

# Molecular structure and biodegradation kinetics of linear alkylbenzene sulphonates in sea water

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**Abstract** The present paper describes the results of the application of the biodegradation test proposed by the United States Environmental Protection Agency (USEPA) “Biodegradability in sea water” Office of Prevention, Pesticides, and Toxic Substances (OPPTS) 835.3160, to Linear Alkylbenzene Sulphonate (LAS), the synthetic surfactant with the highest consumption volume on a world-wide basis. High performance liquid chromatography (HPLC) has been employed for the separation and quantification of the different homologues and isomers of the surfactant. Water from the Bay of Cádiz (South–West of the Iberian peninsula) has been used as test medium. The results indicate how both lag and  $t_{50}$  time shows a significant linear relationship with the length of the alkyl chain of the homologue; the effect of this is that the homologues of longer chain length not only begin to degrade first but also degrade at a faster rate. Regarding the isomeric composition, it is observed that as the percentage of biodegradation increases, there is an increase in the proportion of internal isomers, in comparison

with the isomeric relationships of the original test substance

**Keywords** Biodegradation · Surfactant · Linear alkylbenzenesulfonate · Molecular structure · Kinetic · Isomeric composition · Bay of Cádiz

## Introduction

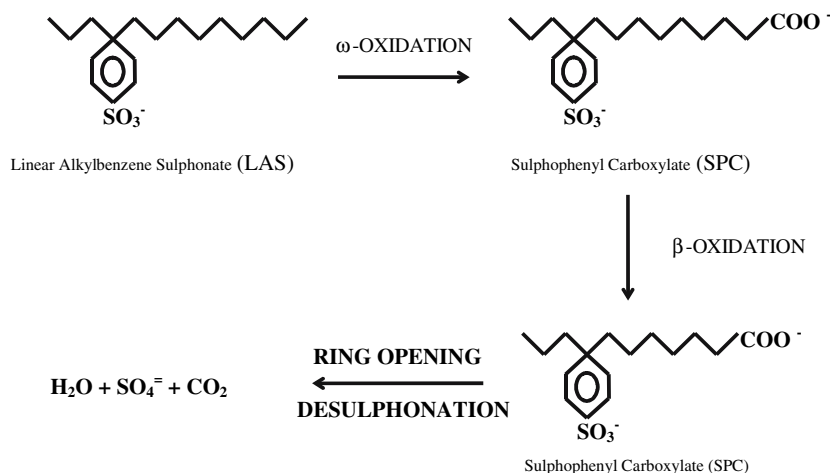
Although, on a world scale, soap is still used more than surfactants, the wide variety of processes in which surfactants are incorporated has resulted in a spectacular increase in their consumption (Granados 1996). Linear alkylbenzene sulphonates (LAS) constitute the quantitatively most important group of xenobiotic surfactants used today, and the use of these compounds will likely increase in the years to come (Cavalli et al. 1999). LAS are mainly used in laundry detergents and cleaning agents. Because of the widespread and high volume use of commercial LAS, significant quantity of it reaches the environment.

Linear alkylbenzene sulphonate consist of an alkyl chain attached to a benzene ring in the para position to the sulfonate group (Fig. 1). Linear alkylbenzene sulphonate are manufactured as a mixture of homologues in which the length of the alkyl chain varies between 10 and 14 carbon atoms ( $C_{10}$ -LAS– $C_{14}$ -LAS). The proportions of these five homologues in the various commercial

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**Fig. 1** LAS  
Biodegradation pathway



formulations depend on the specific application of the detergent product.

Each homologue presents a set of isomers in which the sulphophenyl group may be attached to different C atoms from C<sub>2</sub> to C<sub>7</sub> but not to C<sub>1</sub> of the alkyl chain. The number and proportion of these isomers is a factor that depends on the conditions of the synthesis of the surfactant (Valtorta et al. 1989).

Some authors (Swisher 1963; Wickbold 1964) have found that the isomers of LAS more degradable are those in which the terminal methyl group is positioned furthest from the sulphophenyl group. The explanation for this phenomenon, known as the “distance principle”, is based on the steric effect that the aromatic group exerts over the methyl terminal end of the alkyl chain where the process of biodegradation commences (Roberts 1991; Schoberl 1989) (Fig. 1).

Several authors (Perales et al. 1999; Roberts 1991; Bayona et al. 1986; Pecenic et al. 1984) have subsequently confirmed this effect. Furthermore, LAS monitored in natural waters (Terzic et al. 1992; Nishigaki 2004) have been shown to present a predominance of short-alkylchain homologues. Similar effects on the distribution of homologues and isomers of LAS in natural waters are found to be the consequence of physico-chemical processes of partition between sediments or sludges and the aqueous phase (Amano and Fukushima 1992, 1993; Garcia 2002).

