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BIODEGRADATION KINETICS OF SURFACTANTS IN SEAWATER

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ABSTRACT

In this paper, a general kinetic model for degradation processes of surfactants is proposed. The model equation is $v = K_2 S^2 + K_1 S + K_0$, where v is the substrate consumption rate in the biodegradation process, S is the surfactant concentration in the medium and K_2, K_1, and K_0 are kinetic constants. From this general expression, different simplified equations can be obtained (where $K_0 = 0$; K_2 and $K_0 = 0$; $K_2 = 0$; K_2 and $K_1 = 0$), which are representative of the process for different operating conditions. This model was tested by measuring the degradation of two different surfactants (Sodium dodecyl benzene sulfonate, LAS; and Sodium dodecyl sulfate, DSNa) under two different temperatures (5 and 20°C). Values predicted by the model are close to experimental data obtained.

1. INTRODUCTION

Although removal of an organic chemical from an environmental compartment can take place by abiotic processes such as hydrolysis, photolysis, adsorption onto particular matter and volatilisation into the atmosphere, its complete conversion into mainly inorganic products is invariably due to microbial activity. This process, termed ultimate biodegradation, results in a conversion of the compound into CO_2, H_2O, inorganic salts, new microbial biomass and organic products associated with the normal metabolic processes of bacteria.

The biodegradation of surfactants has been studied for many years [1, 2] and is still being investigated [3, 4]. However, most studies of the biodegradation of this type of substance deal with the

determination of the metabolic degradation pathway [5, 6], the relation between structure and biodegradability, [7, 8, 9], the behaviour in Waste Water Treatment Plants (WWTP) [10, 11] or the levels in the environment [12, 13]. Nevertheless, very few authors have undertaken a study of the basic kinetic parameters of surfactant degradation, which would provide fundamental information for the study of their environmental behaviour. However, biodegradation data are important factors in the development of mathematical models for predicting the distribution and concentration of synthetic chemicals in the environment [14, 15, 16].

In this paper, the effect of test conditions on the kinetics of for generating such data will be discussed. Lastly, an experimental approach for producing environmentally realistic kinetic information will be outlined.

2. KINETIC MODELS FOR DESCRIBING BIODEGRADATION PROCESSES

The kinetics of biodegradation have been described by a variety of mathematical expressions, increasing in complexity as they attempt to accommodate more of the many variables which can affect the rate of biological removal of an organic chemical in the environment.

The kinetic biodegradation process can be described from two different perspectives, by means of : a) microorganism growth models ; and b) substrate consumption models

2.1 Microorganism growth models

The degradation kinetics of particular substrates have been studied by many authors [17,18], by relating them to the growth dynamics of the microorganism population. The classic method suggested by Monod [19] is widely accepted because it is simple; using only two parameters it can predict the transition between zero order and first order kinetics, which occurs in fermentation processes after a decrease in the level of substrate present in the medium, and the parameters used have a clear physical interpretation.

However, Monod's model is not based on a clear mechanism and the equation implies that the specific growth rate is not equal to zero for any finite value of substrate concentration, that the influence of the initial concentrations on the growth rate is not taken into account, the physiological state and the population history of the microorganisms are not taken into consideration and the Monod's model does not take full account of all phases of microorganism growth (the lag phase and the death phase are not well described).

All these factors have led to a large number of bacterial growth models [20,21] based on Monod's original approach, which include some of the factors mentioned above.

So-called logistic models are also among the classic models. They are based on Malthus' Law [17]. The best known logistic model is that of Pearl & Reed [22] who introduced a factor of inhibition of

population growth to Malthus' equation. This model has been less widely used than Monod's model for microorganism growth, for the following reasons: it does not consider the mechanisms of the biological reactions implied ; the relationship between μ (exponential growth rate) and S is not defined; bacterial growth is expressed in terms of the viable biomass present in the medium (this parameter is difficult to determine experimentally) and it does not enable the prediction of population decrease phase, following the stationary phase, when all available nutrients have been consumed.

This last deficiency was remedied by Volterra [17] by incorporating an integral term which includes the influence of the population history on the growth rate, a factor which is difficult to evaluate.

As we have seen, these microorganism growth models show some inconveniences. They are not based on a mechanistic approach. Furthermore, depending on the operating conditions, the type of substrate used, the microorganisms involved, one particular model must be selected. None of them describe generally the phenomenon of biodegradation.

