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Effect of LAS (Linear Alkylbenzene Sulphonates) on organic matter biodegradation

LAS (linear alkylbenzene sulphonates) are still the main surfactants used in formulated detergent products and are an important component of urban sewage. Surfactants could be toxic for microorganisms responsible for the degradation treatment and to organisms present in natural ecosystems. However, sewage treatment plants are not designed to eliminate surfactants. It is interesting therefore to study the effect of LAS on microorganisms in microbiological treatment units to determine the residual LAS concentration reaching the collector channels. This article discusses the effect of LAS on the purification efficiency of aerobic sewage treatment plants, and LAS biodegradation itself in such plants. The results of this study suggest that LAS influence pH self-regulation capacity in aerobic degradation of organic matter when its concentration is greater than 20 ppm. For this, external neutralisation is required in such cases. Likewise, LAS influence the degradation capacity of both organic matter and itself in aerobic processes, reducing the biodegradation rate and increasing the residual COD and LAS concentrations obtained.

LAS (Lineare Alkylbenzolsulfonate) stellen immer noch den größten Anteil aller Tenside in Wasch- und Reinigungsmittelformulierungen dar und sind ein wichtiger Bestandteil städtischer Abwässer. Tenside können sowohl für die Mikroorganismen in der Kläranlage als auch für Organismen in natürlichen Ökosystemen toxisch sein. Kläranlagen sind jedoch nicht darauf ausgelegt, Tenside zu eliminieren. Es ist daher wichtig, den Einfluß von LAS auf die Mikroorganismen in den biologischen Stufen der Kläranlagen zu untersuchen, um die Restkonzentration an LAS, das in die Sammler gelangt, zu bestimmen. In dieser Arbeit wird der Einfluß von LAS auf das Leistungsvermögen aerober Kläranlagen untersucht, ebenso der eigentliche Bioabbau von LAS. Aus den Ergebnissen dieser Studie geht hervor, daß LAS das Selbstregulierungsvermögen für den pH-Wert beim aeroben Abbau organischer Substanzen beeinträchtigt, wenn seine Konzentration 20 ppm übersteigt. In diesem Fall ist eine zusätzliche Regulierung des pH erforderlich. Bei aeroben Prozessen wirkt sich zunehmende LAS-Konzentration auf die Abbauleistung für organische Substanzen und auf den eigenen Bioabbau aus. Die Bioabbaurate sinkt, während die Restwerte für CSB und LAS-Konzentration steigen.

1 Introduction

Urban sewage contains highly toxic compounds. The main source of synthetic chemicals discharged into urban sewage are the surfactants present in detergent formulations (*Berna,* 1989). Linear alkylbenzene sulphonates (LAS) are the most widely used anionic surfactants, accounting for more than 25 % of the world total. Commercially available products are very complex mixtures containing homologues with alkyl chains ranging from 10 to 14 carbon units (C_{10}-C_{14}-LAS). Furthermore, since the phenyl group may be attached to any atom of the alkyl chain, each homologue contains 5–7 positional isomers.

Several authors (*Brown,* 1978; *Wagener,* 1987; *Painter,* 1989) have discovered harmful effects of surfactants on the microorganisms present in natural ecosystems (death of microorganisms, reduction in surface tension, reduction of marine fauna, sublethal effects such as respiration problems, delays to development and reproduction, reduction in the degree of absorption of dissolved oxygen necessary for depuration, etc.). These compounds must therefore be eliminated before reaching the collector channels.

Microbiological processes have been shown to be the most suitable for removing both organic compounds and surfactants (*Painter,* 1987). However, sewage treatment plants are not designed to eliminate the surfactants present, even though their inhibiting effects on microorganisms responsible for microbiological treatments are well-known (*Abbot,* 1968).

As is shown in the literature, the pH of the surfactant degradation experiments progresses to acid values (*Bock,* 1987) affecting the kinetic parameters of the biodegradation process and the predominant microorganism species in the medium (*Lora,* 1978).

