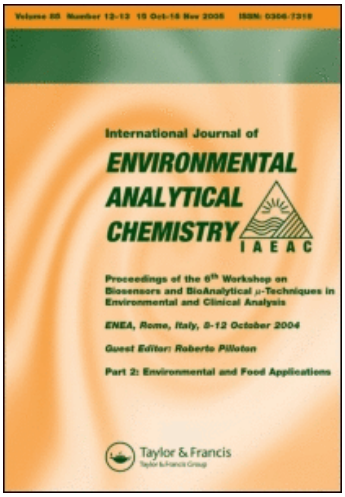

International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Determination of Las in Wastewater Treatment Plants: Comparative Study Between Conventional Biodegradation Testing and an Alternative Photo-Oxidation Method

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To cite this Article Boudenne, Jean-Luc , Coulomb, Bruno , Djellal, Leila and Théraulaz, Frédéric(2001) 'Determination of Las in Wastewater Treatment Plants: Comparative Study Between Conventional Biodegradation Testing and an Alternative Photo-Oxidation Method', International Journal of Environmental Analytical Chemistry, 81: 1, 55 — 72
To link to this Article: DOI: 10.1080/03067310108044358
URL: http://dx.doi.org/10.1080/03067310108044358

Intern. J. Environ. Anal. Chem., Vol. 81, pp. 55–72
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DETERMINATION OF LAS IN WASTEWATER TREATMENT PLANTS: COMPARATIVE STUDY BETWEEN CONVENTIONAL BIODEGRADATION TESTING AND AN ALTERNATIVE PHOTO-OXIDATION METHOD

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(Received 5 August 2000; In final form 22 June 2001)

The aim of this paper was to study the reaction pathway of the biodegradation and photodegradation of Linear Alkylbenzene Sulphonates (LAS), and to propose an analytical method, faster and more reliable, to predict the evolution of LAS in a sewage treatment plant. For this purpose, a $20\,mg\,L^{-1}$ LAS solution was treated on the one hand by a conventional biodegradation method using microorganisms, taken directly from aeration tanks of a sludge treatment plant, and on the other hand by UV radiation. This second method allows a rapid determination of LAS and its by-products by means of a UV signal treatment. This alternative method seems thus to be more suitable for on-line analysis.

Keywords: LAS; Biodegradation; UV photodegradation; Wastewater treatment

INTRODUCTION

The study of tensioactive biodegradation has developed significantly as a consequence of the evolution occurred in the detergent industry. Despite

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the advantages of the new products, they are viewed with certain misgivings by some operators of sewage treatment plants. The synthetic surfactants began to be noticeable in wastewaters, treated sewage, and the receiving waters because of the same properties which had led to their success: they retain their foaming properties in natural waters at concentration as low as approximately $1\ mg\ L^{-1}$.

These foams may cause different kinds of problems:

- poor aeration of activated sludges, preventing harder flocculation of particle size matter and the normal process of decantation [1,2];
- an anaerobic phenomenon in digesters and in septic tanks;
- they are said to be significantly involved in the formation of greases in sewers [3];
- in windy conditions, they are dispersed over large distances, spreading toxic products (especially metals) [4].

Despite this, chemical foams, arising from the so-called "biodegradable" detergents, have been relatively studied. The aim of this paper is to propose a new methodology for the study of organic molecules which are responsible for the chemical foaming observed in the field of water treatment, which particular reference to Linear Alkylbenzene Sulphonates (LAS), which are the most widespread detergents [5], and their by-product compounds.

Analytical methods commonly used present two main drawbacks:

- the standard method [6,7] called MBAS (Methylene Blue Active Substance) is non-specific to LAS and is subject to many interferences [8];
- alternative methods, such as GC-MS or HPLC techniques, are more relevant for the precise determination of LAS, but they are time consuming (owing to a necessary step of extraction) and expensive, and are unsuitable for *in-situ* analysis.

In fact, few techniques exist which take into account not only the disappearance of LAS but also the impact of LAS by-product compounds [9–11].

A simpler and quicker procedure for on-site analysis is thus required. This paper presents the elaboration of a new procedure: UV-spectrophotometry, coupled with a UV-spectra deconvolution step, will be used and applied to the study of the behaviour of LAS and their by-products, during two kinds of degradation, biodegradation and photodegradation. This procedure will be validated on the one hand by determination of by-products with the use of High Liquid Phase Chromatography (HPLC) coupled with UV detection,

and on the other hand by a simple and rapid method based on the study of UV spectra obtained at different stages of the degradation.

EXPERIMENTAL

Material

Ultrapure water (Milli-Q, $\rho = 18\,M\Omega\,cm^{-1}$) was used throughout the work. Samples were mainly collected from the aeration tanks of the activated sludge treatment plant located in Aix-en-Provence (Bouches-du-Rhône, France). Natural water samples were taken at the outlet of the treatment plant.

The model tensioactive studied was LAS (a mixture of isomers and homologous compounds, Fig. 1), purchased from Fluka Chemika (Buchs, Switzerland), was prepared from a $1\,g\,L^{-1}$ solution.

UV spectra acquisition was carried out with an Anthelie SECOMAM spectrophotometer (Domont, France) between 200 and 350 nm, with a 10 mm optical pathlength.

