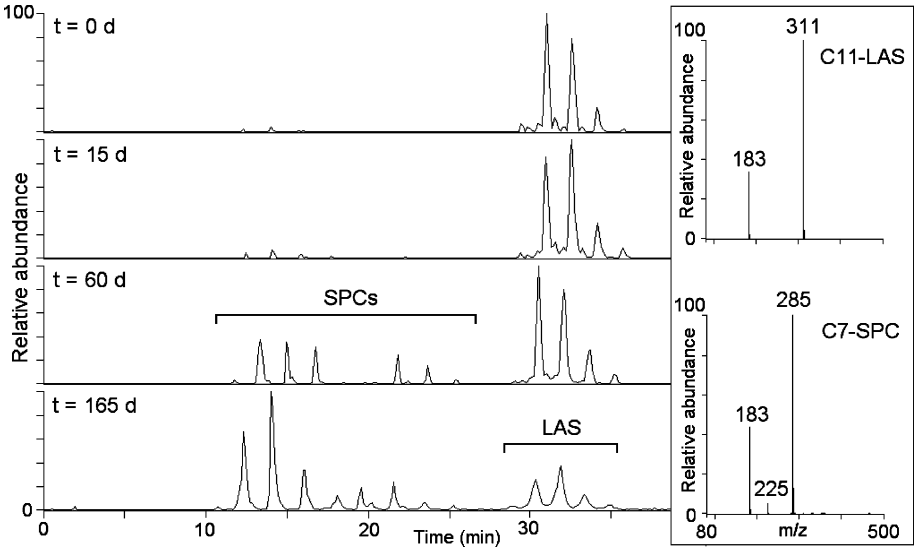

FIGURE 1. LC−ESI−MS extracted-ion chromatogram from water samples showing the evolution of the relative intensity of specific fragment ion *m/z* = 183, which belongs to the homologues of SPCs and LAS, for the spiked LAS amount of 2.3 μmol (10 ppm) in the 1−10 cm depth layer during the complete experiment (0−165 days). Full-scan negative ion mode mass spectra for C_7-SPC and C_{11}-LAS homologues are also shown as examples.

such studies (*24*−*26*) thanks to the introduction of the new molecular biology techniques based on the 16S rRNA. This approach allows assessment of the microbiota present in such habitats in a comprehensive manner, as it targets the great majority of the microorganisms present and not only the minor, culturable fraction (*27*). Our main object in the present work was therefore to re-examine the possible fate of LAS in the marine sediments mentioned above, in order to determine the following: (a) if anaerobic degradation of this surfactant is really taking place, (b) if the metabolic pathway implies the formation of SPCs, as in the case of aerobic degradation, and (c) which types of microorganism carry out this process.

Experimental Section

Materials and Standards. See Supporting Information.

Sample Collection and Microcosm Establishment. Sediment and water samples were collected at the Sancti Petri channel (Cádiz, Spain), located at the geographical coordinates 36° 28.48′ N, 6° 10.71′ W, by means of 50 cm length PVC cores and 2.5 L glass bottles, respectively. Two different depth sections were selected from the sediment cores: 1−10 cm and 10−20 cm. We prepared sediment slurries in an anaerobic chamber (filled with N_2 and CO_2 in a 80:20 v:v proportion) by mixing sediments with anoxic seawater in a 1:3 v:v proportion. Serum bottles (300 mL) were filled with the mixture, leaving 20 mL of gas space. Commercial LAS was then added in quantities of 2.3, 4.6, and 11.5 μmol to reach an overall concentration of approximately 10, 20, and 50 ppm, respectively, and bottles without LAS addition were employed as blanks. The microcosms were kept anaerobic (based on resazurin indicator) throughout the experiment in an incubation chamber 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. To account for abiotic degradation, two sets of duplicate bottles spiked with LAS were sterilized with formaldehyde from the beginning and analyzed after 165 days. After 65 days of operation a mixture of nutrients was supplied to the microcosms (4 g COD L^{-1}): formate, acetate, propionate, butyrate, lactate, ethanol methanol, and sucrose (1:2:2:2:3:2:4). A total number of 72 bottles was employed. Sediments were then centrifuged at 5000*g* to separate them from water and later were dried at 70 °C. Finally, 2 aliquots of 100 mL of water and 3 aliquots of 3 g of sediment were taken from each microcosm.

Sulfide and Methane Measurement. See Supporting information.

LC−MS Analysis of LAS and SPCs. See Supporting Information.

