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Aerobic biodegradation of linear alkylbenzene sulfonates and sulfophenylcarboxylic acids for different salinity values by means of continuous assays

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Abstract Aerobic biodegradation of linear alkylbenzene sulfonates (LAS) and sulfophenylcarboxylic acids (SPCs) in water, at different salinity values, has been studied. Three experiments have been carried out employing a staircase model system with continuous dosage of LAS to the system and using concentrations of LAS of the same order as those detected in littoral waters receiving urban wastewater discharges. LAS biodegradation was observed to be almost complete (showing a great extent), and in all cases exceeds 98.4%. At the very low concentration values of LAS utilized in the experiments, no significant variations in the biodegradation of LAS due to the effect of the different salinity values assayed were observed. The biodegradation intermediates detected for all the cases were sulfophenylcarboxylic acids with carboxylic chains of between five and 13 carbon atoms. The detection of C13-SPC (which is only produced by C13-LAS) confirms the existence of ω-oxidation. The total disappearance of SPCs in all cases indicates that mineralization of LAS at the concentrations tested was complete.

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Introduction

Littoral systems are areas that are particularly affected by human activity, since a high proportion of the total population lives in or near coastal zones (Cohen et al. 1997). Therefore, large quantities of surfactants reach the marine medium both directly and indirectly via rivers through discharges of treated and untreated urban and industrial wastewaters.

Apart from the soaps, linear alkylbenzene sulfonates (LAS) are the surfactant most widely used on a global scale (Berna and Cavalli 1999). Because the amounts of LAS entering the aquatic environment via wastewater discharges are so vast, their environmental fate and distribution have been the subject of thorough research for about 30 years (e.g., Schöberl and Bock 1980; Larson and Payne 1981; Berna et al. 1989); but this research is mainly related to continental environments (rivers, wastewater treatment plants,...). Although the coastal environment can generally be considered the final destination of all industrial and urban wastewater effluents, studies of the biodegradation of LAS in this compartment have been scarce until recently. The presence of LAS

and their biodegradation intermediates in marine water has been demonstrated, thanks to a limited amount of field research works (e.g., González-Mazo et al. 1997; Marcomini et al. 2000; León et al. 2002).

The most widely accepted biodegradation pathway of LAS consists of the ω-oxidation of the terminal carbon atom of the alkyl chain, followed by successive β-oxidations. The sulfophenylcarboxylic acids (SPCs) formed as a result of this process constitute the degradation intermediates of LAS (Swisher et al. 1978). The process finishes with the desulfonation and rupture of the aromatic ring (Schöberl 1989). As the final biodegradation of SPCs (ring cleavage) is the limiting step in LAS mineralization, the disappearance of SPCs indicates that LAS biodegradation has been completed.

Several authors have performed various laboratory assays to assess LAS biodegradation. Most of these experiments have been carried out in static reactors in which LAS is injected into the system in a single discrete quantity. In this way, Vives-Rego et al. (2000) and Perales et al. (2003) have studied LAS biodegradation in seawater, but employed relatively high concentrations of LAS. León et al. (2004) employed environmentally representative LAS concentrations, but they did not detect SPC of chain length longer than nine carbon atoms because these degrade too fast. To detect SPC of long chain length, continuous injection of LAS into the reactor is necessary.

Konopka et al. (1996) carried out an experiment in which a continuous injection of LAS was employed (to assess its biodegradation in synthetic wastewater), although with not realistic LAS concentrations. More sophisticated test systems for simulating the removal of LAS in fresh surface waters were developed by Schöberl et al. (1998) and Boeije et al. (2000) based on a staircase model. In most instances, a reasonable rate of LAS primary degradation was observed which was dependent on the history of exposure among other parameters. In environments with continuous exposure to LAS residues, e.g., coastal areas with direct discharges of domestic sewage, the microbial communities were rather well adapted to the substrate, and advanced rates of degradation were observed. It has been observed that the extent of LAS primary biodegradation varies with the initial concentration of the compound and with the temperature and generally reaches more than 95% in estuarine and marine systems (Swisher 1987; Sales et al. 1987; Terzic et al. 1992a).