Experimental procedure

The experimental procedures used in this paper were of two kinds. The first was a biodegradation test aimed to reproduce the natural conditions of LAS degradation either in a treatment plant or in natural medium. This method has been used by many other authors, as explained below, in order to mimic natural conditions. The second was a photo-oxidation test which may be

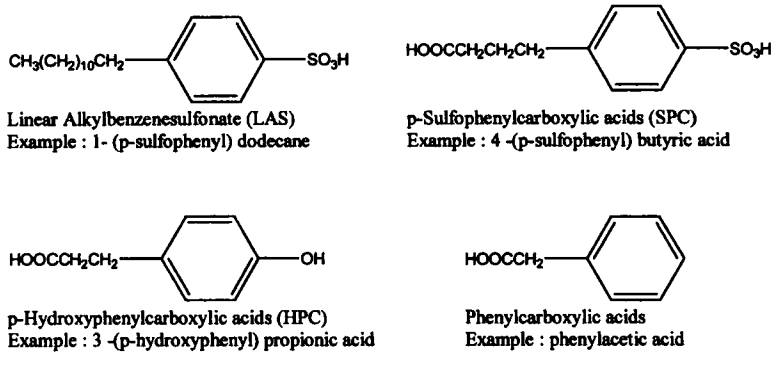


FIGURE 1 Structure of LAS and its potential sulfonated and unsulfonated metabolites.

proposed as an alternative method to the conventional biodegradation test, or at least as a complementary experiment.

Biodegradation test

Among the many biodegradation tests [4,12], a modification of the Joret and Levi test was chosen [13]. It was used to assess the amount of degradable carbon, first biologically, and secondly, by means of UV spectrophotometry, with the aim of characterizing the Biodegradable Dissolved Organic Carbon (BDOC) [14]. This study was used to monitor LAS biodegradation. It consists in comparing the growth of microflora on an inert sandy support with that of the organic load of the sample to be analyzed.

Two kinds of sands were used. The first was a "Fontainebleau sand", with a grain size from 150 to 210 μm (PROLABO, France), and the second was a siliceous sand from a beach located in Marseille (Bouches-du-Rhône, France) with an average grain size of 0.5 mm. Sands were cleaned in three steps before use: first a 24 h wash with a mixture of ultrapure water and hydrogen peroxide (80/20, v/v), secondly with a 24 h wash with hydrochloric acid and finally, a rinse with ultrapure water until a flat UV spectrum was obtained. Sands were then dried in an oven at 100°C.

Afterwards, the sands were mixed with the bacterial flora taken from the treatment plant by a continuous shaking for a period of 17 h. No evidence of any change in microorganism adsorption onto the support after a longer duration was observed. Bulks used for this sowing step were 350 mL of effluent for 60 g of sand. The sand thus activated was placed in flasks with a capacity of 500 mL, with a proportion of 50 g of sand for 150 mL of LAS to be degraded.

Photodegradation tests

These tests were carried out by means of a photochemical reactor which consisted of:

- a low pressure mercury UV lamp (PenRay, UVP). The lamp (radiating part) was 22.86 cm in height and 0.95 cm in diameter. Power generated at 254 nm was 42 $\mu W\,cm^2$ at a distance of 30 cm. Electrical current was 18 mA, which provided 90% of the ray at 254 nm. The remaining 10% was composed of rays at 185, 313, 365, 405, 436 and 546 nm. The photonic flux was determined by actinometry and was 2.299×10^7 Einsteins sec^{-1}.

- a quartz loop (Suprasil quality), which allowed the use of the ray at 185 nm, consisting of a solenoid 23 cm in height and with an inner diameter of 2 mm. The radiation loop and UV lamp both were covered with an aluminium hood.
- a flowing system in the form of a peristaltic pump with a flow rate of 8.1 mL min^{-1}.

The flowing pipes connecting the different elements of the system had an inner diameter of 0.8 mm. The total volume of each sample was 9.2 mL. The radiation time of the sample was 55 s, as compared to the 68 s required for the sample to flow through the whole system.

Before each experiment, the flow system was rinsed with hydrochloric acid 1 M for nearly one hour. Before starting up the experiment, the zero value of the spectrophotometer was adjusted with ultrapure water which flowed through the system.

Degradation kinetics was monitored by automatic acquisition of UV spectra recorded at regular intervals.

Analytical methods

UV spectrophotometry analysis of LAS

The analytical tool used for the determination of amounts of LAS was a semi-deterministic method for UV spectral deconvolution, described elsewhere [15,16].

As already mentioned, the commercial LAS product chosen as a reference product was made up of a mixture of isomers and homologous compounds. However, the UV spectrum of this product possess only two specific wavelengths, 223 nm (maximal absorbance) and roughly 255 nm, irrespective of the alkyl chain length and/or position of the aromatic ring [4,17].

HPLC analysis of LAS

This method has the advantage to determine accurately the amounts of LAS and, with some adaptations, is able to provide indications concerning the formation of metabolites (SPC) [8–10]. The columns used were a Waters μ-Bondapak C-18, with a length of 30 cm and an inner diameter of 3.9 mm, and a granulometry of 10 μm for LAS determination, and an Aminex HPX-87H (Biorad, France) styrene divinyl-benzene ion exclusion column with 0.05 M sulphuric acid as the mobile phase and flow rate of 0.5 ml min^{-1} for carboxylic acids.

Elution was carried out by means of a linear gradient between two eluents: $NaClO_4$ 0.15 M and $NaClO_4$ 0.15 M with CH_3CN/H_2O (70:30, v/v) and a flow rate of 1 mL min^{-1} for LAS determination. The injection value (Rheodyne) allowed the constant injection of a 5 mL sample into the column. To optimize the resolution of the eluted products, a Pye Unicam multichannel detector (at 223 nm) coupled with a Shimadzu C-R3A integrator was used.

