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Removal of linear alkylbenzene sulfonates and their degradation intermediates at low temperatures during activated sludge treatment

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Abstract

The degradation of linear alkylbenzene sulfonates and their degradation intermediates (sulfophenylcarboxylic acids) has been characterized at 9 °C in an activated sludge pilot plant. After an adequate adaptation period (20 days), LAS primary degradation exceeds 99% and takes place preferentially for long alkyl chain homologues and external isomers. LAS homologues in the reactor are preferentially sorbed onto particulate matter, while sulfophenylcarboxylic acids (SPCs) are present predominantly in solution, due to their lower hydrophobicity. During the adaptation period the most abundant LAS biodegradation intermediates were long chain sulfophenylcarboxylic acids (SPCs) (C_9–C_{13}SPC). However once this system is fully adapted, the microorganisms are capable of degrading SPCs efficiently. SPCs with 7–9 carbon atoms in the carboxylic chain predominate due to their degradation being slower than for the rest of the SPCs. The presence of C_{13}SPC confirms that LAS degradation in wastewater starts with a ω-oxidation on the alkylic chain. A preferential degradation of SPC isomers of the types $2\phi C_n$SPC to $6\phi C_n$SPC was also detected, as shown by the relatively higher SPC concentrations of the remaining ones.

Keywords: Biodegradation; LAS; Sulfophenylcarboxylic acids; Wastewater treatment; Temperature effect

1. Introduction

The extensive use of linear alkylbenzene sulfonates (LAS) in the formulation of domestic and industrial detergents (e.g. in Spain the average consumption of LAS per inhabitant per day is 5 g, Berna and Cavalli, 1999), results in thousands of tons of this contaminant being incorporated into wastewaters; then with or without prior treatment in sewage plants, these quantities generally reach the natural environment. Given this situation, numerous studies have been undertaken directed towards the degradation of LAS in wastewater treatment plants, since degradation is the only process able to eliminate LAS.

Commercial LAS consists of a mixture of homologues, in function of the length of the alkylic chain (from C_{10} to C_{14}), and of isomers, in function of the position of the sulfophenyl group link with the alkylic chain. In the present paper the isomers of LAS and SPCs are expressed as $m\phi C_n$LAS and $m\phi C_n$SPC, where "m" and "n" denote the site of benzenesulfonate substitution on the alkyl chain and the length of the alkyl chain, respectively (Fig. 1). Note that in the case of LAS, $m = 1$ denotes the C unit at the end of the alkyl chain which is the nearest to the benzenesulfonate, and in the case of SPCs, $m = 1$ corresponds to the C unit at the COOH group. In the course of the text we have named the LAS isomers with $m = 4$–6 and the SPCs isomers with

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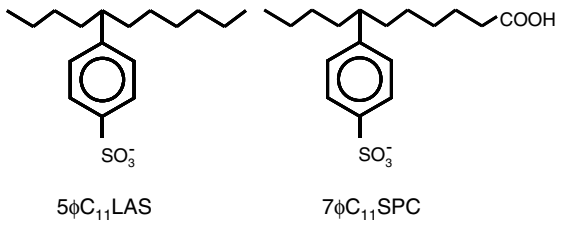

Fig. 1. Chemical structure of $5\phi C_{11}$LAS and $7\phi C_{11}$SPC.

$m < 6$ as internal isomers, with the rest being named as external isomers. The degradation of LAS (Fig. 1) is initiated with the ω-oxidation of the terminal methyl group of the alkylic chain of LAS (Schöberl, 1989; Cook and Hrsak, 2000), and this generates a sulfophenylcarboxylic acid (SPC) with the same number of carbon atoms as the predecessor molecule (Fig. 1). Next, new SPCs are generated by the shortening of the alkylic chain basically through a β-oxidation (Swisher, 1987; Cook and Hrsak, 2000), in which the chain is reduced by two carbon atoms, and as a secondary pathway through α-oxidations, which shorten the chain by one carbon atom (Schöberl, 1989; Cavalli et al., 1992; León et al., 2004). Smaller quantities of sulfophenyldicarboxylic acids (SPDC) can also be generated (Di Corcia et al., 1999); these are degradation intermediates that present two carboxylic groups, one at each end of the hydrocarbon chain, probably from the internal isomers of LAS(4–7ϕ). The degradation continues with desulfonation and the rupture of the aromatic ring, although the sequence in which these two processes occur is still the subject of discussion. Lastly the resulting molecules are mineralized or incorporated into the cellular biomass of the organisms responsible for the degradation. LAS degradation may be performed by specific microorganisms that use it as a sole carbon source (Divo and Cardini, 1980) or as a co-metabolic transformation (Hrsak, 1996).

LAS are rapidly and comprehensively degraded in wastewater treatment plants under aerobic conditions (Berna et al., 1989; Prats et al., 1997). Specifically the reduction of the concentration of LAS in the wastewater (degradation + physical processes) in plants with trickling filter treatment exceeds 85% (Trehy et al., 1996; Holt et al., 1998) and in activated sludge plants, which are the type most commonly utilized, the reduction reaches 95–98% (Trehy et al., 1996; Prats et al., 1997). The removal of LAS from the wastewater by physical processes (sorption, sedimentation, etc.) during its treatment is controlled by the hardness of the water, and this represents less than 10% of total LAS removal in soft waters and up to 30–35% in hard waters (Prats et al., 1997) due mainly to the higher $Ca(LAS)_2$ precipitation at higher Ca^{2+} concentrations (Verge et al., 2001). Recently in a pilot-scale municipal activated sludge plant, it has been found that less than 0.5% of LAS was discharged with effluent (Temmink and Klapwijk, 2004).