Likewise, special attention has been paid to determine how LAS behave in biodegradation processes. Most studies of these compounds report that the LAS biodegradation rate constants have been calculated assuming first order degradation kinetics (*Hrsak,* 1981; *Berna,* 1989; *Trzic,* 1992). However, *Sales* and *Quiroga* (1991) developed a non-linear kinetic model, verifying that it adjusts the experimental results of surfactant degradation in seawater. This model suggests that the degradation rate corresponds to a second-grade polynomial with respect to the surfactant concentration as such.

Since LAS affect the activity and viability of the mixed bacterial culture present in sewage treatment plants and modify the pH of the medium, it is particularly interesting to consider the inhibiting effect of this compound as a consequence of the modification it exerts on acidity. Moreover, this article reports on a study of the effect of the LAS them-

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selves as substances inhibiting bacterial flora, and the biodegradability of these compounds in water which contains biodegradable organic matter. All this information might be of value in the selection of operating and control criteria in sewage treatment plants.

2 Experimental details

A number of experiments were carried out to study the aerobic degradation of synthetic sewage inoculated with activated sludge from an urban sewage treatment plant. The samples contained glucose as carbon source and different LAS concentrations.

2.1 Equipment

All the experiments were carried out in aerobic digestion units of 2 litres capacity and an effective volume of 1.8 litres. The temperature was kept constant by placing the reactors in thermostatic cabinets controlled to within ± 1 °C.

The medium was homogenised by magnetic agitators. Aerators were fitted to provide the microorganisms with the oxygen needed for their survival. The oxygen was supplied at a constant rate of 10 litres/min. The air entered the medium through two microporous stainless steel diffusers made by metal powder sintering which enhanced oxygen transfer to the medium by reducing the bubble size.

2.2 Characterisation of feeds

Synthetic feeds were used, prepared from a mineral nutrient solution (according to the official Spanish method for determining surfactant biodegradation, Boletín Oficial del Estado, B.O.E., num. 260, pag. 34266), a carbon source (glucose) and different concentrations of the substance under examination (LAS).

A typical commercial LAS mixture was used for all the experiments. The concentrations of the different LAS homologues used were: C_{10} = 3.9 %; C_{11} = 37.4 %; C_{12} = 35.4 %; C_{13} = 23.1 %; C_{14} = 0.2 %, with an average chain length of 11.7 carbon units.

All other chemicals used were of analytical grade.

2.3 Inoculum

The inoculum used was the effluent from a secondary reactor of a biological municipal sewage treatment plant located in Puerto Real (Cádiz, España). This plant operates according to the so-called activated sludge process.

In order to conserve the inoculum for use in subsequent experiments, 2L inoculation reactors were used. Initially, the reactors were filled with a mixture composed of the inoculum (1 L) and a synthetic medium (1 L) prepared with the nutrient mineral solution and glucose as carbonated source (3 g COD/L). The reactors were kept at 25 °C and were fed daily with 500 mL of synthetic feed.

Periodic checks of the process showed that the inoculation system operated adequately, supplying an inoculum of constant characteristics suitable for further inoculations.

2.4 Analytical methods

To characterize the degradation process, the following parameters were analysed: pH, using a combined electrode; COD by dichromatometry followed by spectrophotometric detection; volatile solids (VSS) were determined according to "Standard Methods for the Examination of Water and Wastewaters" (*Clesceri*, 1989); volatile fatty acid concentration (VFA), calculated by gas chromatography; LAS were determined by reversed-phase HPLC. The concentrations of individual homologues were determined under isocratic conditions using a column (250 × 4 mm) packed with irregularly shaped octylsilica particles 7 mm in diameter (Lichrosorb RP-8) and fitted with an 11 mm long filtration precolumn containing the same packing material (C-8). The mobile phase (1 mL/min) consisted of a mixture of methanol and water (80:20) containing 10 g/L sodium perchlorate. Concentrations of LAS were determined using a spectrophotometer at 225 nm or by a spectrofluorimetric method, using an excitation wavelength at 230 nm and emission wavelength of 295 nm. The volume of sample injected was 50 mL.

