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Biodegradation kinetics of LAS in river water

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Abstract

This article reports the biodegradation kinetics of linear alkylbenzene sulfonates (LAS) in river water. The authors used the 'river die-away' test method and high performance liquid chromatography to monitor LAS concentrations as functions of time in a series of tests systems. Controlled variables included initial LAS concentrations and incubation temperature. The kinetic parameters computed from the experimental data demonstrated strong correlations ($r^2 > 0.99$) with theoretical values computed from the kinetic model presented in this paper. The proposed model accurately predicts concentrations of non-biodegradable substrate and the maximum specific microbial growth rate.

Keywords: Surfactant; Biodegradation; LAS; Kinetic; River water

1. Introduction

One of the key factors for the evaluation of the environmental impact associated with the use of specific products is the prediction of environmental concentrations. The identification and characterization of the biodegradation processes and the kinetic equations that govern the process are pieces of information that are required to investigate the fate of a contaminant in a given ecosystem.

Linear alkylbenzene sulfonates (LAS) are one of the xenobiotic compounds found most frequently in urban waste waters. LAS world consumption is 1.5×10^6 tons p.a., making LAS the surfactant type with the highest level of consumption (Granados, 1996). In spite of the huge quantity of this product discharged into the natural environment, and although its primary biodegradability is an undisputed fact (Swisher, 1987; Painter and Zabel, 1987), few attempts were made to define a unique kinetic expressions that describe its biodegradation process under different conditions.

Yoshikuni et al. (1992) established zero order kinetic expressions to describe the biodegradation process of LAS, whereas other authors employed first order (Larson and Payne, 1981) and even pseudo-first order kinetic equations (Larson, 1983). In all cases, the authors find good fits between the experimental data and those predictable with the model proposed. However, the biodegradation tests were performed under different conditions; Yoshikuni et al. (1992) used high concentrations of LAS, others used much lower concentrations (Larson and Payne, 1981; Larson, 1983). Furthermore, Yoshikuni et al. (1992) dealt with the primary degradation process of LAS, whereas Larson and Payne (1981) and Larson (1983) investigated the LAS mineralization process.

The biodegradation process of a compound might comprise a prolonged period of acclimatization when change of concentration, type of substrate, temperature, pH, etc. occurs. These periods do not apply when the experiments use previously-acclimatized microbiota (Yoshikuni et al., 1992) or low surfactant concentrations (Larson and Payne, 1981; Larson, 1983).

However, when it occurs, the prolonged acclimatization period affects the graphical representations of the substrate concentration over the test time. The

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curve takes a definite 'S' shape, which does not fit kinetic expressions of zero, first or pseudo-first order. A general kinetic model applicable to situations in which the biodegradation process may or may not undergo prolonged phases of latency would therefore be very useful.

In the present study, the model proposed by Quiroga and Sales (1991) and by Romero et al. (1997) is applied to the experimental data obtained from a biodegradation test of LAS in river water under which was done to influence the length of the period of induction under different conditions of temperature and initial surfactant concentration, in which both long and short periods of induction are required.

2. Kinetic model

Based on Volterra's modifications of the Pearl and Verlhust's unstructured mathematical model of bacterial growth in batch reactors (Bayley and Ollis, 1980), and taking into account the approximation of Gaden (1959) for fermentation processes, Quiroga and Sales (1991) have proposed a surfactants degradation kinetic model expressed by the following equation:

$$-\frac{dS}{dt} = K_2 \cdot S^2 + K_1 \cdot S + K_0. \quad (1)$$

This is a second degree polynomial equation from the integration of which is obtained the following relationship between the surfactant concentration and the degradation time:

$$S = \frac{h \cdot (S_0 - q) - q \cdot (S_0 - h) \cdot e^{p.t}}{(S_0 - q) - (S_0 - h) \cdot e^{p.t}}, \quad (2)$$

in which:

$$p = |\sqrt{K_1^2 - 4 \cdot K_2 \cdot K_0}|,$$

$$q = \frac{(-K_1 + p)}{2 \cdot K_2},$$

$$h = \frac{(-K_1 - p)}{2 \cdot K_2},$$

K_2 is the coefficient of S^2 in the second degree polynomial,
K_1 is the coefficient of S in the second degree polynomial,
K_0 is an independent term of the second degree polynomial,
t is the degradation time,
S_0 is the initial concentration of the surfactant,
S is the concentration of the surfactant at the instant t.

