Influence of Linear Alkylbenzene Sulphonates (LAS) on Microbial Activity of Activated Sludge

Mª D. Coello Oviedo*, D. Sales Márquez, and J. Mª. Quiroga Alonso
Department of Chemical Engineering and Food and Environmental Technology. Faculty of Marine and Environmental Sciences. Cadiz University. 11510 Puerto Real. Cadiz Spain.

Original scientific paper
Received: July 9, 2003
Accepted: May 24, 2004

The purpose of the present work was to study the influence of the anionic surfactant sodium linear alkylbenzene sulphonate, LAS, on the microbial population present in a activated sludge unit of a wastewater treatment plant. Both, traditional control methods to measure volatile suspended solids and chemical oxygen demand, and specific techniques for the measurement of microbial activity (dehydrogenase activity and specific oxygen uptake rate), were used and the results were compared. It is shown that activity measurements are more adequate for the control of biological system, concretely, the results obtained showed that the specific oxygen uptake rate is the simplest and quickest method to carry out a routine control. Three concentrations of LAS have been assay in the experiments (25, 50 and 100 mg L^{-1}). Inhibitions of microorganisms have been observed when the biggest mass concentration (100 mg L^{-1}) was added, although before this a maximum inhibition of microbial activity was observed. Likewise, LAS influences the degradation capacity of, both, organic matter and itself in aerobic processes, increasing the residual COD and LAS mass fraction obtained, and diminishing LAS biodegradation rate. When comparing biodegradation rates of the individual homologues, the highest rate was observed for those with the longest alkyl chain.

Key words:
microbial activity, activated sludge, dehydrogenase activity, LAS, specific oxygen uptake rate.

Introduction

In recent years there has been a widespread tendency to install wastewater treatment plants, in order to stop the progressive deterioration of natural sources of water supplies.

These plants receive mainly wastewater with a high organic load and carry out biologically based treatments, including those using activated sludge which is the most suitable. The effluent quality of the plant is therefore going to depend on the smooth running of this unit.

Wastewater normally contains organic compounds which can render the microbiota of the activated sludge systems inactive. These include surfactants, a basic component of detergents, such as the anionic surfactant linear alkylbenzene sulphonate (LAS), which can reach concentrations of up to 20 mg L^{-1} in the sewage treatment plants influent.[1,2]

Linear alkylbenzene sulphonate is the most widely used surfactant on an international scale. In 1995, 1493 × 10^6 Tm were used[3] which accounts for more than 18 % of world surfactant production. It is one of the synthetic substances that has most frequently been investigated in recent years[4,5] in various aspects: toxicity[6,7], environmental occurrence[8, 9] and biodegradation.[10]

In the present study, the effect of various concentrations of LAS on the microbiota in a pilot scale activated sludge unit applying, both, traditional control methods and specific techniques was investigated, in order to establish the most suitable method for routine control of the waste water treatment.

Materials and methods

Laboratory pilot scale plant reactor

A secundary sewage treatment laboratory system consisting of an aeration tank (volume: 3.5 L at normal operating conditions) and a clarifier (volume 2.5 L) was employed in this study (see Fig. 1).

The reactor was fed continuously with the culture medium (Table 1). As well as the synthetic nutrient, various concentrations of LAS (20, 50 and 100 mg L^{-1}) were added in order to study the possible effect of this on the microorganisms in the reac-

* Corresponding author: Mailing address : Department of Chemical Engineering and Food and Environmental Technology, Cadiz University, Polígono Río San Pedro s/n, 11510 Puerto Real, Cadiz. Spain. Phone: 34–56016158; Fax: 34–56016040. Electronic mail address: dolores.coello@uca.es