However, no studies have been carried out of LAS biodegradation and their primary biodegradation intermediates employing environmentally representative LAS concentrations and a continuous input of LAS to the reactor, at different salinity values. For these reasons, a study has been conducted of the aerobic biodegradation of LAS and SPCs through a series of experiments employing a staircase model system with continuous injection of LAS to the system from a hypothetical discharge point, to assess the longitudinal variation of LAS and SPC concentrations at increasing distances from this discharge point and at different salinity values.

The specific objectives of this research are:

- to characterize LAS and SPCs biodegradability (at environmentally realistic concentrations) in water bodies of different salinity values.
- to assess the evolution of LAS biodegradation intermediates, with particular attention to the detection of long-chain intermediates (C_{11}-SPC, C_{12}-SPC, and C_{13}-SPC), through careful design of these experiments (long-chain intermediates detection confirms the existence of ω-oxidation and, as a consequence, the existence of primary biodegradation).

Material and methods

Experimental device

The dynamic simulation system employed for this study consists of eight tanks (each made of Plexiglass, cylindrical, and of 12 L capacity: 10 L of water and 2 L of air) interconnected under a hydrodynamic regime and placed at ascending levels (staircase model; Fig. 1). The highest tank is supplied continuously with water of a fixed salinity value (drawn from a larger container that acts as a reservoir) by means of a peristaltic pump. When the first tank is full (with 10 L of water), the excess

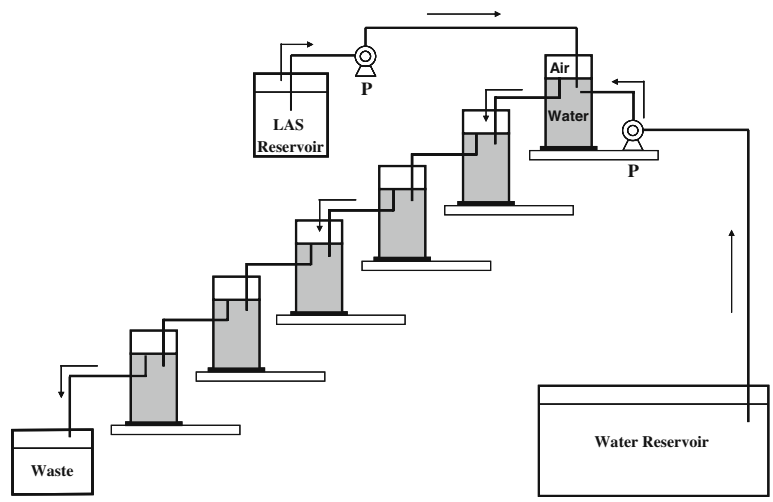

Fig. 1 Schematic representation of the water simulation method (real system consists of eight tanks)

water overflows into the tank below. The same process happens in the second tank and so on. The lowest and last tank overflows to drainage, eliminating excess water and maintaining a constant water volume of 10 L in each tank.

The temperature in each tank is controlled by means of coated dip heaters. To avoid "preferential directions" in the sampling of the tanks, variable-velocity mechanical stirrers are used. The tanks are aerated by means of a blower system, with submerged diffusers, to ensure the correct oxygenation of the water in all the simulations performed.

The control of water flow and temperature is carried out by means of a personal computer (connected to a data acquisition system) running a specially developed software. The data acquisition system consists of an AID 21-bit translation card that incorporates control loops to adjust the functioning of the system. Thus, flow and temperature control loops are intended to adjust real values of flow and temperature (achieved by the peristaltic pump and eight coated dip heaters, respectively) to their expected theoretical values. The system is able to correct flow or temperature values if necessary, adjusting them to the expected values.

Description of experiments

Three experiments (labeled 1, 2, and 3) have been performed to characterize biodegradation of LAS at different salinity values. The assays were performed utilizing natural water and an inoculum, and begun immediately after sampling the water.