The degradation of LAS takes place preferentially on the homologues of greater molecular weight (Swisher, 1987); these homologues in turn are also the ones that present a greater capacity for adsorption (Hand and Williams, 1987). In the wastewater between 50% and 70% of the LAS is associated with the solids in suspension (Cavalli et al., 1993; Prats et al., 1997), and this association can reduce its bioavailability, particularly that of the homologues of longer alkylic chain, and thus also limit its degradation. However, in a recent study, degradation higher than 99% for C_{12}LAS has been detected in an activated sludge plant, despite 92–98% of this C_{12}LAS being found adsorbed onto the sludge (Temmink and Klapwijk, 2004). Sorption also has an indirect positive effect on the biodegradation efficiency, i.e. it provides LAS with a much longer retention time that is related to SRT rather than to HRT.

Another factor that can affect the degradation of LAS is the temperature; when this is higher the metabolic processes and consequently the degradation take place more rapidly. The degradation of LAS is promoted by the temperature of the surrounding environment (Swisher, 1987; León et al., 2004); this affects the kinetics of the process but not its extent after an adequate acclimatization period that is longer at lower temperatures (León et al., 2004). In fact, in actual treatment plants and in assays with model activated sludge units, it has been confirmed that the temperature has little or no effect on the extent of LAS removal between 9 and 20 °C (Painter and King, 1978; Painter and Zabel, 1989). The presence of material in suspension can also promote degradation, by increasing the density of microorganisms present in the medium (Larson et al., 1993), but at the same time, this can also reduce the bioavailability of the LAS if it is strongly adsorbed (Angelidaki et al., 2000).

Despite the numerous studies carried out on the degradation of LAS in wastewater treatment plants (e.g. Prats et al., 1997; Temmink and Klapwijk, 2004), to date only a few authors have considered the degradation intermediates in their study (Trehy et al., 1996; Di Corcia et al., 1999). Trehy et al. (1996) estimated that more than 97% of the degradation intermediates of LAS were eliminated in the various different wastewater treatment plants studied. Later Di Corcia et al. (1999) developed a specific analytical procedure for LAS and SPCs of short chain (LC/IS/MS), and studied especially the fate of LAS co-products, such as dialkyl tetralinsulfonates (DATS) and methyl-branched isomers of LAS, and their biodegradation intermediates. These authors found that the microorganisms in activated sludge plants were capable of completely degrading all the degradation intermediates originating from LAS but not all those from DATS. However, as far as we know, there are no other studies that characterize the evolution of the homologues and isomers of LAS and SPCs in an activated sludge treatment process. The present study describes the monitoring of the concentration of LAS and SPC homologues and isomers at low temperature, conditions at which the degradation may be slowed down. The interest of this study resides not only in the accurate determination (identification and quantification) of LAS and its

principal degradation intermediates, but also in the characterization of this degradation process under conditions of unfavourable temperature. The specific objectives of the present study are: (i) to characterize the process of degradation of LAS both in the effluent and in the sludge generated in an activated sludge pilot plant at 9 °C; (ii) to estimate the period of adaptation necessary and the efficiency of the LAS degradation under these conditions; and (iii) to characterize the evolution of the different homologues and isomers of LAS and SPCs generated during the course of the experiment.

2. Materials and methods

2.1. Test chemicals

Linear alkylbenzene sulfonates (LAS) with an average alkyl chain of 11.6 (CAS number: 68411-30-3) were supplied by PETRESA (San Roque, Spain). The homologue distribution was 12.1%, 34.1%, 30.6% and 23.2% respectively for C_{10}LAS to C_{13}LAS. The content of this mixture in DATS and iso-LAS is below 1%, because this LAS has been produced utilizing HF as catalyzer of the alkylation of the LAB. A complete set of monocarboxylic SPC standards (C_3–C_{13}SPC) is available, some having been donated and the rest synthesized at the University of Cádiz.

2.2. Laboratory simulation of CAS sewage treatment

Three Husmann units (EEC, 1988; ISO, 1995; OECD, 1996) were used for this study, each comprising a 3 l aeration vessel and a 2 l settling tank; sludge was returned to the aeration vessel at a rate of 1 l h^{-1}. The units were operated as follows: two for LAS and one unit as control. The units were inoculated with a composite inoculum (secondary effluent + soil + natural surface water) from the Alicante area (Spain). The plants were operated with a hydraulic residence time (HRT) of 6 h using a constant influent flow of 13.9 ml min^{-1} and at a sludge retention time (SRT) of 10 days. Synthetic wastewater was prepared daily adding to each litre of water the following substances: 160 mg peptone, 110 mg meat extract, 30 mg urea, 7 mg NaCl, 4 mg $CaCl_2 \cdot 2H_2O$, 2 mg $MgSO_4 \cdot 7H_2O$ and 28 mg K_2HPO_4 as recommended by the International Organization for Standardization (ISO, 1995).

Throughout the entire period of experimentation, the units were in operation inside a thermostatic chamber at 9 ± 1 °C, the lowest temperature conditions that are expected in the Mediterranean zone. This assay has been developed starting from the system adapted to 15 °C and to the presence of LAS (10 mg l^{-1}). The temperature was taken down to 9 °C, reducing it at a rate of 1 °C day^{-1}, without adding LAS in this stage, in order to facilitate the adaptation of the microorganisms to the new assay conditions. The LAS concentration in the influent of the units was 10 ± 0.7 mg l^{-1}; this concentration is similar to or less than those detected in real systems (Field et al., 1995; Prats et al., 1997). In any case this value was established bearing in mind that the concentration recommended by the OECD is between 10 and 40 mg DOC l^{-1} (ISO, 1995; OECD, 1996).

