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# Anaerobic biodegradation of linear alkylbenzene sulfonate (LAS) in upflow anaerobic sludge blanket (UASB) reactors

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**Abstract**

The anaerobic biodegradation of Linear Alkylbenzene Sulfonate (LAS) was studied in Upflow Anaerobic Sludge Blanket Reactors (UASB). One reactor was fed with easily degradable substrates and commercial LAS solution during a period of 3 months (Reactor 1), meanwhile a second reactor was fed with a commercial LAS solution without co-substrate (Reactor 2) during 4 months. Both reactors were operated with an organic loading rate of 4–5 mg-LAS/l*day and a hydraulic retention time of one day. The LAS biodegradation was determined by full mass balance. LAS was analysed by HPLC in the liquid phase (influent and effluent streams of the reactors) as well as in the solid phase (granular sludge used as biomass). The results indicate a high level of removal (primary biodegradation: 64–85%). Biodegradation was higher in the absence of external co-substrates than in the presence of additional sources of carbon. This indicates that the surfactant can be partially used as carbon and energy source by anaerobic bacteria. Under the operating conditions used, inhibition of the methanogenic activity or any other negative effects on the biomass due to the presence of LAS were not observed. The methanogenic activity remained high and stable throughout the experiment.

## Introduction

Linear Alkylbenzene Sulfonate is, after soap, the most widely used surfactant in detergents and cleaning products worldwide. Commercial LAS contains an aromatic ring sulfonated at the *para* position and attached to a $C_{10}$–$C_{13}$ linear alkyl chain at any position except the terminal carbons (Figure 1). Estimated world LAS consumption in 2000 was around 2.5 million tons. Once used in detergents or other cleaning products, LAS can reach the environment as a component of the effluents of sewage treatment plants, or as an ingredient in non-treated sewage discharges. Consequently, it is important to assess its behaviour in the different environmental compartments.

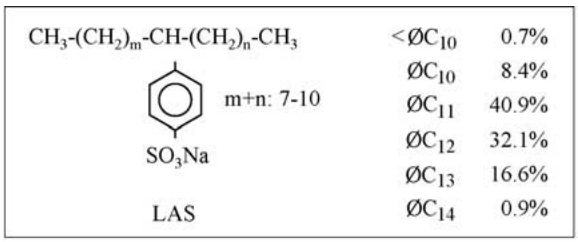


*Figure 1.* Chemical structure of Linear Alkylbenzene Sulfonates (LAS). Benzene ring is randomly distributed in all positional isomers except 1-phenyl. Sulfonate group is located at *para* position. Average alkyl chain length: 11.6 carbons. Average molecular weight: 342. Homologues distribution of the used LAS is shown.

The aerobic biodegradation of LAS has been extensively studied, both in the laboratory and in the environment. An excellent and comprehensive survey of the fate of LAS under various environmental conditions was published in 1989 in Tenside Surfactants Detergents (Berna et al. 1989; Bresan et al. 1989; Gerike et al. 1989; Giger et al. 1989; Painter & Zabel 1989). Since then numerous publications have provided additional information concerning the en-

vironmental safety of LAS under various conditions (Feijtel et al. 1999; Matthijs et al. 1999; Plassche et al. 1999). The efficient removal of LAS in waste water treatment plants (Moreno et al. 1998; Waters & Feijtel 1995) with and without anaerobic digestion of sludge has also been well described in the scientific literature.

It is generally assumed however, that in anaerobic systems LAS is not degraded (Giger et al. 1989; Painter & Zabel 1989; Sarracin et al. 1999), using $CO_2$+$CH_4$ production as an indication of mineralization according to the screening method usually practised in the laboratory (ECETOC, 1988). The ECETOC method however has some important drawbacks: (i) due to the stringent conditions, positive results of mineralization are indicative of a similar behaviour under environmental conditions, while negative results should not be necessarily interpreted as inherent anaerobic recalcitrance; (ii) the method determines only complete mineralization, and no specific analytical method is used to determine eventual disappearance of the test substance not determined by gas formation, therefore primary biodegradation (transformation of the substrate in other products but not necessarily implying complete mineralization) is not accounted for (Birch et al. 1989); and (iii) due to the low concentrations of the tested substance added to the inoculum, no significant increase in gas production is to be expected. Using a specific HPLC analysis, different removal degrees (30 to 85%) of LAS in batch tests (Prats et al. 2000; Sanz et al. 1999) or in continuous reactor systems (Angelidaki et al. 2000; Haagensen et al. 2002) have been reported. In all cases the experiments were conducted under prolonged retention times (HRT = 15 to 250 days) and no identification of potential metabolites was performed. Only under thermophilic conditions benzenesulfonic acid and benzaldehyde were detected in the effluent of an anaerobic reactor treating 2 mg/l*d of LAS with a HRT of 12 hours and with recirculation (Mogensen & Ahring 2002).