Catalyzed Reporter Deposition−Fluorescent In Situ Hybridization (CARD−FISH) and Total Cell Counts. See Supporting Information.

DNA Extraction, Amplification, Library Construction, and Screening. See Supporting Information.

PCR-DGGE. See Supporting Information.

Results and Discussion

Evolution of LAS and SPCs During the Anaerobic Degradation Assays. Marine sediments used to carry out the experiment showed a sharp decrease of the redox potential from −250 mV at their surface to −400 mV at between 10 and 50 cm depth, so the entire sedimentary column was confirmed to be anoxic. Under these conditions, the appearance of polar metabolites associated with a decrease in the LAS concentration during the experiment was verified in all reactors by liquid chromatography−mass spectrometry (LC−MS) (Figure 1), except those employed as abiotic controls, where no change in LAS concentrations nor generation of SPCs were detected. This technique, LC−MS, has been successfully employed in previous studies during the monitoring of LAS aerobic degradation (*6*, *28*) as it permits the effective quantification of each LAS homologue as well as the unequivocal identification of the metabolites generated. In our case, the presence of sulfophenyl carboxylic acid (SPC) homologues of variable alkyl chain length was confirmed by the agreement between SPC standards and samples in the retention times and mass spectra of the different chromatographic peaks. It can be observed from mass spectra shown in Figure 1 that not only quasimolecular ions $[M - H]^-$ corresponding to the different SPC homologues (C_7−SPC is presented as an example) were present but also their specific fragment *m/z* = 183, previously described by González-Mazo et al. (*28*), was detected. Generation of SPCs as degradation products of LAS is evident in aerobic water (*6*) and soils (*7*), but it has been only suggested in anoxic environments (*3*, *22*) where this surfactant is commonly considered as recalcitrant. The present study proves for the

TABLE 1. Evolution of the Average Concentration of LAS and SPCs in Water (ng mL^{-1}) (n = 4) and Sediment (ng g^{-1}) (n = 6) during the Complete Experiment, in the 1−10 cm Depth Layer

	LAS				SPCs			
	water		sediment		water		sediment	
time (d)	mean	SD	mean	SD	mean	SD	mean	SD
				Blank				
0	6	1	2407	167	50	10	n.d.[a]	
15	7	1	2438	96	52	13	n.d.	
60	3	0	1679	240	141	61	n.d.	
165	6	1	693	473	253	44	n.d.	
				2.3 μmol				
0	67	5	12438	2448	16	8	n.d.	
15	63	21	12340	3250	47	6	n.d.	
60	74	8	8924	3435	429	202	n.d.	
165	58	12	3615	465	824	440	77	62
				4.6 μmol				
0	192	19	21167	1001	30	3	n.d.	
15	154	96	17643	4528	219	99	n.d.	
60	158	103	8208	1330	2157	850	332	17
165	133	44	5777	388	3117	503	662	192
				11.5 μmol				
0	443	115	53286	2853	85	16	n.d.	
15	270	36	50825	3610	304	30	n.d.	
60	315	51	51106	2816	417	83	57	60
165	793	51	44051	8881	484	241	67	42

[a] n.d. = nondetected.

first time that biotransformation of LAS into sulfophenylcarboxylic acids in marine sediments is possible in the absence of oxygen. This novel finding should not be considered surprising taking into account that now it has been recognized that even the more recalcitrant n-alkanes and alkylbenzenes such as toluene, *m*-xylene, or ethylbenzene are degraded by anaerobic routes where carboxylated intermediates are involved (*17*, *18*).

Table 1 shows the distribution between the aqueous and particulate phases for both target compounds (LAS and SPCs) throughout the experiment at the 1−10 cm depth. Notable differences can be observed when comparing the concentration values for LAS and SPCs between the aqueous and particulate phases. Almost all of the LAS added was found attached to the sediments (99.2% ± 0.5) due to its hydrophobic character and its high affinity for the organic carbon (K_d ranges from 40 to 469, depending on the particular homologue), as previous studies have pointed out (*8*). On the other hand, the addition of a carboxylic group, as well as the progressive shortening of the alkyl chain, results in the LAS metabolites tending to remain in the aqueous phase, where the highest concentrations are detected, due to their much lower hydrophobicity and sorption capacity (K_d values from 1 to 7 have been reported for long-chain SPC homologues) when compared with LAS (*8*). During the experiment there was a noticeable decrease in LAS concentration in sediments from blanks, the 2.3 μmol (10 ppm) and 4.6 μmol (20 ppm) tests, which was associated with a large increase in the values of SPCs in water. It should be also noted that there were no significant changes in LAS concentration in water, while the presence of SPCs in sediment also was scarce. One explanation for this degradation process may be the existence of a mechanism whereby the LAS in solution, due to its higher bioavailability, is being actively biotransformed to SPCs, while the remaining quantities of LAS are being gradually desorbed from the sediment to the water in order to reach equilibrium again.

