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Biodisposition of linear alkylbenzene sulphonates and their associated sulphophenyl carboxilic acid metabolites in sea water

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Abstract

The present paper describes the results of the application of the biodegradation assay proposed by the United States Environmental Protection Agency "Biodegradability in sea water" Office of Prevention, Pesticides, and Toxic Substances 835.3160, in shaked flask, to linear alkylbenzene sulphonate (LAS), the synthetic surfactant with the highest consumption volume on a world-wide basis. The concentration of sulphophenyl carboxylic acids (SPCs) which are the main metabolites of LAS have also been monitored by high performance liquid chromatography. The experiment were conducted using water originating from the Bay of Cádiz (in the South West of the Iberian peninsula). The results indicate that LAS was rapidly biodegraded. SPCs were transiently produced during the process and they were biodegraded to levels below the limits of detection (136 μg/l) after 42 days of assay. The kinetic of biodegradation of the surfactant and its biointermediates have been modeled; half-life times of 6.2 days for the primary biodegradation of LAS and of 9.6 days for the mineralization of the biointermediates were obtained.

Keywords: Biodegradation; Surfactant; Linear alkylbenzene sulphonate; Biointermediates; Sulphophenyl carboxylic acids; Sea water

1. Introduction

Although in terms of surfactant tonnage used worldwide, soap is still used more than synthetic detergents, the great variety of end products in which the latter are involved shows just how spectacular their process development has been.

Of the 18 million tonnes of surfactant consumed in the world in 1995, just over 50% (9.5×10^6 mT) corresponded to soap, with the rest (8.5×10^6 mT) being synthetic detergents (Granados, 1996). Among the synthetic detergents, the linear alkylbenzene sulphonates (LAS), are the most utilized in the world, with a production volume in 1995 of some 1.5×10^6 mT, and constituting 18% of the total synthetic surfactants manufactured in the world.

LAS also accounts for a considerable proportion of the total xenobiotic compounds discharged into hydric medium. Consequently, it has been widely studied in the last decade, and its process of biodegradation has been investigated under a wide range of conditions in many media (Painter and Zabel, 1988).

The most widely accepted biochemical mechanism for the biodegradation of LAS consists of the ω-oxidation of the terminal carbon atom of the alkyl chain, followed by successive β-oxidations. As a result of this process, sulphophenyl carboxylic acids (SPCs) are formed; these constitute the degradation intermediates of LAS (Swisher et al., 1978), and the process finishes with the desulphonation and rupture of the aromatic ring (Schöberl, 1989) (Fig. 1). Investigations to determine the biodisposition pathway of LAS and its metabolites are critical to assess the ecotoxicological risks associated to LAS on marine ecosystems.

During the late 1970s, Leidner et al. (1976) and Pitter and Fuka (1979) obtained data suggesting that the aromatic ring of LAS is persistent to biocatalytic cleavage. Both groups based their conclusions on the fact that the aromatic ring metabolites accumulated when LAS were used as source of carbon in "die-away" assays to assess biodegradability. Pitter and Fuka (1979) concluded that LAS could not be classified as totally mineralizable compounds, and that, if biodegradation occurs, it would be after a long period of time, hence the use of the term degradable to describe LAS would be very questionable.

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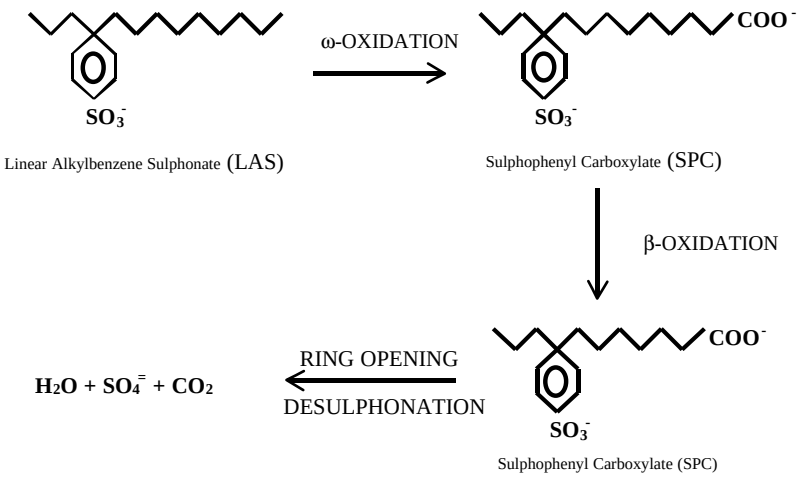

Fig. 1. Metabolic biodegradation route of LAS.