Complete mineralization of LAS under realistic environmental anaerobic conditions has not been described, probably due to the poor bioavailability of such molecules in systems where adsorption/complexation with organic matter or precipitation as insoluble salts are likely to occur. Tan et al. (2001) did not observe degradation of sulfonated aromatic amines under anaerobic conditions with different inoculation sources. Anaerobic desulfonation of azo dyes by *Clostridium* sp. has been reported, although no products were identified (Denger et al. 1996). Recently, a $\gamma$-proteobacterium able to use LAS as sulphur source under anaerobic conditions has been described (Denger & Cook 1999). Sulfo-phenyl carboxylates (SPCs), intermediates of aerobic LAS biodegradation have been found in anaerobic marine sediments in the Bay of Cádiz (Spain) (González-Mazo et al. 1997; León et al. 2000). Therefore, the possibility of primary biodegradation of LAS under anaerobic conditions should be taken into consideration.

In the present work the results of a study on the anaerobic biodegradation of LAS in UASB reactors are reported. The reactors were inoculated with granular sludge from a sugar plant and a solution of LAS has been continuously fed during a period of three months, both in absence and in presence of co-substrates.

## Materials and methods

### *Chemicals*

The LAS used throughout the experimental work was a typical European cut product (CAS No. 68411-30-3) supplied by Petresa (Madrid, Spain). LAS was obtained from a Linear Alkylbenzene (LAB) produced by Petresa in San Roque (Cádiz, Spain) after sulfonation in a commercial multitubular falling film sulfonation unit (Ballestra, Milan, Italy) and neutralisation with NaOH. The structure and characteristics of the LAS are shown in Figure 1.

The various reagents and chemical compounds used to prepare the feed to the UASB reactors as well as for the analysis of the test samples (COD, LAS) were all of analytical grade and supplied by Merck (Darmstadt, Germany) and Aldrich Chemical Co. (Milwaukee, MI, USA).

### *Analytical methods*

Chemical Oxygen Demand (COD) was determined according to Standard Methods for the Examination of Water and Wastewater (Clesceri et al. 1995) (Method 5220 D) using a Hach COD Reactor equipped with a DR/700 colorimeter. Total Solids (TS) and Volatile Solids (VS) were also determined according to Standard Methods (Methods 2540B and 2540E). pH was measured with an Orion 420A pH-meter. Methane was measured by gas chromatography (Sanz et al. 1997).

LAS was determined using an HP-1090 chromatograph and a well validated specific HPLC method (Matthijs & De Henau 1987). The method comprises

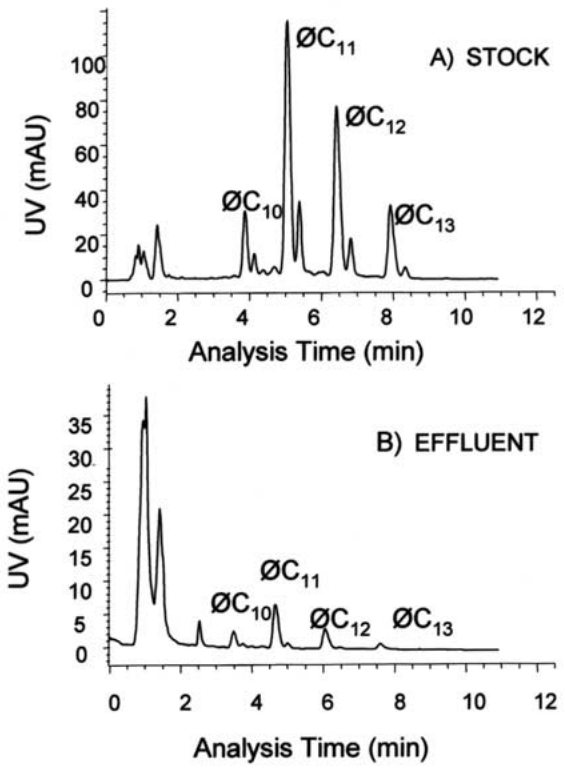


*Figure 2.* Typical chromatogram of commercial LAS. Each peak in the chromatogram corresponds to the different homologue series of the commercial LAS employed. The analytical technique detects all the homologues with equal sensitivity. The quantification was done by using external standards.