2.5 Experimental conditions

This article reports on the effect of surfactants on the purification efficiency of aerobic treatment plants and LAS biodegradation itself in such plants. Control experiments were conducted in each of the test series and were carried out under the same conditions as the biodegradation experiment, but without the addition of LAS.

The following experimental conditions were used: aerobic conditions; discontinuous feed; initial substrate concentration of 10 g COD/L (Glucose); initial LAS concentrations included in the range of 0 and 400 ppm; operation temperature 25 °C; volume of inoculum added: 100 mL/2 L medium.

Initially, in stage 1, several experiments were carried out to study the effect of LAS on the acidity of the medium. In stage 2 and 3, biodegradation studies of both substrate and LAS were conducted for different LAS concentrations in the medium.

3 Results and discussion

3.1 Stage 1 – Effect of LAS concentration on pH

At the start of the experiment, LAS was added to the samples at concentrations between 0 and 35 ppm to study the effect of the LAS on the acidity of the medium. Seven experiments were conducted in the range 0, 5, 10, 20, 25, 30 and 35 ppm LAS. This study was carried out on mediums which were initially at pH values around 7 because the feed added to digesters was neutral since the synthetic mineral solution used includes several phosphate salts with a certain buffer capacity. The pH of the systems was allowed to evolve freely.

The main results obtained from these studies enabled two different behaviours to be distinguished. Fig. 1 shows plots of the analysed parameters vs. time, namely pH, COD (mg/L) and VSS (g/L) in the range 0–20 initial LAS concentration.

With initial LAS concentrations under 20 ppm, the medium initially tended to acidify. Subsequently, the systems neutralised spontaneously and the pH stabilised at values in the range 7–8 (Fig. 1a). Substrate degradation, reflected in COD decrease, was similar in all these experiments. The initial phase corresponded to the period of adaptation of the flora to the medium, which was followed by a sudden drop in values for this parameter coinciding with the spontaneous neutralisation of the medium (Fig. 1b). Microbial flora growth (represented by the increase of volatile solids, VSS) in the different experiments is shown in Fig. 1c. The shape of the volatile solids curve, as an index of micro-organism con-