2. 2 Substrate consumption models

If we accept the fact that microbial growth kinetics cannot be used to predict biodegradation rate constants, what other rate functions can be used instead?. One possibility is to study the kinetics of the degradation process itself i.e. by proposing a mechanism for the reaction as a whole, and then developing the corresponding kinetic expression. This model has only one parameter "S" which is easily quantifiable. However, in principle, the development of this type of models is extremely complicated since the degradation of organic material is a heterogeneous process, and the microorganisms involved are usually not pure cultures

Some of the mathematical equations which have been proposed to describe the kinetics of the substrate degradation process, are based on curve fitting of the experimental substrate consumption or CO_2 production during biodegradation [23].The simplest approximation used by the majority of authors is a curve fitting of the decrease of the substrate concentration using a first order equation. Some authors [24] consider this approach to be complete, taking in the account an initial acclimatisation term.

While first order kinetic biodegradation equations are applicable to laboratory experiments, the reliable use of such rate constants for the environment is doubtful, for there is also evidence that discrete alterations of some chemicals in surface water samples are highly dependent on population size, making "k" a second or pseudo first order constant. Thus, pseudo first order kinetics are sometimes observed during degradation in the laboratory, as indicated by the S shape of the curve of concentration versus time. Larson [25] explains this shape of curve by the fact that the rate of product formation or substrate consumption is a function not only of the concentration of product or substrate, but also of time.

Larson's explanation of the S shape of the substrate/time function is feasible, but the procedure is not strictly correct, because time is considered as a function of the number of microorganisms. It is merely an artificial means of producing a quadratic equation. If dS/dt really is a function of substrate and bacteria

concentration, then these two variables, not time, should be included in the second term of the equation, and the resulting differential equation should be integrated.

2.3 Proposed Model

Therefore, it would be better to have a new model with a coherent theoretical base, consistent with the experimental data obtained, and should be capable of simulating the various more specific equations proposed by other authors to predict degradation kinetics under specific operating conditions..

Thus, considering a discontinuous reactor to which an organic substrate (S) is added, a slow substrate conversion rate is observed at the beginning of the test. But because the microorganisms multiply during the course of the process, the reaction rate increases until a maximum is reached. Then substrate consumption gradually decreases and this rate falls to zero. This is clearly autocatalytic behaviour, observing the following equation of reaction rate:

$$S + n \Longrightarrow \alpha P + \sigma n \Longrightarrow V = d S/d t = K_v S n$$

According to the stoichiometry of the reaction, for each molecule of S consumed, α molecules of P are formed, and σ microorganisms are produced (σ should be >1). If the initial concentration is S_0 and the initial number of microorganisms is n_0, when part of the substrate has been consumed and the amount of substrate remaining in the medium is S, then the result will be "n" microorganisms given by the expression $\sigma(S_0 - S)$. Thus the number of microorganisms in the medium at a given moment (when the experimental measurement is made) is $\mathbf{n = \sigma(S_0 - S) + n_0}$. Substituting this for n in the rate equation gives :

$$-\frac{dS}{dt} = K_v S [n_0 + \sigma(S_0 - S)] \qquad (1)$$

$$-\frac{dS}{dt} = K_v \sigma [(\frac{n_0}{\sigma} + S_0) S - S^2] \qquad (2)$$

These equations assume that all the substrate is biodegraded by the microorganisms ($S = S_T = S_B$). But there is usually a proportion not metabolized by the microorganisms. Therefore, given that:

$$S_T = S_B + S_{NB} ; \qquad S_{0T} = S_{0B} + S_{0NB} ; \qquad S_{NB} = S_{0NB}$$

where: S_T = total substrate concentration ; S_B = biodegradable substrate concentration

S_{NB} = nonbiodegradable substrate concentration ; S_T = initial (t = 0) total substrate concentration

S_B = initial biodegradable substrate concentration ; S_{NB} = initial nonbiodegradable substrate concentration

Substituting in (2)

$$-\frac{dS}{dt} = K_v \sigma \{[\frac{n_0}{K} + (S_{0T} - S_{0NB})] (S_T - S_{NB}) - (S_T - S_{NB})^2\} \qquad (3)$$