a Soxhlet extraction of the LAS from solid samples using methanol, followed by a clean-up step and a specific UV detection (230 nm) and quantification by HPLC (Figure 2). In the case of liquid samples, the Soxhlet extraction was not carried out since LAS was extracted using a SPE (Solid Phase Extraction). A Merck Lichrocart column (10 cm length, and 0.5 cm internal diameter coated with 5 $\mu$m of RP-8) was used. The mobile phase was a mixture of 45% $H_2O$ and 55% AcetoNitrile (ACN) – water (80 : 20) with $NaClO_4$ 0.1 M (isocratic) which was run at a flow rate of 1 ml/min.

*Biomass and reactors experimental design*

Two UASB reactors of 5.4 litre each were used (Figure 3). They were inoculated with granular sludge from a full-scale UASB reactor of Ebro Agrícola, a sugar factory located at La Bañeza (León, Spain). The characteristics of the sludge were as follows: 0.7 g COD/g VS $*$ d of methanogenic specific activity, 9.9% volatile solids (VS) content and 13.1% total solids (TS) content. LAS concentration in the sludge was 0.18 mg/g dry matter. The methanogenic activity assays were carried out according to Sanz et al. (1996).

Both reactors were inoculated with 130 g of VS of granular sludge and fed with a mix of co-substrates: acetate, propionate, butyrate, lactate, methanol, ethanol and sucrose (1 : 1 : 1 : 1 : 1 : 1 : 1 COD ratio), macronutrients and trace elements (Sanz et al. 1996). The reactors were kept anaerobic throughout the experiments (based on resazurin indicator). A steady state was reached after 20 days. The Organic Loading Rate (OLR) at that moment was 9 g COD/l $*$ day and the COD removal 90% (80% based on methane production). At that moment the feeding with LAS solution (4–5 ppm) was started and was continued over a period of 3 months (Reactor 1) and 4 months (Reactor 2) respectively. The Hydraulic Retention Time (HRT) was 24 h and the average temperature during the experiments was kept at 30 $\pm$ 2 °C.

Reactor 1 (R-1) was fed with the former mixture of co-substrates and LAS solution during 3 months, and after that period half of the sludge was removed to evaluate the mass balance of the LAS during this period. The rest of the biomass was thereafter exclusively fed with the co-substrates mixture, without LAS, during 2 additional months. The pH of the reactor was between 7.2 and 7.4 during the whole experiment. COD removal and CH4 production were monitored to assess the performance of the reactor. In reactor 2 (R-2) the addition of co-substrates was stopped once the steady state was reached. Thereafter, the only feed to the reactor was an isotonic solution of LAS (LAS in 0.9 wt % NaCl solution, without any co-substrate) during 4 months. In this reactor the pH was kept in the range of 6.5–7.0.

In both reactors the LAS concentration was periodically measured in the liquid phases (influent and effluent) as well as in the sludge. In all cases the LAS mass balance was calculated taking into account the total LAS fed with the influent, the total amount collected in the effluent and the remaining product in the sludge (adsorbed, precipitated). In the case of the liquid streams the total LAS mass balance was calculated taking into account the corresponding concentrations and the flow rates. The amount of biomass showed a growth of 25% in reactor R-1 and a reduction of 11.5% in R-2, as discussed in the Results section. We assume both rates to be constant during the duration of the experiments. The final LAS mass balance in the biomass was calculated after removing