Ionic Chromatography (IC) analysis of sulphate ions

During the degradation, sulphate ions can be generated by oxidation of the sulphonate group. To determine the concentration of these ions, a Dionex DX-100 Ion Chromatograph, equipped with an Ionpac AS4A-SC column with a length of 25 cm and an inner diameter of 4 mm was used. The eluent was a mixture of 1.8 mmol of Na_2CO_3 with 1.7 mmol of $NaHCO_3$, which flowed through the column at a rate of 2 mL min^{-1}. Detection was made by means of conductimetry. The injection valve had a capacity of 20 μL.

RESULTS

LAS biodegradation test results

Two following tests were included in the experimental setup:

(a) (experimental test) performed with the activated support (sand + microorganisms) and the LAS solution to be degraded.
(b) (negative control test) made with the activated support and ultrapure water, and used to monitor the activity of the bacterial flora.
(c) (positive control test) made with the support and ultrapure water.

Evolution of control tests

The control tests (b and c) were carried out in the same conditions as the experimental test, i.e. at room temperature, in day light and in a non sterile atmosphere. Figures 2 and 3 show the evolution of UV spectra control tests, from the beginning to the end of the experiment after 20 days.

At initial time, UV spectra of (b) and (c) samples presented almost no absorbance in the considered range of wavelengths. As far as the experiment

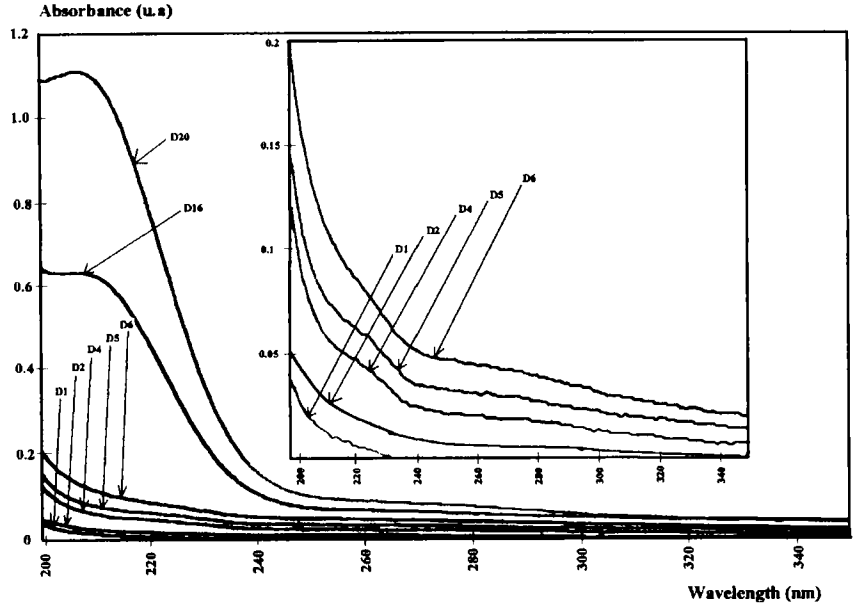


FIGURE 2 Evolution of UV spectra during negative control test (D1: after 1 day; D2: after 2 days; etc...).

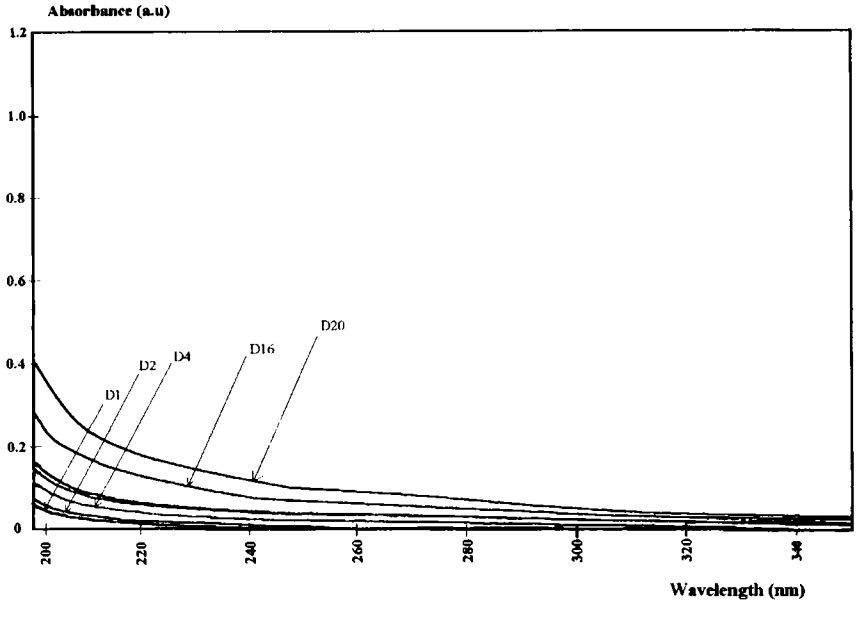


FIGURE 3 Evolution of UV spectra during positive control test.

progressed, an increase of absorbance appeared between 200 and 240 nm. These absorbance readings were relatively low, only 0.2 a.u. (absorbance unit), until the sixth day. Some differences appeared between the two tests towards the end of the experiment as from the sixteenth day. While the (c) absorbance UV spectra continues to increase in a similar manner up to 0.4 a.u., the shape of (b) UV spectra was modified with a sizeable increase of absorbance between 200 and 240 nm, reaching 0.65 a.u. after sixteen days and 1.1 a.u. after twenty days.