Later, Larson and Payne (1981) found that when the concentration was below 10 mg/l, the complete mineralization of LAS did take place, but that above this level mineralization did not take place due to the bacteriostatic effects of the surface agent. The incomplete biodegradation of LAS was also demonstrated by Yoshimura et al. (1984), who detected the presence of SPCs after 25 days in an assay of river water. Kölbener et al. (1995) identified degradation products of a commercial LAS treated on a laboratory trickling filter.

Di Corcia et al. (1994), used HPLC to monitor the concentration of LAS and its intermediates in raw and treated waste waters, and identified various SPCs of short chain length (less than seven carbon atoms) in concentrations that varied between 22 and 0.6 mg/l. Berna et al. (1993) performed a similar monitoring at seven wastewater treatment plants of the Autonomous Region of Madrid, Spain. They found concentrations of SPCs that varied between 0.85 and 0.25 ppm in raw wastewaters, and between 0.7 and 1.2 ppm in treated waters.

Other biodegradation studies (Huddleston and Nielsen, 1979; Steber, 1979; Swisher, 1981) were conducted with ^{14}C-labelled commercial LAS. These investigations were done using settings that simulated biological treatment plants for wastewaters operating on a semi-continuous basis. Data clearly showed that over 60% of LAS was converted to CO_2.

Other authors have evaluated the mineralization of LAS from the release of SO_4^{2-} based on the assumption that the rupture of the aromatic ring together with its desulphonation are the final stages of the mineralization route of the surface agent.

In a study conducted in a laboratory-scale activated sludge plant, Cordon et al. (1968) found that the sulphate released after 21 days of assay represented 89% of the total theoretical amount added to the reactor as LAS. Pecenik et al. (1984) obtained similar results using ^{35}S-labelled LAS.

Data obtained from wastewater treatment plants indicated a degree of elimination above 98% of LAS (Giger et al., 1989; Berna et al., 1993), although in those cases, only 80% of this disappearance is biological, given that between 18% and 20% of the LAS in the influent water is sorbed or precipitated (Giger et al., 1989; Berna et al., 1993).

Gonzalez-Mazo et al. (1997), have identified some long chain SPC produced from the biodegradation of LAS in the marine environment. To our knowledge there is only one report of investigation conducted to identify and quantify SPCs produced from LAS in marine environment, despite this being a medium with less purifying capacity than either continental water systems or wastewater treatment systems.

The purpose of this paper was to verify if LAS can be degraded in a marine environment and to identify some of the metabolites produced.

2. Material and methods

All the biodegradation assays conducted in the present study followed the OPPTS guideline 835.3160 “Biodegradability in sea water” (USEPA, 1998). These directives cover the assays in closed flasks or in shake flasks. The latter method has been employed in the present study. This method is a variant for sea water of the modified test assay of the OECD (OECD, 1992) that was developed by the Danish institute of Water Quality for the European Union as a result of an exercise of intercalibration (Nyholm and Kristiansen, 1987). This assay is not designed to estimate the easiness of biodegradability, but can be used to verify if a given compound is biodegradable in a marine environment.

The substrate used in this study was a sodium salt of LAS with the following characteristics and properties:

Supplier: Fluka Chemie A.G.; product: Dodecylbenzenesulphonic acid sodium salt; product *no.*: 44200; empirical

Table 1
Molar and weight proportions of the different homologues of the LAS utilized

	C_{10}-LAS	C_{11}-LAS	C_{12}-LAS	C_{13}-LAS
MW (g/mol)	297	311	325	339
% Mol ($n = 18$, CI95%)	19.91 ± 0.56	31.19 ± 0.46	27.82 ± 0.33	21.08 ± 0.37
% Weight ($n = 18$, CI95%)	18.59 ± 0.54	30.50 ± 0.45	28.43 ± 0.34	22.47 ± 0.39

C_{10}-LAS: decylbenzenesulphonate; C_{11}-LAS: undecylbenzenesulphonate; C_{12}-LAS: dodecylbenzenesulphonate; C_{13}-LAS: tridecylbenzenesulphonate

Fig. 2. Picture of the Bay of Cadiz showing the zone selected to sample the seawater used for the biodegradation assays.

formula: $C_{18}H_{29}NaSO_3$; molecular weight: 34,848 g/mol; aspect: pale yellow powder; solubility in water (20°C): 50 g/l; purity: 80.2%; content in carbon: 48.17%; composition: 80.2%; active matter, ≈17% sodium sulphate, < 3% water. The proportion of the different homologues present in the mixture utilized as determined in the laboratory is given (Table 1).