Developing and simplifying this equation:

$$-\frac{dS}{dt} = K_2 S_T^2 + K_1 S_T + K_0 \quad (4)$$

where K_2, K_1 and K_0 are:

$$K_2 = -K_v \sigma \quad (5)$$

$$K_1 = K_v \sigma\left(\frac{n_0}{\sigma} + S_{0T} - S_{0NB} + 2S_{NB}\right) \quad (6)$$

$$K_0 = -K_v \sigma\left(\frac{n_0}{\sigma} S_{NB} + S_{0T} S_{NB} - S_{0NB} S_{NB} + S_{NB}^2\right) \quad (7)$$

By separating variables and integrating into equation (4), the relationship between organic material and degradation time is obtained:

$$\int_0^t dt = \int_{S_0}^{S} -\frac{dS}{K_2 S^2 + K_1 S + K_0} \quad (8) \qquad \text{and therefore:} \qquad S = \frac{h(S_0 - q) - q(S_0 - h)e^{pt}}{(S_0 - q) - (S_0 - h)e^{pt}} \quad (9)$$

where: $p = |(K_1^2 - 4K_2K_0)^{1/2}|$ $\qquad q = (-K_1+p)/2K_2$ $\qquad h = (-K_1-p)/2K_2$

By plotting substrate concentration against time as given by equation (9), the curve shown in Figure 1a. is obtained.

The meaning of the kinetic parameters p, q and h can be deduced from the mathematical equations used earlier. From equation (4) it can be deduced that the substrate consumption rate is zero when the substrate concentration in the medium is equal to q or h, because these are the solutions of this equation. The implication of the solution $S = q$ is that q represents the minimum possible value for the organic material concentration Therefore q is the level of nonbiodegradable organic material for this type of microorganism. The solution S = h implies that h corresponds to the maximum amount of organic material available in the medium for the formation of biomass. Romero [26] found that h can also be given by the equation:

$$h = \frac{X_{v_0}}{Y_{x/v}} + S_0 \quad (10)$$

where: X_{Vo} = initial concentration of microorganisms ; $Y_{x/v}$ = growth yield and S_O = initial concentration of substrate.

Lastly, the term p represents the maximum rate and it can be shown that it is the product of the rate constant observed, multiplied by the maximum concentration of microorganisms that it is possible to reach in the medium [27]. In other words, it represents the maximum specific growth rate of those microorganisms responsible for the biodegradation process, and it is that maximum rate possible when the term K_2 from the equation for p is equal to zero ($K_2 = 0$).

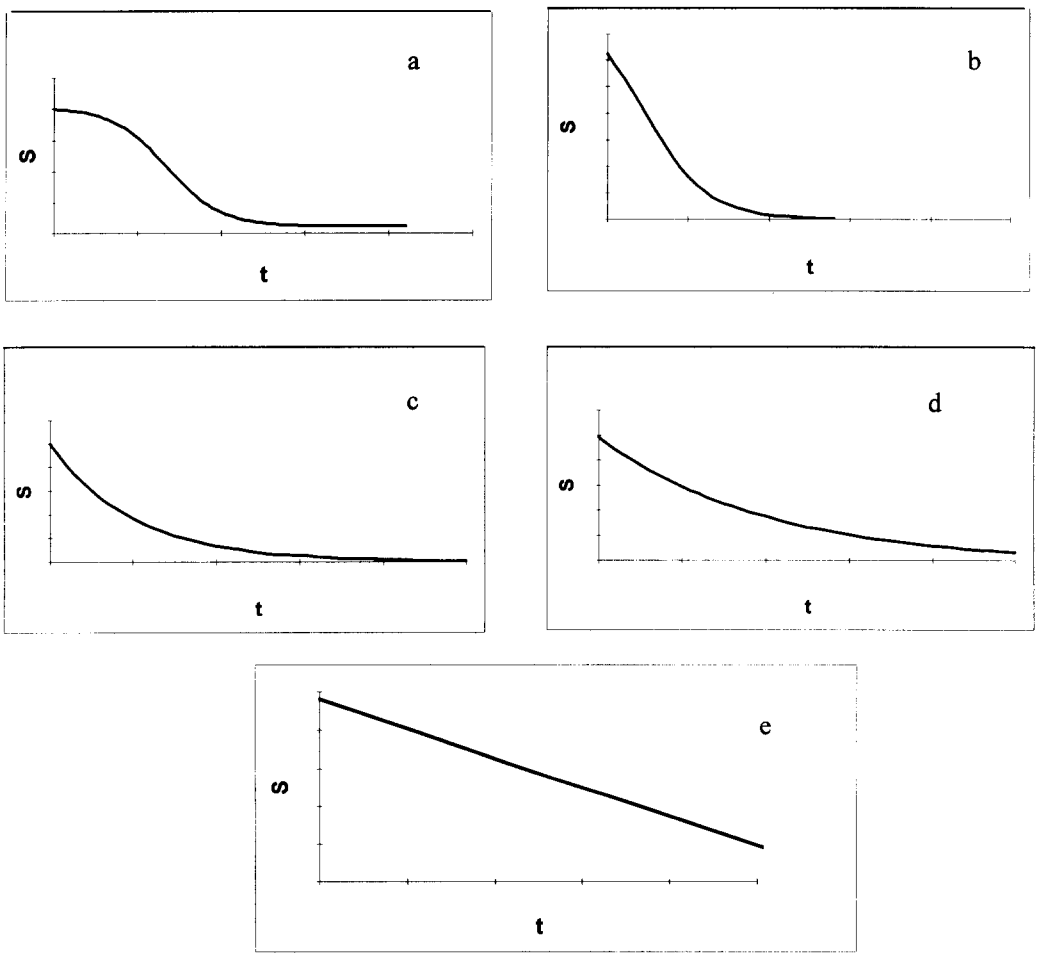
On the basis of our general equation (4) and by eliminating some terms, other equations can be deduced which correspond to different conditions possible in the reactor.