A mass balance in the entire microcosm (water + sediment) is shown in Table 2 for both target compounds throughout the experiment at the two depths of 1−10 and 10−20 cm. This enables a more accurate description of the degradation process to be established. First, it is noticeable that an average background LAS contamination of approximately 2.5 ppm (0.6 μmol) was detected in the blank reactors due to the proximity of the sampling point to a wastewater discharge outlet, as it was previously observed from past field samplings (*3*, *4*). During the first 15 days an acclimation period was observed, with LAS levels remaining fairly constant in almost all cases and generation of SPCs being scarce. It was noticeable that this situation changed 2 months after the start: there was a notable decrease in the LAS amount in the blanks, the 2.3 and 4.6 μmol tests, and this was associated with a large increase in values of SPCs. In the 11.5 μmol test, however, LAS degradation appeared to be slower as can be observed by a lower generation of SPCs when compared with the other concentrations tested. There were no significant differences between the two sediment depths (1−10 and 10−20 cm) selected, except for those detected in the microcosms containing 4.6 μmol of added LAS, where a slower disappearance of LAS can be observed at 10−20 cm compared with 1−10 cm (Table 2). However, this observation should be taken with caution due to the standard deviations found, because of the different degradation rates shown by the two microcosms used as replicates. The decrease of LAS continued until the end of the experiment (day 165) as SPCs showed increasing values during the entire test period, reaching total concentrations up to 0.25 ppm (0.2 μmol), 1.80 ppm (1.5 μmol), 3.78 ppm (2.9 μmol), and 0.55 ppm (0.5 μmol) for the blanks and the microcosms with 2.3, 4.6, and 11.5 μmol of added LAS respectively. No significant generation of SPCs or reduction in LAS levels were detected in any of the abiotic controls after 165 days. Figure 2 shows that the total number of nmol (LAS + SPCs) remained practically unaltered during the initial days as the losses of LAS were replaced by generation of SPCs. However, the total amount of these two compounds decreased further throughout the experiment so it was clearly below 100% for the blanks and the 2.3 μmol tests on the last sampling days. This suggests that the LAS and/or SPCs may also be degraded by formation of other metabolites (Mogensen et al. (*14*) have suggested benzaldehyde and benzosulfonic acid as possible LAS intermediates under anaerobic degradation in UASB reactors, although, if these were formed, they were not detected in our case, probably due to their low retention in the SPE cartridges.) and/or it is even possible that LAS is being mineralized. On this point, it can also be observed (Table 2) that when 2.3 μmol of LAS were spiked, there were significant differences between the two layers at the end of the experiment: while the percentage of nondetected intermediates was approx 40% in the 1−10 cm layer, it was only 20% in the 10−20 cm layer, which suggests lower biodegradation kinetics in the deeper layer after spiking.

Independently of the real kinetic followed by this surfactant during this process, and taking into account that a lag phase was detected, we estimate that a half-life value of 90 days (3 months) could be considered reasonable for LAS degradation in anoxic marine sediments under the incubation conditions selected for the experiment ($[O_2]$ = 0 ppm, E_h = −300 to −400 mV and T = 30 °C). This value is much higher that those previously reported for this surfactant during the performance of aerobic degradation tests in waters and soils (*6*, *7*), which usually range from 1 to 4 days, due to the much lower efficiency of the anaerobic degradation pathways in sediments. Complete primary biodegradation of LAS is also reached when tests of this type, in the presence of oxygen, are performed. Although the decrease of LAS continued until the end of the experiment (day 165), that objective was not reached in the anaerobic case, when 66−79% of LAS had been removed in blanks and reactors with 2.3 μmol (10 ppm)