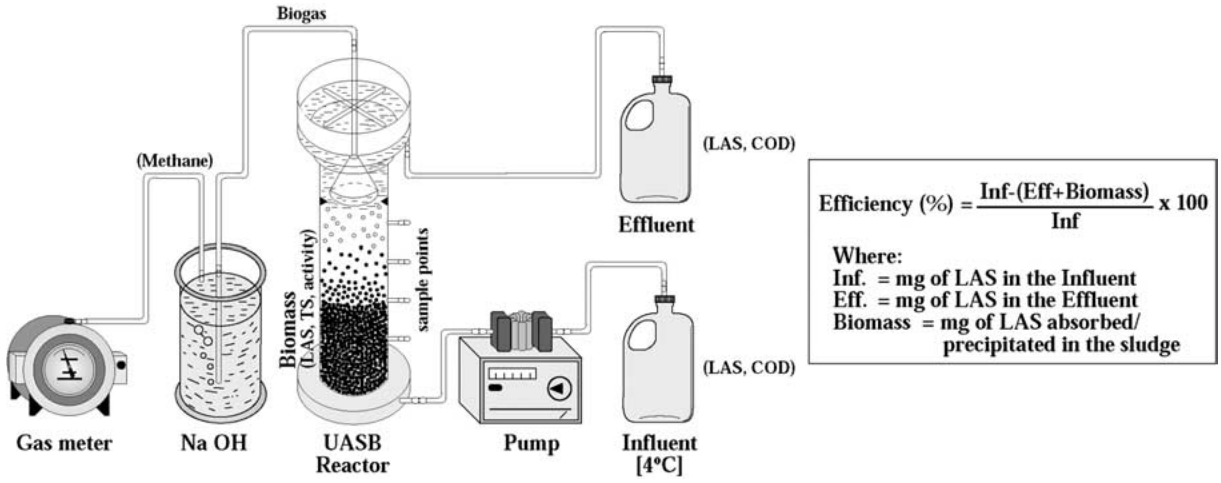


*Figure 3.* Schematic diagram of the laboratory experimental set-up used in the work. The parameters measured in the liquid, solid and gas phases are indicated between parenthesis. The LAS removal efficiency was calculated according to the equation shown.

and analysing the sludge from the reactors at the end of experiments. The LAS removed with the samples of sludge taken for other analyses (monitoring LAS content and methanogenic activity) was also taken into account in the final mass balance. The LAS removal efficiency was calculated according to the equation shown in Figure 3. Biomass was also periodically sampled to monitor the specific methanogenic activity.

## Results

### *Reactor 1*

The LAS concentrations in the influent, effluent and sludge at different times during the experiment are given in Figure 4. Figures about LAS removal profile (4 to 6), refers to data from day 20th after the start-up of the reactors, when the LAS dosage was initiated. The LAS concentration (4.7 mg/l average) in the influent was kept constant during the first 100 days of the test. Thereafter, the LAS feed was discontinued. LAS concentrations in the effluent and in the sludge show a different profile in four well-defined zones. During the first two weeks of LAS feeding to the reactor no LAS was detected in the effluent stream. The LAS concentration in the sludge – due to sorption or precipitation – after the two weeks was 0.326 mg/g dry matter. Considering that total LAS added with the influent was 329 mg (4.7 mg/l × 5 l/d × 14 d), that the LAS retained in the sludge was 42.3 mg (0.326 mg/g dry matter × 130 g dry matter) and that no LAS was

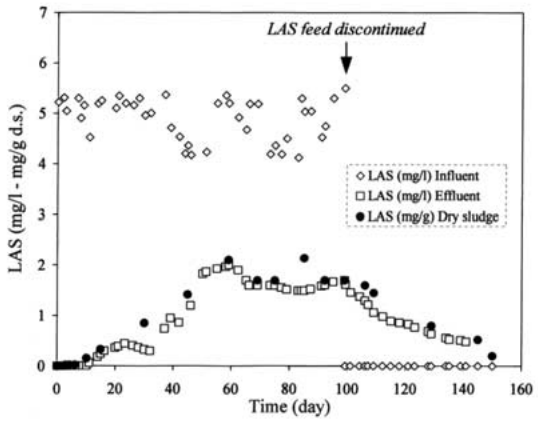


*Figure 4.* LAS concentrations in the influent (◇), effluent (□), and sludge (●) of the UASB reactor fed with a solution of LAS and a mixture of co-substrates. The OLR was about 9 g COD/l*d, the HRT 1 day and the performance of the reactor kept on 85–90% of COD removal over the experiment.

detected in the effluent, than the LAS removal after that period was 87%.

From day 14th until approximately day 50th, the LAS concentration increased in both the effluent stream (up to 1.5–2.0 mg/l) and in the sludge (up to 1.5–2.0 mg/g dry matter). These values remained more or less constant during the time when LAS solution was fed to the reactor – about 2 months – (day 100). Thereafter, LAS feeding solution to the reactor was stopped. It can be assumed that, in a more prolonged test, a stable situation would have been maintained because of a steady state (considering both LAS and COD removal) was already reached. The LAS mass

*Table 1.* Final LAS mass balance in Reactors 1 (with co- substrates) and 2 (without co-substrates). Removal data are given for individual homologues and total LAS mix.