The modification of the shape of (b) spectra could be attributed to bacterial activity, probably because of exudates and of dead organism degradation compounds, and because of oxidized forms of nitrogen. Indeed, proteins, nucleic acids from microorganisms and nitrate ions absorb in the UV region between 220 and 240 nm.

Slight modification of (c) spectra can be attributed to ambient contaminations, or even to a redissolution of sand-containing minerals.

LAS biodegradation study

Formation of LAS by-products

UV spectra acquired during the first eight days of the (a) experimental test (Fig. 4) represent a very important step, because of the first modification of

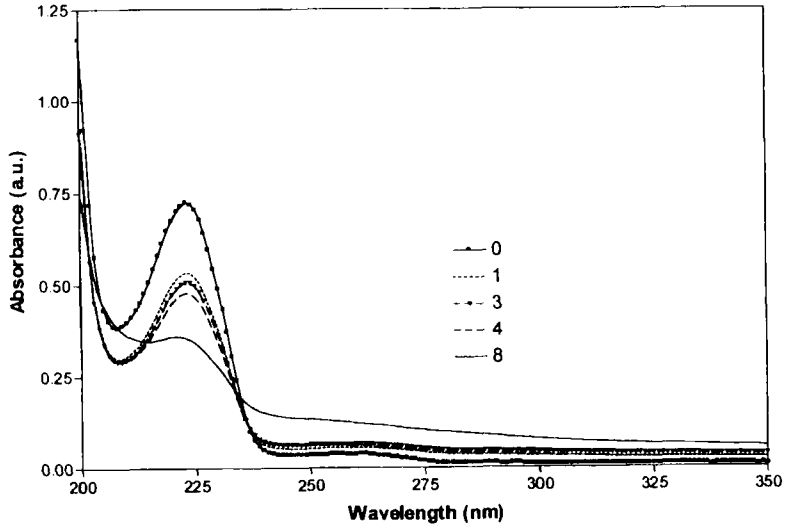


FIGURE 4 Evolution of UV spectra during the first eight days of LAS biodegradation.

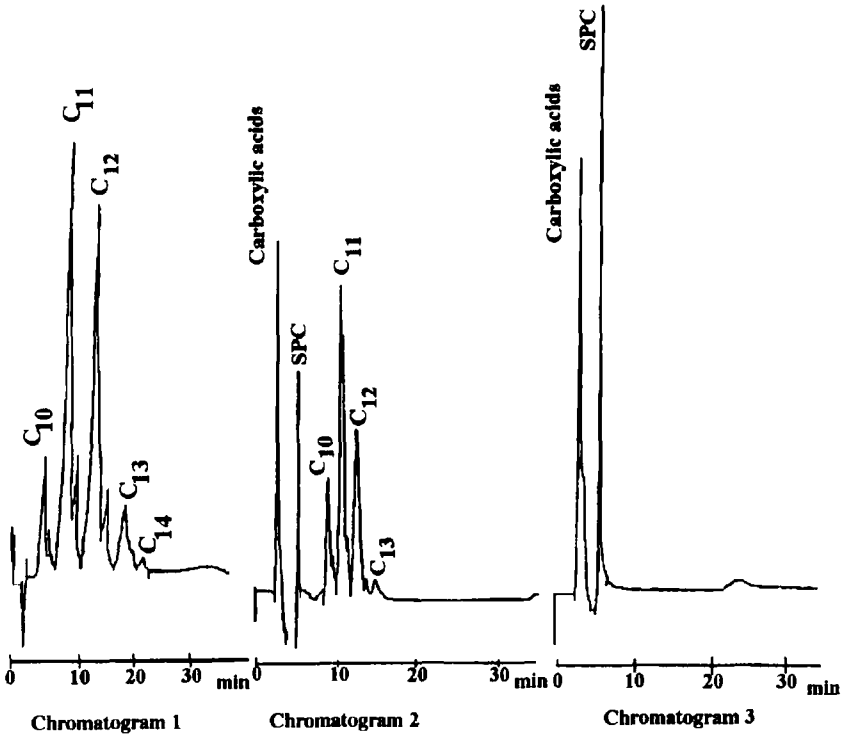


FIGURE 5 LAS biodegradation followed by HPLC analysis.

the LAS molecule. All spectra present a common point at 235 nm. This isobestic point reveals the presence of two compounds, or groups of compounds, in the reaction medium [18]. In order to characterize these different products which absorb at the same wavelength, HPLC analysis was used.

Figure 5 shows the chromatograms corresponding to samples taken from the flasks before and after LAS biodegradation. Before degradation (Chromatogram 1), peaks relative to the different LAS homologous (C_{10} to C_{14}) were observed. During degradation (Chromatogram 2), two new peaks appeared. The first peak at shorter retention time (2 min) may be carboxylic acids which are all eluted at the same time. The second, at 5 min, corresponds to sulphophenyl carboxylic acids (SPC), which are the only derivatives detected during biodegradation [19]. They are formed by ω and β-oxidation which leads to a decrease in the alkyl chain length (up to 4 or 5 carbon atoms).