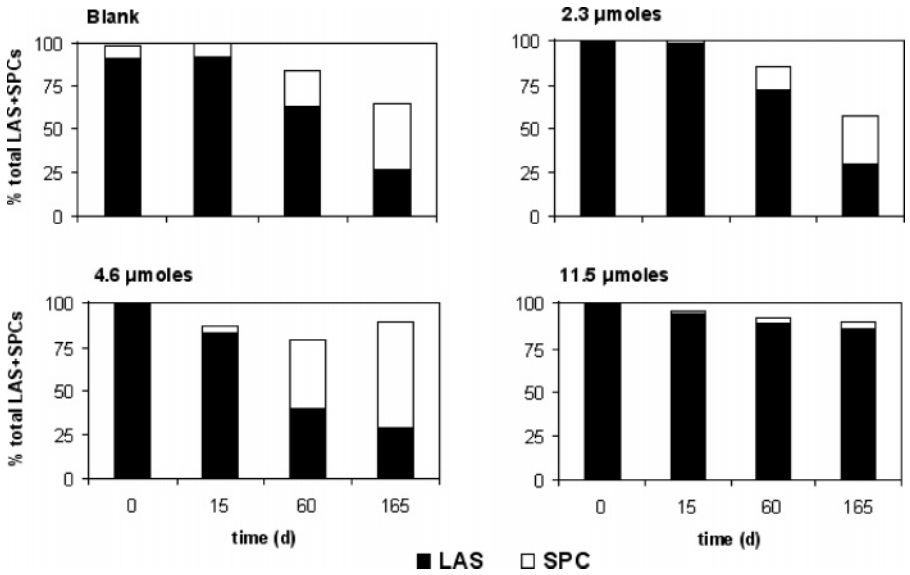

FIGURE 2. Mass balance of LAS and SPCs found in the microcosms in the 1−10 cm depth layer during the complete course of the experiment (0−165 days).

TABLE 2. Mass Balance of the Total Amounts (Water + Sediment) of LAS and SPCs during the Complete Course of the Experiment, in the 1−10 cm and 10−20 cm Depth Layers

	1−10 cm					10−20 cm				
	LAS (nmol)		SPC (nmol)			LAS (nmol)		SPC (nmol)		
time (d)	mean	SD	mean	SD	total	mean	SD	mean	SD	total
					Blank					
0	519	36	40	9	559	632	72	45	7	677
15	528	21	43	11	571	605	72	70	6	676
60	362	52	118	50	480	202	46	96	11	298
165	153	101	220	37	373	132	54	149	55	281
					2.3 µmol					
0	2751	524	13	7	2764	2884	36	22	3	2905
15	2737	689	40	5	2777	2428	532	28	2	2456
60	2000	744	367	172	2367	1582	107	703	218	2285
165	828	107	755	403	1582	990	437	1482	51	2472
					4.6 µmol					
0	4761	175	24	3	4785	4834	844	62	8	4896
15	3968	1004	163	88	4130	4533	1330	184	4	4717
60	1894	232	1868	727	3761	4138	1754	159	42	4297
165	1374	32	2887	400	4261	3616	150	572	213	4188
					11.5 µmol					
0	11983	769	71	12	12053	11961	797	72	10	12033
15	11318	801	202	25	11520	11041	1444	175	37	11216
60	10626	1067	364	83	10990	10811	820	166	54	10977
165	10267	1953	449	209	10715	10462	1556	248	76	10710

of added LAS, 25−71% in those with 4.6 µmol (20 ppm) added, and only 13−14% in the tests with 11.5 µmol (50 ppm) added. Furthermore, it should be noted that degradation is reduced when the amount of LAS in sediment exceeds 20 ppm, so this concentration could be considered toxic to certain marine microorganisms. However these LAS values are unlikely to occur in aquatic ecosystems except for specific locations near untreated wastewater discharges. Longer half-lives (several years) should be expected for this surfactant in these cases. In this context, toxicity for the anaerobic microbial community has been reported when the concentration of LAS is around several tens of ppm of LAS (*12*, *13*). Moreover, negative results regarding anaerobic degradation of LAS and generation of SPCs were obtained in another test carried out by García et al. (*11*) using LAS concentrations much higher than 50 ppm (our top value) although methanogenic activity was still present.

Microbiological Activity. Sulfate reduction and methane production were measured to monitor the overall microbiological activity in the sediments. Both processes are placed at the end of the food chain and therefore they are the most representative of the anaerobic metabolic activities. For sulfate-reducing activity, the media was amended to 4 g L^{-1} sulfate (natural concentration in seawater: 2.7 g L^{-1}) which was completely depleted and converted to sulfide within 30 days (see Figure S1, Supporting Information). While no differences were detected in the methane production rates throughout the sediment column (6 mL CH_4 kg^{-1} dry sediment day^{-1}), the total cell number as determined by 4′-6-diamidino-2-phenylindole (DAPI) staining, was twice as high in the first ten centimeters (4×10^8 cells g^{-1} dry weight) as in the 10−20 cm layer of the sediment (2×10^8 cells g^{-1} dry weight), which could probably be one explanation of the differences found in the degradation percentages with depth