The microorganisms required a prior period of acclimatization (data not shown) to the test temperature (9 °C); the total duration of this period was 16 days. Once this period had elapsed, we can differentiate two stages, one of adaptation to LAS presence at these conditions until the efficiency of the DOC elimination exceeds 80%, and the second in which the system efficiently degrades the organic matter. This last stage was maintained for 40 days to determine the capacity of an activated sludge system to degrade LAS and SPCs at 9 °C during a sufficiently long continuous period.

The samples of effluents were taken from the homogeneous composite mixture every 24 h. The samples for the analysis of surfactants were preserved with 3% (v/v) formalin (37% formaldehyde) at the time of collection. The samples for other parameters were kept at 4 °C until they were analyzed (within 24 h).

2.3. Analytical methods

The analyses performed in this study include the characterization of the influent, the effluent and the mixed liquor throughout the entire assay, and of the sludge at the end of the assay (Fig. 2). The dissolved organic carbon content (DOC) was determined in the influent and the effluent. The concentration of dissolved oxygen (DO), concentration of suspended solids (SS), total solids and volatile solids were monitored in the mixed liquor, together with the temperature and the bacterial populations present in the system. The organic carbon content in the sludge was also determined.

The analyses corresponding to solids, temperature, DO and DOC were conducted in order to monitor the

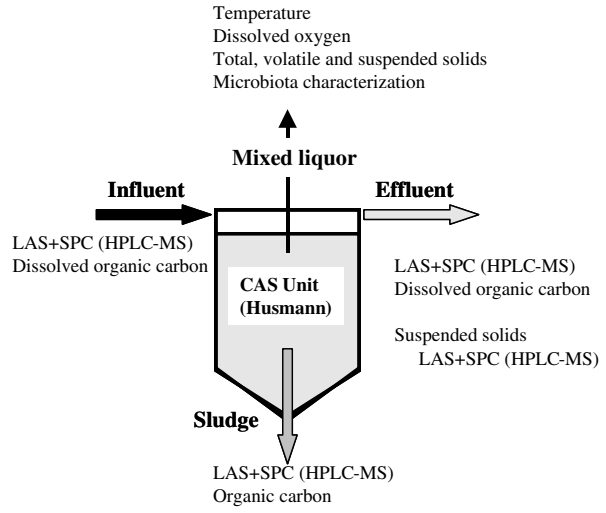

Fig. 2. Scheme showing analysis performed in every fraction studied.

operation of the units in the test during the experiment. The total solids, suspended solids and volatile solids were determined by gravimetry up to a constant weight, at 105 °C and 550 °C respectively (APHA, 1998). The dissolved oxygen and the temperature in the biological reactors were determined using specific electrode and thermometer. The dissolved organic carbon was obtained from the difference between the total carbon and the inorganic carbon, once the samples had been filtered through a 0.45 μm filter. For this purpose, an analyser of total organic carbon (Shimadzu TOC-5000A), based on catalytic combustion and subsequent and non-dispersive detection, was used (Standard Method 5310B).

In order to check the operation of the units during the biodegradation process, the dynamics of the most important microbial groups (bacteria, protozoa and metazoa) were also monitored. A weekly count of the viable total heterotrophic bacteria was done by means of the plate count bacteria culture technique, from the mixed liquor of each unit throughout the various tests, and the abundance of the protozoa and metazoa microfauna was determined using phase contrast microscopy (10X and 40X ocular was used).

The concentrations of LAS and SPCs were determined in the influent and the effluent throughout the entire the assay, and for solids in suspension and the sludge in the final stage of the assay. In the case of the solid samples, the analytes of interest were extracted using a Soxhlet extraction with MeOH for 6 h. The extract obtained was brought to dryness in a rotavapor and redissolved in water, and then was submitted to the same treatment as the liquid samples. In all the cases LAS and SPCs were simultaneously preconcentrated and purified using a specific solid phase extraction method (León et al., 2000), and for their quantification, liquid chromatography with mass spectrometry (González-Mazo et al., 1997) was employed, utilizing external standards treated in the same way as samples, LAS and SPC analyses were performed in duplicate.

The HPLC system consisted of a Spectrasystem liquid chromatograph with autosampler, and with the injection volume set at 20 μl. The chromatographic separation was performed using a reversed-phase C-18 analytical column LiChroCART Superspher 100 RP-18 of 125×2 mm and 3 μm particle diameter (Merck). Water (with 5 mM of acetic acid and triethylamine added), solvent A, and a mixture of acetonitrile and water in a ratio of 80:20, solvent B, were used as solvents in the following gradient (flow = 0.15 ml min^{-1}): initial conditions 100% A, linearly decreased to 0% A in 40 min and kept isocratic for 10 min. The detection was carried out using an LCQ ion-trap mass spectrometer (Thermo), equipped with an atmospheric pressure ionization source with electrospray interface (ESI). All extracts were analyzed using ESI full-scan negative ion mode in order to identify LAS and SPCs homologues, scanning the mass/charge (m/z) range between 75 and 500. Quantification was performed using selected ion monitoring (SIM) mode to achieve a better sensitivity and lower limits of detection (between 1 and 5 μg l^{-1} per compound, calculated using three times the standard deviation of the blank). Other MS parameter values were ion fragmentation energy, −40 V; needle tip voltage, 4.5 kV; gas stealth flow, 60 ml min^{-1}; and ion source temperature 220 °C. Concentrations were determined for each homologue of LAS and SPCs by monitoring its quasi-molecular ion $[M–H]^-$ and measuring their peak areas. External standard solutions containing all the LAS and SPCs homologues (0.1–10 mg l^{-1}) prepared in methanol/water 80:20 and C_{16}LAS as internal standard (1 mg l^{-1}) were used to carry out the quantification.