The seawater utilized was sampled from a point in the Bay of Cádiz (South West of the Iberian Peninsula) (Fig. 2). The seawater was sampled with a Ruttner oceanographic-type bottle at a depth of 0.5 m. The samples were stored in polyethylene containers of 25 l capacity and were transported at room temperature to the laboratory immediately after sampling.

Once in the laboratory, the water was filtered to remove the coarse particles on a fibreglass filter of 1 μm nominal pore size (Micron Separations Inc.). The characteristics of the water utilized are listed in Table 2.

The reactors employed in the biodegradation assays had a capacity of 2.5 l, were of borosilicate glass, and amber in colour to ensure that the biodegradation took place under diffuse light. The reactors were stoppered with a plug that permitted the exchange of gases with the atmosphere. A volume of 1.5 l of filtered seawater was added to each reactor with the objective of leaving sufficient empty space to allow the correct reoxygenation of the medium.

Next a volume of 1.5 ml of each of the four stock solutions of nutrients was added (Table 3). Having been agitated for 15 min, the reactors were stoppered and kept in a precision incubator (Hot–Cold GL 4000700: J. P. Selecta S. A.) at 20 ± 1°C and in darkness for 24 h before the compounds to be assayed were added; the purpose of this was to allow the preconditioning of the microorganisms to the assay conditions. The initial concentration of LAS used was approximately 20 mg/l.

LAS concentration was determined by reverse phase high performance liquid chromatography (HPLC), following the method proposed by Nakae et al. (1980). The LAS metabolites were determined by HPLC following the method proposed by Cavalli et al. (1996). The chromatograph comprised of two pumps, a Waters model 510 alternating double piston type and a Waters model 501 simple piston

Table 2
Result of the analyses conducted to characterize the waters samples utilized in the assay of biodegradation ($n = 3$, 95% confidence interval)

Parameter	Mean	Standard deviation
pH	8.11	—
Salinity	36	—
Solids in suspension (mg/l)	224.0	56.0
Volatile solids (mg/l)	31.5	6.4
% Volatile solids	14.2	0.8
Total carbon (mg/l)	29.76	—
Inorganic carbon (mg/l)	24.72	—
Dissolved org. carbon (mg/l)	5.04	—
Nitrites (μg/l NO_2^-)	28.6	3.1
Nitrates (μg/l NO_3^-)	20.6	0.9
Ammonium (μg/l NH_4^+)	20.3	2.1
Total phosphorus (μg/l P)	114.9	4.2
Silicates (μg/l Si)	257.4	25.5
Greases and oils (mg/l)	< 2	—
Anionic surfactants (μg/l MBAS)	< 40	—
Phenols (μg/l C_6H_5OH)	< 25	—
(μg/l Cla)	105	—
Chlorophyll (μg/l Clb)	nd	—
(μg/l Clc)	nd	—
Fecal streptococci (UFC/100 ml)	6700	—

Table 3
Composition of the stock solutions used in the biodegradation test medium

Stock solution no. 1
8.5 g/l KH_2PO_4
21.75 g/l K_2HPO_4
33.3 g/l $Na_2HPO_4 \cdot 2H_20$
0.5 g/l NH_4Cl
Stock solution no. 2
27.5 g/l $CaCl_2$
Stock solution no. 3
22.5 g/l $MgSO_4$
Stock solution no. 4
0.25 g/l $FeCl_3 \cdot 6H_2O$

type. The HPLC was equipped with a manual type model U6K injector, and Waters model 470 fluorescence type detector.

The various sulphophenyl carboxylic acids (SPCs) were identified based on their retention times relative to internal standards of C_2-SPC (sulphophenyl acetic acid), C_5-SPC (2-(4-sulphophenyl) pentanoic acid), C_9-SPC (2-(4-sulphophenyl) nonanoic acid) and C_{11}-SPC (2-(4-sulphophenyl) undecanoic acid).