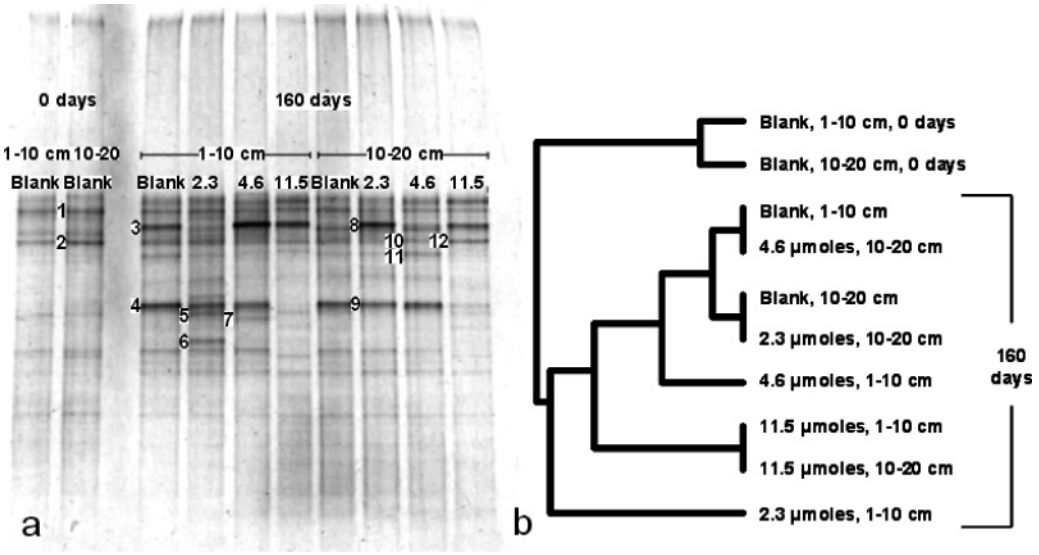

FIGURE 3. (a) DGGE of bacterial 16S rDNA amplified from the microcosm sediments. Numbered bands were excised, reamplified, and sequenced. Closest microorganisms: (1) uncultured firmicute, (2) *Kribbella sandramycini* (*Actinomycetales*), (3) and (8) *Chlorobium phaeovibrioides* (*Chlorobiales*), (4) and (9) *Sedimentibacter sp.* (*Clostridiales*), (5) *Clostridium sp.* (*Clostridiales*), (6) *Tepidibacter sp.* (*Clostridiales*), (7) *Natronoanaerobium halophilum* (*Clostridiales*), (10) *Anoxybacillus gonensis* (*Bacillales*), (11) *Bacillus sp.* (*Bacillales*), (12) *Rhodothermus marinus* (*Sphingobacteriales*). (b) Dendrogram showing similarities of the DGGE banding patterns for the microcosm samples.

in the 4.6 μmol tests and the percentages of nondetected intermediates in the 2.3 μmol tests. After 65 days of operation, a mixture of nutrients was supplied to maintain the biological activity (see Figure S2, Supporting Information). The cell numbers increased tenfold following the feeding of the microcosms and were similar (5×10^9 cells g^{-1} dry weight) regardless of LAS concentration or the original sediment depth. Methane production rates reached values of up to 60 mL CH_4 kg^{-1} dry sediment day^{-1} at 125 days of operation and then decreased with a new depletion of nutrients.

Identification of Possible Candidates for LAS Degradation. Sulfate-reducing bacteria were the most abundant microbial subgroup in the observed samples, comprising up to 12% of the total prokaryotic biomass, as quantified via catalyzed reporter deposition−fluorescent in situ hybridization (CARD−FISH) and accounting for 25% of all clones sequenced: *Desulfobacterium* sp., *Desulfocapsa* sp., and *Desulfosarcina* sp. (*Desulfobacterales*). These bacteria, using sulfate as the terminal electron acceptor, have been described in connection with the anaerobic biodegradation of hydrocarbons (*17*). The clone library we created displays a high phylogenetic variety in the sampling area, with the presence of members of the *Alphaproteobacteria* (genera *Roseobacter*, *Laktonella*, *Rhodovulum*), *Gammaproteobacteria* (genera *Pseudomonas*, *Stenotrophomonas*, *Marinobacter*), and an entire cluster of Epsilonbacteria-related sequences, which have no cultured representative yet, but are moderately similar to a recently discovered species dwelling in sea sediments (*29*). Furthermore we detected the presence of bacteria related to different *phyla* like the *Firmicutes*, *Verrucomicrobia*, *Actinobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes*. Within this set of different sequences and phylogenetic affiliations we encountered species closely related to microorganisms reported as being capable of anaerobic biodegradation of molecules similar to LAS-moieties. One clone identified was closely related to the genus *Desulfosarcina*, exhibiting a 97% similarity with the described hydrocarbon degrading strain oXyS1 (*30*), a microorganism that grows on crude oil and degrades xylene and toluene. Furthermore we detected the presence of *Pseudomonas* (*Gammaproteobacteria; Pseudomonadales*) and *Geobacter* (*Deltaproteobacteria; Desulfuromonadales*), two genera embracing various representatives that degrade hydrocarbons equivalent to the linear alkylbenzene chain of LAS in the absence of oxygen (*18*). Species of *Geobacter* have also been reported to degrade more recalcitrant organic compounds like benzene and benzoate under anaerobic conditions (*31*, *32*).