3. Results and discussion

3.1. Hussmann unit efficiency

The concentration of solids in suspension in the mixed liquor was approximately 2 g l^{-1} in the units with LAS and 2.5 g l^{-1} in the control unit. These values are lower than those detected in real systems (Berna et al., 1989), because the use of synthetic wastewater means that there was no initial supply of solids in suspension in the influent; however, the values are similar to those obtained in other laboratory assays (Tian et al., 1994). The concentration of dissolved oxygen was above 3 mg l^{-1} during the entire assay; therefore the aeration of the system has been adequate to maintain aerobic conditions. In respect of the concentration of bacteria, this was some 2.9×10^6 CFU ml^{-1} on average during the period of assay.

The elimination of the DOC at 9 °C exceeded 90% in both the control and the duplicates, once the system had been adequately adapted. Therefore according to the criterion of the OECD, this system has operated efficiently (OECD, 1996). When the assays are conducted at low temperatures relative to the standard assay proposed by the OECD (30 days, for 18–25 °C), it should be noted that the time of duration of the assay must be increased. When working at 9 °C, it is necessary to increase the acclimatization time and the time for adaptation to the conditions of the experiment to four weeks, against the three weeks proposed as a maximum by the OECD for temperatures higher than 18 °C. At 9 °C more time is required to reach the critical biomass of microorganisms capable of efficiently carrying out the degradation.

3.2. Total LAS and SPC concentrations

The mean concentration of LAS in the effluent during the stage of adaptation to the assay conditions is 1029 ± 129 and 948 ± 108 μg l^{-1} in reactors 1 and 2 respectively (Fig. 3), and no significant variations to these values were detected over the course of this period, since the elimination of LAS in this stage is already about 90%. The high efficiency in degradation capacity found in these reactors from the beginning of the assay is due to the system having

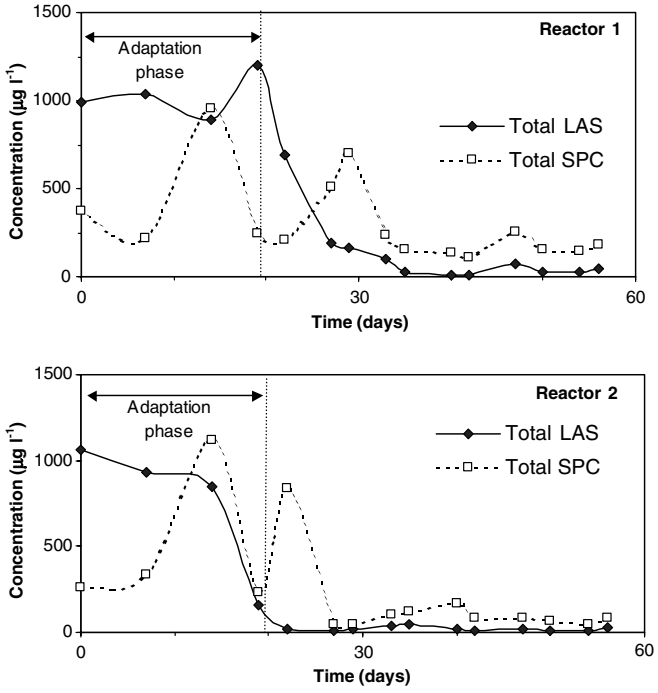

Fig. 3. Evolution of the total concentration of LAS and SPCs in the effluent of the two duplicates during the period of assay. Concentration of LAS in influent: 10 mg l^{-1}.

been previously acclimatized to the degradation of LAS at 15 °C. After the period of adaptation, the concentration of LAS in the effluent decreases in all the cases to levels below 50 µg l^{-1}, and to below 25 µg l^{-1} in most cases. Therefore, the elimination of LAS at 9 °C exceeds 99.5% in all the cases. The extent of the elimination at particular times even exceeds 99.8%, especially in reactor 2, which proved to be slightly more efficient in the degradation of LAS than reactor 1. At the end of the test high LAS removal is detected, and taking into account low LAS concentration in sludge and in effluent, the removal process mainly responsible for this must be primary biodegradation (Fig. 4). These data are similar to those obtained by other authors (Prats et al., 1997; Temmink and Klapwijk, 2004). Therefore, it has been confirmed that LAS removal is higher than 98% in activated sludge treatment even at 9 °C. In fact, in previous research, it has been observed that a temperature of between 9 and 20 °C does not affect the elimination of LAS (Painter and King, 1978).

After the period of adaptation, the total concentration of SPCs in the effluent (Fig. 3) is normally below 0.4 µg ml^{-1}, except on days 15 and 30 of the assay, when higher concentrations were reached (but always below 1.2 µg ml^{-1}), probably as a consequence either of the degradation efficiency of SPC by the microorganisms not yet being optimum. In general, the degradation of the SPCs during the adaptation stage normally exceeds 88%, and exceeds 98% in most of the cases once this stage has been completed, considering that 100% of the SPCs corresponds to the total generated from the LAS. These data confirm the observations made previously by Trehy et al. (1996) in this type of system; these authors estimated the degradation of the intermediates of LAS at more than 97%.

The behaviour of the DATS and their degradation intermediates has not been characterized in this study, since the LAS utilized is one with a low content (<1%) in DATS, which corresponds to the type of LAS that is currently most produced (with HF used as catalyzer). This explains why significant concentrations of these compounds have not been detected. As previously commented, other authors who have utilized LAS with a greater initial content of co-products have observed that the microorganisms in activated sludge plants were capable of completely degrading all the degradation intermediates originating from the LAS, but not those of the DATS (Trehy et al., 1996; Di Corcia et al., 1999).