Experiment 1 was performed employing seawater with a salinity value of 37.6. This seawater was taken in the Atlantic Ocean, in an area of low contamination (close to Bay of Cádiz, SW Spain), and the inoculum was taken from the interior of Sancti Petri Sound (inner Bay of Cadiz, SW Spain), in a zone close to the discharge point of untreated urban wastewaters from the town of San Fernando (Cádiz). Employed inoculum was previously acclimatized to the salinity value of this first assay. The channel is 18-km long and receives the untreated urban effluent waters from a population of about 95,000 inhabitants. Tidal flows from the Atlantic Ocean and from within the bay enter by both mouths of the channel. Average current velocity of Sancti Petri Sound is about 0.4 $m \cdot s^{-1}$.

Experiment 2 was performed employing water with a salinity value of 19.6. The inoculum employed was the same as in Experiment 1.

Experiment 3 was performed employing water with a salinity value of 3.4. In this case, the inoculum employed was obtained from the influent of the wastewater treatment plant of Puerto Real (Cádiz; average population, 38,000 inhabitants), with a salinity value similar to the water employed in this third assay.

In all experiments, a concentrated solution of LAS was injected in a continuous flow using another peristaltic pump in the first tank of the system (Fig. 1). Calculating correctly, it is possible

Table 1 Average values of salinity, pH, alkalinity, duration of the assay, accumulated residence time, and initial concentration of LAS quantified in the three experiments performed

Experiment	Salinity	pH (SWS)	Alkalinity (mM)	Duration (d)	Residence time (d)	[LAS] ($\mu g \cdot L^{-1}$)
1	37.6	8.17	2.396	9	8.20	117.1
2	19.6	7.93	1.230	10	8.20	10.9
3	3.4	7.12	0.378	10	8.20	13.8

to obtain a desired concentration (at $\mu g \cdot L^{-1}$ level) of LAS in the first tank. After this, LAS begins to circulate through the system. Table 1 shows the values of salinity, pH, alkalinity, duration of the assay, accumulated residence time, and initial concentration of LAS quantified in the three experiments.

In each assay, salinity presented a constant value in all tanks. So, knowing the hydraulic residence time of the tanks (13.4 h) and the value of the littoral drift (close to 40 $cm \cdot s^{-1}$ in the Bay of Cádiz), each tank can be considered to represent one sampling station situated a certain distance from the discharge point of LAS. Consequently, in a body of water with a certain salinity value, it can be established how the LAS biodegradation changes with the distance from the discharge point. Thus, the second tank would represent a station situated 19.3 km from the discharge point; the third tank, 38.6 km, and so on.

To avoid undesirable sorption processes (due to particulate matter), the water employed in all experiments was filtered to 0.45 μm. LAS adsorption–desorption equilibrium is reached in a few hours (Rubio et al. 1996). The hydraulic residence time of the tanks was 13.4 h, and the average duration of the three experiments was 9.5 days; the samplings were done at the end of each assay. Therefore, LAS sorption onto tank walls was not a problem, and its influence on the LAS concentration at the end of the experiment can be ignored. The duration of the assays guaranteed the steady state in all cases.

All the assays were performed at 20°C. In all experiments, a reasonable inoculum population, ranging from 10^4 to 10^5 colony-forming units (CFU), was maintained stable over time (González-del Valle, personal communication). Isolates were identified by using MIDI (Microbial ID, Newark, DE, USA) system. This system identifies microorganisms based on fatty acid methyl ester (FAME) patterns of microorganisms. FAME profiles are then compared to a standard database, and most likely hits are then presented to the user (if similarity index > 0.5, identification can be considered as valid; if $0.3 <$ similarity index < 0.5, identification can be considered as possible). The identified strains were: *Anthrobacter globiformis* (similarity index 0.661), *Micrococus liale* (subgroup A; S.I. 0.655), *Bacillus halodesnitrificans* (S.I. 0.353), *Pseudomonas haloplanktis haloplanktis* (S.I. 0.132), and *Flavobacterium johnsoniae* (S.I. 0.018). The last three strains have been identified in several studies as capable to biodegradate LAS (León 2001).

Other authors have performed assays of similar characteristics with abiotic control (Perales et al. 2007). Authors reported that LAS biodegradation in abiotic conditions can be considered as negligible.