This finding, taken together with the fact that some authors have found deviations in the actual

biodegradation of LAS from that predicted by the distance principle (Divo and Cardini 1980; Larson 1990), leads one to consider that this principle may be dependent in some way on the conditions of the test medium or on the variables of laboratory tests.

In a previous study (Perales et al. 1999), the authors found a dependence of this principle on the biodegradation conditions in a test using river water. The aim of the present paper is to determine in a rigorous way whether any dependence exists between the molecular structure and the kinetic of biodegradation. A second objective is to determine if there are deviations in the distance principle when the biodegradation test medium is seawater, given that all the previous studies reported on this subject were carried out with continental waters or wastewaters.

## Materials and methods

All the biodegradation tests conducted in the present study follow the guideline OPPTS 835.3160 “Biodegradability in sea water” (USEPA 1998). This guideline covers two possible test methods, that of the closed flask and that of the shaken flask; the latter method has been employed in the present study. This method is a variant for sea water of the modified OECD test (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 Kristensen 1987). The results of this test should not be taken as indicators of easy biodegradability, but can be used specifically to obtain information on the biodegradability of chemicals in marine environments.

The following are the characteristics and properties of the surfactant tested:

Supplier: Fluka Chemie A.G.; Product: Dodecylbenzenesulphonic acid sodium salt; Product N°: 44200; Empirical formula:  $C_{18}H_{29}NaSO_3$ ; Molecular weight: 348,48 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,  $\approx$ 17% Sodium sulphate, <3% Water. The proportion of the different homologues in the mixture were determined by High performance liquid chromatography (HPLC), the results obtained being given in Table 1.

The biodegradation test was carried out at 20°C in 2.5 L reactors containing 1.5 L of filtered, pre-conditioned and nutrient enriched natural sea water. This sea water came from a point in the Bay of Cádiz (South West of the Iberian Peninsula). More information about the sampling point, water quality characteristics and the test procedure can be found in a previous work (Perales et al. 2006).

The experiment conducted consisted of a biodegradation test of LAS, in quintuplicate, and with an initial concentration of surfactant material close to 20 mg/L (tests T1, T2, T3, T4 and T5).

The determination of linear alkylbenzene sulphonates LAS was by means of HPLC in reverse phase, following the method proposed by Nakae et al. (1980) for homologues analysis and Nakae et al. (1981) for isomers analysis. The equipment was a chromatograph consisting of two pumps, one a Waters model-510 alternating double piston type and the other a Waters model-501 simple piston type. The injection unit was a manual type

model U6K, and the detection system was of the fluorescence type, Waters model-470.

Instead of utilizing the method for the data treatment proposed in the USEPA guideline (graphical calculation of the values of the lag time,  $t_L$ , and of the time starting from the end of the lag phase to reach 50% of biodegradation,  $t_{50}$ ), the experimental data obtained was fitted by means of the Quasi-Newton method of non-linear regression, to the kinetic models proposed by Simkins and Alexander (1984). Having selected the simpler of the models that were more closely fitted to the experimental results, the values of  $t_L$  and  $t_{50}$  were calculated, making use of the kinetic parameters obtained, in accordance with the definitions proposed in the guideline. The estimation conditions and procedure is described in a previous work (Perales et al. 2006).

## Results and discussion

### Influence of the length of the alkyl chain

Shown as Fig. 2 by way of example are four chromatograms of one of the five experiments conducted (T3) and corresponding to successive stages of the biodegradation process. It can be observed that, at the beginning of the test, the signals appear of the four homologues that constitute the mixture of the LAS utilized; after 12 days (Fig. 2B), and having reached a level of 51% biodegradation, it is observed that all the chromatographic signals show a reduced intensity. This reduction is particularly notable for the fourth signal corresponding to the homologue of longest chain length ( $C_{13}$ -LAS).