| | Reactor 1 | Reactor 2 |
|---|---|---|
| Added LAS in the influent (mg) | 1998 | 2630 |
| Recovered LAS in the effluent (mg) | 433 | 166 |
| Recovered LAS in the sludge (mg) | 286 | 236 |
| Removal (%) | | |
| Homologues distribution: | | |
| Ø $C_{10}$ | 68 | 91 |
| Ø $C_{11}$ | 75 | 93 |
| Ø $C_{12}$ | 83 | 95 |
| Ø $C_{13}$ | 87 | 96 |
| Total | 78 | 94 |
| Biodegradation (%) | | |
| Homologues distribution: | | |
| Ø $C_{10}$ | 61 | 87 |
| Ø $C_{11}$ | 64 | 87 |
| Ø $C_{12}$ | 66 | 84 |
| Ø $C_{13}$ | 63 | 77 |
| Total | 64 | 85 |

balance at day 100 indicates a removal of 64%. At the moment when the LAS feeding was discontinued to the reactor, the total LAS amount in the sludge was 14% and the amount recovered in the effluent 22% of the total amount supplied to the system (Table 1).

The last part of Figure 4 (day 100 to 150) shows the evolution of the surfactant concentration in both the effluent and the sludge, indicating a steady and simultaneous decrease. The LAS adsorbed/retained in the sludge is steadily desorbed/redissolved and at the end of that period the sludge was almost free of LAS. The mass balance at the end of this period shows an average LAS removal of 75%. The evolution of the LAS removal efficiency during the overall period, when the surfactant was present in the influent, is represented in Figure 5.

COD removal efficiency throughout the experiment was maintained fairly constant between 85 and 90%. The methanogenic activity of the biomass after 150 days was 0.67 g COD/g VS*d, which is nearly the same as the initial activity of the inoculum. Because of the use of co-substrates as carbon and energy sources, the total biomass increased 25% approximately .

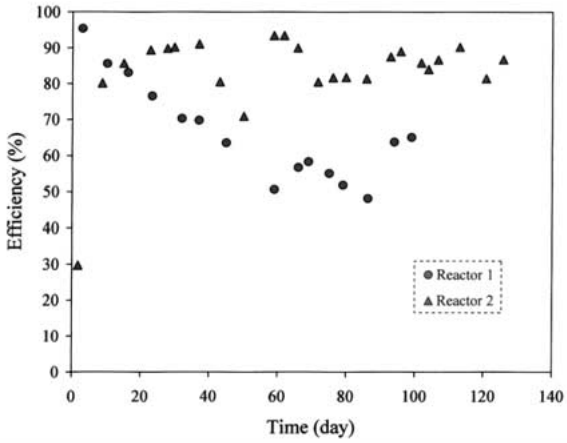


*Figure 5.* LAS biodegradation in reactor 1 (with co-substrate) and reactor 2 (without co-substrate) throughout the experiment. The rate of biodegradation in each point has been calculated considering the total LAS supplied, total LAS recovery in the effluent and total LAS accumulated in the sludge, according to the expression including in the Figure 2.

*Reactor 2*

The LAS concentrations in the influent, effluent and sludge at different times in the experiment are depicted in Figure 6. Once the steady state was reached, reactor 2 was exclusively fed with an isotonic solution (0.9% NaCl) of LAS during 125 days. Because of the starving carbon conditions used, at the end of the experiment a total biomass decrease of 11.5% was detected, in parallel with a decrease in specific activity to 0.55 g COD/VS*d. At the start of feeding with LAS solution (average concentration of 4.3 mg/l in the effluent), the residual surfactant concentration in the sludge was 0.18 mg/g dry matter.

During the operation of the reactor two well-differentiated zones could be distinguished. During the first 70 days, there was no LAS in the effluent of the reactor and the total amount retained in the sludge until day 40th was low. Consequently, most of the LAS must have been degraded indicating removal degrees in the order of 90% and higher. After day $70^{th}$, the amount of LAS in the effluent increased in the same proportion as the quantity retained by the sludge. From day 80 (effluent) and day 50 (sludge), the LAS concentration remained almost constant until the end of the experiment with average concentrations of 0.9 mg/l in the effluent and 1.9 mg/g dry matter in the sludge. The LAS removal efficiency was high during the whole period. The mass balance at the end of the experiment (Table 1), shows that 6% of the total LAS added was