At the end of the assay, with an efficiency of degradation higher than 95%, the concentrations of LAS and SPCs in the reactor were determined, for both the dissolved and the particulate phases. Thus it was confirmed that 75–80% of the LAS present in the reactor was adsorbed onto the particulate matter (of which 40% was constituted by C_{13}LAS), and consequently the remaining 20–25% was dissolved LAS. In the case of the SPCs, 70–80% of the total present in the system are dissolved, and only 20–30% of the SPCs are associated with the solids in suspension. The oxidation of the alkylic chain reduces the surfactant character of LAS, making the molecule more polar, which facilitates the presence of these compounds in solution by reducing their hydrophobic character.

3.3. Concentrations of LAS and SPC homologues and isomers

The distribution of the homologues of LAS in the reactor is different from that of the commercial standard, since there is a preferential accumulation of the homologues of longer alkylic chain on the sludge, especially the homologue of C_{13}LAS (Fig. 5). In fact, the mean values of the distribution coefficient (ratio between the compound concentration in sludge and in water) determined at the end of the assay are 448, 848, 1284 and 6500 l kg^{-1} for C_{10}LAS, C_{11}LAS, C_{12}LAS and C_{13}LAS, respectively. These data show the preferential adsorption of the homologues of longer alkylic chain, and are similar to those detected previously by other authors in assays of adsorption with activated sludge (Games, 1982). Sorption could reduce the bioavailability of LAS homologues and consequently their degradation, especially for longer alkylic chain ones, but could also increase their retention time in the reactor.

The homologues of greater molecular weight also undergo preferential degradation by the microorganisms (Fig. 4), since the degradation efficiency is slightly higher for C_{12}LAS and C_{13}LAS than that detected for C_{10}LAS and C_{11}LAS. As a consequence of both processes, a decrease of the mean length of the alkylic chain in the

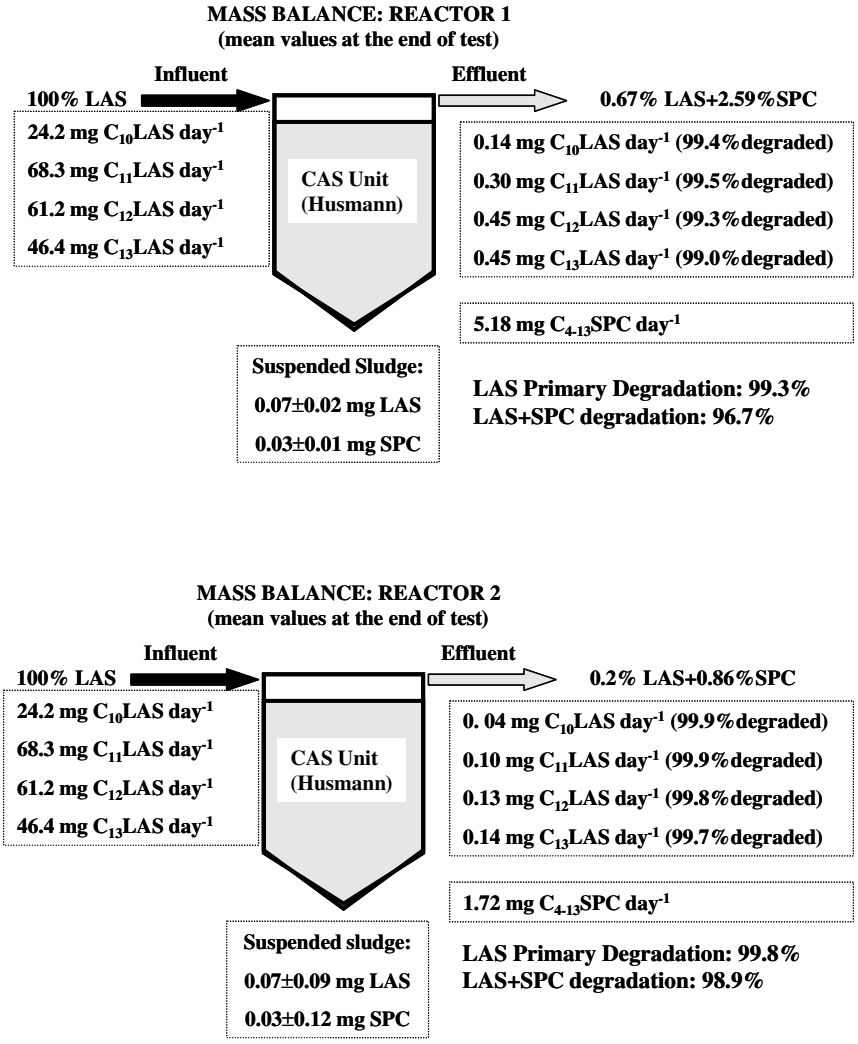

Fig. 4. LAS homologue mass balance (mg day^{-1}) at the end of the experiment (mean of last three days) in reactor 1 and 2, indicating the LAS and SPC removal percentages.

effluent is detected. In fact, during the period of assay, C_{10}LAS increases from constituting 12.1% of the total LAS in the influent to 32% in the effluent, and C_{13}LAS decreases from 23% to 12% of total LAS (Fig. 5). The distribution of homologues of LAS is similar during the period of adaptation and during the period of assay, with a slight increase in the proportion of C_{13}LAS at the expense of that of C_{11}LAS (Fig. 5), probably as a consequence of the reduced availability of the adsorbed portion of the homologues of greater molecular weight when the degradation is more efficient.

