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Biodegradation of Linear Alkylbenzene Sulfonates and Their Degradation Intermediates in Seawater

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A study has been made of the aerobic biodegradation of linear alkylbenzene sulfonates (C_{12} and C_{11} homologues) and sulfophenylcarboxylic acids (C_5 and C_{11} homologues) in seawater at concentrations of the same order as those detected ones in coastal waters influenced by wastewater effluents, at different temperatures, and both with and without the addition of an inoculum adapted to the presence of linear alkylbenzene sulfonate (LAS). The biodegradation of C_{12}LAS, C_{11}LAS, C_5SPC, and C_{11}SPC exceeds 99% in all tests performed and can be satisfactorily fitted to a second-degree polynomial without an independent term. The kinetic of degradation of LAS presents a clear seasonal component, since the process is considerably inhibited at lower temperatures; it is also kinetically enhanced by the presence of the inoculum. The intermediates detected for all the cases are sulfophenylcarboxylic acids (SPCs), the most abundant being those intermediates produced by the ω- and β-oxidations of the parent compound, although intermediates produced by the α-oxidation have also been detected. The kinetic of the SPCs generated can be described using a model composed of two terms that represent the formation and the degradation of these intermediates. The total disappearance of the SPCs in all cases indicates that the degradation of LAS in seawater at the tested concentrations in aerobic conditions is complete.

Introduction

Large quantities of surfactants reach the marine medium through discharges, both direct and indirect, via rivers, of treated and untreated urban and industrial wastewaters. Apart from the soaps, linear alkylbenzene sulfonate (LAS) is the surfactant most widely used on a global scale (*1*). Its presence in the marine medium is a known fact (*2*, *3*). However, LAS levels in the marine environment are low as a consequence of their high rate of removal in sewage treatment plants (*4*), in sewers (*4*), and in the environment (*5*).

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Nevertheless, although the aerobic biodegradation of LAS in seawater is evidently relevant, studies of this process are scarce (e.g., refs *6*−*13*); those conducted are limited to monitoring its primary biodegradation (i.e., the disappearance of the surfactant) or to its mineralization in estuaries (*14*). The primary degradation and mineralization kinetics for LAS under aerobic conditions have previously been characterized in continental (*5*, *15*), estuarine (*14*, *16*), and marine waters (*8*−*11*). Some of the mathematical equations that have been proposed to describe the kinetics of the process are based on curve fitting of the experimental substrate consumption or CO_2 production during biodegradation (*16*). The simplest approach for LAS biodegradation, used by the majority of authors, is a curve fitting of the decrease of the LAS concentration using a first-order equation (*11*, *16*−*18*). The extrapolation to the natural medium of the kinetic constants obtained from this approach is questionable, principally because of the alteration that takes place in the bacterial populations while assays are being performed (*19*). For this reason, to model the degradation of LAS, other authors have applied second- or third-order kinetics for the mineralization of LAS in soils (*20*) and the logistic model (*13*) or kinetics of variable order in assays performed with seawater (*10*). In particular, Sales Marquez et al. (*10*) have proposed a surfactant degradation kinetic model expressed by the following equation:

$$-\frac{\partial C}{\partial t} = K_2 C^2 + K_1 C + K_0 \qquad (1)$$

where K_2 is the coefficient of C^2 in the second-degree polynomial, K_1 is the coefficient of C in the second-degree polynomial, K_0 is an independent term of the second-degree polynomial that represents the non-biodegradable substrate concentration in the medium (*19*), and C is the concentration of surfactant at the instant t.

The studies conducted to date show that the kinetic of aerobic biodegradation of LAS depends on the concentration of the initial substratum (*21*), on the presence and density of microorganisms adapted to LAS (*22*), on the availability of nutrients (*9*), on the homologue/isomer LAS composition (*21*), on the temperature (*5*, *10*−*12*, *14*, *15*), and on the bioavailability of the compound (*23*). In the marine medium, values have been obtained for LAS half-life that range between 6 and 9 d (*8*, *10*, *13*), similar to those obtained for estuarine systems and higher than those detected in continental waters due to the more limited activity of marine microorganisms (*5*). Assays performed with natural seawater present considerable variability in the half-life found for LAS (3.4−13.8 d) as a consequence of the spatial and temporal variability of the microbial populations (*9*).

To date, the biodegradation route of LAS in seawater is still unknown, but it should be analogous to that found in continental systems (*15*, *24*) given that sulfophenylcarboxylic acids have been identified in the marine medium (*2*, *25*). The presence of SPC has been demonstrated both in laboratory assays with continental waters (*26*−*32*) and in the environment (*2*, *25*, *33*, *34*), although unfortunately the quantification of those homologues for which standards are not available has been performed by assuming a response analogous to that of LAS or to that of one of its homologues (*2*, *33*, *35*) or by utilizing mass spectrometry (*2*, *28*, *32*, *36*).

However, to date, there have been no studies dealing with the aerobic biodegradation route of LAS and of their biodegradation intermediates in the marine medium, nor have any kinetic models been proposed that explain the

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TABLE 1. Percentage by Weight of Homologues and Isomers of the Various Standards of C_{11}LAS and C_{12}LAS

	active index (%)	distribution of homologues (%)					distribution of isomers (%)		
		C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	2ϕ	3ϕ	4−5ϕ
C_{11}LAS I[a]	47.0		94.9	5.1					
3ϕC_{11}LAS	15.05		98.7	1.3			10.8	73.8	14.1
C_{12}LAS I	5.0		6.3	92.1	1.6		39.7	38.7	13.7
C_{12}LAS II[a]	42.0		1.3	97.5	0.4				
2ϕC_{12}LAS	33.0		0.1	98.9	0.4		86.8	12.1	0.6

[a] Unknown isomeric composition.

processes of formation and degradation of sulfophenylcarboxylic acids (SPCs). For these reasons, a study has been conducted of the aerobic biodegradation of LAS (C_{11} and C_{12}) and SPC (C_{11} and C_5) in seawater, applying a specific method for the simultaneous analysis of all the homologues of LAS and all monocarboxylic SPC (*37*). We have evaluated the effects on the biodegradation kinetic on the presence of inoculum, temperature, and the nature of the substrate subjected to degradation. The objectives of our work can be specified as follows: (i) to determine the biodegradation of C_{12}LAS, C_{11}LAS, C_{11}SPC, and C_5SPC at concentrations of the same order as those detected in coastal waters; (ii) to characterize LAS and SPC degradation routes in seawater, (iii) to model the experimental degradation results of the majority LAS homologues (C_{11}LAS and C_{12}LAS) and SPC homologues (C_{11}SPC and C_5SPC) and to develop a kinetic model for the formation−degradation of the SPCs generated from LAS; and (iv) to determine the influence of the presence of microorganisms adapted to LAS, the temperature, and the composition of LAS on this biodegradation kinetic.