Reagents

LAS was supplied by Petroquímica Española S.A. (PETRESA), and its composition is shown in Table 2. Our research group has collected a complete set of monocarboxylic SCP standards (C_3–C_{13} SPC) with the exception of C_7 SPC (some have been donated and the rest synthesized at the University of Cádiz). After synthesis, in all

Table 2 Composition of the linear alkylbenzene sulfonates employed in experiments performed in this study: Na LAS P550 (purity: 99.2%)

Ph C10	Ph C11	Ph C12	Ph C13	Ph C14	<5 Ph C10	2Ph Alkane	Parafines	Active index (%)	M.W.
8.4	38.3	33.6	17.9	1.0	0.8	15.6	0.1	10	240.5

cases, the purity was higher than 96%. The structure of the compounds was confirmed by ^{1}H and ^{13}C NMR.

The methanol was of chromatography quality, supplied by Scharlau (Barcelona, Spain), and water was MilliQ quality. Tetraethylammonium hydrogen sulfate (TEAHS) was supplied by Sigma-Aldrich (Steinheim, Germany). Sodium chloride was supplied by Scharlau (Barcelona, Spain) and potassium di-hydrogen phosphate by Panreac (Barcelona, Spain).

Analysis of LAS and SPC

Three replicates were taken in each tank in all experiments. All the samples were treated following the procedure proposed by León et al. (2000) for the simultaneous determination of LAS and SPCs. This method consists of a solid phase extraction over a Bound Elut C18 minicolumn (Varian) and then over a SAX (Supelco), and its subsequent analysis by high-performance liquid chromatography with fluorescence detection (HP 1050). For the quantification, external standard solutions were used (ocean water spiked with LAS and/or SPC standards); these were treated in the same way as the samples. In those cases in which it was necessary to validate the assignation of peaks obtained by the previously described procedure, or when standards were not available (C_7 SPC), liquid chromatography with mass spectrometry was applied (LCQ ThermoQuest, S.A. of the Central Services of Science and Technology of the University of Cádiz) in accordance with the conditions proposed by González-Mazo et al. (1997).

Results and discussion

Behavior of LAS

Figure 2 shows the evolution of the concentration in dissolved phase of LAS in the three experiments. For all the experiments, a very considerable extent of the LAS biodegradation can be observed. Thus, a decrease of the surfactant concentration from the first tank can be found in all three assays, and from the second tank, almost all LAS has been biodegraded. In this tank, the percentage of LAS biodegradation was around 98.4% in all experiments. The very high LAS concentration detected for the first tank in experiment 1 showed a slower biodegradation due possibly to the inoculum having more difficulty in acclimatizing to a high salinity value.

In degradation experiments performed with commercial LAS by other authors (Swisher 1987; Terzic et al. 1992b; Perales et al. 1999), a preferential degradation of homologues with longer alkyl chain can be observed. However, in this study, the extent of the degradation observed did not present significant variations with the length of the alkyl chain, since it is difficult to find these differences in assays that are carried out with very low

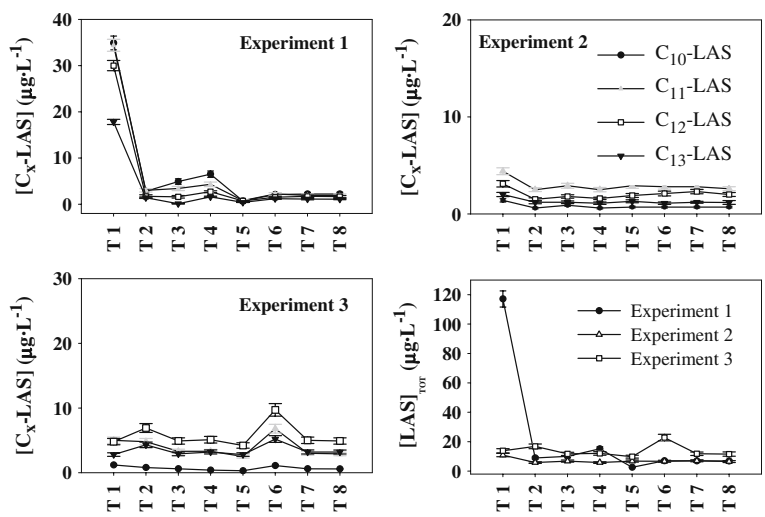

Fig. 2 Evolution of the concentrations in dissolved phase of the four LAS homologues (C_{10}-LAS, C_{11}-LAS, C_{12}-LAS, and C_{13}-LAS) and for total LAS, in the three experiments

LAS concentrations. A similar behavior has previously been described for seawater degradation by León et al. (2004) employing environmentally representative LAS concentrations.