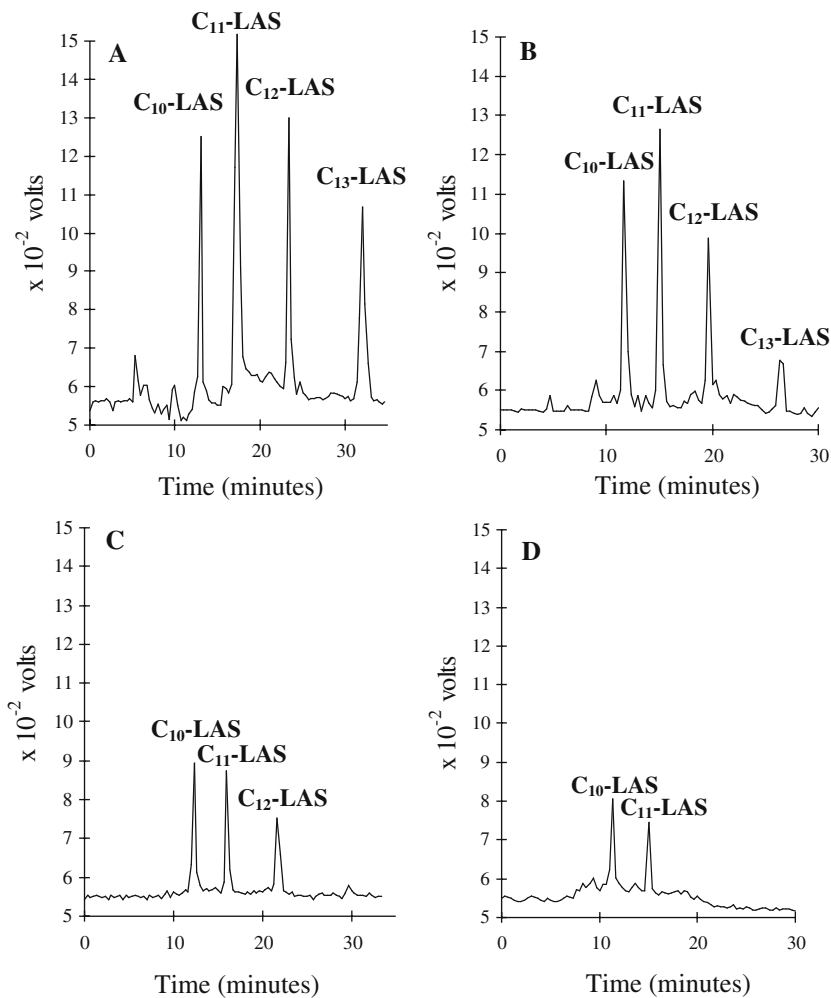
After 18 days, Fig. 2C, the signal corresponding to the homologue  $C_{12}$ -LAS, that at the beginning of the test showed a signal intensity

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

	$C_{10}$ -LAS <sup>a</sup>	$C_{11}$ -LAS <sup>b</sup>	$C_{12}$ -LAS <sup>c</sup>	$C_{13}$ -LAS <sup>d</sup>
MW (g/mol)	297	311	325	339
% MOL ( $n = 18$ , CI95%)	19.91 $\pm$ 0.56	31.19 $\pm$ 0.46	27.82 $\pm$ 0.33	21.08 $\pm$ 0.37
% WEIGHT ( $n = 18$ , CI95%)	18.59 $\pm$ 0.54	30.50 $\pm$ 0.45	28.43 $\pm$ 0.34	22.47 $\pm$ 0.39

<sup>a</sup>  $C_{10}$ -LAS: decylbenzenesulphonate; <sup>b</sup>  $C_{11}$ -LAS: undecylbenzenesulphonate; <sup>c</sup>  $C_{12}$ -LAS: dodecylbenzenesulphonate;

<sup>d</sup>  $C_{13}$ -LAS: tridecylbenzenesulphonate.



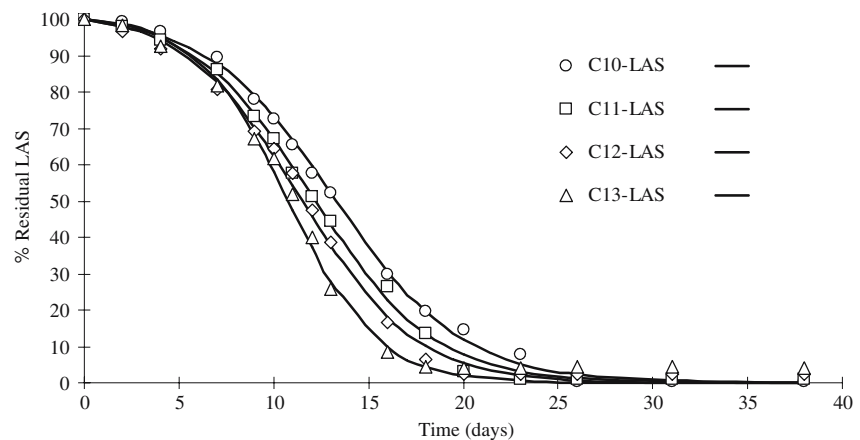
**Fig. 2** Chromatograms corresponding to the analysis of homologues of test T3 at day 0 (A), day 12 (B), day 18 (C) and day 20 (D)

similar to that of the homologue  $C_{10}$ -LAS, now shows a lower intensity. It is also observed that the signal corresponding to the homologue  $C_{13}$ -LAS has almost completely disappeared; the homologue  $C_{12}$ -LAS is now the minority component of the residual mixture. Lastly, in chromatogram D, corresponding to day 20, by which time a biodegradation percentage of 94.5% has been reached, it is observed that the signal corresponding to the homologue  $C_{12}$ -LAS has also disappeared, while the intensity of the homologue  $C_{11}$ -LAS, originally the highest of the four, has now fallen to below that of the homologue of shortest chain length ( $C_{10}$ -LAS).