Materials and Methods

Reagents. Undecylbenzene sulfonate (C_{11}LAS) and dodecylbenzene sulfonate (C_{12}LAS) were supplied by Petroquímica Española S.A. (PETRESA), and their compositions are shown in Table 1. The content in DATS and ramified LAS of these mixtures is less than 1%. Our research group has collected a complete set of monocarboxylic SPC standards (C_3−C_{13} SPC), with the exception of C_7SPC (some have been donated, and the rest have been synthesized at the University of Cádiz). When the phenylcarboxylic acids were available, they were synthesized by sulfonation (*38*). The others were synthesized by a five-stage procedure: a Wittig reaction (*39*); conversion to methyl esters, which were subsequently reduced and hydrolyzed to obtain the corresponding carboxylic acids; and finally these were sulfonated (*38*). The structure of the compounds was confirmed by ^{1}H and ^{13}C NMR.

The methanol was of chromatography quality, purchased from Scharlau (Barcelona, Spain), and water was Milli-Q quality. Tetraethylammonium hydrogen sulfate (TEAHS) was purchased from Sigma-Aldrich (Steinheim, Germany). Sodium chloride was purchased from Scharlau (Barcelona, Spain), and potassium dihydrogen phosphate was from Panreac (Barcelona, Spain).

Procedure. Natural marine water spiked with 1 mg/L LAS/SPC and inoculum was utilized for the assays, which were begun immediately after sampling the seawater (<1 h). The seawater was taken from the Atlantic Ocean, in an area of low contamination (*40*), and the inoculum was taken from the interior of Sancti Petri Sound (Cadiz Bay, SW Spain), in a zone close to the discharge point of untreated urban wastewaters from the town of San Fernando (Cádiz). The principal properties of the seawater and of the inoculum are given in Table 2. The concentrations of LAS and SPC in the inoculum are high, but this was diluted with seawater by a factor of 250 for the experiment and represents less than 3% of the LAS added at the beginning of the assay.

TABLE 2. Characteristics of Seawater and of Inoculum Utilized in the Various Assays Performed

	assay C	assay D/E	assay F
Water			
salinity	35.1	36.0	37.0
pH (SWS)	7.95	8.21	8.12
alkalinity (mM)	2.35	3.08	2.82
PO_4^{3-} (μM)	0.37	0.89	1.03
NH_4^+ (μM)	0.88	2.29	3.01
NO_3^- (μM)	0.26	0.64	0.74
NO_2^- (μM)	0.08	0.20	0.21
SiO_2 (μM)	1.22	4.80	4.50
LAS (μg L^{-1})[a]	35	55	30
SPC (μg L^{-1})[a]	5	<1	<1
Inoculum			
LAS (μg mL^{-1})[a]	5.23	9.65	3.12
SPC (μg L^{-1})[a]	150	57	102

[a] Addition of the concentrations of all detected homologues.

The assays were performed in glass bottles of 3 L capacity that were kept in a thermostatically controlled chamber with saturation of O_2 in darkness and with continuous agitation by means of a magnetic stirrer. The aliquot of the corresponding homologue and either the inoculum (10 mL) or the formaldehyde (100 mL in the controls) were added to each reactor, which was then filled with seawater to a final volume of 2.5 L. The sampling (50 mL) was conducted every 30 min, and its frequency was reduced in line with the time elapsed in the assay, the sampling sequence being matched to the monitoring performed by direct injections into the HPLC. The samples of water were fixed with formaldehyde (2%) and were kept at 4 °C until their analysis.

The assays were performed in duplicate and with an abiotic control, prepared in the same way but with formaldehyde (2%) added, which enabled the characterization of the reduction of the concentration of LAS as a result of the physicochemical processes (adsorption, ...) exclusive of the biodegradation itself. The principal characteristics of each series of assays, designated by a capital letter C−F, are given in Table 3. Separate assays were performed for each compound. The effect of the presence of adapted microorganisms on the kinetic of degradation has been evaluated in assay C (Table 3). The effect of temperature, and thus of the seasonality, has been studied at two extreme temperatures for the seawater of littoral systems situated at latitudes similar to that of the Bay of Cádiz. One experiment was conducted at 25 ± 0.2 °C (test D), the upper temperature limit of the summer period, and the other was conducted at 10 ± 0.2 °C (test E), representing the winter lower limit. Last, an evaluation has been made of the influence of the composition in terms of the isomers and homologues of LAS, considering different isomeric mixtures and distributions of homologues C_{11}LAS or C_{12}LAS (tests C, D, and F).

Analysis of LAS and SPC. All the samples (50 mL) were treated following the procedure proposed by León et al. (*37*) for the simultaneous determination of LAS and SPC. This method consists of a solid-phase extraction (SPE) over a Bond Elut C18 minicolumn (Varian) and then over a SAX (Supelco) and its subsequent analysis by HPLC 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_7SPC),

TABLE 3. Principal Characteristics of the Assays Performed

	C[a]	D	E	F
compound	C_{12}LAS I C_{11}LAS I	C_{12}LAS II 3ϕC_{11}LAS	C_{12}LAS II 3ϕC_{11}LAS	2ϕC_{12}LAS 3ϕC_{11}LAS 11ϕC_{11}SPC 5ϕC_5SPC
LAS (μg L^{-1})[b]	56	94	94	42
SPC (μg L^{-1})[b]	6	<1	<1	<1
T (°C)	25	25	10	25

[a] Assays performed both with and without inoculum. The rest of the assays were all performed with inoculum. [b] Concentration provided by the seawater and the inoculum at the start of the assay (not included in the nominal concentration).