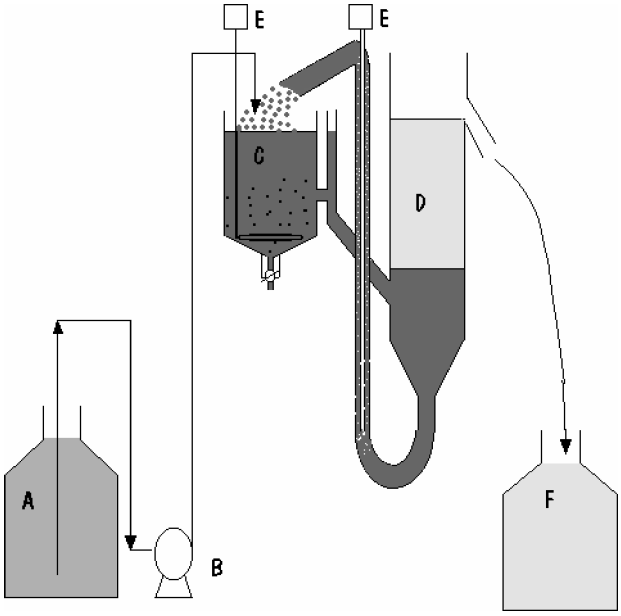


Fig. 1 — *Diagram of the laboratory–scale activated sludge treatment plant. A. Influent. B. Peristaltic pump. C. Aeration tank. D. Sedimentation tank. E. Compressed air pumps. F. Effluent*

Table 1 – *Composition of mineral nutrient solution*

Compaund	Mass concentration γ/mg L^{-1}
Peptone	160
Urea	30
Beef Extract	110
NaCl	7
$CaCl_2 \cdot H_2O$	4
$MgSO_4 \cdot 7H_2O$	2
K_2HPO_4	28

tor. The concentrations of 50 and 100 mg L^{-1} are higher than those normally found in the treatment plant. This is because our aim has been to study the possible inhibiting effect of the anionic surfactant, and not to reproduce the concentrations that are normally found in waste water.

The LAS was a typical commercial blend with a homologues composition which is reported in Table 2.

Analytical procedures

The functioning of the pilot plant was followed over time by periodic measurements of volatile supended solids (VSS) and by determinating the chemical oxygen demand of the effluent (COD), according to the Standard Methods.[11]

Table 2 – *Characteristics and composition of the substance (LAS) used in the biodegradation assays*

Reference Activity Molecular mass	
Composition	
$<C_{10}$ LAS	0,9 %
C_{10} LAS	14 %
C_{11} LAS	51.6 %
C_{12} LAS	31.8 %
C_{13} LAS	1.5 %
C_{14} LAS	0 %

Microbial activity was calculated by measuring dehydrogenase activity (DHA) as well as the specific oxygen uptake rate (SOUR).

The dehydrogenase activity was recorded by the method of Awong[12] consisting in the preparation of a solution of 3.95 mmol L^{-1} of INT 2-(ρ-iodophenyl)-3-(ρ-nitrophenyl)-5-phenyl-tetrazolium) chloride. Triplicate 5 mL samples were amended with two 3 mL of INT and 4 mL of solution buffer. After 60 min. of incubation in the dark, the reaction was interrupted by adding 100 mL of concentrated sulphuric acid.

The samples were then centrifuged at 1500 rpm for 10 min. The pellets which formed were extracted by consecutive ethanol (96 %) rinses in the dark, the extracts were combined together, and their optical density measured at 480nm. Dehydrogenase activity was calculated in oxygen equivalents (O_2*) using the following equation proposed by *Awong*:[12]

$$a_{\mathrm{DHA}} = \frac{1024 A_{480} v_{\mathrm{e}}}{V_{\mathrm{s}} \gamma_{\mathrm{VSS}} t D}$$

where:

$a_{\mathrm{DHA}} = a_{\mathrm{INT}}$ – dehydrogenase activity (mg O_2 g^{-1} VSS d^{-1}).

A_{480} = absorbance at 480 nm.

v_{e} = final volume of dissolvent used to extract formazan, mL.

V_{s} = volume of reactive used and sample treated, mL.