The results obtained in Fig. 2 appear to indicate that a preferential biodegradation has taken place of the homologues of longer alkyl chain length, relative to those of shorter chain length. To confirm this conclusion, a study was conducted of the kinetic of biodegradation of the four homologues separately, in the different biodegradation tests conducted.

Figure 3 shows the percentages of remaining substrate concentration for the different homologues of LAS in one of the tests conducted (T3). The points represent the experimental values, while the continuous line represents the values obtained from the model.

**Fig. 3** Evolution of the percentage of remaining surfactant concentration for each of the four homologues of LAS, in the biodegradation test T3



It can be seen that the rate of biodegradation of all the homologues (given by the slope of the curves) seems to be ordered from slower to faster rate, following the length of the alkyl chain. In respect of the lag times, from Fig. 3 it is not possible to appreciate if any differences exist between the different homologues.

It has been found from the kinetic modeling of the biodegradation that the most appropriate model is the logistic (Simkins and Alexander 1984), the equation for which is given by the expression:

$$S = \frac{(S_0 + B_0)}{1 + \left(\frac{B_0}{S_0}\right) \cdot e^{[k \cdot (S_0 + B_0) \cdot t]}} \quad (1)$$

where

$B_0$  = concentration of substrate required to produce the initial population of biomass.

$S_0$  = initial concentration of surfactant

$S$  = concentration of surfactant at a moment of time  $t$

$K$  = kinetic constant

$T$  = time

To obtain an expression to calculate the value of the period of acclimatization ( $t_L$ ), it is sufficient to substitute in equation 1 the term  $S$  by  $0.9 \times S_0$ , whereas to determine  $t_{50}$  it is sufficient to subtract the lag time ( $t_L = t_{10}$ ) from the half life or time required to reach 50% of biodegradation ( $t_{1/2}$ ,  $S = 0.5 \times S_0$ ) (Perales et al. 2006).

These two expressions enable lag and  $t_{50}$  times to be calculated for the different homologues in the five biodegradation tests conducted. The values obtained are given in Table 2. Taking these values, two Students  $t$ -test by pairs were conducted, with five replicates, to check whether significant differences of lag and  $t_{50}$  times exist between the different homologues. The results of the tests are summarized in Tables 3 and 4.

The values underlined in Tables 3 and 4 are the  $t$  values that are lower than the critical value of  $t$  for a confidence level of 95%, i.e. those comparisons that present significant differences. In respect of the lag times, it can be observed in Table 3 that there are significant differences between the values calculated for the higher homologues ( $C_{12}$  and  $C_{13}$ ) and those obtained for the lower homologues ( $C_{10}$  and  $C_{11}$ ). However, significant differences were not found between the lag times of the homologues of 10 and 11 carbon atoms, nor between the homologues of 12 and 13 carbon atoms. In respect of the results of the  $t$ -test for the  $t_{50}$  times (Table 4), these indicate that significant differences do exist between the rates of biodegradation of the different homologues, with the exception of the  $t_{50}$  times obtained for the homologues of 11 and 12 carbon atoms.

Having confirmed that significant differences exist in respect of the lag and  $t_{50}$  times between the different homologues, or at least between those of longest and shortest chain length, the

**Table 2** Kinetic parameters (logistic model), lag and  $t_{50}$  times, and for the different homologues in the five LAS biodegradation tests conducted

	T1			T2			T3			T4			T5		
	C <sub>10</sub>	C <sub>11</sub>	C <sub>13</sub>	C <sub>10</sub>	C <sub>11</sub>	C <sub>13</sub>	C <sub>10</sub>	C <sub>11</sub>	C <sub>13</sub>	C <sub>10</sub>	C <sub>11</sub>	C <sub>13</sub>	C <sub>10</sub>	C <sub>11</sub>	C <sub>13</sub>
	$t_L$ (days)	7.7	7.4	6.5	7.5	6.6	5.6	6.3	5.7	5.7	6.3	6.6	5.9	6.9	6.9
$t_{50}$ (days)	6.7	6.3	5.7	7.4	7.0	6.9	6.9	6.2	5.0	8.2	7.2	7.1	7.0	6.5	6.2
$B_0$ (mg L <sup>-1</sup> )	0.044	0.062	0.083	0.064	0.115	0.145	0.083	0.127	0.048	0.131	0.124	0.139	0.127	0.083	0.099
$K$ (mg·L <sup>-1</sup> ·d <sup>-1</sup> )	0.076	0.055	0.061	0.068	0.047	0.054	0.072	0.096	0.056	0.056	0.046	0.052	0.068	0.052	0.059
$r^2$	0.994	0.996	0.996	0.993	0.999	0.997	0.994	0.991	0.995	0.996	0.994	0.996	0.997	0.996	0.997