Romero et al. (1997) liken the behavior of a batch degradation process to that of an autocatalytic reactor, represented by the following expression:

$$S + n \rightarrow \alpha \cdot P + \sigma \cdot n \Longrightarrow -V_s = -\frac{dS}{dt}$$

$$= K_v \cdot S \cdot n - \frac{dS}{dt} \quad (3)$$

$$= K_v \cdot \sigma \cdot \left[\left(\frac{n_0}{\sigma} + S_0 \right) \cdot S - S^2 \right],$$

where

P is the product concentration,
S is the substrate concentration at the instant t,
S_0 is the initial substrate concentration,
n is the microorganisms concentration at the instant t,
n_0 is the initial microorganisms concentration,
K_v is a global kinetic constant,
α and σ are stoichometric constants.

Similar to the empirical equation of Quiroga and Sales (1991), these equations corresponds to a second degree polynomial with respect to the substrate concentration present in the medium.

Thus based on Romero et al.'s hypothesis, it is possible to define the parameters p, q and h (Romero et al., 1997). Thus 'p', which is a term for velocity or the rate at which the process takes place, represents the substrate degradation rate by the microorganisms; 'h' is a quantity term representing the substrate maximum quantity available in the medium to form biomass and includes the term relating to the initial microorganisms concentration. The term 'q' is also a quantity term for the substrate, it represent the concentration of substrate that the microorganisms cannot metabolize.

3. Material and methods

The biodegradation tests were performed according to the 'river die-away' method (Swisher, 1987), which uses water sampled close from the river source (in this case, the River Guadalete, SW of the Iberian Peninsular).

Six individual tests were conducted, using glass flasks of 6 l total capacity, each filled with 4 l of previously decanted river water collected on the day of commencement of the tests. Various amounts of surfactant were then added, the flasks were stoppered with hydrophobic cotton and left in the dark at the corresponding temperatures.

The test conditions selected were: Test 1: 5 mg LAS/l, 21°C; Test 2: 10 mg LAS/l, 21°C; Test 3: 20 mg

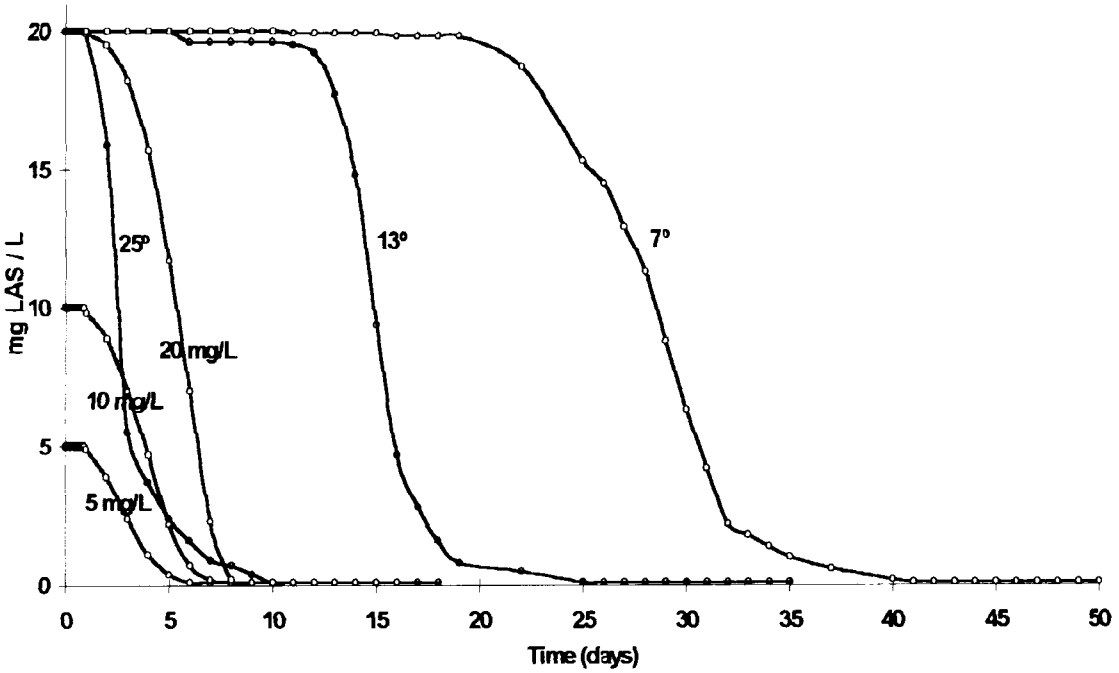

Fig. 1. Evolution of LAS concentration found in the six tests conducted.