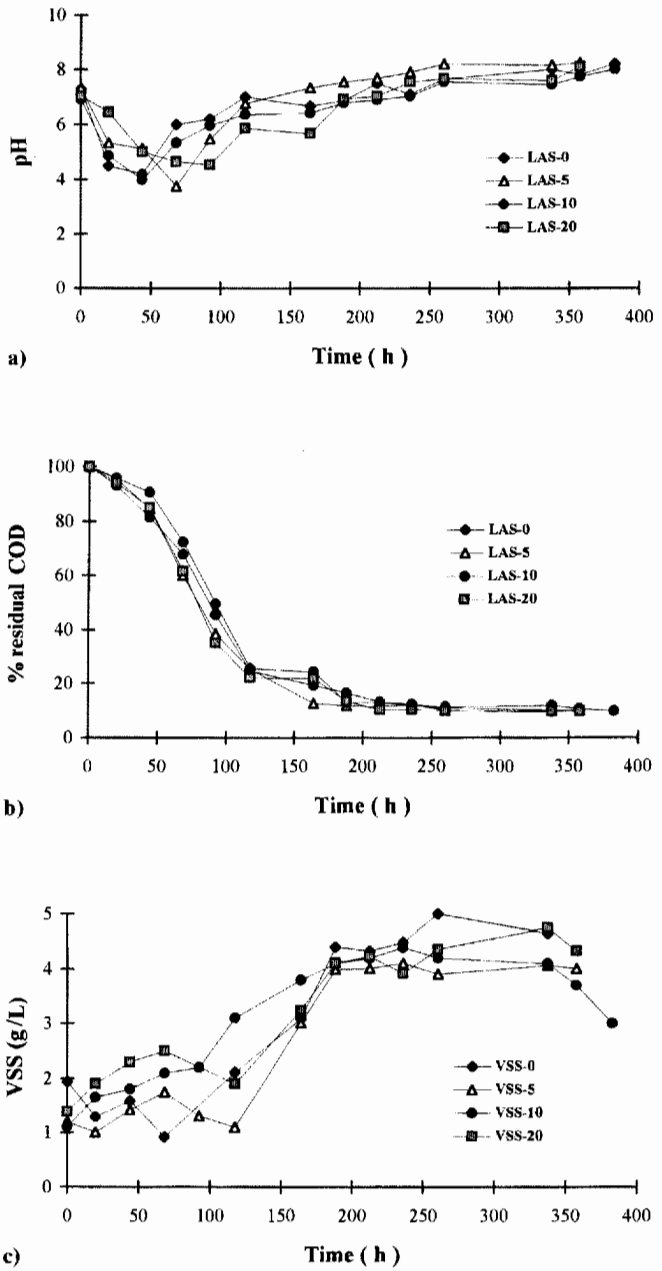


Fig. 1. Development of: a) pH; b) residual COD and c) VSS (mg/L) with time, in experiments with initial LAS concentration of 0–20 ppm

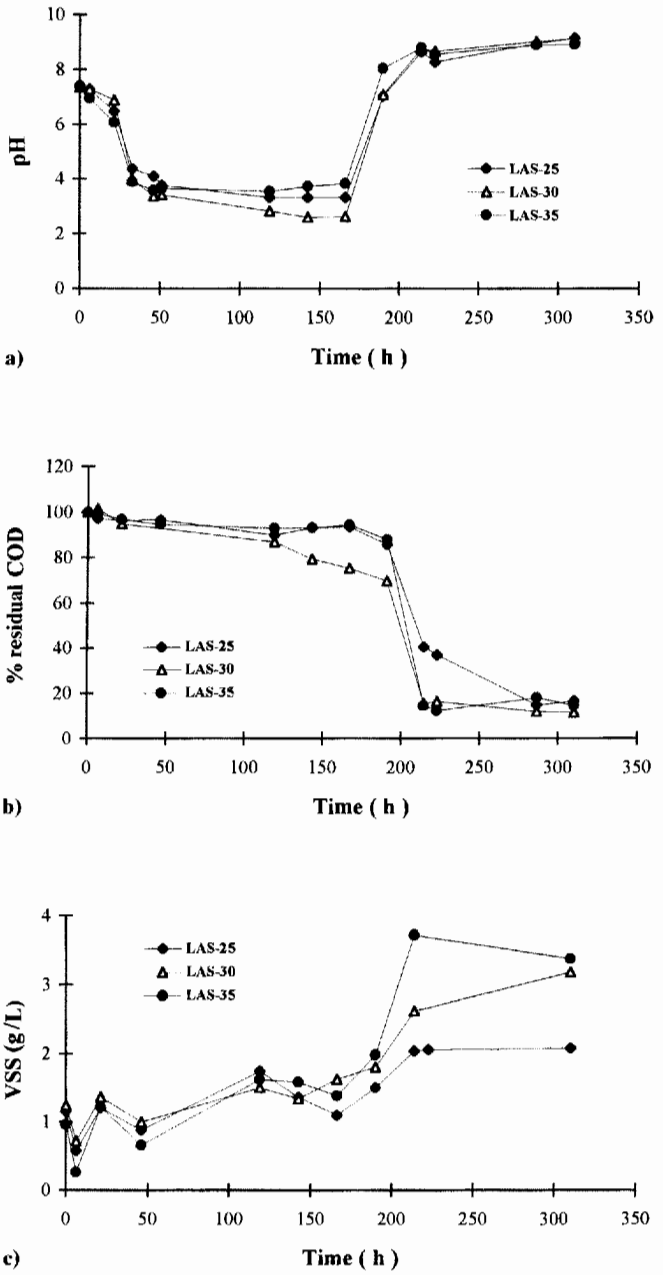
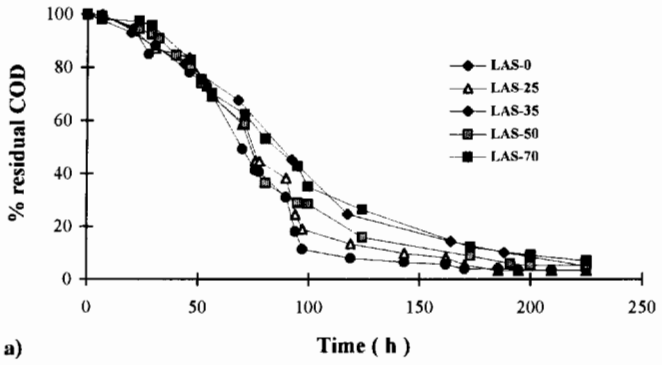