**Table 3** Results of the Student  $t$ -test by pairs of lag time and the homologues alkyl chain length

$t_L$	C <sub>10</sub> -LAS	C <sub>11</sub> -LAS	C <sub>12</sub> -LAS	C <sub>13</sub> -LAS
C <sub>10</sub> -LAS	1	0.244	0.013	0.010
C <sub>11</sub> -LAS	0.244	1	0.002	0.017
C <sub>12</sub> -LAS	0.013	0.002	1	0.105
C <sub>13</sub> -LAS	0.010	0.017	0.105	1

**Table 4** Results of the Student  $t$ -test by pairs of  $t_{50}$  time and the homologues alkyl chain length

$t_{50}$	C <sub>10</sub> -LAS	C <sub>11</sub> -LAS	C <sub>12</sub> -LAS	C <sub>13</sub> -LAS
C <sub>10</sub> -LAS	1	0.004	0.008	0.003
C <sub>11</sub> -LAS	0.004	1	0.299	0.013
C <sub>12</sub> -LAS	0.008	0.299	1	0.005
C <sub>13</sub> -LAS	0.003	0.013	0.005	1

next step was to determine the direction of the effects. For this, two matrices of correlation between the mean values of the lag and  $t_{50}$  times of the five replicated tests and the length of the alkyl chain of the homologue were produced. The results of these correlation matrices are given in Table 5.

In the results of the correlation matrices given in Table 5, it can be seen that both time parameters present a significant linear relationship ( $p < 0.05$ ) with the length of the alkyl chain of LAS homologue, with the slope of the correlation curve being negative in both cases. This means that when the length of the alkyl chain is greater, the lag and  $t_{50}$  times are lower, i.e. that not only do the homologues of longer chain length begin to degrade sooner but also that they degrade at a faster rate.

The explanation for this selective degradation of the homologues of higher molecular weight is known as the “distance principle” (Swisher 1987). According to this principle, this selectivity is due to the effects of steric impediment by the

**Table 5** Correlation matrix between lag and  $t_{50}$  times, and the homologues alkyl chain length

	Mean	Standard Deviation	$r^2$	P	Slope	Intercept
Length	11.5	1.291				
$t_L$	6.2	0.696	0.976	0.012	-0.533	12.358
$t_{50}$	6.6	0.551	0.930	0.035	-0.412	11.347

aromatic group on the extreme methyl terminal of the alkyl chain where the  $\omega$ -oxidation, which is the start of the biodegradation process, takes place. This would explain the fact that in the homologues of greater chain length, the aromatic group presents less of a steric impediment, and therefore a greater susceptibility to  $\omega$ -oxidation (Fig. 1).

Considering therefore that the longer chain homologues are more easily biodegradable, it is to be expected that the short chain homologues will be more predominant in natural waters, that is the LAS that biodegrade more slowly. This is certainly found to occur, as demonstrated by the determinations of the distribution of the homologues of LAS in natural waters carried out by Kikuchi et al. (1986), Terzic (1990) and Gonzalez-Mazo et al. (1998). Similar effects on the distribution of LAS homologues in natural waters are obtained as a result of the processes of physico-chemical partition between the sediments and the dissolved phase (Rubio et al. 1996), although this could be attributed also to processes of biodegradation. It is therefore difficult to separate the respective contributions of the physico-chemical and biological mechanisms to the distribution of LAS homologues in the medium.

In contrast to the results obtained in this study, and to those of the authors previously cited, Larson et al. (1993) studied the rates of degradation of five LAS homologues in river water, and found little difference in the rates of mineralization of the different homologues, the  $t_{50}$  times of which were  $20\text{h} \pm 3\text{h}$ . The difference between the results obtained by Larson and those presented here could be in the different end-points of biodegradation measured (primary biodegradation/mineralization) and in the differences in concentration employed ( $\mu\text{g/l}$  as against  $\text{mg/l}$ ).