γ_{VSS} = concentration of volatile solids in the sample, mg L^{-1}.

t = incubation time, min

D = dilution rate, d^{-1}

The determination of the Specific Oxygen Uptake Rate (SOUR) was made on triplicate samples by filling a 50 mL bottle, inserting the electrode (CRISON OXI92), and recording dissolved oxygen concentration as a function of the time over a pe-

riod of approximately 20 min. Samples were assayed at room temperature. Bottles were sealed to prevent oxygen transfer and were stirred by a magnetic mixer. SOUR was calculated according to the following equation:[12]

$$\mathrm{SOUR} = \frac{1440 \cdot r}{\gamma_{\mathrm{VSS}}}$$

where:

γ_{SOUR} = Specific Oxygen Uptake Rate, mg O_2 g^{-1} VSS d^{-1}

1440 = factor converting minutes into days.

γ = oxygen uptake rate, mg O_2 L^{-1} d^{-1}

γ_{VSS} = biomass concentration in the sample, g L^{-1}

Determination of LAS

The anionic surfactant LAS was determined by a specific procedure based on reversed–phase high performance liquid chromotography (HPLC). The Fig. 2 shows the chromatogram of the results.

The method used was based on the Nakae method[13] and the working conditions employed were the following:

column: lichrosorb C18, d_0 = 5 μm, d_i = 4.6 mm, l = 250 mm

eluent: acetonitrile/water (45:55) 0.1 mol L^{-1} sodium perchlorate.

flow rate: Q_v = 1 mL min^{-1}

elution: isocratic

injection volume: 100 μL

detector: Fluorescence (emission wavelength: 290 nm, excitation wavelength: 232 nm.).

Results and discussion

Influence of different LAS mass concentrations on the microbial activity of the activated sludge

20 mg L^{-1} of LAS

Figure 2a shows the evolution of the volatile suspended solids (VSS) for a concentration of 20 mg/L of LAS. It can be seen that the level falls slightly during the first days of contact, until adaptation of the microbiota takes place on the third and fourth day. From this point, the VSS values remain practically constant for the rest of the assay.

As regards the COD values of the effluent, these increased in the first few days due to the elimination of part of the microbiota, and then fell in accordance with the decrease of volatile suspended solids. This implies a lower COD in the effluent, and therefore a greater elimination of the latter (Fig. 3a). This is easily explained by the fact that the reduction of superior microorganisms allows the bac-

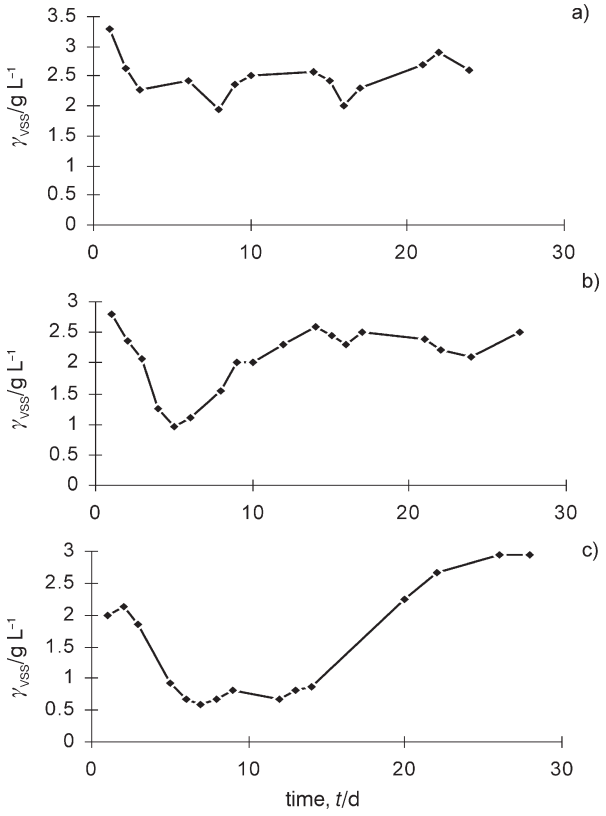


Fig. 2 – *Evolution of VSS for the different LAS mass concentration LAS used: a) 20 mg L^{-1}, b) 50 mg L^{-1} and c) 100 mg L^{-1}*