In relation to the proportions of homologues, in all cases, the most abundant homologues were C11-LAS and C12-LAS (Fig. 3). The same behavior has been described by León et al. (2002) for natural samples from several Iberian littoral ecosystems. C10-LAS was the homologue that presented an enrichment with regard to the commercial formulation of LAS employed, above all in experiment 1. C13-LAS was the homologue whose proportion was kept closest to the proportion found in the commercial formulation of LAS employed. Nevertheless, it is difficult to try to elucidate trends in experiments 2 and 3 owing to the very low values of residual LAS obtained, close to the limits of detection.

Behavior of SPCs

SPC homologues with five to 13 carbon atoms in their carboxylic chains were detected in all three experiments (Fig. 4). Taking into account that SPCs are highly reactive, the detection of these intermediates was possible only because LAS was continuously injected into the system. Identification of long-chain length SPCs has been especially significant. In static laboratory assays, long-chain SPCs have not been detected, probably due to the rapid kinetics of LAS biodegradation (León et al. 2004). SPCs with the same chain length as the originating LAS homologue (C13-LAS) have even been detected (in experiments 1 and 2); since C13-SPC can only be derived from C13-LAS, its detection confirms the existence of ω-oxidation and, consequently, the existence of primary biodegradation.

Figure 4 shows, by way of example, the evolution of SPCs quantified in the simulation system in experiments 1 and 3; these experiments represent the highest and the lowest salinity values simulated in this study. In all the assays, a decrease of the SPC concentrations was detected with time, confirming at the end of the experiments the biodegradation of LAS. Biodegradation of various SPC homologues is not as fast as biodegradation of LAS homologues, showing an exponential decrease from the first to the last tank (Fig. 4).

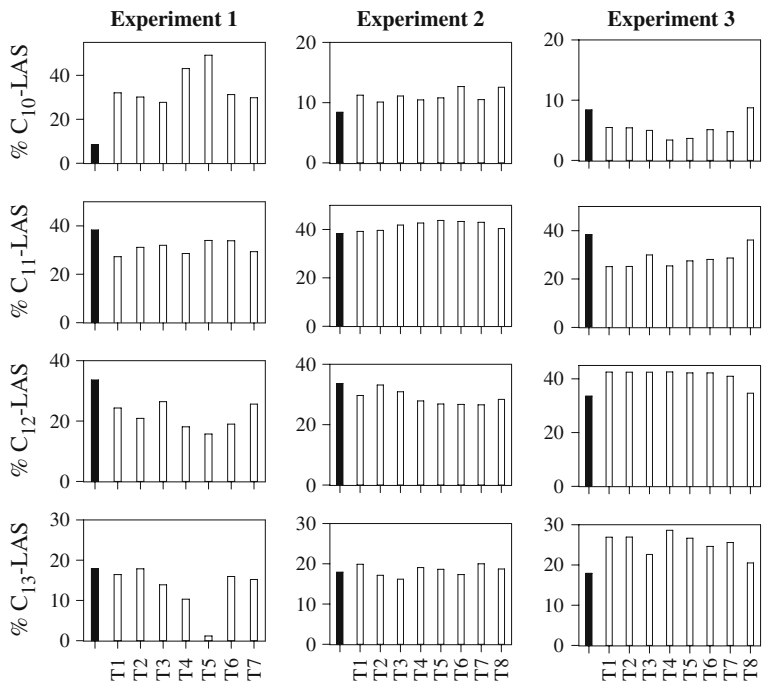

Fig. 3 Variation of the percentages of the four LAS homologues over the eight tanks of the system, in the three assays. *Black bars* represent percentage of each homologue in the commercial LAS employed (see Table 2)