For example, when there is no nonbiodegradable substrate concentration in the medium ($S = S_T = S_B$), the polynomial second-grade equation becomes:

$$-\frac{dS}{dt} = K_2 S_T^2 + K_1 S_T \qquad (11)$$

The graphic representation of equation (11) is given in Figure 1b.

FIGURE 1. Plot of substrate concentration against time as given by equations 9 (a), 11 (b), 12 (c), 13 (d) and 14 (e).



Considering a process in which not only S_{NB} is zero but the concentration of active microorganisms remains high and practically constant, it can be shown that the coefficients K_2 and K_0 in the initial rate equation (4) are negligible. Thus this equation can be simplified to :

$$V = - \frac{dS}{dt} = K_1 S \tag{12}$$

The graphic representation of equation (12) is given in Figure 1c.

If not all the substrate is biodegradable, then n will again remain constant and the equation will become :

$$- \frac{dS}{dt} = K_1 S_T + K_0 \tag{13}$$

The concentration of S against time given by equation (13) is represented in Figure 1d.

When K_2 and K_1 are zero, equation (4) is transformed into

$$- \frac{dS}{dt} = K_0 \tag{14}$$

where the degradation rate of the organic material remains constant and independent of concentration. For this condition, the representation of substrate concentration against time given by equation (14) is shown in Figure 1e.

Our basic equation (4) can also be derived from other theoretical considerations presented by the same authors in an earlier paper [27]. There, a comparison is made with the models of Volterra and Monod referred to in the bibliography.

2. MATERIAL AND METHODS

In order to validate the mathematical model proposed here, a series of experiments were carried out with various substrates (vinasses [27], both anionic [28] and non-ionic surfactants [4]) and under various experimental conditions (different temperatures [29], concentrations [4], salinity [30], darkness [31], light [31]). The assays were carried out in duplicate and, in some cases, in triplicate and the results obtained in all experiments showed an excellent fit of the experimental data to the kinetic model proposed. This paper presents the results obtained for two anionic surfactants in order to show the operating conditions corresponding to the general equation of the model or to some of its simplifications.

The test method used was the "river die-away" test, fully described in the bibliography and used by many authors [7, 30]. Briefly, the test consisted of adding a concentration of 20 ppm of the surfactant into sea water at two representative temperatures. This concentration has been found by various authors [32] near the discharge outlets of untreated urban waste-water. At pre-set intervals of time, samples were taken for analysis. A preservative was added to the sample and it was then refrigerated at 5° C until its analysis.

The surfactants chosen were linear alkylbenzene sulfonate (LAS), and sodium dodecyl sulfate (DSNa), which are the most representative anionic surfactants. LAS is the most widely used surfactant on a worldwide scale; the volume of production in 1995 being approximately $1{,}5x10^6$ t [33]. DSNa is used as a standard due to its high level of purity and biodegradability. The temperature conditions selected were 20°C and 5°C for LAS and 20°C for DSNa, as typical extremes at which surfactant-containing waste waters are usually discharged. All the experiments were carried out in duplicate.