#### Influence of the position of the aromatic group

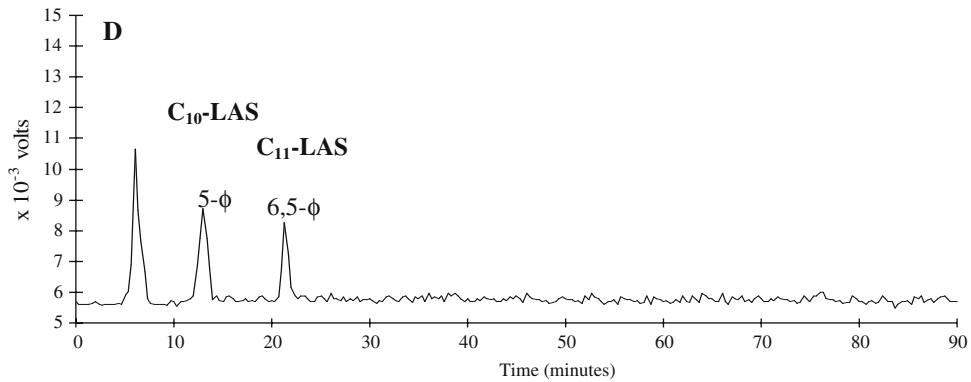
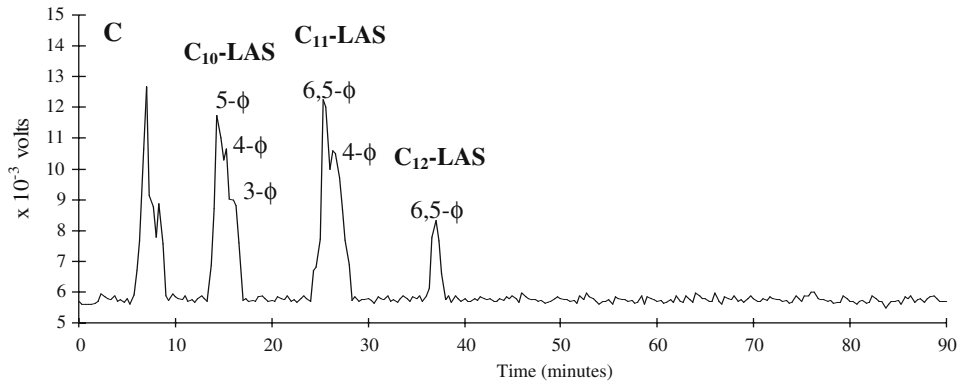
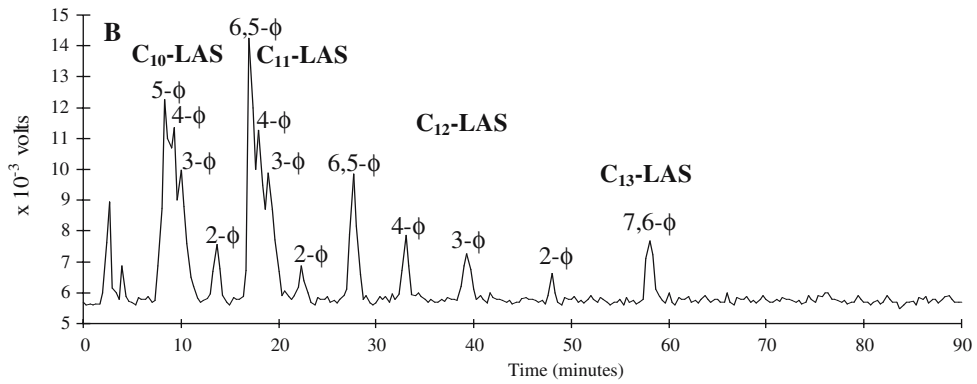
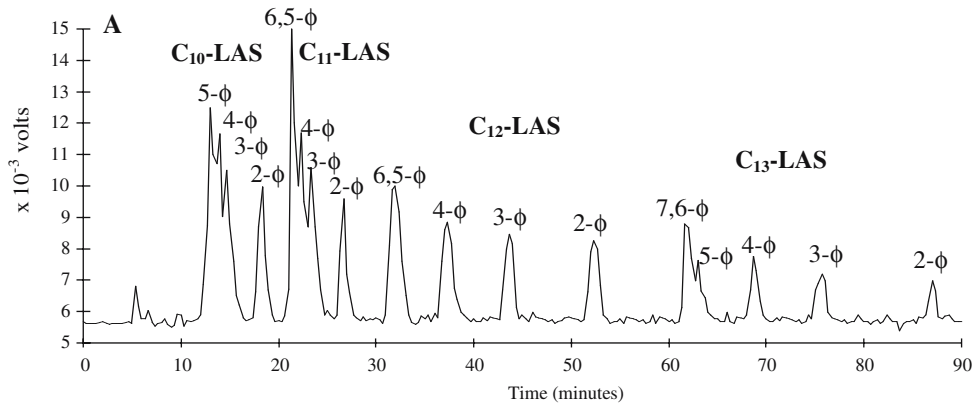
Figure 4 shows four chromatograms corresponding to different stages of the process of biodegradation of LAS in one of the five tests conducted (T3).