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. (*2*).

Development of the Model. The evolution of the concentration of LAS with time for each assay (Table 1) has been fitted to a second-degree polynomial model, proposed previously by Sales Marquez et al. (*10*) for the degradation of LAS in seawater.

Fit of the Primary Degradation of LAS to the Second-Degree Polynomial Model. The primary biodegradation involves the disappearance of LAS as a molecule and its transformation into various degradation intermediates. In our assays performed in the marine medium, the biodegradation is complete. Therefore in this case, according to the interpretation proposed by Quiroga et al. (*19*), $K_0 = 0$; consequently, the expression (eq 1) proposed by Sales Marquez et al. (*10*) can be simplified to

$$\frac{\partial[\mathrm{LAS}]}{\partial t} = K_2[\mathrm{LAS}]^2 + K_1[\mathrm{LAS}] \quad (2)$$

the integrated expression of which is

$$[\mathrm{LAS}](t) = \frac{K_1}{\left(K_2 + \frac{K_1}{B}\right)e^{K_1 t} - K_2} \quad (3)$$

where K_1, K_2, and B are parameters of fit, specifically K_1 and K_2 are the coefficients of the first- and second-order term, respectively, and B is the initial concentration of LAS. From the interpretation proposed by Quiroga et al. (*19*) for the solutions of the second-degree polynomial model, we can deduce that, for this particular case ($K_0 = 0$), K_1 is a term for the velocity or rate at which the process takes place, this parameter representing the rate of substrate degradation by the microorganisms, and $-K_1/K_2$ is a quantity term representing the maximum quantity of substrate available in the medium to form biomass and includes the term relating to the initial concentration of microorganisms.

The experimental data have been fitted utilizing the SigmaPlot 4.0 program, applying a maximum of 300 iterations and 10^{-4} as convergence criterion. In all cases, the concentration is expressed in micrograms per liter and time is given in hours. The initial parameters utilized in the fits were $K_1 = 0.01$, $K_2 = 0.01$, and $B = 1000$ μg L^{-1}.

The LAS and SPC primary degradation data have also been fitted to the first-order kinetic model to permit the comparison of our results with those of previous studies, since this latter model has commonly been applied in the past.

Fit of the SPC Formation and Degradation. In the experiments conducted, the nondetection of SPCs of more than nine carbon atoms has made it impossible to carry out a differentiated study for each of the individual reactions that sequentially shorten the alkylic chain of LAS; therefore, it was decided to characterize the variation of the total concentration of SPC over time (the combined process that has been termed formation−degradation).

The total concentration of sulfophenylcarboxylic acids present in the medium at any particular instant is the result of their formation from the primary biodegradation of the LAS and of their own subsequent degradation, as indicated by the following expression:

$$[\mathrm{SPC}] = [\mathrm{SPC}]_{\mathrm{formation}} - [\mathrm{SPC}]_{\mathrm{degradation}} \quad (4)$$

The variation of the total concentration of SPCs formed can be obtained from the expression of the degradation of the LAS (eq 2), using a coefficient of proportionality ($A \leq 1$) that must be introduced because part of the SPCs generated cannot be detected in the analysis. The expression corresponding to the degradation of the SPCs is assumed to be analogous to that of LAS (eq 2), where K_3 and K_4 for SPCs have meanings analogous to those of K_1 and K_2 for LAS. Therefore the variation of the total concentration of SPCs with respect to time will be given by

$$\left(\frac{d[\mathrm{SPC}]}{dt}\right)_{\mathrm{total}} = A(K_2[\mathrm{LAS}]^2 + K_1[\mathrm{LAS}]) - K_4[\mathrm{SPC}]^2 - K_3[\mathrm{SPC}] \quad (5)$$

where the concentration is expressed in micrograms per liter and the time is expressed in hours.

The analytical solution of this expression is very complex, and for this reason, the kinetic parameters corresponding to the degradation of the SPCs have been determined by means of a numerical approach. The specific method utilized is an iterative numerical fit by finite differences, with the data obtained for the concentration of SPC taken as starting point. The Microsoft Excel calculation program has been used to seek the best fit (the maximum correlation coefficient, r^2), starting with the values of K_1 and K_2 (first- and second-order constants) obtained for LAS and utilizing A, K_3, and K_4 as parameters of fit that enable the degradation of the SPC to be characterized. Thus for each instant $i + 1$, the concentration of SPC considering the finite differences approach would be

$$\mathrm{SPC}_{i+1} = \mathrm{SPC}_i + \left(\frac{\Delta \mathrm{SPC}}{\Delta t}\right)_i \Delta t \qquad t_{i+1} = t_i + \Delta t \quad (6)$$

in such a way that the concentration of SPC at each instant of time is equal to that of the preceding instant plus the increase calculated from the general expression proposed for these compounds (eq 5). In all the cases, the fit has been made for a time increment of 0.15 h, which is a short enough period to obtain a good fit.

Results and Discussion

Primary Biodegradation of the LAS. Figure 1 shows, by way of example, the evolution of the concentration of 2ϕC_{12}LAS and 3ϕC_{11}LAS during the biodegradation assays and in the corresponding abiotic controls. During the first 100 h of assay,

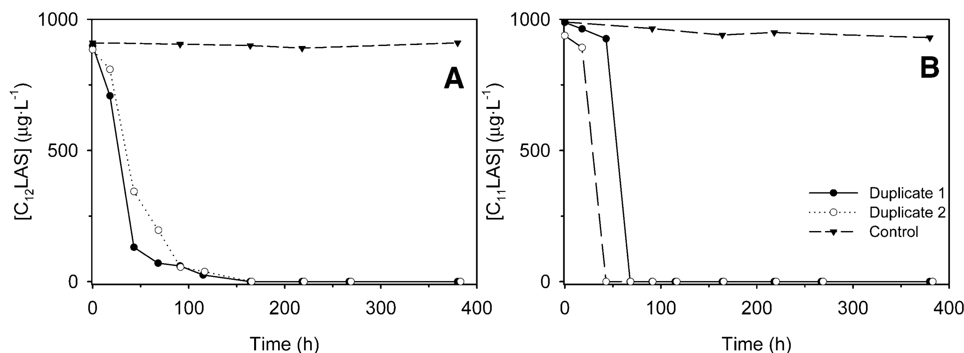

FIGURE 1. Disappearance of $2\phi C_{12}LAS$ (A) and $3\phi C_{11}LAS$ (B) under aerobic conditions for both duplicates and control assays (2% formaldehyde).