The other crucial step for complete LAS-biodegradation is the desulfonation of the benzene ring. Only a few prokaryotes have so far been described as performing this reaction under anaerobic conditions (*19*, *33*). In our experiment detectable amounts of LAS disappear but cannot be traced as SPCs, therefore we assumed the possibility of secondary degradation taking place in the microcosms. Using molecular ecology methods, we detected microorganisms affiliated to species known to be capable of performing the desulfonation step. One clone, for example, showed a similarity of 94% with *Sulforhopalus singaporensis*, a sulfate-reducing bacterium that ferments taurine (a hydrocarbon sulfonate) generating sulfide (*16*). Furthermore, various members of the *Firmicutes/Clostridiales* bacterium which cover a wide range of xenobiotics as possible substrates and electron acceptors (*19*, *34*), were detected. These should be considered possible candidates for anaerobic LAS degradation, given that various species of clostridia are confirmed to be able to desulfonate alkyl- and arylsulfonates, like *Clostridium beijerinckii* strain EV4 (*19*) and, recently published, the *Clostridium pasteurianum* strain DSM 12136, using the sulfonic group of 4-toluenesulfonate as the sole sulfur source (*34*).

The assumption that the clostridia participate in LAS degradation is strengthened by sequencing and identifying the bands generated via denaturing gradient gel electrophoresis (DGGE) analysis of the microcosm sediments (Figure 3a); it was found that the majority of these belong to microorganisms of the phylum *Firmicutes*. It can also be seen that the overall microbial diversity of the sediments increased compared to the beginning of the experiment and was not disturbed by low concentrations of LAS. After 160 days of operation all the microcosms generated a very similar banding pattern, regardless of sediment depth or LAS concentration, except for the samples incubated at 50 ppm (11.5 μmol) where the *Sedimentibacter*-band disappears

(Figure 3a, bands 4−9), probably due to the surfactant's inhibitory effects. This observation is in accordance with LAS-removal and SPC-production data (Table 2). The similarities between samples are visualized in the dendrogram generated from the banding patterns of the DGGE gel (Figure 3b). It can be seen that the two original microcosm samples retrieved at $t = 0$ d are similar to each other, though taken at different depths of the sediment cores. They form a cluster apart from the rest of the samples, all of which were taken at the end of the experiment, and which again show banding patterns similar to each other, including identical patterns for the two microcosms incubated with 11.5 μmol LAS, the only incubated samples in which the *Sedimentibacter* band is not present. Other samples are also either identical or very similar, except for one microcosm (2.3 μmol, 1−10 cm core depth), which has several additional bands, two of them being identified as belonging to the *Clostridiales* order. The *Sedimentibacter*-affiliated band is especially interesting, because of its appearance in all microcosms except for the ones incubated with 11.5 μmol LAS, which are the samples in which LAS degradation took place to a lesser extent. *Sedimentibacter*, as a member of the order *Clostridiales*, a group of microorganisms that contains various representatives capable of anaerobic degradation of organic molecules, could be a possible candidate for LAS biodegradation in the examined marine sediments, although this is stated with some reservation.

Acknowledgments

We express our gratitude to E. de Miguel (SCCYT UCA) for his technical support with the LC−MS system. We also thank PETRESA for supplying us with the surfactants standards. This study was carried out within the CICYT R&D Project REN2001-2980-C02-01.