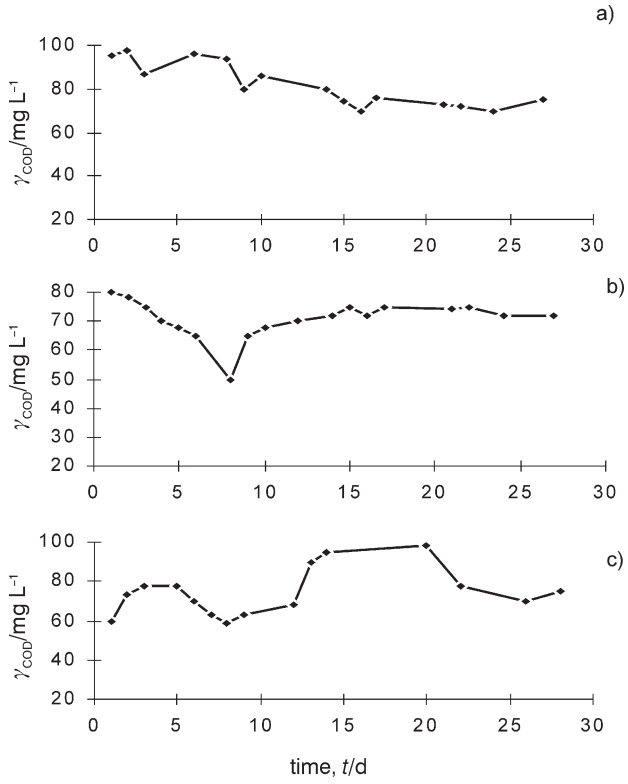


Fig. 3 – *Evolution of COD in the effluent for the different LAS mass concentrations used: a) 20 mg L^{-1}, b) 50 mg L^{-1} and c) 100 mg L^{-1}*

teria, which are the microorganisms that are mainly responsible for the elimination of organic material to develop better, resulting in a more numerous and active population which can improve effluent quality. However, the plant is not operating adequately, as good clarification does not take place due to the effluent's loss of biomass resulting from the lack of superior microorganisms. Once this period has passed, the efficiency of depuration returns to normal values, revealing at this point a certain adaptation of the microorganisms in the reactor to the presence of the surfactant.

Figure 4a shows the development of the dehydrogenase activity and the specific oxygen uptake rate under the conditions previously described. During the first three days of the microbiota's exposure to the surfactant, the microbial activity increases considerably. This effect has already been described by other authors[14] who observed that the initial increase in activity caused by contact with the toxin is followed by a decrease in activity due to the inhibition resulting from the exposure of the microbiota to the toxic compound.