Fig. 2. Development of: a) pH; b) residual COD and c) VSS (mg/L) with time, in experiments with initial LAS concentration of 25–35 ppm

centration, indicates that cellular growth follows trends similar to those suggested by the logistic model (*Bayley,* 1977). The final reduction of the solids level could have been due to phenomena of cellular lysis and over-oxidation or even endogeneous metabolism, since this phase begins when the level of organic matter is virtually the same as the non-biodegradable COD.

However, when initial LAS concentrations in the experiment were more than 20 ppm, a sudden drop in the pH of the medium was noticed which, unlike in the previous studies, is maintained until stabilising at limit pH 3–4 (Fig. 2a). This phenomenon has a significant effect on microbial activity and viability and so on purification efficiency and growth as indicated in Fig. 2b. The medium acidity influences substrate utilisation kinetics: biodegradation of the organic matter slows down, indicating that the bacterial flora is inhibited in these pH conditions at such LAS concentrations. VSS values are less than those found in experiments where initial LAS concentrations were less than 20 ppm, indicating smaller population sizes (Fig. 2c).

The results of this study suggested neutralisation of the medium in those experiments where the pH was seen to drop and stabilise at acid values. As can be seen in Fig. 2a, the necessary amount of NaOH 3M was added (163 hours after the start of the experiment) to render the pH neutral. Fig. 2b shows that, after this time, the degradation capacity of the system rose and COD values fell sharply to residual values. For these experiments at LAS concentrations greater than 20 ppm, the VSS was significantly enhanced only after neutralisation, as can be seen in Fig. 2c. In the last case, the bacteria responsible for the observed biodegradation began to proliferate after external neutralisation since the system is incapable of doing this itself. A very interesting comparison between bacterial population originating from samples where



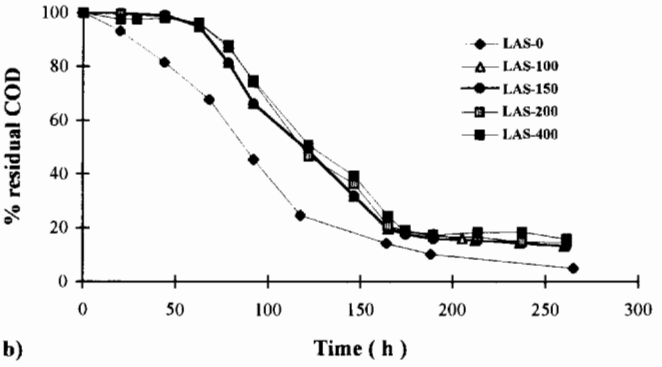


Fig. 3. Development of residual COD with time, in experiments with initial LAS concentrations of: a) 25–70 ppm and b) 100–400 ppm

LAS concentration was less or over 20 ppm LAS can be made on the basis of Figs. 1c and 2c.

As a result, in experiments with LAS concentrations over 20 ppm the medium must be neutralised externally. New biodegradation experiments were carried out subsequently, with daily neutralisation of the trial medium, with the aim of analysing only the effect on the biodegradation process of adding increasing quantities of LAS (in the range between 25 and 400 ppm).