Chromatogram 3 obtained after eight days of biodegradation shows the disappearance of the different LAS components, and the remaining of the intermediate by-products (SPC and carboxylic acids).

Like LAS, SPC have a maximum absorption at 223 nm but their absorbance peak at about 235 nm is higher than that of LAS [20]. It is thus possible to highlight the primary biodegradation of LAS and the formation of its by-products by UV spectrophotometry.

Formation of ultimate residues

The most characteristic spectra of the main stages of the degradation of a 20 mg L^{-1} LAS solution are represented in Fig. 6. The D16 spectrum

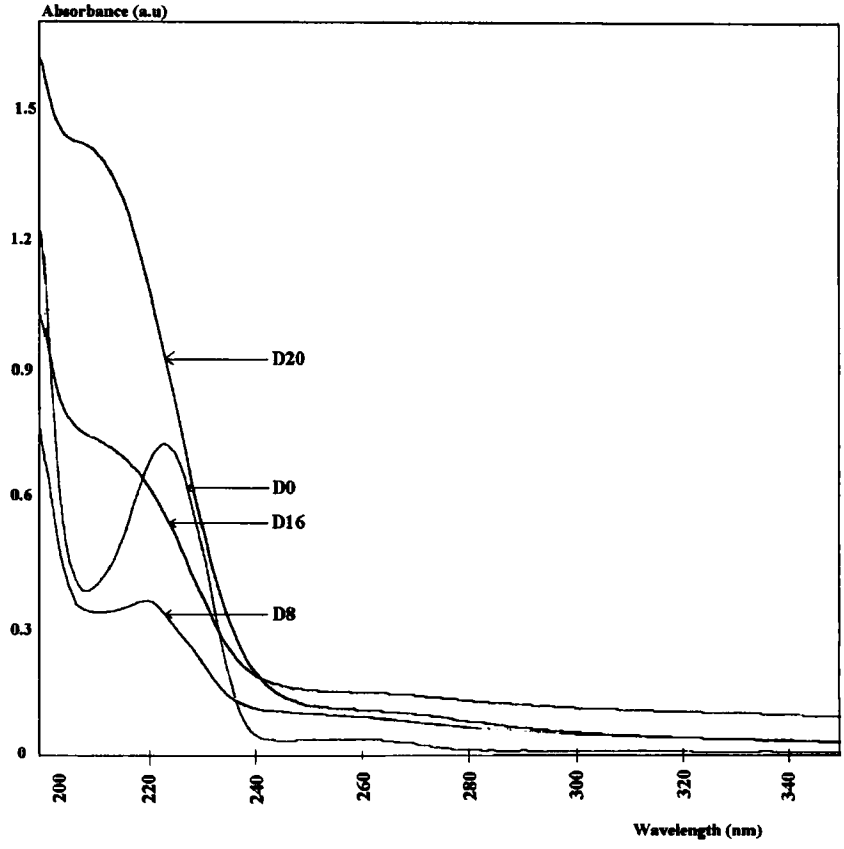


FIGURE 6 Following of LAS biodegradation by UV spectrometry (D0: initial time, D8: after 8 days, D16: after 16 days; D20: after 20 days).

presents a different shape with an increase in absorbance between 200 and 240 nm. The D20 spectrum presents a shape similar to the previous spectrum, with an increase of absorbance between 200 and 240 nm. These two spectra present a shape comparable to those acquired during the control test (b) (Fig. 2), and it may be assumed that D16 and D20 spectra result from more intensive bacterial activity than that observed during control test (b).

IC Chromatograms after 16 and 20 days of biodegradation show the presence of nitrites, nitrates and sulphates. Chromatograms obtained by HPLC after 16 and 20 days show the presence of SPC. In the experimental conditions used, mineralization of LAS was thus not complete.

Until the eighth day of biodegradation, no sulphate ions were detected by IC in solution. After 16 and 20 days, the concentration of sulphate ions in solution was high. According to Karsa [20], Schöberl [21], and Swisher [4], the cleavage of sulphonate groups can only occur after ring-opening of aromatic cycles. In view of all these considerations, a reaction pathway of LAS biodegradation may be proposed (Fig. 13).

This biodegradation method leads to three major conclusions:

- the results of LAS biodegradation are consistent with those found by many other authors, despite the fact that the present procedure was not exactly the same as those used elsewhere [4,20,21];
- after 8 days, mineralization of LAS had not been completed which shows that detergents are not fully degraded in a wastewater treatment plant, and that biological methods conventionally used to quantify surfactants always underestimate the real amount of these organic pollutants;
- results obtained by UV spectrophotometry were confirmed by HPLC analysis. UV spectrophotometry is thus an interesting and a rapid analytical tool to monitor the primary and ultimate biodegradation of LAS. It can be supposed that chromatographic methods could be advantageously substituted by UV spectorphotometry, for on-line monitoring LAS biodegradation in a sewage network or in a biological treatment plant.