LAS/l, 21°C; Test 4: 20 mg LAS/l, 7°C; Test 5: 20 mg LAS/l, 13°C; Test 6: 20 mg LAS/l, 25°C.

LAS used was a commercial formulation of homologues and isomers supplied by PETRESA, Spain, with the following molar distribution of homologues:

Sodium decadecylbenzene sulfonate (C_{10}–LAS): 18%;
Sodium undecylbenzene sulfonate (C_{11}–LAS): 51%;
Sodium dodecylbenzene sulfonate (C_{12}–LAS): 31%.
The average molecular weight was 337.8 g/mol.

The surfactant material was analysed by reversed phase High Performance Liquid Chromatography (RP–HPLC), according to the protocol of Nakae et al. (1981). The equipment consisted of a Waters 510 liquid chromatograph fitted with a Waters Model U6K injector and a Waters 470 fluorescence detector. A volume of 50 µl of sample was injected into a 250 × 45 mm Lichrospher 100 RP18 (5 µm) column. The material was eluted with a mixture of acetonitrile/water (45:55) 0.1 M sodium perchlorate at a flow rate of 1 ml/min. The excitation wavelength was set at 232 nm and the emitted light was measured at 290 nm.

HPLC quality acetonitrile and Milli-Q quality water were used.

The computational process used to determine the kinetic parameters was a nonlinear estimation (user-specified regresion), of the Quasi-Newton method. The estimation conditions were: convergence criterion, 10^{-3}; maximum number of iterations, 10^2; start values for all the parameters (p, q and h), 0.1 and initial step, 0.01. The software used was Statistica 4.0 (Statsoft, Inc. 1993).

4. Results and discussion

Fig. 1 shows the evolution of LAS concentration in the six tests conducted. Under all conditions of temperature and initial concentration tested, more than 90% of the surfactant degraded. As expected, the acclimatization period increased at higher initial concentration and lower temperature.

The various kinetic parameter values were obtained by fitting the experimental data of Fig. 1 into Eq. (2).

The correlation coefficient values (r^2), shown in Table 1, indicate how the theoretical values predicted

Table 1
Values of the various kinetic parameters obtained by fitting the experimental data to the authors' model. T^a: Temperature

T^a	21°C	21°C	21°C	7°C	13°C	25°C
$[LAS]_0$	5 mg/l	10 mg/l	20 mg/l	20 mg/l	20 mg/l	20 mg/l
p	1.30	1.09	1.07	0.39	0.77	1.84
q	0.08	0.05	−0.05	−0.04	0.04	0.54
h	5.22	10.26	20.07	20.09	20.09	20.47
r^2	0.999	0.999	0.999	0.998	0.997	0.995

from Eq. (2) are close to the experimental values. This correlation applies to the tests with prolonged latency periods, such as Test 4 (20 mg/l, 7°C) as well as those with short acclimatization phases, such as Test 1 (5 mg/l, 21°C).

The values obtained for the parameter '*h*' in the various tests are found to be very close to the initial concentration of LAS used. This parameter is defined by Eq. (4) (Romero et al., 1997):

$$h = S_0 + \frac{X_{V0}}{Y_{x/s}}, \tag{4}$$

where:

S_0 represents the initial surfactant concentration,
X_{V0} represents the initial biomass concentration,
$Y_{x/s}$ represents the yield coefficient of biomass production/substrate.

The $\frac{X_{V0}}{Y_{x/s}}$ term demonstrates the existence of a low initial concentration of biomass capable of biodegrading the surfactant, which in our experimentation was determined in the test water at the commencement of the test to be 8400 CFU (Clesceri et al., 1989).