3.2 Stage 2 – Effect of initial LAS concentration on organic matter biodegradability

Several experiments were carried out at 0, 25, 35, 50, 70, 100, 150, 200 and 400 ppm of LAS (with daily neutralisation of the medium) to study the influence of different LAS concentrations on the organic matter biodegradation process.

The decrease of the COD with time is shown in the Figs. 3a and 3b for the experiments carried out in this range. The development of this parameter is similar in all the experiments with initial LAS concentration less than 70 ppm. However, when the initial LAS concentration was more than 100 ppm, an initial induction period was noted which increases with the concentration of added LAS. This could be explained by the fact that these LAS concentrations influence their assimilation capacity or produce population inhibition phenomena. The CO_3^{2-}/CO_3H^- buffer formed by absorption of CO_2 in the medium from cellular respiration is unable to modify the pH to neutrality. Likewise, other data indicate that the surfactant is absorbed on the cellular membrane, so blocking the transfer of matter (*Aiba*, 1973; *Bailey*, 1977; *Bu'lock*, 1991).

Following the period of adaptation, a virtually linear decrease is observed in the substrate concentration with time. Finally, a COD stabilisation phase is reached. The last

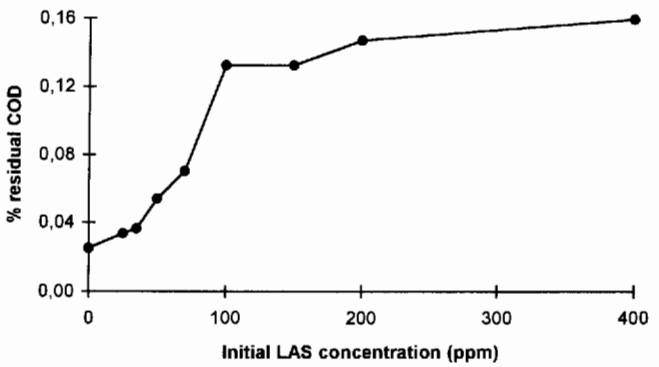


Fig. 4. Development of % COD removal at different initial LAS concentrations

value, corresponding to the concentration of non-biodegradable material in the medium, increases with the LAS concentrations in the experiment. The decrease of the percentage of COD removal at different initial LAS concentration (Fig. 4) shows two defined tendencies: initially, an almost linear dependence is noted of the parameters shown up to a concentration less than 150 ppm, from which level the data adjust to a less sloping straight line (constants values over 85 % COD removal).

3.3 Stage 3 – homologues and LAS biodegradation

After analysing the effects of LAS on organic matter degradation, it is interesting to examine how this evolves in clarification processes. The residual LAS during the biodegradation experiments was determined in order to resolve the biological activity of the microbial culture. LAS compete unfavourably with the carbonated matter, so that its biodegradation may be related to the biological activity of the LAS-degrading bacteria in the bacterial population.

As can be seen in Figs. 5a–5b, when the initial LAS concentrations increase, longer times are shown for adaptation to the substrate and, therefore, the curves are significantly modified, adapting to the shape of the curves proposed by *Sales* and *Quiroga* (1991, polynomial degradation kinetics). Thus, in experiments at lower concentration, all LAS degraded after approximately 50 hours: this time increases progressively as LAS concentrations rise, to values of 150 hours for the experiment carried out at 400 ppm (the exponential slope diminishes). After this phase, the curve adopts a virtually zero slope and the biodegradation process ends.

The residual LAS concentrations in each experiment rise with LAS concentrations fed to the reactor similar to the decrease found for the carbonated substrate (Fig. 6a), although with no direct proportionality. Initially, an almost linear relationship is noted up to a concentration of 100 ppm from which level the data around 25 ppm non-biodegradable LAS (Fig. 6b).