It can be observed in the chromatogram corresponding to the start of the test (A) that there are a total of 17 chromatographic signals corresponding to the different isomers that constitute the surfactant mixture utilized. These signals are found grouped together in four sets corresponding to the homologues  $C_{10}$ ,  $C_{11}$ ,  $C_{12}$  and  $C_{13}$ . Within the set for each homologue, the first signals correspond to the isomers with the aromatic group in intermediate positions in the alkyl chain, while the last signals correspond to the isomers with more terminal positions of the sulphophenyl group. Comparing this first chromatogram with the second, corresponding to a sample taken after 12 days, it can be seen that in all the homologues the signal corresponding to the most terminal isomer, 2- $\phi$ , shows a considerable reduction, this being more marked in the homologues of greater chain length. In the case of the homologue  $C_{13}$ , not only has the signal corresponding to this terminal isomer disappeared, but also the signals corresponding to isomers 3-, 4- and 5- $\phi$  have disappeared, too; these are isomers that show reduced intensity in the rest of the homologues but are still present after 12 days.

In the third chromatogram (C), corresponding to the 18th day, only the signals corresponding to the homologues  $C_{10}$ ,  $C_{11}$  and  $C_{12}$  appear, and there is no signal at all for the homologue  $C_{13}$ . It should be noted that the shorter the chain length of the homologue, the fewer the number of signals; thus, for  $C_{12}$  only the signals corresponding to the centrally-placed isomers (5- and 6- $\phi$ ) appear, while among the signals of  $C_{11}$  that corresponding to the isomer 4- $\phi$  is found. Lastly, in the case of the homologue  $C_{10}$ , the signals that appear also include the isomer 3- $\phi$ .

After 20 days of test have elapsed, and with a biodegradation percentage of 94.5%, we can see in the fourth chromatogram that the only signals to appear correspond to the internal isomers of the homologues of shorter chain length, specifically the isomers 5- $\phi$ - $C_{10}$ -LAS, and 5 and 6- $\phi$ - $C_{11}$ -LAS.

From this pattern of evolution of the isomers over the course of the test, it can be deduced that the isomers in terminal positions are most readily biodegraded, in contrast to those with the aromatic group placed in more central positions of the alkyl chain.





◀ **Fig. 4** Chromatograms corresponding to the analysis of isomers of LAS in the biodegradation test T3, after 0 (A), 12 (B), 18 (C) and 20 (D) days

These results are in agreement with those reported by various authors, (Pecenic et al. 1984; Yoshimura et al. 1984; Bayona et al. 1986; Yoshimura 1984; Takada and Ishiwatari 1990; Terzic et al. 1992), who show, as in our study, that in the process of LAS biodegradation a systematic isomeric alteration takes place that could be translated into a selective microbial degradation of the more external isomers.

In an analogous way to that, which occurs with the distribution of homologues in natural waters, Marcomini and Giger (1988) observed significant differences in the distributions of the isomers of LAS in water before and after treatment in an activated sludge unit, and found that at the outlet, the isomers with the aromatic group placed in central positions were predominant. Similar behavior has been reported by Trehy et al. (1990) in a trickling filter wastewater treatment plant. Those findings clearly support the result obtained in the present study regarding the greater biodegradability of the external isomers of LAS.

From the results obtained in the five tests conducted, Fig. 5 shows the evolution of the isomeric ratio  $(I/E)^*$  and the percentage of total LAS biodegradation reached in the tests, for the four homologues.

The  $(I/E)^*$  ratio is simply the proportion existing between the internal and external

isomers of each homologue. It has been calculated from the following expression:

$$\begin{aligned} \left(\frac{I}{E}\right) &= \frac{\sum \text{Isomers}_{\text{int}}}{\sum \text{Isomers}_{\text{ext}}} \\ &= \frac{\text{Area}(7\phi) + \text{Area}(6\phi) + \text{Area}(5\phi)}{\text{Area}(4\phi) + \text{Area}(3\phi) + \text{Area}(2\phi)} \end{aligned} \tag{2}$$

where:

Area ( $i\phi$ ) = area of the chromatographic signal corresponding to the isomer  $i\phi$

If the I/E ratio is normalized, the resulting expression is:

$$\left(\frac{I}{E}\right)^* = \left(\frac{I/E}{I/E_0}\right) \tag{3}$$

where:

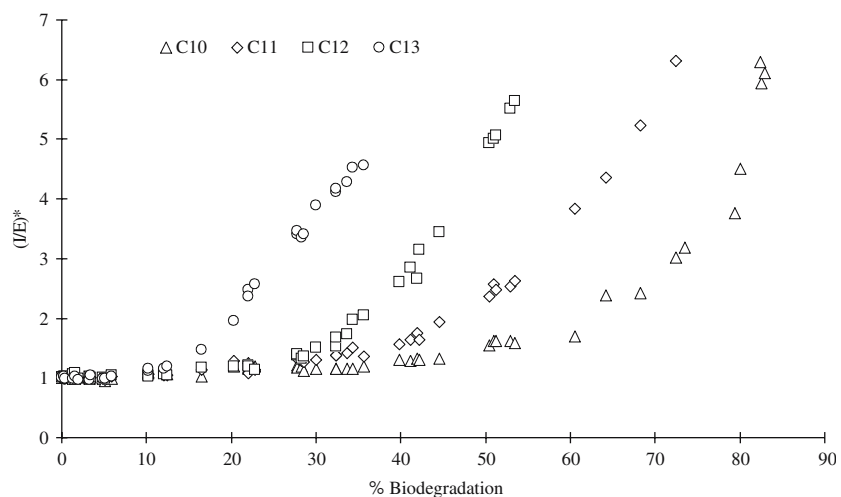
$\left(\frac{I}{E}\right)^*$  = normalized isomeric ratio