Abbot's Blue Methylene [34] method was selected from among the alternatives available (HPLC, CG, DOC) and employed in these experiments since it is the method specified in Spanish legislation for monitoring surfactant biodegradation [35].

Sea-water used for the tests came from an inlet close to the Bay of Cádiz (SW of the Iberian Peninsular). Its main physico-chemical characteristics were : salinity (33.8 g/L), pH (8.10) and dissolved oxygen (7.1 mg/L). Micro-organisms were counted in accordance to the procedure of Harrigan and MacCance [36]. The number of aerobic microorganisms was $6x10^3$ colonies/ mL. Anionic surfactant matter (MBS) was 90 μg /L.

3. RESULTS AND DISCUSSION

Figures 2a, 2b, and 2c show for the 3 different experiments carried out, the experimental values obtained for the residual surfactant material, S, against the test duration time (individual points). In addition, the resulting curve (solid line) from the application of the proposed kinetic model in its general form or in one of the simplifications. The good agreement between theoretical and experimental values, and the value of the correlation coefficients r^2 in Table I, corroborate the validity of the proposed model. Table I shows also the values of the kinetic parameters corresponding to each of these tests, together with the corresponding correlation indices.

TABLE I. Kinetic parameters and correlation coefficients obtained from the application of the various equations of the Quiroga and col. kinetic model to the experimental data.

TEST A	p	q	H	K_2	K_1	K_0	r^2
LAS, 20 °C	0.62	1.12	20.28	-0.03	0.69	-0.73	0.998

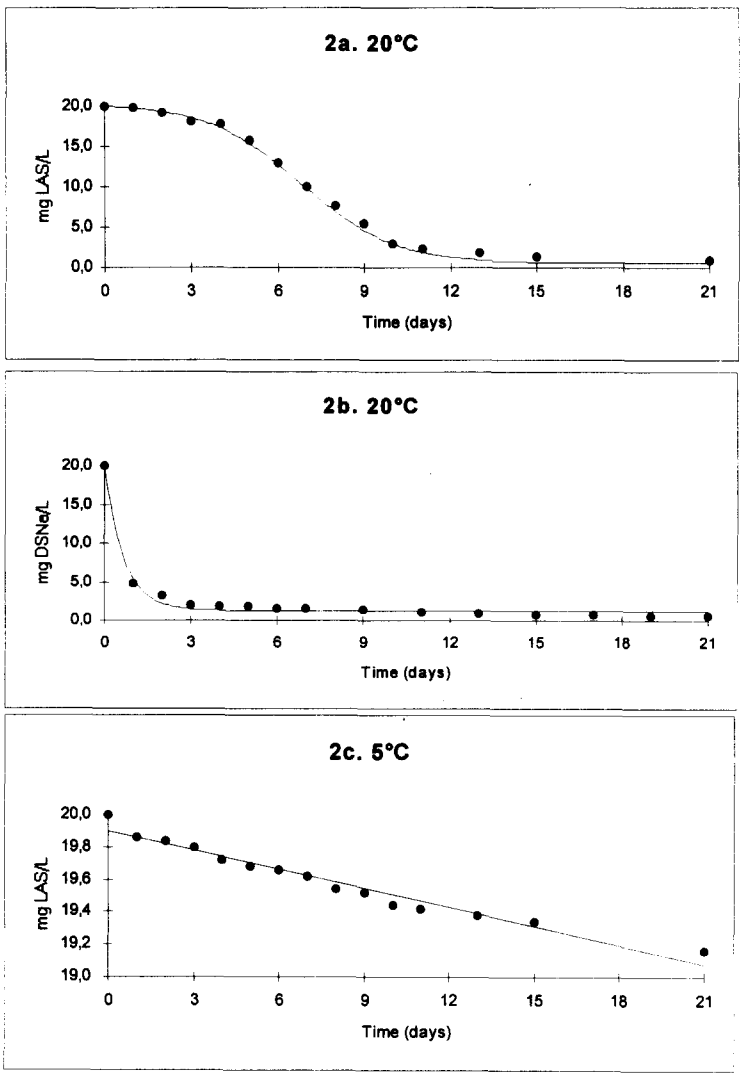
TEST B	K_1	K_0	r^2
DSNa, 20 °C	-1.52	1.91	0.987

TEST C	K_0	r^2
LAS, 5 °C	-0.05	0.995

It can be seen that, for the test with LAS at 20 °C (Fig. 2a), the theoretical result that corresponds most closely is that for the second-grade polynomial equation (4), ($dS/dt = K_2 S^2 + K_1 S + K_0$) while at 5°C (Fig. 2c), it is equation (14), ($dS/dt = K_0$) that most closely fits the data.