In the evolution of the different homologues it is observed that, at low concentrations, the curves described are similar to those encountered for total LAS, and all of them adjust to decreasing exponentials (first order kinetics) which become similar to those described by *Quiroga* (1991) as the initial LAS concentration is increased in the test.

For each particular homologue, a different evolution was noted. Although homologue C_{10} was found in a small percentage in comparison with the total initial LAS feed in each experiment, the curve defining its evolution kept the initial feed value constant, indicating small or zero degradation of this homologue.

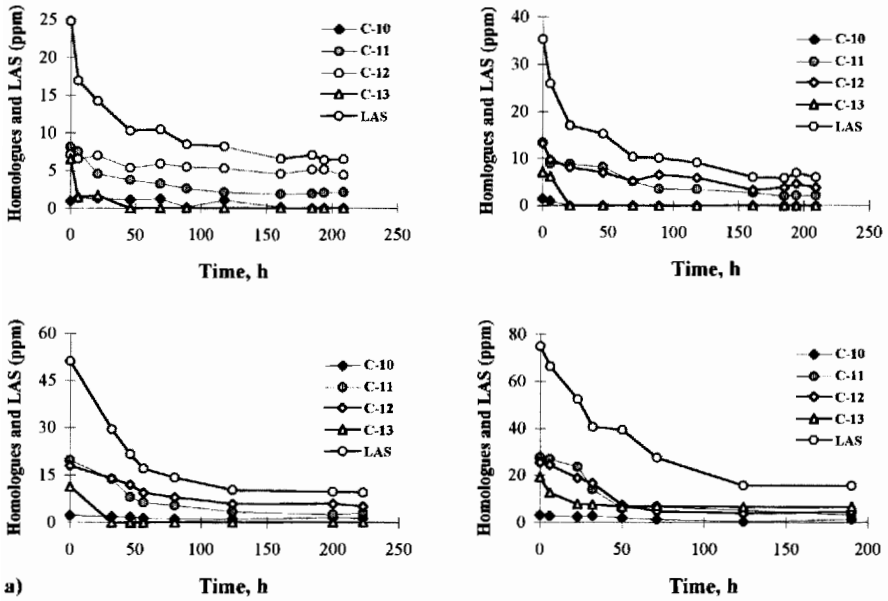


Fig. 5a. Development of homologues and LAS (ppm) in experiments with initial LAS concentrations: 25, 35, 50 and 70 ppm

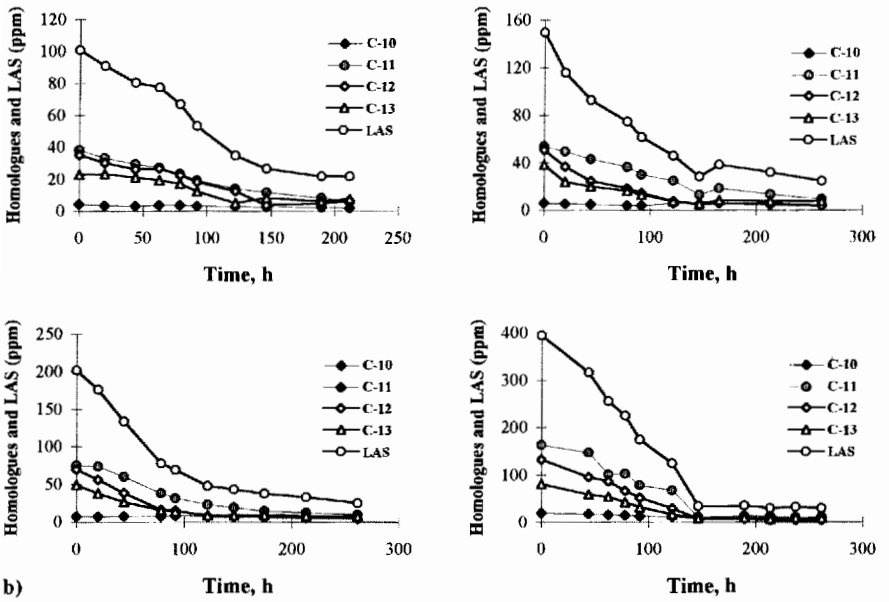


Fig. 5b. Initial LAS concentrations 100, 150, 200 and 400 ppm

Homologues C_{11} and C_{12} are present in greater relative concentration than other isomers. With low total LAS concentrations in the medium, C_{12} homologue degrades most slowly and contributes most to the total residual LAS concentration. However, as a 70 ppm total LAS, the C_{11} homologue contributes the largest quantity to the residual LAS.