LAS photodegradation results

Photodegradation of 20 mg L^{-1} LAS solution

The photodegradation test used, coupled with an UV spectrophotometer, allows the acquisition of numerous UV spectra at frequent intervals. Spectra obtained during the first two minutes of the degradation are

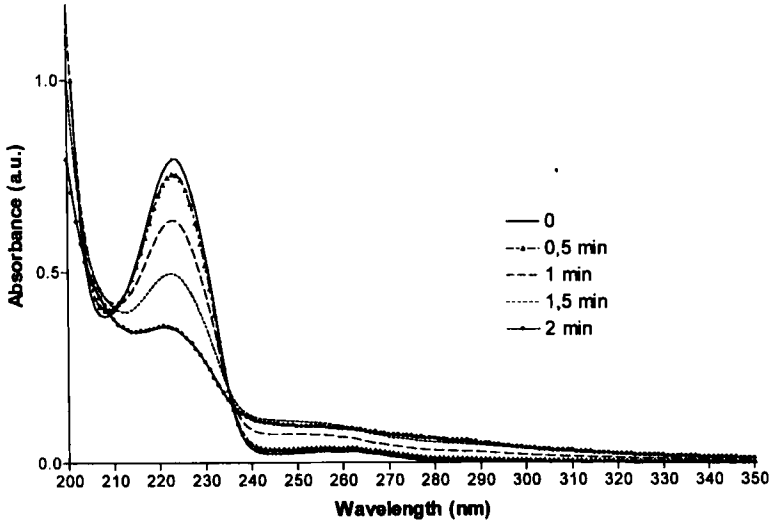


FIGURE 7 UV spectra of 20 mg L^{-1} LAS submitted to UV radiations during 2 min.

shown in Fig. 7. All these spectra presented an isobestic point at 235 nm. A continuous decrease of absorbance at the shoulder at 223 nm was observed, while a continuous increase of absorbance beyond the isobestic point is also observed.

The presence of an isobestic point revealed that two compounds, or groups of compounds were in solution. HPLC analysis confirmed this observation (Fig. 8). These results are comparable to those obtained during the biodegradation test. Indeed, in both cases, an isobestic point was observed at 235 nm. In this experiment, a LAS degradation product was formed after only 0.5 min. The appearance of SPC was coupled with the formation of carboxylic acids, as during the biodegradation test.

The difference between the two tests is the reaction time: sulphate ions are formed after only a few minutes and two minutes suffice to degrade LAS, as compared to 8 days during the biodegradation test.

The photodegradation reaction can be monitored by the evolution of the concentration of sulphate ions, according to the following reaction:

$$C_{18}H_{30}SO_3^- + 25.75\,O_2 \longrightarrow 18\,CO_2 + 14.5\,H_2O + SO_4^{2-} + H^+$$

Figure 9 shows the evolution of the concentration of sulphate ions obtained by IC. After 10 min, the concentration of sulphate ions was 6.1 mg L^{-1}, which corresponds to the maximum concentration possible with a 20 mg L^{-1} initial LAS solution.

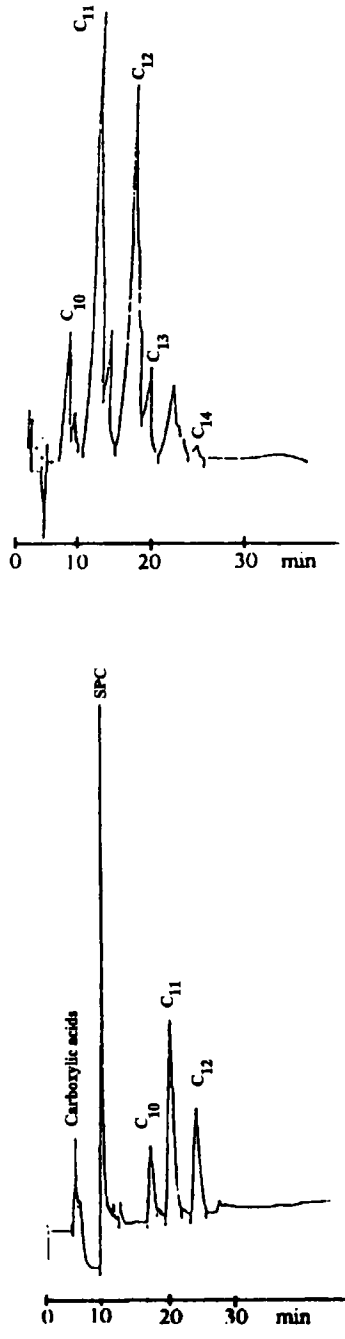


FIGURE 8 HPLC Chromatograms obtained before (a) and after 2 min of UV radiations (b).

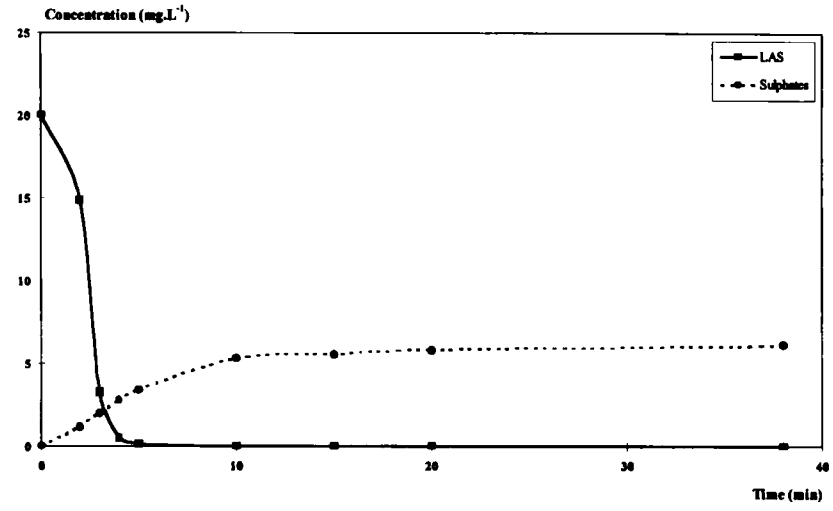


FIGURE 9 Evolution of LAS and sulphate ions amounts during the photo-oxidation of 20 mg L^{-1} LAS.