The parameter '*q*' which constitutes the concentration of non-biodegradable substrate, shows values close to zero, which implies that all the substrate used (the surfactant) can be considered as primarily biodegradable material. However, the length of time required to achieve a maximal level of biodegredation varies from one experiment to another (Fig. 1). For example, in Test 1 (5 mg/l, 21°C), 98% biodegradation is reached after 6 days, whereas in Test 6 (20 mg/l, 7°C) it takes 38 days to reach this level of biodegradation.

Parameter '*p*', values variy proportionately with the initial concentration of LAS in the tests. This dependence of the rate of biodegradation on the initial concentration of the surfactant material is sharper on passing from 5 to 10 mg/l, whereas between the tests conducted with 10 and 20 mg/l of LAS, the differences in the rate of biodegradation are much less notable. This behavior may be due to a bacteriostatic effect of the surfactant on the microbiota (Swisher, 1987) which begins to become apparent at concentrations higher than 10 mg/l.

However, the temperature is the variable which has the major influence on the rate of biodegradation of LAS. Thus, the values of '*p*' Table 1, for Tests 4, 5, 3 and 6 (7°C, 13°C, 21°C and 25°C, respectively) were, respectively, 0.39, 0.77, 1.07 and 1.84 days^{-1}. These results are in agreement with those obtained by other authors (Quiroga et al., 1989) and they indicate that the process of biodegradation could have an important seasonal component.

Table 2
Values of the kinetic parameters obtained for the various homologues in the six tests

	21°C5 mg/l			21°C10 mg/l			21°C20 mg/l		
	C_{10}	C_{11}	C_{12}	C_{10}	C_{11}	C_{12}	C_{10}	C_{11}	C_{12}
r^2	0.997	0.998	0.998	0.996	0.999	0.998	0.995	0.998	0.999
p	1.12	1.23	2.05	0.71	1.11	1.48	0.86	1.1	1.29
q	0.08	0.08	0.07	0.07	0.07	0.07	0.06	0.06	0.02
h	0.82	2.71	1.63	1.81	5.40	3.14	2.61	10.80	6.62
	7°C20 mg/l			13°C20 mg/l			25°C20 mg/l		
	C_{10}	C_{11}	C_{12}	C_{10}	C_{11}	C_{12}	C_{10}	C_{11}	C_{12}
r^2	0.993	0.997	0.992	0.992	0.997	0.982	0.984	0.998	0.999
p	0.32	0.38	0.36	0.59	0.72	0.75	0.64	1.23	4.40
q	0.07	0.06	0.3	0.09	0.03	0.19	0.13	0.22	0.03
h	2.74	11.02	6.40	2.77	10.67	6.87	3.84	10.88	6.60

4.1. Biodegradation kinetics of the homologues

The Quiroga–Sales kinetic model has been applied to the concentration values of the different LAS homologues obtained in the six tests conducted during the study. The purpose was to obtain the kinetic parameters required to predict their fate.

Table 2 gives the values found for the parameters '*p*', '*q*' and '*h*', together with the correlation coefficients obtained for the various homologues in the six tests.

The correlation coefficients obtained from the application of the Quiroga–Sales kinetic model to the experimental values are close to unity, which shows that the model is useful for predicting the behavior of the various homologues under the test conditions.

The values for '*q*' were close to zero, which confirms the fact that all the LAS used may be considered as primarily biodegradable material, although at different rates.

Taking into account the initial quantity of surfactant material used in each test and the percentages of each homologue, the calculated values of '*h*' were consistently slightly higher than the initial concentration used Table 1.

The values for '*p*', reported in Table 2 follow a pattern similar to the one of Table 1. Thus, in the tests conducted at different temperatures, the values of '*p*' increase proportionately with the temperature; in the tests conducted with different initial concentrations, the value for this parameter is higher when the initial concentration is lower. Data can also show that the rate of degradation '*p*' is higher for the homologues of longer alkylic chain. This indicates that the homologues of longer alkylic chain not only begin to degrade sooner, as predicted by the distance principle (Swisher, 1987), but that they also degrade at a faster rate.