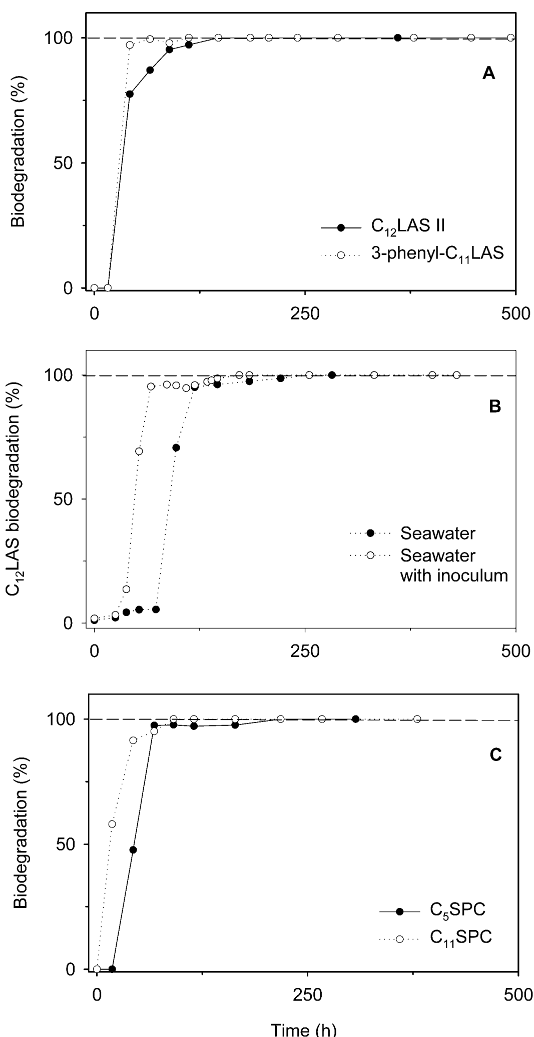

FIGURE 2. Percentage of primary biodegradation at 25 °C for (A) $3\phi C_{11}LAS$ and $C_{12}LAS$ II; (B) $C_{12}LAS$ I, with and without inoculum; and (C) C_5SPC and $C_{11}SPC$.

the LAS is fully eliminated, with the exception of those controls in which its concentration remains constant (a decrease of less than 10% of the initial concentration). From these results, it can be concluded that the process responsible for the removal of the LAS from the medium is the biodegradation and that the decrease observed in the controls is due to the process of adsorption onto the walls of the receptacle.

The primary biodegradation of $C_{11}LAS$ and $C_{12}LAS$ in seawater is more than 99% complete (Figure 2 A,B), as indicated by the very low values of residual LAS below the level of detection (0.4 μg L^{-1}) obtained at the end of the assays. This percentage of biodegradation is higher than that detected previously by other authors (*8*, *10*) and demonstrates the capacity of the marine microorganisms to degrade LAS. The extent of the degradation in seawater observed for the homologues of LAS separately did not present significant variations with the length of the alkyl chain (Figure 2A), as has previously been described for freshwater degradation (*16*, *41*). The extent of the primary biodegradation of LAS is also independent of the addition of inoculum. However the addition of inoculum reduces the phase of latency and kinetically stimulates the process of degradation (Figure 2B) as a consequence of the introduction to the medium of microorganisms adapted to LAS. In fact during the first 48 h, the number of viable microorganisms is 30−40% higher for the test with inoculum as compared to that without (data not shown). Regarding the extent of the primary biodegradation of $C_{11}LAS$ and $C_{12}LAS$, it has been confirmed that this is not affected by the temperature (Figure 4), since even at 10 °C more than 99% of the LAS present in the medium is degraded. These results apparently differ from those reported previously by other authors (*10*), who did not detect a significant primary biodegradation of LAS at 5−10 °C after 21 d of assay, probably as a consequence of the high initial concentrations (20 ppm) utilized, which could have inhibited or reduced the microbial activity and/or the short duration of the test at low temperatures. The biodegradation of C_{11}-SPC and C_5SPC (Figure 2C) is more than 99%, and as in the preceding cases, they are also very fast processes. Until now, SPC degradation has not been characterized adequately; consequently, there are no previous data with which to compare our results.

TABLE 4. Degradation Intermediates Detected from Different LAS and SPC Homologues

homologue	major intermediates	minor intermediates
$C_{11}LAS$	C_5SPC, C_6SPC, C_7SPC	C_9SPC
$C_{12}LAS$	C_6SPC, C_4SPC	C_7SPC, C_8SPC
$3\phi C_{11}LAS$	C_5SPC	C_6SPC, C_7SPC
$2\phi C_{12}LAS$	C_4SPC, C_6SPC	
$C_{11}SPC$	C_5SPC, C_7SPC, C_3SPC	C_4SPC, C_6SPC
$5\phi C_5SPC$	C_3SPC	

Transformation of $C_{11}LAS$ and $C_{12}LAS$ and Their Degradation Intermediates ($C_{11}SPC$ and C_5SPC). The intermediates detected are SPCs whose chain length varies in function of the originating homologue and of its purity (Table 4). In general, the most abundant SPCs generated from $C_{12}LAS$ (major intermediates) have an even carboxylic chain length (C_4SPC and C_6SPC) whereas those from $C_{11}LAS$ have an odd chain length (C_5SPC and C_7SPC), suggesting that probably the ω- and β-oxidation are the major mechanism for the transformation of the alkyl chain of LAS, as has been reported previously in assays with freshwater (*15*, *24*, *29*−*30*, *42*−*44*). Nevertheless, intermediates of even chain length have been

detected from an odd chain length homologue and vice versa. When a mass balance is considered, it is shown that at least a 10% of the chain shortening is performed by α-oxidation, generating C_6SPC and C_7SPC from C_{11}LAS and C_{12}LAS, respectively. The identification of these intermediates was confirmed by HPLC/MS [following the procedure proposed by González-Mazo et al. (*2*)]. This result coincides with that previously reported by some authors for continental waters (*15*, *24*) but differs from that of other authors who do not consider this oxidative route (*32*). From C_{11}SPC, the SPCs of odd chain length (C_7, C_5, and C_3) were also obtained as the most abundant metabolites (major intermediates); C_6 and C_4 were obtained as minority metabolites (minor intermediates); and C_3SPC was obtained from C_5SPC (Table 4).