Given the fact that all the SPCs exhibit an identical molar sorption coefficient (Rothman, 1982; Marcomini et al., 1989), C_5-SPC was taken as the reference, such that

$$C_i = \frac{A_i M_i}{m_5 M_5}, \quad (1)$$

Table 4
Calibration curve drawn for the C_5 sulphophenyl carboxylic acid

C_5-SPC	
Slope (b) (area/ppm)	79208
Intercept (a) (area)	31379
r^2	0.999
Detection limit (mg/l)	0.136
$SD_{y/x}$	3585
SD_a	2404
SD_b	189

$SD_{y/x}$ standard deviation of the target. SD_a = standard deviation of the intercept. SD_b = standard deviation of the slope.

Table 5
Mean molecular weight of the sulphophenyl carboxylic acids delimiting the various zones of the chromatogram

Zone	Mean number of atoms of carbon in the alkyl chain	Mean molecular weight (g/mol)
1	4	273
2	3.5	236
3	7	285
4	10	327

where C_i is the mass of the SPC to be quantified (g), A_i the area of the chromatographic signal to be quantified, M_5 the molecular weight of the C_5-SPC (257 g/mol), m_5 the slope of the calibration curve for the C_5-SPC acid (Table 4) and M_i the molecular weight of the SPC to be quantified.

In order to calculate M_i (Eq. (1)), the molecular weight of the SPC to be quantified, four molecular weight standards were used to delimit four zones on the chromatogram (Table 5).

In the case of the sulphophenyl dicarboxylic acids (SP2C), and considering the distance principle, the double oxidation of the alkyl chain will not take place until the β-oxidation is unable to continue its course due to steric impediments of the sulphophenyl group, so they will be short chain intermediates. The mean alkylic chain length considered for dicarboxylic metabolites has been 4 carbon atoms. It should also be mentioned that the quantity of sulphophenyl dicarboxylic acids produced during the biodegradation did not represent more than 14% of the total sum of the areas of the chromatographic signals, in any of the samples analysed.

For the kinetic modelling, the experimental data obtained in the different assays were fitted using the quasi-Newton method of non-linear regression to the kinetic models proposed by Simkins and Alexander (1984). Having selected the simplest model, and the one that best fits the experimental results, the kinetic parameters obtained were used to calculate the values of t_L and $t_{1/2}$, in accordance with the definitions proposed in the USEPA directive (USEPA, 1998).

3. Results and discussion

In Fig. 3 are shown the chromatograms corresponding to the analysis of SPCs in one of the experiments conducted.