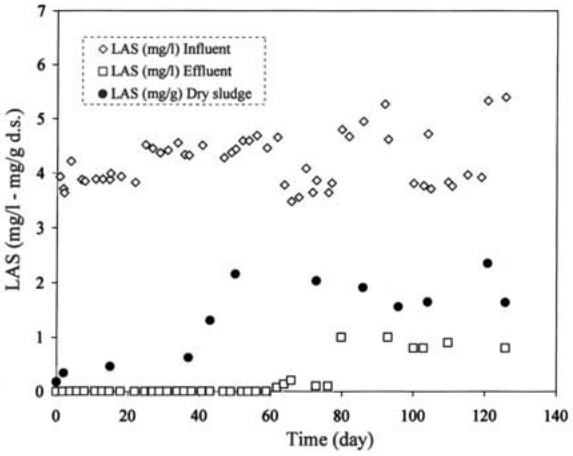


*Figure 6.* LAS concentrations in the influent (◇), effluent (□), and sludge (●) of the UASB reactor fed exclusively with an isotonic solution of LAS.

recovered in the effluent while 9% was retained in the sludge. This means that, 85% of the total LAS added to the reactor was removed.

## Discussion

The presence of LAS in the range of concentrations used in the study (4–5 ppm) over a period of 3 to 4 months did not inhibit the anaerobic activity of the biomass of UASB reactors. In fact, the reactor 1 showed both LAS and COD (data not shown) removal in the range of 85 to 90% with stable and high methanogenic activity (0.7 g COD/g VS*d). This is not surprising taking into account previously reported $IC_{50\%}$ values of 40–150 mg LAS/l for the methanogenic activity (Sanz et al. 1999).

The LAS concentration profile throughout the experiment was complex. Since several mechanisms and processes take place: ad-/ab-sorption, precipitation of insoluble LAS salts, and degradation to unidentified metabolites with unknown kinetics. The results from reactor 1 showed an equilibrium between LAS concentrations in the effluent and the sludge once the system had reached the steady state. The relationship between such concentrations was constant according to the Freundlich isotherm described for LAS and activated sludge (Verge & Moreno 1996). The influence of alkaline ions (Ca, Mg) or even heavy metals, although similar in concentrations in both reactors, can not be ignored as a key parameter since they may affect the bioavailability of LAS. LAS in the sludge was most probably either adsorbed/bound to organic matter or precipitated as insoluble salt, in both cases resulting in an extremely limited bioavailability. It is generally assumed that only the soluble fraction is bioavailable, and it is noteworthy to mention that several data indicate that only the bioavailable fraction of LAS is transformed by anaerobic digestion (Haagensen et al. 2002). LAS in the influent was present in soluble form and therefore more accessible to the microorganisms. Similar observations have been reported in batch tests (Prats et al. 2000). The desorption of LAS from the sludge is clearly shown in Figure 4 (forward day 100) indicating that the LAS desorbed from the sludge – approximately 45% – is biodegraded while the balance is recovered in the liquid effluent.

The anaerobic biomass used in the tests was able to degrade LAS in the presence of co-substrates as well as in absence of added external co-substrates. In their absence the efficiency was considerably higher. At the end of the experiment a loss of 11% of biomass was observed in reactor 2. The partial degradation of this biomass produced available debris that could be used as co-substrates in the degradation of LAS. It is, however, remarkable that the removal of LAS was always higher in the absence of alternative sources of carbon (Figure 5). This could be interpreted as an indication that the primary biodegradation of LAS did not occur through co-metabolism but that LAS was used as a source of energy and carbon. A similar effect has been described for the aerobic biodegradation of LAS where the surfactant was more rapidly degraded in the absence of methane by a methane-utilising consortium or in absence of glucose by *P. putida* (Hrsak 1995; Marques et al. 1997) than in the presence of the co-substrates. Despite this fact, no increase of LAS-degrading activity was observed. We estimate that the total amount of LAS added was insufficient to provoke an enrichment in the population of LAS-degrading bacteria.

The results presented in this work strongly suggest that LAS is degraded under methanogenic conditions, although no mineralization was observed from the biogas production measurements. Nevertheless, it must be taken into account the low LAS loading rate (4–5 mg LAS/l*d) compared to the co-substrate loading rate (9 g COD/l*d). Obviously, the amount of methane from the hypothetical mineralization of LAS (approximately 4 ml $CH_4$/d) is not significant in comparison to the methane generated from co-substrate (3650 ml $CH_4$/d). In the case of Reactor 2, although the estimated contribution of LAS to the total generated

methane was higher (4%, assuming the biomass lost was mineralised) than in Reactor 1, such percentage is not relevant in an open systems like UASB reactors. Despite the significant removal observed, we had not been able to identify metabolites generated from the disappearance of the parent molecule. The complexity of the effluent as well as the lack of information of the potential pathway makes such identification a challenging task.

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