In none of the assays were any intermediates of longer chain length (C > 9) detected, which indicates that the first oxidations are very rapid processes. However, in the marine environment these intermediates have been detected close to untreated wastewater effluent points (*2*, *25*) or in sediments with low oxygen concentration (*45*).

The order of appearance and the concentration of the degradation intermediates also depend on the purity of the originating homologue as well as on its isomeric composition. In Figure 3 are shown the RP-HPLC analyses of samples during the experiment with C_{12}LAS I. In the first 53 h, the concentration of C_{12}LAS has noticeably diminished, and the intermediates detected are 5ϕC_6SPC and 3ϕC_4SPC, which must be generated by the ω- and β-oxidations of the most external isomer 2ϕC_{12}LAS (40%); therefore, the first oxidations must be very rapid processes (*30*). Also observed is the intermediate 4ϕC_6SPC that will have originated from the corresponding ω- and β-oxidations of the 3ϕC_{12}LAS, the percentage of which is 38.7%. In turn there appear faint signals of the C_8SPC probably originating from the previous external isomers of 4ϕC_{12}LAS, the kinetic of which is slower (*15*). The intermediates of odd chain length, C_7SPC and C_5SPC, must originate from the C_{11}LAS (6.3%) that forms part of the homologue C_{12}LAS or from the α-oxidation of any of the intermediates from the C_{12}LAS. At 109 h of assay, the degradation continues with some 5% of the C_{12}LAS remaining, and there are increases in all of the signals; even the homologue C_9SPC appears, possibly originating from the more internal isomers of C_{11}LAS. At this time, in other experiments conducted with the C_{12}LAS, a peak corresponding to C_7SPC is also detected, and the intensity of this peak can only be explained by an α-oxidation. After 134 h when the C_{12}LAS had totally disappeared, the remaining intermediates were 4ϕC_6SPC and C_7SPC, the oxidation of which would not be stimulated due to steric impediment (*15*) and probably the rupture of the ring takes place, as confirmed by the finding that at 332 h these SPCs have completely disappeared. For the case of the 2ϕC_{12}LAS, the only intermediates to appear are the homologues of 6 and 4 atoms of carbon. A large part of the 5ϕC_6SPC passes to 3ϕC_4SPC, but a small portion remains as a result of its slow degradation, in consequence of the higher steric impediment presented by this isomer. Finally this intermediate has completely disappeared after 9 d of assay.

In all the experiments conducted in this study, the result observed has been the total disappearance of all the SPCs. In continental waters, this process takes place via the rupture of the aromatic ring (*46*). On this point, it can be concluded that the degradation of LAS in seawater, in aerobic conditions, and at concentrations similar to those detected in marine environments influenced by wastewater effluents is complete. Other possible metabolites such as sulfophenyldicarboxylic acids (SPDC) or other nonsulfonated compounds have not been detected by HPLC/MS. In fact, Perales et al. (*13*) detected very low SPDC concentrations from commercial LAS in the marine environment and concluded that these intermediates constitute a minority intermediate form. However some of these, specifically C_5SPDC and C_6SPDC, were detected by Di Corcia et al. (*28*) in concentrations of less than 10% of the initial mass of the parent compounds. The authors suggested that the main origin of these compounds was the degradation of the most internal isomers of LAS, which in the present study exist in very low proportions. Neither were DATS nor their intermediates of degradation detected since their proportions in the original LAS were less than 0.5%.

LAS and SPC Biodegradation Kinetics. The variation of the concentration of LAS over time for the complete series of assays performed cannot be satisfactorily explained by a first-order kinetic model. However the high values of the correlation coefficient (above 0.95 for all cases) obtained by fitting the experimental data to the integrated expression of a second-degree polynomial without an independent term (eq 3) indicate that the theoretical values predicted are close to the experimental values. The sigmoid form obtained from the proposed velocity equation allows three phases for the degradation of LAS in seawater to be clearly defined (Figure 4A): an initial phase of adaptation of the bacterial flora present in the medium during which the concentration of the surfactant does not vary appreciably with time, next a sharp and rapid decrease in the concentration of the substratum, and then a phase where the degradation is slower until the compound finally disappears. The same behavior has been detected by other authors (*9*, *10*) and in other environmental matrixes (*47*). The degradation of the SPCs (C_{11}SPC shown as an example in Figure 4B) displays a behavior analogous to that of LAS, and the second-degree polynomial model also satisfactorily fits the evolution of the concentration of SPC.

In respect of the behavior of the sulfophenylcarboxylic acids, the correlation coefficients obtained from the application of the proposed model (eq 5) to the experimental values of total concentration of SPC from the biodegradation of LAS or SPC are close to unity (Figure 4). In general, during the initial hours of assay, a slight increase is observed in the concentration of SPC; later, as a consequence of the rapid degradation of LAS, the maximum concentration of SPCs is reached; and finally, a gradual decrease of its concentration is detected until it is eliminated. These results constitute the first kinetic characterization of the biodegradation of the sulfophenylcarboxylic acids generated during the degradation of LAS.