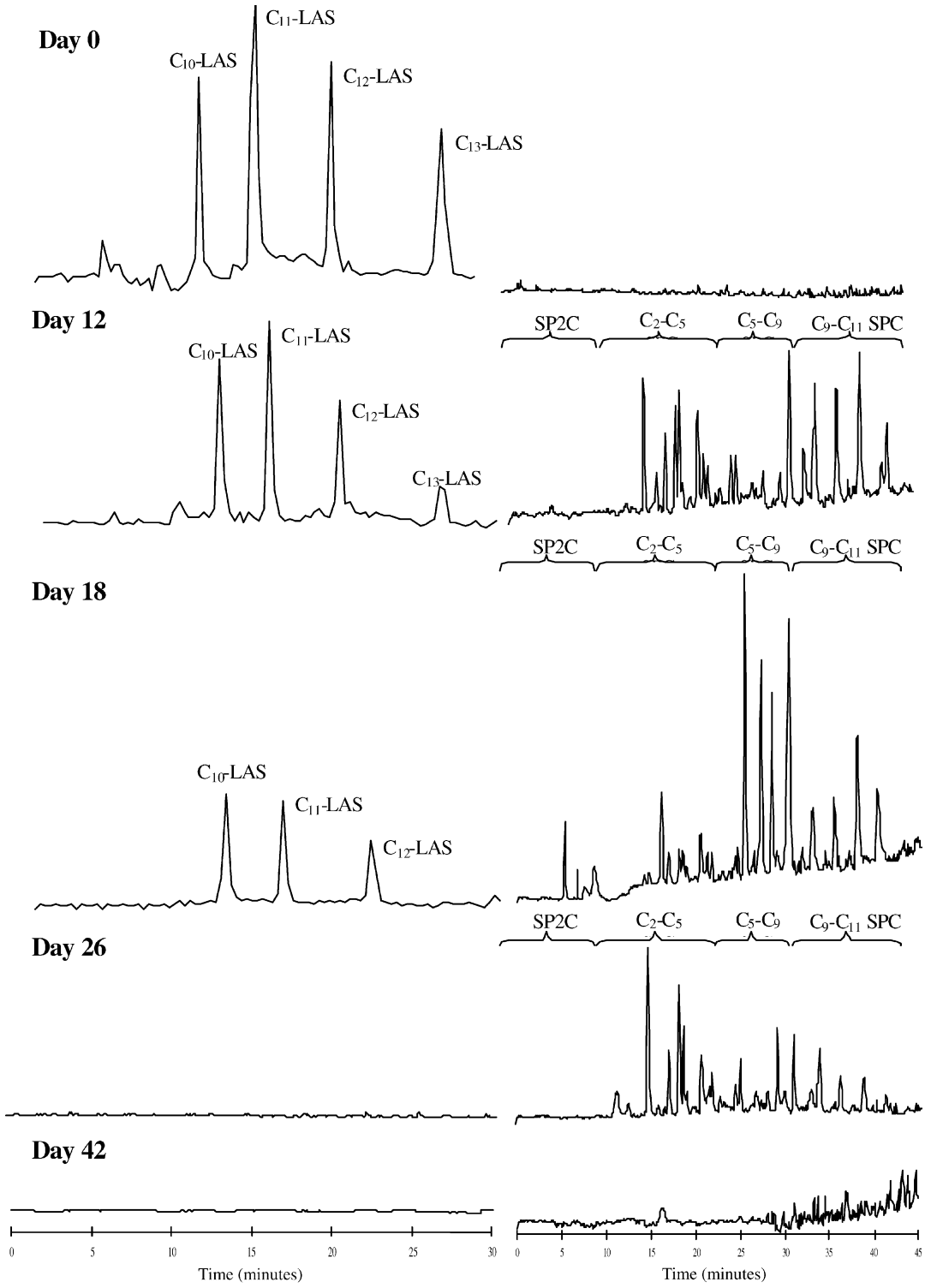

Fig. 3. Chromatograms corresponding to the analysis of the surfactant material and the sulphophenyl carboxylic acids over the course of the biodegradation assay.

Many metabolites were generated from LAS because the surfactant material employed was a mixture of four types of homologue, each of which possesses between 4 and 6 different isomers, representing a total of 20 compounds of different structure. Furthermore, the various mode of catalytic oxidation of the alkyl chain have to be considered (Fig. 1).

Data presented in Fig. 3 show the transients production of LAS metabolites. SPCs were detected after 12 days of assay.