A 3D spectral representation allows a visualization of the evolution of spectra (Fig. 10). The initial spectrum of LAS solution, obtained before UV radiation, presents a characteristic shoulder at 223 nm. As soon as the LAS solution is submitted to UV radiation, this absorbance decreases. After 37 min of degradation, the spectrum obtained is almost flat.

Determination of the reaction pathways

From the observation of the UV-graph presented above, four characteristic spectra can be selected (Fig. 11), each of them representing an important stage in LAS biodegradation:

- S1: LAS solution spectrum, before UV radiation;
- S2: spectrum obtained after 2 min of UV radiation, taken from the whole of the spectra which have an isobestic point with the first spectrum;
- S3: spectrum obtained after 6 min of UV radiation and presenting a sharp decrease of absorbance;
- S4: spectrum of nearly zero absorbance, obtained at the end of the degradation.

The first stage is characterized by a significant degradation of LAS (S1), and by the appearance of SPC (S2). S3 represents the disappearance of the isobestic point with all the other spectra after 6 min of UV radiation. This stage was monitored by the evolution of the concentration of sulphate

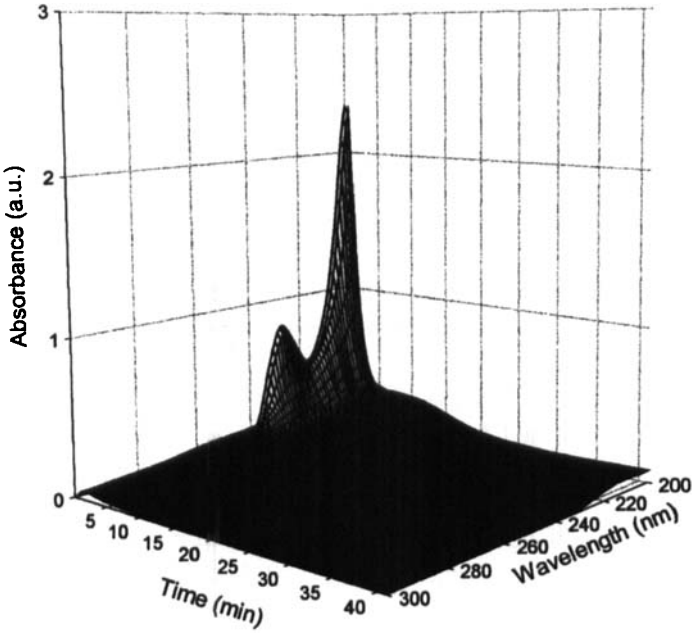


FIGURE 10 3D-UV graphy of the photo-oxidation of 20 mg L^{-1} LAS.

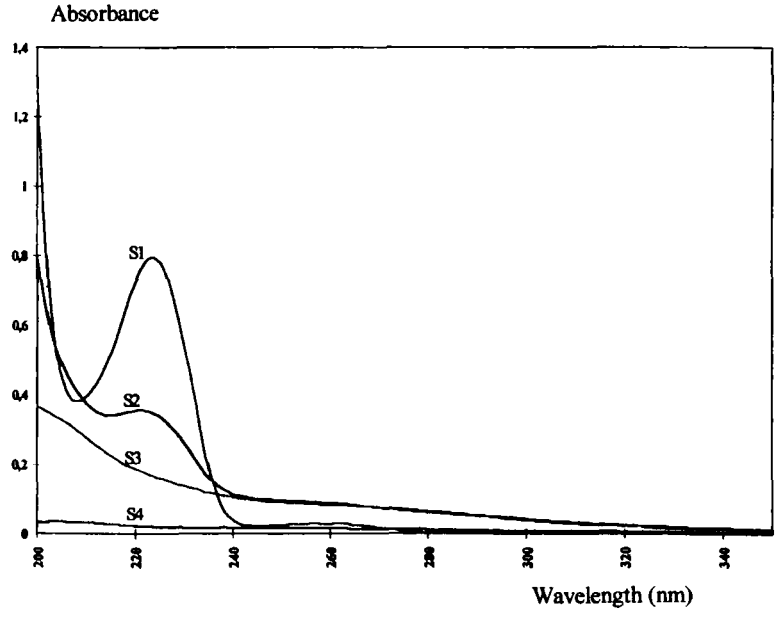


FIGURE 11 The four characteristic UV spectra of the photo-oxidation of LAS.

ions. Indeed, after 5 min of UV radiation, LAS were fully degraded, while only 50% of sulphate ions were formed. This means that the first stage of the reaction is the formation of SPC, which possess a sulphonate group. After 10 min of UV radiation, all the sulphate ions are formed. This means that between 5 and 10 min, a desulphonation reaction occurs before the aromatic ring-opening, contrary to what happens during biodegradation, where the oxidation of sulphonate group is the last stage of LAS degradation.

The aromatic ring-cleavage takes place between S3 and S4, where a mixture of intermediate compounds can be observed, because of the absence of an isobestic point (Fig. 11).