Supporting Information Available

Descriptions of different methodologies used for analysis of LAS and SPCs, methane production, sulfate reduction, and identification of microorganisms; and data on sulfate reduction (Figure S1) and methane production (Figure S2) during the experiment. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Dealmeida, J. L. G.; Dufaux, M.; Taarit, Y. B.; Naccachae, C. Linear alkylbenzene. *JAOCS* **1994**, *71*, 675−694.

(2) Jensen, J. Fate and effects of linear alkylbenzene sulphonates (LAS) in the terrestrial environment. *Sci. Total Environ.* **1999**, *226*, 93−111.

(3) León, V. M.; González-Mazo, E.; Forja Pajares, J. M.; Gómez-Parra, A. Vertical distribution profiles of linear alkylbenzene sulfonates and their long-chain degradation products in coastal marine sediments. *Environ. Toxicol. Chem.* **2001**, *20*, 2171−2178.

(4) González-Mazo, E.; Forja, J. M.; Gómez-Parra, A. Fate and distribution of linear alkylbenzene sulfonates in the littoral environment. *Environ. Sci. Technol.* **1998**, *32*, 1636−1641.

(5) McAvoy, D. C. Dyer, S. D.; Fendinger, N. J.; Eckhoff, W. S.; Lawrence, D. L.; Begley, W. M. Removal of alcohol ethoxylates, alkyl ethoxylate sulfates, and linear alkylbenzene sulfonates in wastewater treatment. *Environ. Toxicol. Chem.* **1998**, *17*, 1705−1711.

(6) León, V. M.; Gómez-Parra, A.; González-Mazo, E. Biodegradation of linear alkylbenzene sulfonates and their degradation intermediates in seawater. *Environ. Sci. Technol.* **2004**, *38*, 2359−2367.

(7) Elsgaard, L.; Pojana, G.; Miraval, T.; Eriksen, J.; Marcomini, A. Biodegradation of linear alkylbenzene sulfonates in sulfate-leached soil mesocosms. *Chemosphere* **2003**, *50*, 929−937.

(8) González-Mazo, E.; Léon, V. M. Surfactant sorption to natural sediments. In *Analysis and Fate of Surfactants in the Aquatic Environment*; Barcelo, D., Ed.; Elsevier: Amsterdam, The Netherlands, 2003; pp 616−617.

(9) Wagener, S.; Schink, B. Anaerobic degradation of nonionic and anionic surfactants in enrichment cultures and fixed bed reactors. *Water Res.* **1987**, *21*, 615−622.

(10) Federle, T. W.; Schwab, B. S. Mineralization of surfactants in anaerobic sediments of a laundromat wastewater pond. *Water Res.* **1992**, *26*, 123−127.

(11) García, M. T.; Campos, E.; Ribosa, I.; Latorre, A.; Sánchez-Leal, J. Anaerobic digestion of linear alkylbenzene sulfonates: biodegradation kinetics and metabolite analysis. *Chemosphere* **2005**, *60*, 1636−1643.

(12) Shcherbakova, V. A.; Laurinavichius, K. S.; Akimenko, V. K. Toxic effects of surfactants and probable products of their biodegradation on methanogenesis in an anaerobic microbial community. *Chemosphere* **1999**, *39*, 1861−1870.

(13) Mösche, M.; Meyer, U. Toxicity of linear alkylbenzene sulfonate in anaerobic digestion: influence of exposure time. *Water Res.* **2002**, *36*, 3253−3260.

(14) Mogensen, A. S.; Haagensen, F.; Ahring, B. Anaerobic degradation of linear alkylbenzene sulfonate. *Environ. Toxicol. Chem.* **2003**, *22*, 706−711.

(15) Denger, K.; Cook, A. M. Linear alkylbenzenesulphonate (LAS) bioavailable to anaerobic bacteria as a source of sulphur. *J. Appli. Microbiol.* **1999**, *86*, 165−168.

(16) Lie, T. J.; Godchaux, W.; Leadbetter, E. R. Sulfonates as terminal electron acceptors for growth of sulfite-reducing bacteria (*Desulfitobacterium* spp.) and sulfate-reducing bacteria: effects of inhibitors of sulfidogenesis. *Appl. Environ. Microbiol.* **1999**, *65*, 4611−4617.

(17) Widdel, F.; Rabus, R. Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Curr. Opin. Biotechnol.* **2001**, *12*, 259−276.

(18) Anderson, R. T.; Lovley, D. R. Hexadecane decay by methanogenesis. *Nature* **2000**, *404*, 722−723.

(19) Chien, C. C. Arylsulfonates as sole source of sulfur for *Clostridium pasteuranium* DSM 12136. *J. Basic Microbiol.* **2005**, *45*, 274−278.