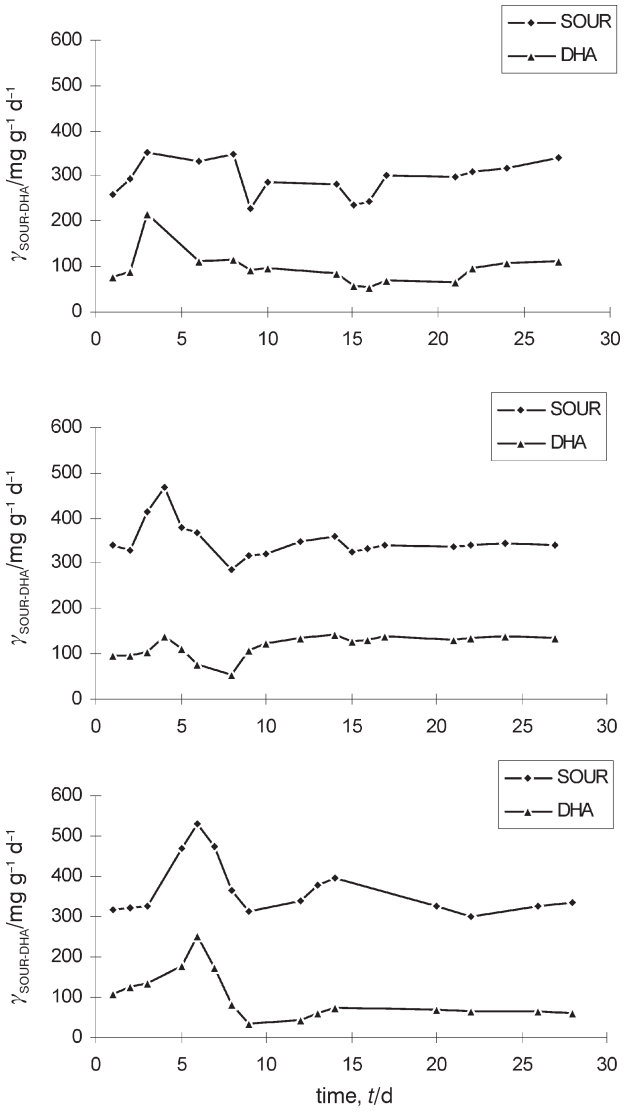


Fig. 4 – *Evolution of microbial activity for the different LAS concentrations used: a) 20 mg L^{-1}, b) 50 mg L^{-1} and c) 100 mg L^{-1}*

Microbial activity seems to be affected by the compound after the eighth day, when activity falls due to the decrease in the total microorganism population in the reactor (coinciding with the second minimum in the VSS values). As time passes, it seems that the microbiota adapts to the surfactant, experiences a recovery, and reaches the activity values that the system had before the surfactant was added.

If Fig. 2a and 3a are compared, it can be observed that the minimum values are obtained on days 9 and 15, coinciding with the beginning and end of the least active phase of the microbiota.

50 mg L^{-1} of LAS

At a higher surfactant concentration, a considerable decrease in the biomass of the system is observed, bringing about a 75 % reduction of the volatile suspended solids (Fig. 2b). This reduction is much greater than that observed with 20 mg L^{-1} of LAS. The system recovers from this minimum value. After 5 days the VSS values are normal (around 2.5 g L^{-1}).

As far as the efficiency of purification is concerned, i.e. the COD values, it can be seen in Fig. 3.b. that there is no significant decrease. The COD values of the effluent, while slightly higher than before the addition of LAS, are not excessively high. This may be due to an adaptation to the compound by the microorganisms as a result of the prior exposure to 20 mg L^{-1}, suggesting that the values of this variable are not affected by the increase in surfactant concentration.

Microbial activity also increases in response to the higher surfactant concentration, as can be observed in Fig 4b. A decrease in activity then takes place around the eighth day.

100 mg L^{-1} of LAS

Although such a high concentration of LAS is quite unrealistic, i. e. not likely to be reached in an activated sludge unit, these experiments were carried out to investigate whether such a high concentration of surfactant is lethal for the microbiota of the system.

Thus in Fig. 2c. it can be seen that the amount of biomass present in the unit during the first few days of contact with the compound decreases significantly, as in only 7 days the figure falls from 2 g L^{-1} to 0.6 g L^{-1}. The values for solids are much

lower than those reached in the previous assays, and the length of time during which the flora is inhibited is also longer. The effect on the microbiota is greater, especially in the superior microorganisms, which disappear as was observed, using optical microscopy. This disappearance causes a fall in flocculation levels, which in turn produces a loss of biomass through the effluent (the concentration of suspended solids in the effluent reaches up to 250 mg L^{-1} at some points). After this long period of inhibition, the flora begins a slow recovery, reaching normal values of suspended solids (2.5 – 3 g L^{-1}) by days 18 – 20. The starting values of biomass are even surpassed as a result of this postive evolution.

The inhibition resulting from the increase of LAS concentration affects the efficiency of the purification process, as is shown in Fig. 3c. The maximum COD value in the effluent occurs later than in the assays at 50 mg L^{-1} (approximately between day 13 and day 15, probably due to adaptation to the toxic compound). These values for the COD of the effluent are the highest reached in all the experiments, which is logical given the higher concentration of surfactant used, which seems to affect the bacterial population as well. The system later recovers, returning to purification efficiency levels of 80 – 90 %.

Figure 4c shows the evolution of dehydrogenase activity together with the specific oxygen uptake rate. The first effect on microbial activity in this assay is an increase in the SOUR (as also occurred with lower concentrations). This increase in activity microbial is followed by a decrease caused by the death and evacuation of microorganisms with the conditions environmental in the reactor. This period of inhibition continues for approximately 7 days, after which time the system recovers and returns to normal values. The microbial of the system shows adaptation to the compound added.