Effect of the Addition of Inoculum on the Biodegradation Kinetics. Table 5 (test C) gives the kinetic parameters obtained for LAS and SPC biodegradation with or without the presence of inoculum by applying the proposed model. As can be seen, the velocity of degradation (K_1) of C_{12}LAS is significantly greater when an inoculum adapted to the presence of LAS is added. The values of K_2 have a negative sign, and this can be interpreted as the inhibiting effect of the substratum on the bacterial flora, a conclusion reached by other authors (*10*). The term K_2 is therefore a correction factor that reflects the lag phase in the activity of the microorganisms while they adapt to the substratum to be degraded. However, its very low value indicates a low inhibition effect at the concentrations employed.

With the object of comparing our data to those obtained previously by other authors, the half-life values have been calculated considering a first-order kinetic. The results for both the experiments conducted without inoculum (1.9 d) and those conducted in the presence of the inoculum (1.4 d) are appreciably lower than those obtained previously by other authors (between 6 and 9 d) in natural seawater (*8*, *13*). In the experiments conducted with inoculum, the velocities of degradation reached are similar to those detected for waters of continental origin (*5*, *47*, *48*), which usually present a greater capacity for degradation than marine waters. It has

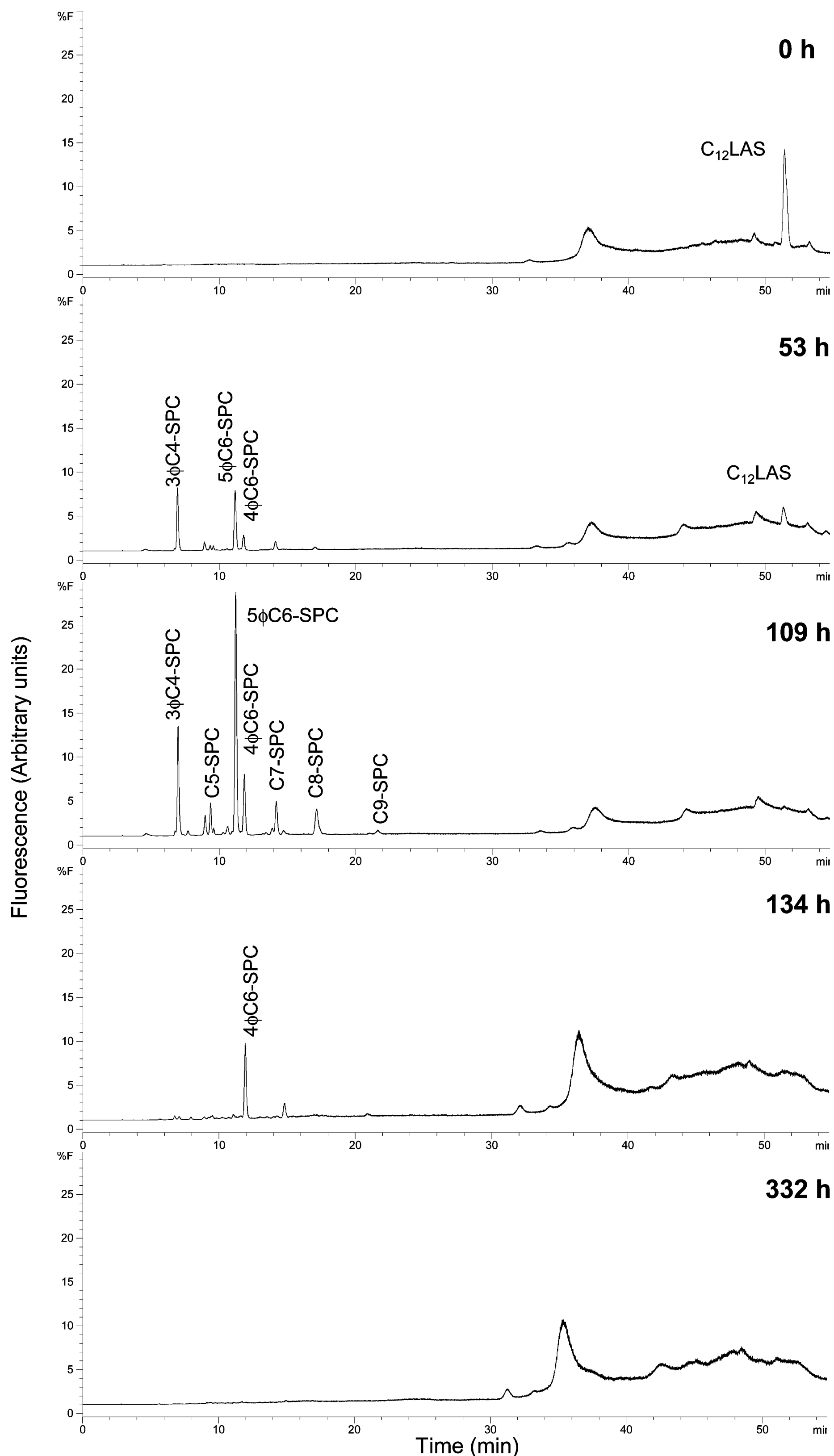

FIGURE 3. Reversed-phase HPLC chromatograms during C_{12}LAS I biodegradation experiments, indicating the peaks of the biodegradation intermediates.

been estimated that the presence of natural inoculum reduces the lag phase by approximately 12 h, and in some cases, this acclimatization phase has not even been detected so therefore it must be less than 6 h (the time difference between taking the first and second samples).

The kinetic parameters obtained by applying the proposed model for the formation–degradation of SPCs (Table 5) demonstrate the influence of the presence of inoculum on the velocity of the process. The values of K_3 are higher for the process that takes place in the presence of inoculum. In

TABLE 5. Kinetic Parameters of the Second-Degree Polynomial Model Obtained for Several Mixtures of $C_{12}LAS$ and $C_{11}LAS$ Together with Those Corresponding to the Proposed Model for the Total Concentration of SPCs Generated in Each Case