(20) Sanz, J. L.; Culubret, E.; de Ferrer, J.; Moreno, A.; Berna, J. L. Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in upflow anaerobic sludge blanket (UASB) reactors. *Biodegradation* **2003**, *14*, 57−64.

(21) Angelidaki, I.; Toräng, L.; Waul, C. M.; Schmidt, J. E. Anaerobic bioprocessing of sewage sludge, focusing on degradation of linear alkylbenzene sulfonates (LAS). *Water Sci. Technol.* **2004**, *49*, 115−122.

(22) Huang, Y.; Latorre, A.; Barceló, D.; García, J.; Aguirre, P.; Mujeriego, R.; Bayona, J. M. Factors affecting linear alkylbenzene sulfonates removal in subsurface flow constructed wetlands. *Environ. Sci. Technol.* **2004**, *38*, 2657−2663.

(23) Elshahed, M. S.; Gieg, L. M.; McInerney, M. J.; Suflita, J. M. Signature metabolites attesting to the in situ attenuation of alkylbenzenes in anaerobic environments. *Environ. Sci. Technol.* **2001**, *35*, 682−689.

(24) Llobet-Brossa, E.; Rosselló-Mora, R.; Amann, R. Microbial community composition of Wadden Sea sediments as revealed by fluorescence in situ hybridization. *Appl. Environ. Microbiol.* **1998**, *64*, 2691−2696.

(25) Ravenschlag, K.; Sahm, K.; Amann, R. Quantitative molecular analysis of the microbial community in marine Arctic sediments (Svalbard). *Appl. Environ. Microbiol.* **2001**, *67*, 387−395.

(26) Polymenakou, P. N.; Bertilsson, S.; Tselepides, A.; Stephanou, E. G. Bacterial community composition in different sediments from the Eastern Mediterranean Sea: a comparison of four 16S Ribosomal DNA clone libraries. *Microbiol. Ecol.* **2005**, *50*, 447−462.

(27) Muyzer, G.; de Waal, E. C.; Uitterlinden, A. G. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **1993**, *59*, 695−700.

(28) González-Mazo, E.; Honing, M.; Barceló, D.; Gómez-Parra, A. Monitoring long-chain intermediate products from the degradation of linear alkylbenzene sulfonates in the marine environment by solid-phase extraction followed by liquid-chromatography/ion mass spectrometry. *Environ. Sci. Technol.* **1997**, *31*, 504−510.

(29) Inagaki, F.; Takai, K.; Nealson, K. H.; Horikoshi, K. *Sulfurovum lithotrophicum* gen. nov., sp. nov., a novel sulfur-oxidizing chemolithoautotroph within the ϵ-Proteobacteria isolated from Okinawa trough hydrothermal sediments. *Int. J. Syst. Evol. Microbiol.* **2004**, *54*, 1477−1482.

(30) Harms, G.; Zengler, K.; Rabus, R.; Aeckersberg, F.; Minz, D.; Roselló-Mora, R.; Widdel, F. Anaerobic oxidation of *o*-xylene, *m*-xylene, and homologous alkylbenzenes by new types of sulfate-reducing bacteria. *Appl. Environ. Microbiol.* **1999**, *65*, 999−1004.
(31) Rooney-Varga, J. N.; Anderson, R. T.; Fraga, J. L.; Ringelberg, D.; Lovley, D. R. Microbial communities associated with anaerobic benzene degradation in a petroleum-contaminated aquifer. *Appl. Environ. Microbiol.* **1999**, *65*, 3056−3063.
(32) Wischgoll, S.; Heintz, D.; Peters, F.; Erxleben, A.; Sarnighausen, E.; Reski, R.; Van Dorsselaer, A.; Boll, M. Gene clusters involved in anaerobic benzoate degradation of *Geobacter metallireducens*. *Mol. Microbiol.* **2005**, *58*, 1238−1252.
(33) Denger, K.; Cook, A. M. Assimilation of sulfur from alkyl- and arylsulfonates by *Clostridium* spp. *Arch. Microbiol.* **1997**, *167*, 177−181.
(34) Cao, X.; Liu, X.; Dong, X. *Alkaliphilus crotonatoxidans* sp. nov, a strictly anaerobic, crotonate-dismutating bacterium isolated from a methanogenic environment. *Int. J. Syst. Evol. Microbiol.* **2003**, *53*, 971−975.

Received for review October 4, 2006. Revised manuscript received January 22, 2007. Accepted March 9, 2007.

ES062372Z