4 Conclusions

The following conclusions may be drawn from the experiments performed:

- Linear alkylbenzene sulphonates (LAS) affect pH self-regulation capacity in aerobic degradation of organic matter. Initial LAS concentrations under 20 ppm only bring about temporary change. Thus microorganism activity itself is able to neutralise the medium, as occurs in LAS-free media. Initial LAS concentrations over 20 ppm do not allow the pH to self-regulate. Fermentation media acidify spontaneously and microbial activity stops. In these cases, the inhibition caused by high LAS concentrations and the acidity of the medium itself on the flora responsible for the process, reduce their activity. External neutralisation of the medium enables the organic matter degradation process to develop irrespective of the added LAS concentration. Moreover, external neutralisation provokes rapid proliferation of the microorganisms responsible for the process, and a sudden drop in COD.
- LAS affect the degradation capacity of organic matter in aerobic processes: LAS concentrations under 100 ppm do not affect the degradation capacity of the process. The aerobic microorganisms inoculated are from municipal sewage treatment plant and are acclimatised to concentrations of this order of magnitude. With initial LAS concentrations over 100 ppm an initial induction period is noted which increases with the concentration of added LAS.
- LAS concentrations added to the medium determine its assimilation capacity. In the range 0–100 ppm, a linear dependence is noted between the concentration added and the residual LAS concentration of the medium. However, at higher initial LAS values, residual concentration stabilises around 25 ppm.

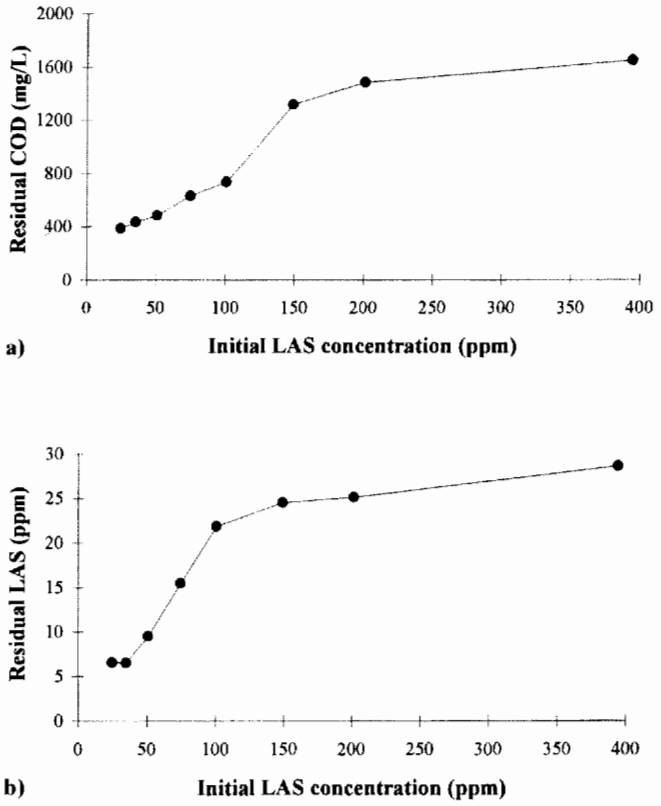


Fig. 6. Development of: a) residual COD (mg/L) and b) residual LAS (mg/L) to different initial LAS concentrations

- Of the four homologues present in the LAS, it is confirmed that those with the shortest chains are the ones with the most marked toxic effect and their initial concentration determines non-biodegradable organic matter concentration.

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