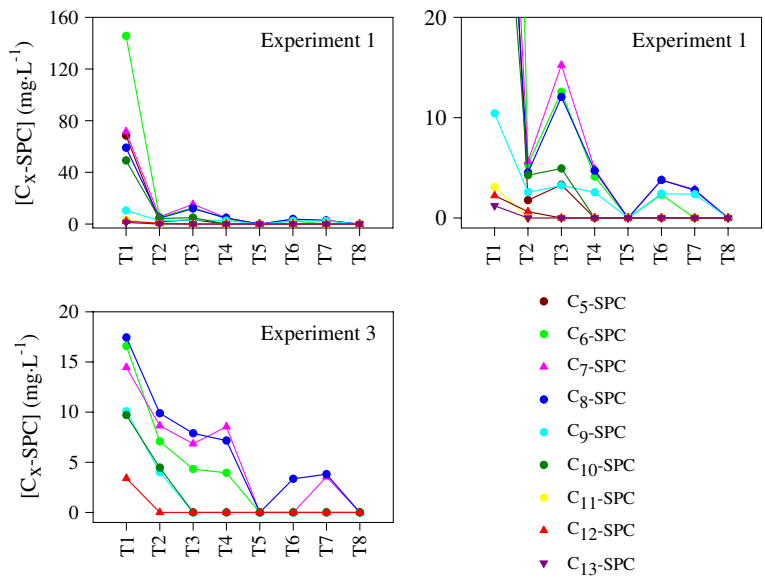

Fig. 4 Evolution of SPC quantified over the eight tanks of the simulation system, in experiment 1 (*two upper graphs*) and experiment 3

In the experiment performed at the highest salinity value, the longest chain length SPC homologues (C11-SPC, C12-SPC, and C13-SPC), produced by the first stages of biodegradation, were detected only in the first and second tanks, and show a sharp decrease in their respective concentrations later. By extrapolating the change between each tank and the time that each represents, the longest chain length SPCs disappear after approximately 29 h.

Medium chain length SPCs (C6-SPC, C7-SPC, and C8-SPC) were the most representative homologues during the experiment, their concentrations decreasing more gradually. This behavior is in accordance with that previously described by others authors (Knepper and Kruse 2000 in experiments with seawater; and Leon et al. 2002 in natural marine samples) who have designated these homologues as "key intermediates," since they are the most persistent in the LAS biodegradation process.

León et al. (2002) reported that total amount of combined C6-SPC, C7-SPC, and C8-SPC constituted more than 70% of total SPC concentration detected in the various Iberian littoral ecosystems studied. In this study, the corresponding percentage ranged from 67% (experiment 3) to 55% (experiment 1). For C5-SPC and shorter, biodegradation was so fast that intermediates of lower chain length have not been detected, as previously reported by León (2001).

Unlike in experiments 1 and 2, in experiment 3 (that with the lowest salinity value) the presence of C13-SPC and C11-SPC was not recorded (due probably to a faster biodegradation in this experiment), nor that of C5-SPC (Fig. 4). The behavior of the rest of homologues is similar to that presented in experiment 1. Concentrations of C12-SPC, C10-SPC, and C9-SPC (long-chain length homologues) decreased sharply from the first tank. In fact, C12-SPC disappeared in the first tank, whereas C9-SPC and C10-SPC disappeared in the second one. In contrast, from the third tank onwards, concentrations of medium chain length homologues (C6-SPC, C7-SPC, and C8-SPC) remained relatively stable in relation to the previous tank. This behavior is due to the rupture of the long-chain length intermediates. These concentrations persisted within a narrow range of values, to decrease later.

Conclusions

The results obtained in the laboratory corroborate the metabolic route of LAS biodegradation proposed by several authors (Swisher 1987; Schöberl 1989), in which the LAS first undergoes

a ω-oxidation of the extreme terminal of the alkyl chain, with the consequent formation of long-chain length SPCs. Subsequently, a successive shortening of the alkyl chain takes place, by means of β-oxidations, giving rise to the formation of shorter chain SPCs. In all experiments, the decrease of SPC concentrations down to levels below the limit of detection indicated that mineralization of LAS at the tested concentrations in aerobic conditions was complete. At the very low concentrations of LAS utilized in the experiments, no significant variations in the biodegradation of LAS due to the effect of the different salinities assayed was observed.

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