The concentration of all the homologues of LAS in the effluent decreases over the course of the assay (Fig. 6) as a consequence of the microorganisms being better adapted or due to an enrichment of the specific microbial populations responsible of LAS degradation, which leads to a greater efficiency of the primary degradation. This efficiency in the degradation also leads to a change in the isomeric composition of the LAS present in the effluent (Fig. 6). The proportion of external isomers (2–3ϕ) of LAS, which is significant during the period of adaptation (day 0), is clearly seen to be reduced after this initial stage, as can be observed in the chromatograms corresponding to days 49 and 58 of the assay. Therefore, after the period of adaptation, in line with the increasing extent of LAS degradation, there is an enriching in the internal isomers (4–6ϕ), probably as a consequence of their degradation being made more difficult by the greater steric impediment that they present (Swisher, 1987).

In respect of the SPCs, three periods can be seen (Fig. 6); the first (example of day 0) in which LAS is degraded with less efficiency and the concentration of SPCs generated is low; the second (example of day 21) in which an efficient degradation of LAS is initiated but SPC degradation is not yet optimum; and the last period (examples of days 49 and 58) in which the degradation of both LAS and its degradation intermediates is efficient, as shown by the low concentrations detected.

Over the course of our assay, homologues of SPC from 4 to 13 carbon atoms in its carboxylic chain have been

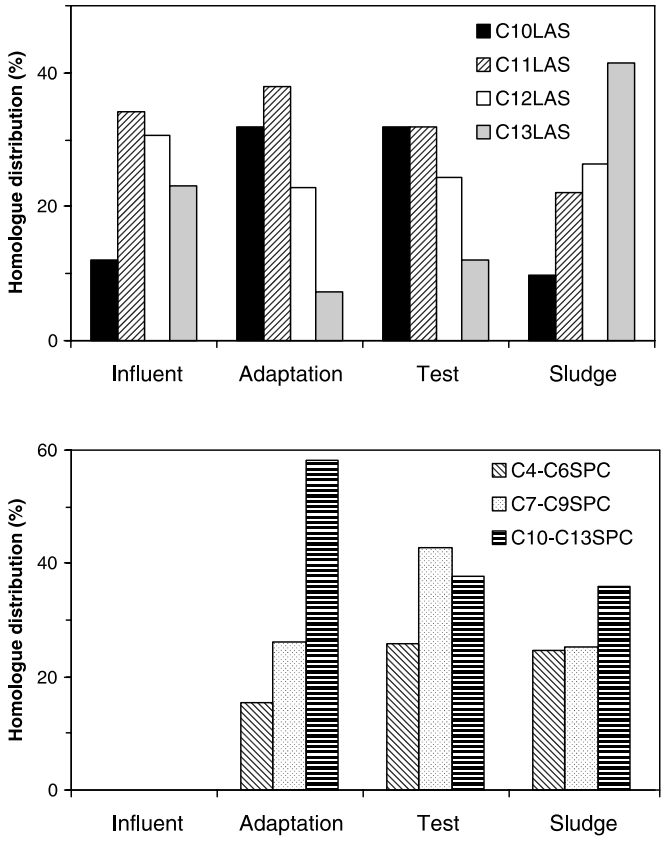

Fig. 5. Average distribution (%) of LAS and SPC homologues in influent, effluent (during adaptation and assay periods) and sludge ($n = 2$).

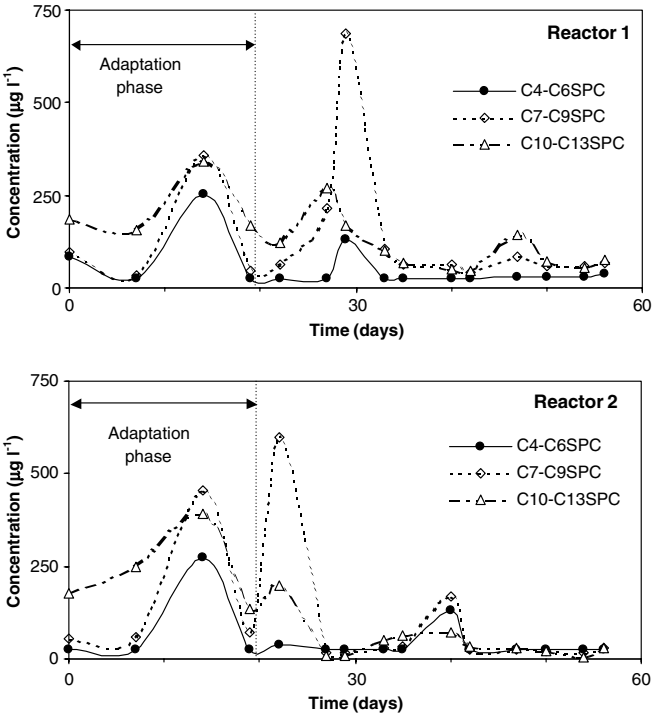

Fig. 7. Evolution of the concentration of the SPC homologues for reactor 1, and of the three groups of homologues (of short, intermediate and long chain length) for reactor 2, over the course of the assay.

detected. The detection of the C_{13}SPC during the treatment of wastewaters confirms that the ω-oxidation is the first reaction that takes place on the LAS, as already shown previously by González-Mazo et al. (1997) in the interstitial water of marine sediments. The SPCs show similar concentrations in water and sludge, due to their adsorption capacity being lower than LAS (González-Mazo and León, 2003) (Fig. 5).