Relation between the residual concentration of LAS and activity microbial

The evolution of substrate consumption (surfactant) in the medium by the microbiota was monitored in order to confirm the results obtained with the measurement of microbial activity.

The relation between the percentage of surfactant elimination and microbial activity is similar for the three LAS concentrations tested. For example, figure 5 represents the evolution of the SOUR with the percentage of biodegradation of the substrate (LAS) in the effluent of the reactor for the assay with 100 mg L^{-1}.

In figure 5 it can be observed that at the beginning of the assay, microbial activity increases when a 100 mg L^{-1} concentration of LAS is added to the pilot scale plant (initial values of COD of 325.7 increase to 531.7 mg L^{-1}).

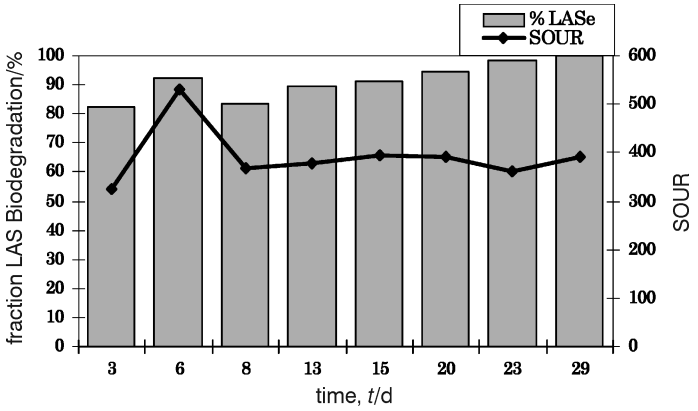


Fig. 5 – *Evolution of biodegradation percentage and microbial activity (SOUR)*

Table 3 – *Rate of biodegradation of LAS and LAS's homologs*

Days	Fraction LAS degradation %	Fraction C10 degradation %	Fraction C11 degradation %	Fraction C12 degradation %
3	82.1	49.69	81.06	96.75
6	92.1	77.4	93.32	96.34
8	83.3	72.14	84.31	84.34
13	89.7	74.68	89.74	95.65
15	90.9	81.51	91.21	93.87
20	94.5	88.09	94.69	97.12
29	99.6	99.32	100	100

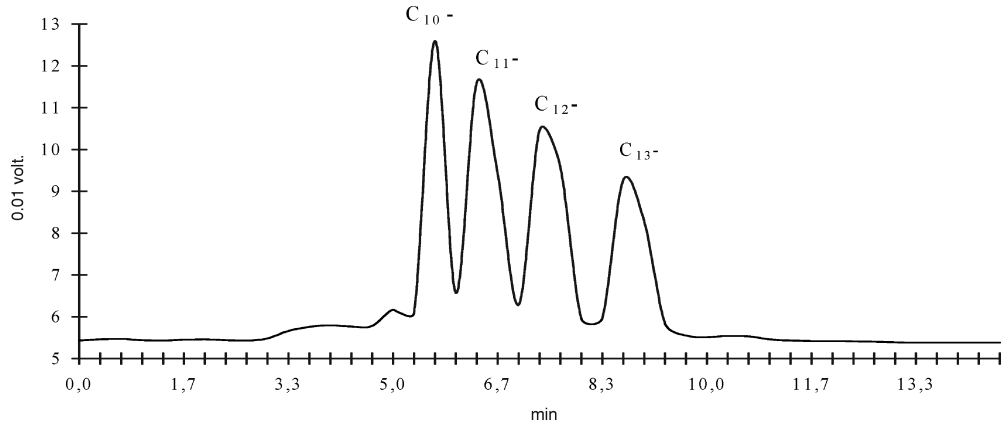
A recovery takes place after this period of inhibition and the microbiota is reestablished, followed by an increase in the SOUR and a decrease in the concentration of residual LAS.

Table 3 gives the percentages of biodegradation of the homologue and isomers of the LAS in the assay with 100 mg L^{-1}.