				LAS primary degradation				SPC degradation			
homologue	test	T (°C)	inoc[a]	K_1	K_2	B (μg/L)	r^2	K_3	K_4	A	r^2
$C_{12}LAS$ I	C	25	no	0.065	-8.2×10^{-5}	810	0.959	0.002	0.5×10^{-5}	0.92	0.850
$C_{12}LAS$ I	C	25	yes	0.115	-14.1×10^{-5}	824	0.987	0.005	1.0×10^{-5}	1.00	0.948
$C_{11}LAS$ I	C	25	yes	0.119	-15.2×10^{-5}	839	0.949	0.003	5.8×10^{-5}	0.74	0.803
$3\phi C_{11}LAS$	D	25	yes	0.220	-23.1×10^{-5}	953	0.996	0.002	4.9×10^{-5}	0.70	0.867
$C_{12}LAS$ II	D	25	yes	0.096	-9.3×10^{-5}	1028	0.993	0.001	4.0×10^{-5}	0.85	0.927
$3\phi C_{11}LAS$	E	10	yes	0.006	-0.6×10^{-5}	967	0.971	$1.0 \cdot 10^{-5}$	1.3×10^{-5}	1.00	0.759
$C_{12}LAS$ II	E	10	yes	0.055	-6.3×10^{-5}	864	0.993	$5.5 \cdot 10^{-5}$	1.0×10^{-7}	0.70	0.772
$3\phi C_{11}LAS$	F	25	yes	0.101	-10.2×10^{-5}	989	0.962	0.009	2.0×10^{-5}	1.00	0.748
$2\phi C_{12}LAS$	F	25	yes	0.104	-11.5×10^{-5}	905	0.979	0.009	2.5×10^{-5}	0.75	0.836

[a] Inoc, inoculum addition.

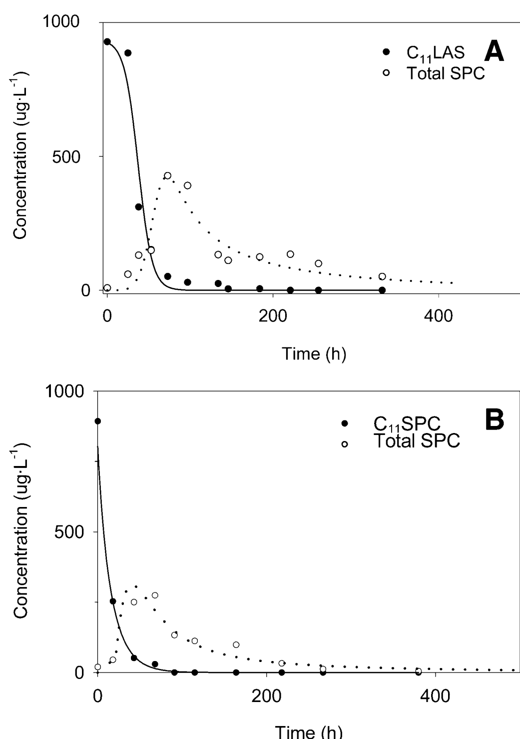

FIGURE 4. Fits with the second-degree polynomial model (A) for an assay performed with $C_{11}LAS$ and inoculum at 25 °C (test C) and (B) for another performed with $C_{11}SPC$ under the same conditions (test F). The evolution of the total concentration of SPC generated and the fit with the proposed model are also shown.

these cases the maximum concentration of SPC is detected when the concentration of LAS is low, and from this moment, the concentration of SPC decreases rapidly and sharply until it disappears. However for the assays without inoculum, although initially the behavior is similar, the formation of the SPCs is a slower process in consequence of the longer lag phase for LAS and the deceleration of the degradation of the SPCs themselves, especially those of lower molecular weight. In both cases, the second-order constant (K_4) is positive, from which it is concluded that this term also contributes to the degradation and is not an inhibiting term as occurs for the case of LAS.

Effect of Temperature on the Biodegradation Kinetics. Table 5 (tests D and E) gives the kinetic parameters obtained for LAS and the biodegradation intermediates generated at two different temperatures, 25 and 10 °C. The kinetics of the process are clearly influenced by the temperature, and this is the variable that has the greatest effect on the velocity of

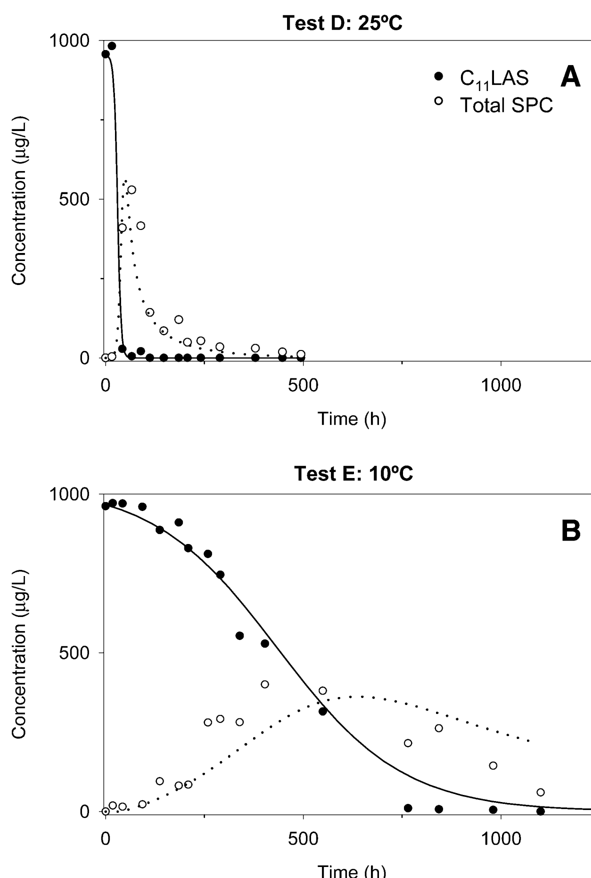

FIGURE 5. Variation of the concentration of $3\phi C_{11}LAS$ and of the total concentration of the sulfophenylcarboxylic acids generated at 25 °C (A; test D) and at 10 °C (B; test E), the line showing the fits obtained with the second-degree polynomial model (LAS) and the model proposed for SPCs in each case.

the process as has been shown in field observations (*49*). The values of the constant of velocity K_1 are appreciably lower in the experiments conducted at the lower temperature, which confirms the results obtained previously in the marine medium by other authors (*10*, *19*) and by Terzic et al. (*11*, *17*) in estuarine systems. In the experiments conducted at 25 °C (Figure 5A), the concentration of LAS falls rapidly and dramatically in the initial hours; however, in the experiments conducted at the lower temperature (Figure 5B), the decrease is slow and gradual. These results agree with those obtained by Terzic et al. (*11*) in estuarine waters where a lag phase of 2 d was detected at 14 °C, and in contrast, this phase was not detected at 23 °C. In respect of the values of K_2, these are very