The evolution, over the course of the assay, of the concentration of the SPC homologues, grouped according to the length of their alkylic chain, is shown for both reactors in Fig. 7. In general, the concentration of the groups of SPC homologues of long, intermediate or short alkylic

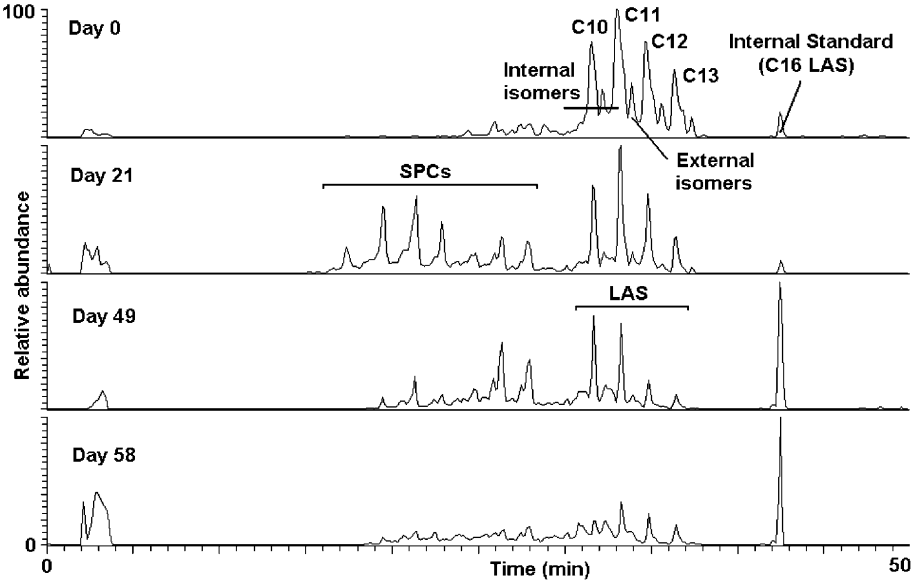

Fig. 6. HPLC/ES/MS chromatograms showing the evolution of the distribution of the LAS and SPC homologues over the course of the assay in reactor 1.

chain is below 250 μg l^{-1}, although occasionally concentrations higher than this have been detected for the intermediate or long chain homologues. The homologues C_9SPC, C_{10}SPC and C_{11}SPC were the most abundant in both reactors throughout the entire the assay, both in the effluent (40–65% of the total SPCs) and in the sludge (35–50% of

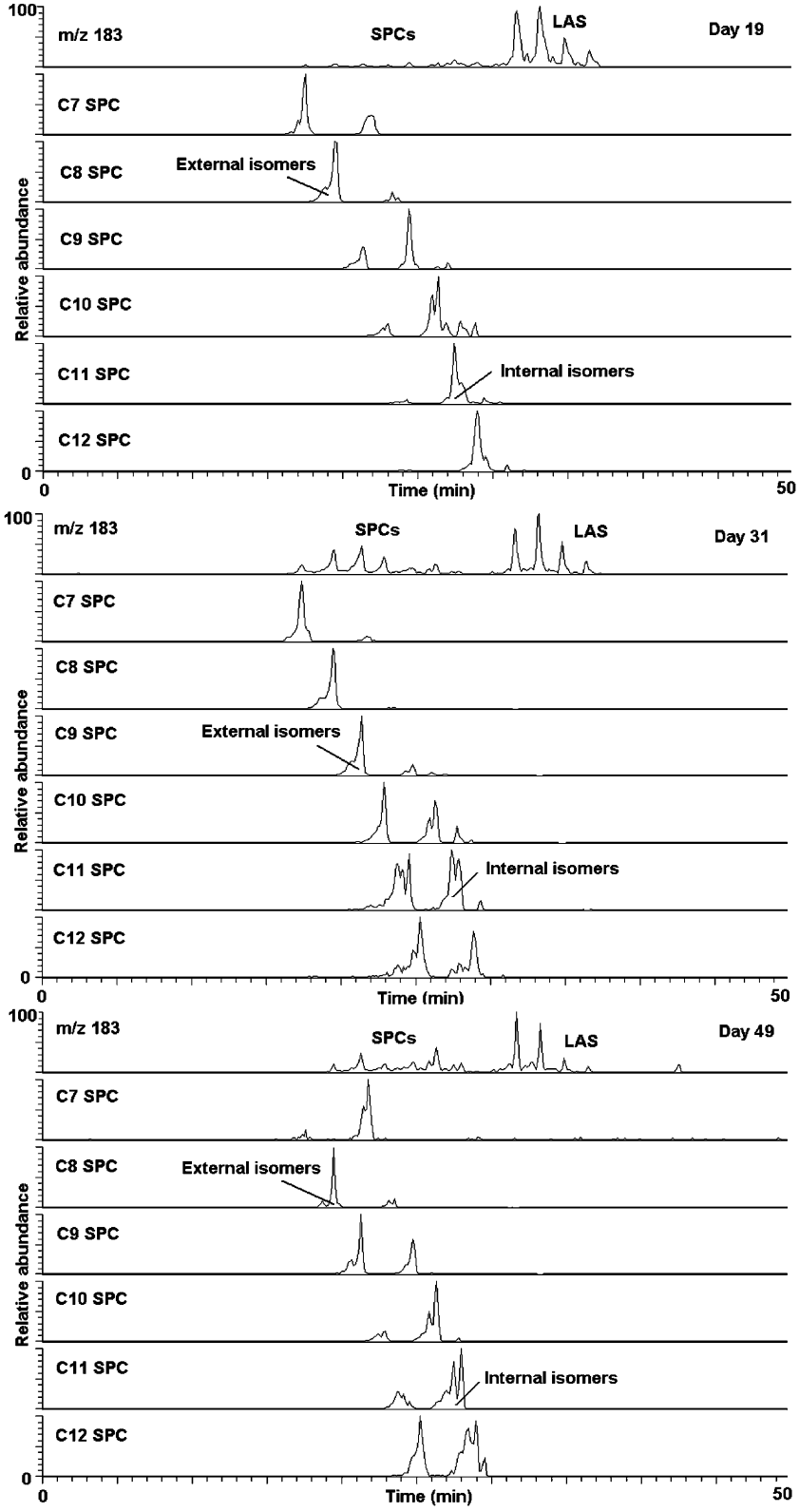

Fig. 8. HPLC/MS (negative ions) selected ion monitoring (SIM) traces from the total ion current chromatogram corresponding to sulfophenylcarboxylates with carboxylic chain of C_7–C_{12} detected in effluent on the 19th, 31st and 49th test days for reactor 1.