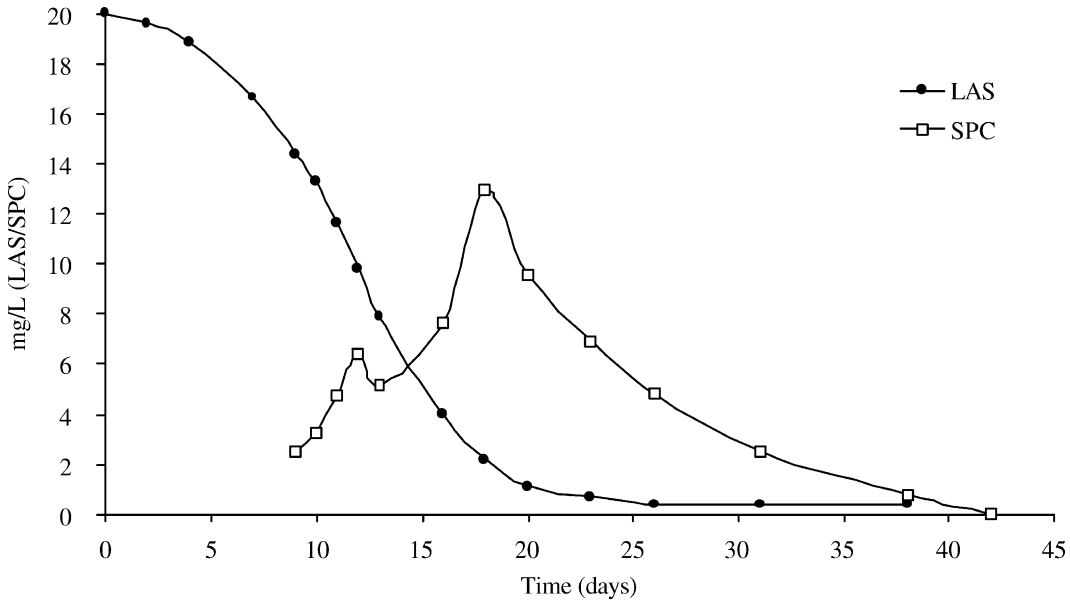

Fig. 4. Evolution of the concentrations of LAS and SPCs over the course of the period of biodegradation.

The most intense chromatographic signals being situated in the zone corresponding to SPCs of long chain length (C_9–C_{11} SPC).

At a later stage of the biodegradation (day 18), the signals corresponding to the SPCs of chain length between 5 and 9 atoms of carbon become predominant.

By day 26 of the assay, when the percentage of biodegradation of the LAS is about 98% completed, the dominant signals are seen to be those of sulphophenyl carboxylic acids with a chain length of between 2 and 5 atoms of carbon. Finally, after 42 days of assay there were no chromatographic signals of appreciable intensity. This would indicate that all the biointermediates, or at least the sulphophenyl carboxylic acids, have been degraded to values below the limits of detection of our analytical setting.

Fig. 4 shows the evolution of the total concentrations of LAS and SPCs. The drawing clearly shows that LAS are biodegraded and SPCs are transiently produced in the medium. The transient accumulation of SPCs is caused by the fact that the rate of primary biodegradation of the LAS is higher than the rate of mineralization of the SPCs. This rate differences can be explained by biochemical considerations. Only one reaction is required (the ω-oxidation of the terminal methyl group) to eliminate LAS from the medium, whereas the mineralization of the SPCs requires several steps, among which are successive β-oxidations as well as the cleavage of the aromatic ring. The maximum concentration of SPCs (13 mg/l) was reached after 18 days of assay, and then the concentration of intermediates began to fall as less LAS were available for biodegradation.

The kinetic of biodegradation of LAS and its metabolites has been modelled based on data presented in Fig. 4. First-order models were well fitted to de data for the SPCs and the logistic for LAS. The kinetic parameters are given in Table 6. where

Table 6
Kinetic parameters obtained from modeling the experimental data of the concentrations of SPCs (from day 18) and of LAS

	SPCs
First-order model (Simkins and Alexander, 1984)	$S = S_0 e^{-K_1 t}$ (2)
K_1(/d)	0.131
r^2	0.995
$t_{1/2}$ (d)	9.607
	LAS
Logistic model (Simkins and Alexander, 1984)	$S = \frac{(S_0 + B_0)}{1 + \left(\frac{B_0}{S_0}\right) e^{[K_{lg}(S_0+B_0)t]}}$ (3)
B_0(mg/l)	0.421
K_{Lg} (mg/l/d)	0.016
r^2	0.999
t_L (d)	5.63
$t_{1/2}$ (d)	6.22

$$K_1 = \mu_{max}\left(\frac{B_0}{K_s}\right) \quad \text{(day/s)},$$

$$K_{Lg} = \frac{\mu_{max}}{K_S}\ \text{(mg/l day/s)},$$

K_s is the concentration of substrate at which the specific growth rate of microorganisms is equal to half of the maximum rate ($\mu_{max}/2$), μ_{max} the maximum specific growth rate (day/s), B_0 the quantity of substrate necessary to produce the initial concentration of microorganisms (mg/l), t the time (days), S_0 the initial concentration of substrate (mg/l of LAS or SPCs), S the concentration of substrate at a time t (mg/l of LAS or SPC).