TABLE 6. Kinetic Parameters of the Second-Degree Polynomial Model Obtained for C_{11}Las, C_{11}SPC, and C_5SPC at 25 °C Together with Those Corresponding to the Proposed Model for the Total Concentration of SPCs Generated in Each Case

homologue	LAS or SPC primary degradation				SPC degradation			
	K_1	K_2	B (μg/L)	r^2	K_3	K_4	A	r^2
3ϕC_{11}LAS	0.101	-10.2×10^{-5}	989	0.962	0.009	2.0×10^{-5}	1.00	0.748
C_{11}SPC	0.203	-21.0×10^{-5}	966	0.999	0.009	0.9×10^{-5}	1.00	0.694
5ϕC_5SPC	0.132	-14.5×10^{-5}	911	0.993	0.002	18.0×10^{-5}	0.60	0.967

low, as in the previous cases, and do not present a clear variation with the temperature.

Again in order to compare our results with those obtained previously by other authors, the mean half-life values of LAS have been calculated according to the first-order model. The mean half-life times at 10 °C are 3.2 and 6.7 d for 3ϕC_{11}LAS and C_{12}LAS, respectively; these fall within the wide range (between 3.4 and 13.8 d) obtained by Vives-Rego et al. (*9*) in assays performed with natural seawater. However, the mean half-life times for 3ϕC_{11}LAS and C_{12}LAS at 25 °C are 0.3 and 1.4 d, respectively; these values are lower than any detected to date in the marine medium and of the same order as those detected in wastewater treatment plants (*21*).

The degradation intermediates (SPCs) are extensively degraded at both 10 and 25 °C (degradation in excess of 97% in all the cases), which confirms that temperature does not affect the extent of the degradation of SPCs either. However, as in the case of LAS, temperature does affect the velocity of the process; the total degradation is completed in 12.5 d at 25 °C and in 42 d at 10 °C (Figure 5). The value of the kinetic constant K_3 is higher for the case of the experiments conducted at 25 °C (Table 5). Although the values of K_4 continue to be very low, they do show a slight decrease with temperature.

The results obtained confirm that the biodegradation of LAS and its degradation intermediates must present a clear seasonal component in littoral systems, the degradation being more efficient in summer than in winter as other authors have previously reported for this surfactant (*10*–*12*, *49*). In any case, the biodegradation is complete at the tested concentrations in the temperature range studied (10–25 °C).

Effect of Homologue and Isomeric Composition on the Biodegradation Kinetics. The kinetic parameters obtained for different isomeric mixtures of C_{11}LAS and C_{12}LAS at 25 °C and with inoculum do not show significant differences (Table 5, tests C and F). Therefore, the length of the alkylic chain does not affect the velocity of degradation as shown by the similarity of the values of K_1. These results match those obtained by Larson et al. (*5*). However, other authors have detected an increase in the velocity of degradation with the length of the alkylic chain during the treatment of wastewaters (*21*) or a preferential biodegradation of those compounds of longer alkylic chain when various homologues are present simultaneously in the medium (*11*, *15*). In our case, when the assays are performed separately for each homologue, the individual kinetics of degradation for each case have been determined without effects of competition or of preferential degradation being observed.

In respect of the purity of the isomer, it has also been confirmed that there are no significant differences between the velocity of degradation (Table 5) of the homologues with a high percentage by weight of an external isomer (74% of 3ϕC_{11}LAS and 87% of 2ϕC_{12}LAS) and that of other isomeric mixtures (mainly isomers 2ϕ and 3ϕ). However, significant differences in the velocity of the process are observed for the SPCs that are generated. The SPCs generated from 3ϕC_{11}LAS and from 2ϕC_{12}LAS are biodegraded completely after 300 h of assay, and the biodegradation is more rapid as indicated by the values of K_3 than for the SPCs generated from other isomeric mixtures (Table 5). The reason for this is that the SPCs generated from the external isomers present a lower steric impediment to the degradation (on the distance principle) and therefore can be degraded more easily than those generated from the internal isomers (*15*).

Degradation of C_{11}LAS Intermediates, C_{11}SPC and C_5SPC. Table 6 gives the kinetic parameters obtained for 3ϕC_{11}LAS and two of its probable degradation intermediates, C_{11}SPC and C_5SPC, by applying the proposed model. The kinetic constant K_1 is lower for C_{11}LAS (0.10 h^{-1}) than for C_{11}SPC (0.20 h^{-1}), which is an indication that the β-oxidation is a more rapid process than the ω-oxidation; this latter oxidation process constitutes one of the limiting steps in the degradation of LAS as explained previously by other authors (*48*). In fact, the value of K_1 for 5ϕC_5SPC is also higher than that for C_{11}LAS due to it being an external isomer of the SPC susceptible of undergoing a β-oxidation (by which process C_3SPC is formed). The rapid degradation observed for C_{11}-SPC indicates that the degradations of long-chain SPCs are very rapid processes that cannot even be detected in discontinuous experiments as confirmed by our experimental results.

The degradation of sulfophenylpentanoic acid (5ϕC_5SPC) has required an acclimatization phase (Figure 2) that has not been detected for its homologue C_{11}SPC. Furthermore, its velocity of degradation is also less than that of C_{11}SPC since the shorter the length of the alkylic chain, the greater the steric impediment that it presents, taking into account the proximity of the benzenosulfonic group to the end of the carboxylic chain (*15*). In respect of the degradation of the intermediates generated, it can be seen that for C_{11}LAS and C_{11}SPC the kinetics of the process are similar ($K_3 \approx 0.01$ h^{-1} in both cases), whereas K_3 of C_3SPC generated from C_5SPC is 4.5 times lower. Therefore, the disappearance of C_3SPC is less favored since it has to be produced by the rupture of the aromatic ring, this being the limiting step in the mineralization of LAS (*24*).

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