the total SPCs). During the adaptation stage, the SPCs of longer carboxylic chain are predominant (Figs. 5 and 7), constituting 60% of the total for the SPCs, while those of intermediate chain length (C7–C9) and shorter chain length constitute 25% and 15%, respectively (Fig. 5). In this stage the degradation efficiency is not optimum, and for this reason the concentration of the degradation intermediates of greater molecular weight is greater than in the rest of the assay, while the concentration of the SPCs of less than nine carbon atoms constitutes less than 28% in the effluent. Once this stage is completed, the proportion of the homologues of greater molecular weight decreases to 30–35%, as a consequence of the microorganisms becoming more efficient at degrading these compounds. In the final part of the assay, the complete set of homologues of intermediate chain length (C_7–C_9SPC) come to constitute around 40% of the total SPCs present in the effluent (Figs. 5 and 7). The reason for this is that, once the microorganisms are adapted, they are capable of rapidly performing the first oxidation reactions of the alkylic chain (León and González-Mazo, 2003), unlike the degradation of the SPCs of intermediate chain length, which is slower because they are subject to a greater steric impediment than the homologues of longer carboxylic chain (Swisher, 1987). Hence these intermediates are considered to be the key intermediates in the process of degradation (Hrsak et al., 1981; Swisher, 1987), and in fact, other authors have also confirmed that the homologues of SPC of intermediate chain length persist longer than the rest in the final stages of the degradation of LAS both in continental systems (Field et al., 1992) and in marine systems (León et al., 2004). The group of SPCs of less molecular weight (C_4–C_6SPC) constitutes the minority fraction in the effluent (15–25%) throughout the entire period of assay.

In respect of the characterization of the sludge at the end of the assay (Fig. 5), it was found that the SPCs that predominate are also those of intermediate and long chain length, with the proportion of C_4–C_6SPC being significantly less. Although the SPC molecules are much more hydrosoluble that those of LAS, the homologues of greater molecular weight present a certain tendency to become associated with the particulate matter, as has been confirmed by the data obtained in the present assay.

In respect of the isomeric composition of the SPCs, Fig. 8 shows some examples of the chromatograms obtained for the specific m/z ratios of the SPCs of 7–12 carbon atoms. During the adaptation stage, the internal isomers ($<6\phi$) of the SPCs (C_9–C_{12}SPC) are the most abundant isomers in the effluent (day 19 in Fig. 8); however, for C_7SPC and C_8SPC, the predominant isomers are the external ones ($>6\phi$). After this initial stage, it is basically the internal isomers for the complete set of SPCs that continue to be detected in the effluent. Therefore, it has been confirmed that the external isomers of SPCs degrade preferentially to the internal isomers, and this finding is especially evident at the end of the assay (day 49). The degradation of the internal SPC homologues is kinetically less facilitated than for the external ones, because a greater steric impediment is presented to their degradation based on the distance principle (Swisher, 1987). For this reason, as the microorganisms in the activated sludge system adapt better to the degradation of LAS and its degradation intermediates, an enriching is detected in the internal isomers for the SPCs, similar to that detected for the case of the LAS. The degradation of the SPCs was found to be efficient throughout the entire assay under conditions that are thermodynamically unfavourable (9 °C), although on occasions temporary increases in the concentration of SPCs (C_7–C_{11}SPC) were detected in the effluent (Fig. 7), in consequence of a slight decrease in the efficiency of their degradation. This reduction in efficiency is also reflected in the increase in the proportion of external isomers of SPCs, as shown for one of these peaks in Fig. 8 (day 31), which then came to represent a significant fraction of the total. Specifically this increase was detected for the SPCs of 7–9 carbon atoms, with respect to what was detected in the rest of cases, as well as for the SPCs of greater molecular weight that also present in this case a significant proportion of external isomers (40–50%).

4. Conclusions

The process of degradation of LAS has been accurately characterized by including the study of its degradation intermediates in an activated sludge system, taking into account not only its isomeric composition but also its distribution by homologues. The primary degradation of LAS during the activated sludge treatment is more than 99% at 9 °C, after an adequate period of adaptation (20 days). The degradation takes place preferentially on the homologues of greater molecular weight and on the external isomers. During the degradation assay, 80% of the LAS present in the reactor was adsorbed on the particulate matter (preferentially the homologues of longer alkylic chain), whereas only 20–30% of the SPCs is associated with this phase, particularly the homologues of greater molecular weight.

The most abundant degradation intermediates found during the period of adaptation were the longer chain SPCs (C_{10}–C_{13}SPC), and those of intermediate chain length (C_7–C_9SPC) and longer chain length (C_{10}–C_{13}SPC) once the process of LAS degradation was operating at optimum efficiency. From the detection of the C_{13}SPC, we can conclude that the degradation of LAS is initiated with the ω-oxidation of the terminal methyl of the alkylic chain in activated sludge systems. Also the preferential degradation of the external isomers has been confirmed for both LAS and the SPCs, since it is these isomers that present the least steric impediment to their degradation.

Considering the difference between the LAS present in the influent and the sum of the LAS and the SPCs detected in the effluent, it can be deduced that the LAS and SPC removal at 9 °C exceeds 96% in all the cases. Therefore, it can be concluded that, after an adequate period of acclimatization, the degradation of LAS and their degradation

intermediates is efficient, even under adverse climatic conditions.

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