Each one of these spectra (S1 to S4) can be used for the restitution of spectra obtained during LAS photodegradation, by a linear combination of these reference spectra. The principle of this method, called "spectral deconvolution" [22] is that the shape of any UV spectrum of water, S_w, can be mathematically decomposed (with a matricial procedure) in a linear combination of reference spectra ($Ref_1, \ldots, Ref_i$) related to characteristic states or compounds of water.

$$S_w = \sum_{i=1}^{p} a_i \times Ref_i \pm r$$

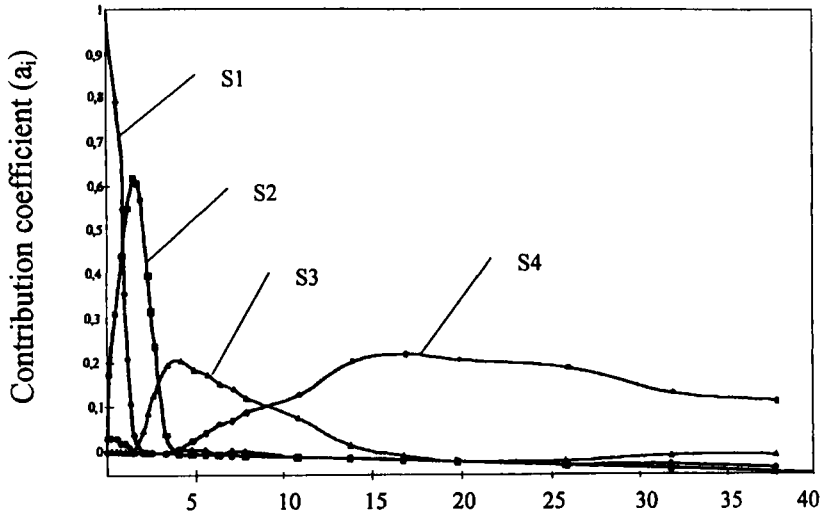
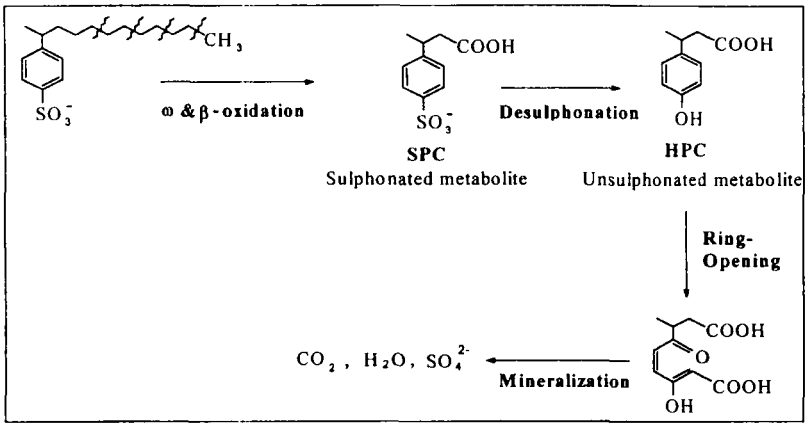
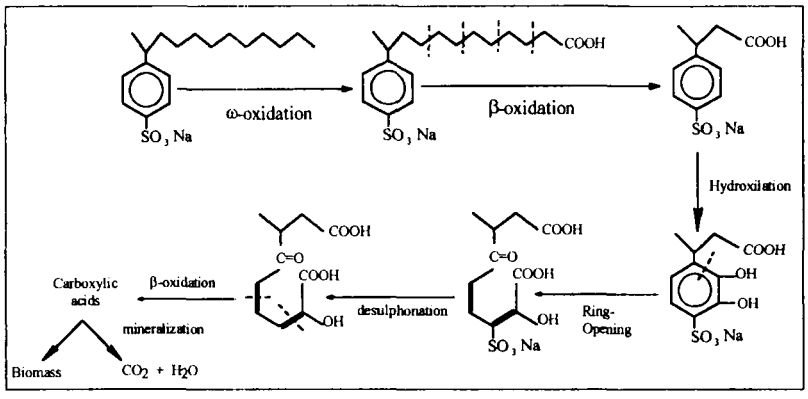


FIGURE 12 Evolution of coefficient contribution of the four reference spectra (S1 to S4) during the photodegradation of LAS.



A



B

FIGURE 13 Reaction pathways for photodegradation (A) and biodegradation (B) of LAS.

where a_i is the contribution coefficient of the reference spectrum concerned and r is the admitted error or restitution.

This semi-deterministic multiwavelength method was applied to the monitoring of LAS photodegradation. The evolution of the contribution coefficient of each spectrum is shown in Fig. 12. The contribution coefficient of S1 decreases very quickly because of LAS degradation while a new group of compounds is formed. The latter disappear after 5 min (S2); these compounds are SPC. Another group of compounds is formed (S3), which are

unsulphonated compounds (HPC), obtained during the SPC desulphonation reaction. The evolution of the contribution coefficient of S4 should correspond to the aromatic ring-opening and to the mineralization of LAS.

The restitution error of each spectrum acquired during the photodegradation of LAS was always below 0.005%. This implies the four reference spectra were chosen correctly and sufficed to explain the reaction pathway of LAS photodegradation: each time the UV spectrum acquired can be explained by a linear combination of the four reference spectra.

By following the evolution of the contribution coefficient of each reference spectrum, LAS photodegradation kinetics, can be monitored, and the LAS reaction pathway proposed is presented in Fig. 13.

The use of this spectral deconvolution procedure can thus replace the use of HPLC analysis, and will allow on-line monitoring of LAS degradation in the near future.

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