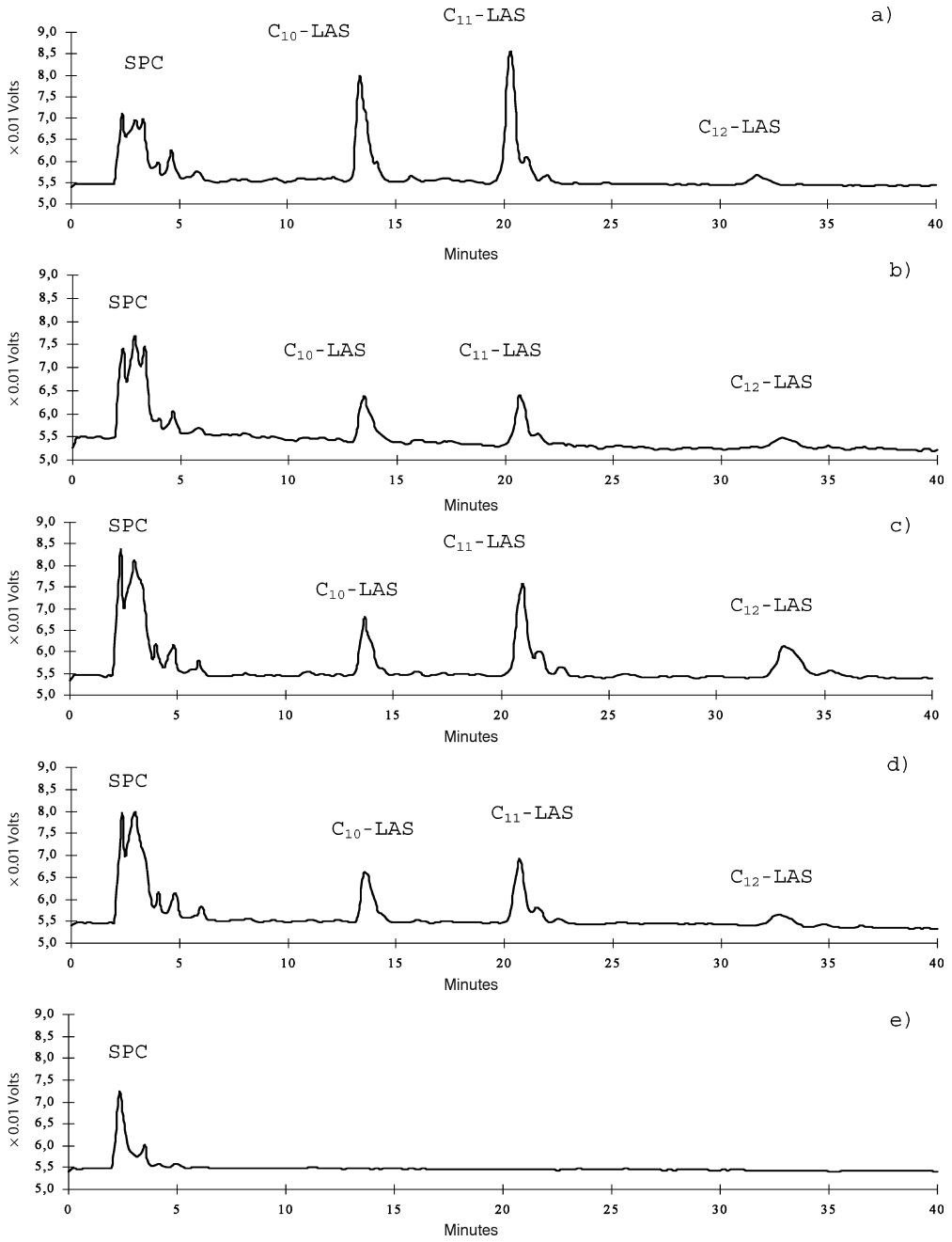
An increase in the percentage of biodegradation of the surfactant is observed when the duration of the assay is extended (as a result of a greater adaptation of the microbiota) except on day 3, due to the impact of the surfactant, and on day 8, which is when microbial activity decreases.