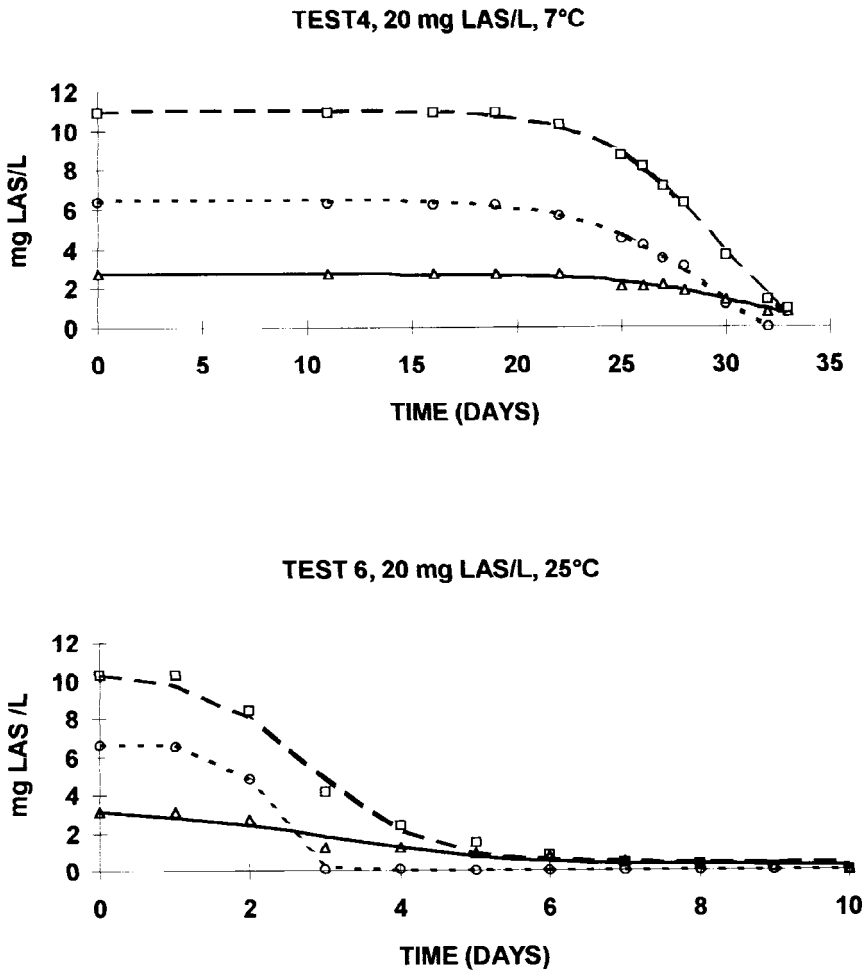

Fig. 2. Evolution for concentration of LAS homologues found in tests 4 and 6. Experimental data (○ C10–LAS; □ C11–LAS; △ C12–LAS). Predicted data (C10–LAS; C11–LAS; C12–LAS).

When the 'p' values for the homologues C_{10} and C_{12} are obtained in the tests at different temperatures, it is found that for the tests conducted at lower temperatures (Test 4, at 7°C and Test 5, at 13°C), the difference between the values is very small (0.04 and 0.16 days^{-1}, respectively); whereas for the tests conducted at higher temperatures (Test 3, at 21°C and Test 6, at 25°C) the difference is greater (0.42 and 2.8 days^{-1}, respectively). From these data, we suspect that at lower temperature, the structure of the compound ceases to be the major factor limiting the rate of biodegradation and it is replaced by the temperature of the medium. This phenomenon is illustrated in Fig. 2, which compares the experimental and predicted data of the different homologues obtained in tests 4, 7°C and 6, 25°C.

Making a similar comparison for the tests conducted at different concentrations, the difference between the values of 'p' of the homologues of longer and shorter chain length is 0.93 days^{-1} for Test 1 (5 mg/l), 0.77 days^{-1} for Test 2 (10 mg/l) and 0.42 days^{-1} for Test 3 (20 mg/l); these data, together with the effect observed at different temperatures, demonstrates that as the rate of the biodegradation process increases, the differences between the rate of biodegradation of the different homologues are sharper.

5. Conclusions

The following conclusions can be drawn from the values obtained by the application of the proposed kinetic model to the experimental results obtained during this work:

1. The kinetic model proposed by Quiroga and Sales (1991) and Romero et al. (1997) is valid for predicting the fate of LAS in river water, under a wide variety of conditions, including those in which long periods of acclimatization are required and those which involve scarcely any period of induction, as confirmed by the values for r^2 being close to unity.