$\left(\frac{I}{E}\right)$  = isomeric ratio corresponding to day  $d$  of the test

$\left(\frac{I}{E}\right)_0$  = isomeric ratio corresponding to day 0 of the test

In Figure 5 can be seen that, as the percentage of biodegradation reached by the different

**Fig. 5** Representation of the isomeric ratio  $(I/E)^*$  and the percentage of biodegradation of LAS for the four homologues, in the five LAS biodegradation tests conducted



homologues in the tests increases, the value of the  $(I/E)^*$  ratio becomes higher, or in other words, there is an increase of the proportion of internal isomers with respect to the isomeric ratio of the original product.

It can also be seen that, in line with increased chain length of the homologues, the isomeric ratio is higher for the same percentage of biodegradation. For homologues of shorter chain length, the difference in respect of biodegradability between internal and external isomers is less than for higher homologues. On the basis of the distance principle already mentioned (Swisher 1987), this behavior means that the differences in respect of the steric impediment are less between internal and external isomers of lower homologues, such that the longer the chain length of the homologue, the sharper are these differences; as a result, the biodegradation is more selective towards external isomers, i.e. that with low percentages of biodegradation, there are high isomeric ratios.

The  $(I/E)^*$  diagrams can be used to estimate the degree of degradation of LAS in environmental samples, on the assumption that the results obtained in laboratory experiments are valid for natural media. This is a reasonable assumption since the medium employed in the experiments (sea water) contains the same microbiota as can be found in this environmental compartment. As a result, the biological processes that take place in the reactors are probably very similar to those taking place in the natural medium.

Of course, other variables such as the temperature of the water, the redox potential, the initial concentrations of LAS, etc., may affect the capacity of the microbiota to accomplish biodegradation. Consequently, the rate of LAS degradation in the natural medium may differ from that observed in the laboratory experiments, but the differences in the microbial activity that could be produced as a consequence of the alteration of variables such as the temperature or the initial concentration of LAS do not affect the relative ratio of biodegradation of the different isomers, as has been demonstrated in biodegradation tests of LAS in river water (Perales et al. 1999).

If we compare the  $(I/E)^*$  values obtained in river water biodegradation tests (Perales et al.

1999) with those observed in this study, they are very similar. For example, in river water, for  $C_{11}$ -LAS,  $(I/E)^*$  values of 2, 4 and 5 are reached when the biodegradation percentage was approximately 40, 65 and 70% respectively. In sea water these percentages were approximately 45, 60 and 67% respectively. Same similarities can be also observed for  $C_{12}$  and  $C_{10}$  homologues in sea and river water. Therefore, the  $(I/E)^*$  diagrams obtained from laboratory experiments can be reliably extrapolated to the natural medium, making them useful as indicators of the degree of degradation of LAS in this aquatic medium, considering also the kinetic of other processes that could alter the isomeric distribution, as adsorption on sediments.

## Conclusions

The following conclusions can be drawn from the results presented in this work:

1. In the biodegradation process of a mixture of LAS homologues in sea water, those with longer alkyl chain not only begin to degrade sooner (lower  $t_L$  values) but also degrade at a faster rate (lower  $t_{50}$  values).
2. The  $(I/E)^*$  diagrams shows that as the biodegradation process progress the initial isomeric composition of the homologues of LAS evolves towards internal isomers,. This means that the isomers with the sulphophenyl group located at terminal positions of the alkyl chain, biodegrades at higher rates.
3. As happens in river water (Perales et al. 1999), this selective degradation in sea water of the homologues of higher molecular weight and terminal isomers confirm the “distance principle” (Swisher 1987). According to this principle, this selectivity is due to the effects of steric impediment by the aromatic group on the extreme methyl terminal of the alkyl chain where the  $\omega$ -oxidation, which is the start of the biodegradation process.
4. These changes towards short alkyl chain homologues and inner isomers during LAS biodegradation can have implications on its environmental risk assessment. One of the

steps of an environmental risk assessment is the calculation of the predicted non effect concentration (PNEC), and traditionally this is made by means of toxicity tests of the chemical, but What about the changes that happens during its transformation in the environment? In future works combined toxicity–biodegradation tests with LAS in sea water will be done in order to study the evolution of the toxicity in the biodegradation test medium and quantify the differences in the effects between the initial surfactant and that partially biodegraded.

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