It can also be observed that the longer chain homologues degrade quicker than the shorter ones, in accordance with Swisher's distance principle.[15]

In the Figure 6 appear the chromatogram of initial LAS, and we can see that in it doesn't show the peak corresponding to sulphophenyl carboxylic acids. The degradation of surfactant hasn't begun



F i g . 6 — *Chromatogram of LAS in the beginning assay*



F i g . 7 – *Chromatograms for the following days of the assay: a) day 3 b) day 6 c) day 8 d) day 13 e) day 29*

yet. Figure 7 shows some of the chromatograms obtained during the assay with 100 mg L^{-1} of LAS. It can be observed that within each homologue, the most external isomers (C_x) are the most easily biodegradable, with ease of biodegradation increasing with the length of the chain (i.e. the homologue C_{12} degrades more easily than the homologue C_{10}). This is in accordance with the principle mentioned above.

As the LAS disappears, the intermediary products of its biodegradation, known as sulphophenyl carboxylic acids (SPC), start to appear. The different peaks correspond to the sulphophenil carboxylic acids formed.

The first maximum activity value, which implies maximum LAS degradation, leads to maximum SPC concentration.

From day 3, the intensity of first peak (C_{10} -LAS) increases at the cost of the others, which disappear almost completely from day 29. This is due to the fact that from this moment, the SPC begin to degrade as the microorganisms present in the reactor are completely adapted to the LAS, which is quickly eliminated.

In conclusion, it can be said that the residual concentration of LAS which is found in the effluent of the pilot scale plant follows a pattern of behaviour which corresponds to the state of the microbiota present in the reactor. The maximum activity corresponds to a maximum LAS elimination, while the amounts of surfactant in the effluent are much greater when the population is inhibited.

List of symbols:

COD – Chemical Oxygen Demand

C_{10}, C_{11}, C_{12}, C_{13}, C_{14} – LAS–homologues

LAS – Linear Alkylbenzene Sulphonates

DHA – Dehidrogenase Activity

SOUR – Specific Oxygen Uptake Rate

VSS – Volatile Suspended Solids

References

1. *Matthijs, E., Henau H. D. E.,*. Tenside Surf. Det. **24** (1987) 193.
2. *Berna, J. L., Moreno, A., Ferrrer, J.,* Ing. Quím. **10** (1992) 151.
3. *Granados, J.,* 4th World Surfactants Congress, 1. 1996. pp 100–123.
4. *Dicorcia, A., Marchetti M., Samperi R., Marcomini* A., Anal. Chem. **63** (1994) 1179.
5. *Cavalli, L., Cassani G., Maraschin C.,* 4th World Surfactants Congress. Barcelona Vol 4, 1996. pp 448–462.
6. *Vives–Rego, J., Martínez, M., Calleja, A.,* Aquatic toxicity of LAS. Tenside Surf. Deterg. **28** (1994) 31.
7. *Romero, J., Ventura, F.,* Environ. Tox. and Wat. Qual. **8** (1993) 383.
8. *Mcavoy, J., Giger W.,* Env. Sci. Technol. **20** (1994) 376.
9. *Rappaport, R., Hopping W., Echoff W.,* Env. Tox. and Chem. **12** (1987) 147.
10. *Quiroga, J., Sales D.,* Tenside Surf. Det. **28** (1991) 27.
11. APHA– AWWA–WPCF. Métodos Estandarizados para el análisis de aguas potables y residuales. Editorial Díaz de Santos S. A. Edición en Español. Madrid 1992.
12. *Awong, J., Bitton G., Koopman B.,* Wat. Res. **7** (1984) 917.
13. *Nakae, A., Tsuji K., Yamanaka M.,* Anal. Chem. **53** (1981) 1818.
14. *Edwards A., Sherrard J.,* Tenside Surf. Det. **20** (1982) 45.
15. *Swisher, R.,* Tenside Surf. Det. **19** (1981) 298.
16. *García–Lara, J., Perón, J., González, Vives–Rego J.,* Biom. Letters **46** (1991) 151.
17. *Kikuchi, M., Tokay J., Yoshida T.,* Wat. Res. **20** (1986) 643.
18. *Romero, J., Ventura, F.,* Environ Tox. and Wat. Qual **8** (1993) 383.