2. None of the variables studied affects the extent of the primary biodegradation. Only the rate at which the process takes place is affected, as confirmed by the different values for 'p' and the similar values for 'q' obtained from the various tests.
3. LAS was primarily biodegraded to more than 99% by a natural microbial flora such as that of river water, under all the conditions tested, even the most severe (7°C, 20 mg/l), as can be deduced from the values of residual substrate found in all the tests ($q < 0.1$ mg LAS/l), and from the experimental data presented in Fig. 1.
4. The differences existing between the values of the rate of degradation ('p'), when the tests conducted with 5 and 10 mg/l of LAS ($\Delta p = 0.14\ d^{-1}$) are calibrated to those with 10 and 20 mg/l of LAS ($\Delta p = 0.02\ d^{-1}$) demonstrate that at concentrations above 10 mg/l of LAS, the process of biodegradation is slowed down, possibly as a result of the bacteriostatic effect of the xenobiotic (Larson and Payne, 1981).
5. The dependence of the rate of the process of biodegradation on the temperature, evidently related to the metabolism of the microorganisms responsible for the degradation, allows one to deduce that the potential contamination represented by effluents containing these surfactants has an important seasonal component. In winter months, in places where the water temperature is below 13°C, the adverse effects caused by the direct discharge of these compounds into water flows will be more severe given their slower rate of biodegradation.
6. The higher homologues of LAS are biodegraded at a faster rate at temperatures above 13°C. The difference in the rate of degradation ('p') between homologues is reduced as the overall rate of the process is reduced, presumably because under the more adverse conditions tested (Test 3, 20 mg/l of LAS; Test 4, 7°C), the limiting factor on the rate ceases to be the molecular structure of the compound but instead becomes the low temperatures or the presence of an inhibiting agent.

References

Bayley, J., Ollis, D., 1980. Biochemical Engineering Fundamentals. McGraw Hill.

Clesceri, L., Greenberg, A., Rodhes, T., 1989. Standard Methods for the Examination of Water and Wastewater, 17th ed. American Public Health Association (A.P.H.A.), American Water Works Association (A.W.W.A.), Water Pollution Control Federation (W.P.C.F.), Washington.

Gaden, E., 1959. Fermentation process kinetics. Journal of Biochemical, Microbiological and Technological Engineering 1, 63–70.

Granados, J., 1996. Surfactant raw materials. Constant evolution and a solid future. 4th World Surfactant Congress, Barcelona 1, 100–123.

Larson, R., Payne, A., 1981. Fate of the benzene ring of linear alkylbenzene sulfonate in natural waters. Applied and Environmental Microbiology 41, 621–627.

Larson, R., 1983. Comparison of biodegradation rates in laboratory screening studies with rates in natural waters. Residue Review 85, 159–171.

Nakae, A., Tsuji, K., Yamanaka, M., 1981. Determination of alkyl chain distribution of alkylbenzene sulfonates by liquid chromatography. Analytical Chemistry 53, 1818–1821.

Painter, H., Zabel, T., 1987. Review of the Environmental Safety of LAS. Water Research Center, Medmenham.

Quiroga, J., Sales, D., 1991. Degradation kinetics of surfactants. Tenside Surfactants Detergents 28, 27–30.

Quiroga, J., Sales, D., Gómez-Parra, A., 1989. Experimental evaluation of pollution potential of anionic surfactants in the marine environment. Water Research 23, 801–807.

Romero, L., Perez, M., Sales, D., 1997. Degradation kinetics of distillery wastewaters by immobilized anaerobic mixed cultures. In: The 8th International Conference on Anaerobic Digestion (AD-97). IAWQ, Japan.

Swisher, R., 1987. Surfactant Biodegradation, 2nd ed. Marcel Dekker Inc, New York.

Yoshikuni, Y., Chizumi, E., Yasuko, M., Hiroshi, K., Kazuaki, M., Kouichi, Y., 1992. Kinetic study on the biodegradation of Linear Alkylbenzenesulfonates (LAS) in well-water. Chemosphere 24, 969–977.