For the tests with DSNa at 20°C (Fig. 2b), the first-grade equation (13) ($dS/dt = K_1 S + K_o$) can be fitted to the experimental data.

FIGURE 2. Comparison between the experimental values obtained for the residual surfactant material, S, against the time, and the application of the most appropriate expression of the theoretical model.



As can be seen from the test result which best fits the equation (4) (Fig. 2a : LAS at 20 °C), the value of the parameter h (20.28) agrees closely with the value of the initial surfactant concentration (20 mg/L). The small difference between these values may be due to the meaning of this parameter given by Romero [26], already discussed, where the initial biomass concentration is also included. The value of q (1.12 mg/L), which represents the nonbiodegradable substrate concentration, is also close to the experimental value

(0.9mg/L). Finally, the value of parameter p, representing microorganism growth rate, (0.62 day^{-1}), is within the values reported in the bibliography for microorganism growth in the degradation of this and other types of organic material [37].

The values of K_2, K_1 and K_0 , also given in Table I, can be calculated from the values of p, q and h. The value for K_2 is negative (-0.03), and can be interpreted as the inhibiting effect of the substrate on the bacterial flor at the concentrations employed. However, its very low value indicates minimal inhibition. The term $K_2 \cdot S^2$ is therefore a correction factor which reflects the lag phase of the microorganisms' activity, while they adapt to the substrate to be degraded.

For the term K_1, the value obtained is positive (0,69). This value increases in proportion to the biodegradation rate, shown by the results obtained by the same authors in other biodegradation studies [38]. Finally, the value for the term K_0 is negative and can be explained by the competition among the bacterial population for the limited amount of substrate remaining in the medium.

From Test C (LAS at 5 °C : Fig.2c), the best fit to the experimental data is not given by the second-grade polynomial, but by the equation $dS/dt = K_0$. The value of the slope of the straight line allows the calculation of the value of K_0. This is very small (0,05 day^{-1}), indicating a process in which practically no biodegradation is taking place.

By comparing the tests on LAS at 5°C and 20°C, it can be observed that the biodegradation rate depends strongly on the temperature ; at 5°C, the metabolic processes of the microorganisms are considerably inhibited. Furthermore, the microorganisms used in the tests came from the Bay of Cádiz, where such a low temperature is not found even in the winter months.

It is established that the contamination potential of this type of substrate is closely related to seasonal factors. Thus in coastal waters with relatively low temperatures, the adverse effect of the discharge of this type of anionic surfactant can be considerable, since it remains in the water for a relatively long time until it is degraded. This contamination is particularly serious in coastal areas with low water renovation rates, such as bays and estuaries, where aquaculture is practised, because of the harmful effect of this type of substance on the larvae and fry of many species of commercial interest [39].

The surfactant DSNa is considered "soft" ; it is much more easily biodegraded than LAS because mineralization of the aromatic ring is not required. The equation which best describes the process is a first-order kinetic (equation (13)). This is in line with the findings of other authors [40].

The nature of the fit resulting from the DSNa test indicates a microorganism induction period of less than one day, as well as the existence of a minimum level of organic matter which the microorganisms cannot biodegrade (Fig.2b).

DSNa is biodegraded by hydrolytic enzymes which are likely to exist in the medium or are synthesized by the microorganisms ; this probably explains why there is no period of acclimatisation. LAS, on the other hand, is more resistant to biodegradation because the carbon link between the aromatic ring and

the sulphur of the sulphonate group is more difficult for the microorganisms to break down, since the enzymes, being more specific, need time to be synthesized.

Also in the case of first-order kinetics, the concentration of nonbiodegradable substrate is given by the ratio $-K_0/K_1$, resulting in a theoretical nonbiodegradable substrate concentration of 1.25 mg/L, whereas the experimental value is lower (0.6 mg/L). This leads us to conclude that if the test had been continued for more than 21 days, the residual surfactant matter would probably have disappeared totally and we would be left with a first-order kinetic in which the term K_0 (case 1c) had disappeared.

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