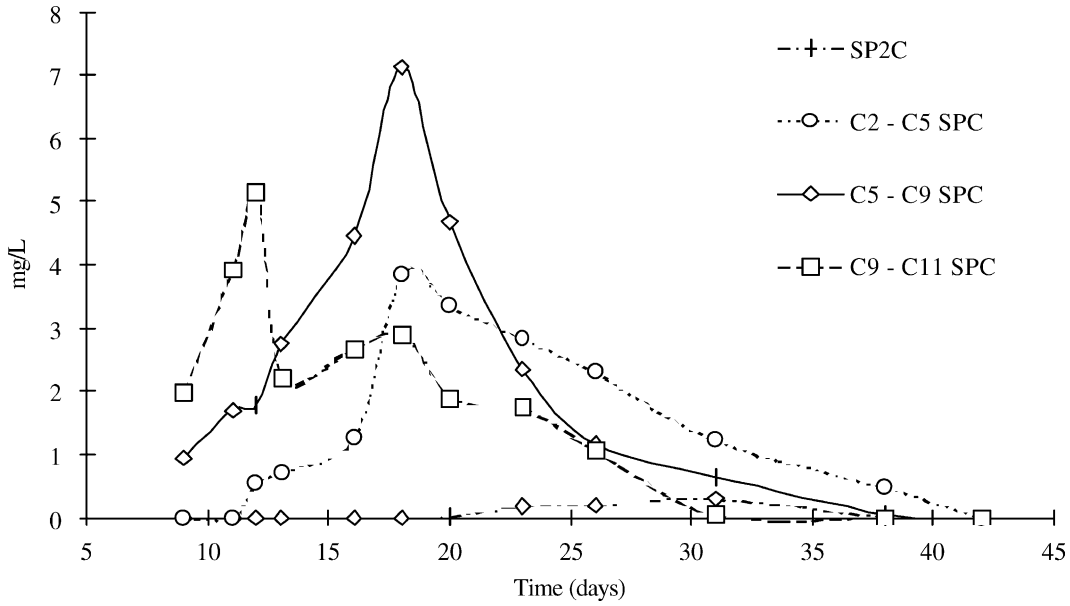

Fig. 5. Evolution of the different groups of sulphophenyl carboxylic acids formed during the course of the process of biodegradation.

By comparing the half-life of the SPCs with that of LAS it appears that SPCs are more persistent than the LAS. This however is not completely justified, because the interpretation of the kinetic parameters of the surfactant and of its intermediates must be subjected to the following reservations:

- In the first place, we are comparing two different types of biodegradation. In the case of LAS, we are considering a single-step biotransformation, whereas the SPCs, are mineralized through a sequence of reactions.
- Secondly, in the process of LAS biodegradation, there is no further input of substrate beyond the amount introduced at the start of the assay, whereas in the case of the SPCs, the rate being evaluated is that which results from the difference between the rate of mineralization of the intermediates and the rate of provision or input of SPCs to the medium (from the primary biodegradation of the LAS).

Nevertheless, the rate of mineralization of SPCs is comparable to the rate of mineralization of aniline ($t_{1/2}$ = 1–10 days) or sodium benzoate ($t_{1/2}$ = 1–7 days). These two compounds are considered as easily biodegradable standard that are often used as reference compounds in biodegradation assays in seawater (Nyholm and Kristiansen, 1987). Thus, SPCs are highly mineralizable by the microbiota present in the seawater utilized in the assays.

Fig. 5 shows the evolution of the concentration of the different SPCs presents in the medium as a function of their alkyl chain length.

Data confirmed that the first intermediates to appear were those of longest alkyl chain, of between 9 and 11 carbon atoms. Next, the SPCs of alkyl chain length between 5 and 9 carbon atoms began to appear after ca. 13 days and reached a maximum around 20 days. Then the SPCs of medium length gave way to the intermediates of shorter chain length (2–5 carbon atoms), which remained in the medium until the complete mineralization of all the biointermediates. It can also be seen that the sulphophenyl dicarboxylic acids do not represent an especially important form of intermediate. The observation that the SPCs with chain lengths of between 5 and 9 carbon atoms are those that reach the highest concentrations in the assay medium, is in agreement with the results reported by other authors (Cavalli et al., 1996) utilizing other assay media.

4. Conclusions

SPCs present net rates of mineralization in seawater that are comparables to those of the compounds utilized as references in biodegradation assays in seawater (aniline, sodium benzoate). Thus, they can be considered highly susceptible to mineralization by the microbiota present in the seawater utilized in the assays.

The results obtained 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 SPCs of long chain. Subsequently, a successive shortening of the alkyl chain takes place, by means of β-oxidations, giving rise to the formation of short chain SPCs.

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