

On the complete aerobic microbial mineralization of linear alkylbenzene sulfonate

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Abstract

The xenobiotic organic compounds linear alkylbenzene sulfonates (LAS) are among the most thoroughly investigated with respect to biodegradability. Previous studies have shown that LAS are relatively rapidly degraded under aerobic conditions, but only very slowly or not at all degraded under anaerobic conditions. In the present work, the degree of primary and ultimate biodegradation of LAS by consortia of aerobic microorganisms was examined. The quantification of primary biodegradation was conducted via analysis of the LAS content in the mixed liquor, which was based on microwave-assisted-extraction (MAE) followed by solid phase extraction with liquid chromatography coupled with a fluorescence detector (HPLC-FD). The ultimate biodegradability of LAS was examined by measuring both the disappearance of the test compound and the formation of carbon dioxide using the OECD method 301B or the sulphate. It was found that the established enriched mixed microbial population was capable for complete mineralization of all LAS, as verified by the sulfate production as well as the application of the OECD method and the carbon mass balances carried out throughout the course of the conducted experiments. In particular, any intermediate compound formed during LAS biodegradation was rapidly decomposed, making any intermediate accumulation temporal.

Keywords: Linear alkylbenzene sulfonates (LAS); Aerobic biodegradation; Biological mineralization; Mixed microbial consortium; OECD

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1. Introduction

Nowadays the issue of broadly used xenobiotic organic compounds is becoming more important worldwide. Xenobiotics are chemicals produced from human activity that may have pathogenic, toxic, or mutagenic impact on the biological life. A main group of these man-made chemicals is surfactants (surface-active-agents). It concerns formulations designed to have cleaning and solubilisation properties. They generally consist of a polar head group (either charged or uncharged), which is readily soluble in water, and a nonpolar hydrocarbon tail, which is not easily dissolved in water. Hence, surfactants combine hydrophobic and hydrophilic properties in a single molecule [1]. Due to their widespread use and high consumption, surfactants and their degradation products have been detected at various concentrations in different environmental compartments [1–3].

Linear alkylbenzene sulfonates (LAS) are the most popularly used synthetic anionic surfactants in commercial detergents. Their basic structure consists of a benzene ring connected to a sodium sulphate group (the hydrophilic end) and an alkyl chain containing 10–13 carbon atoms (the hydrophobic end) [4]. The commercially available LAS are very complex mixtures of various homologues and phenyl positional isomers.

Large amounts of LAS are disposed in municipal wastewaters due to their wide daily use [4]. Based on their physicochemical properties, adsorption and biodegradation are the only processes determining the fate of LAS in environment [5,6]. Biodegradation is an important process responsible for the removal of LAS from both raw wastewater and sludges in sewage treatment plants, while it also enhances the removal of untreated or partially degraded compounds in the environment after their disposal in natural receptors (seas, rivers, lakes etc.), reducing thus their impact on biota. During biodegradation, microorganisms can either utilize LAS as the

sole carbon source or co-metabolize LAS by microbial metabolic reactions [1].

LAS are generally regarded as biodegradable surfactants. Very high levels of biodegradation (>95%) have been reported in some wastewater treatment plants that use aerobic bioprocesses [2,7,8]. In contrast, a negligible primary biodegradation of the LAS homologues and isomers has been detected under anaerobic conditions [9].

Several studies have been conducted to determine the LAS degradation pathways. In overall terms – the way the process should be looked at in nature and in wastewater treatment plants – the LAS breakdown looks as follows:

- (a) Oxidative conversion of one of the two methyl groups of the alkyl chain into a carboxylic group (ω -oxidation).
- (b) Oxidative shortening of the alkyl chain by 2 carbon units (β -oxidation).
- (c) Oxidative ring splitting.
- (d) Cleavage of the carbon-sulphur bond, i.e. sulphate liberation [10].

LAS are not a single chemical entity but a mixture of organic chemicals in which hydrophobic and hydrophilic groups are joined together in the same molecule. It is therefore expected that microbial consortia will play an important role in the biodegradation of these compounds [11]. More specifically, LAS catabolism confronts the microorganisms involved in it with the task of being able to convert a very wide variety of structures, namely the aliphatic chain with a non-uniform number of carbon atoms, the aromatic ring, which is, in addition, distributed randomly over the alkyl chain, and cleavage of the carbon-sulphur bond on the benzene ring. It is therefore not surprising that mixed cultures are frequently more efficient than single-cell cultures with respect to LAS mineralization [10,12–14]. Consortia of microorganisms may be constructed on the basis of commensalistic

(benefit to one microorganism) and synergistic relationships [11]. Such interactive effects lead to more effective biodegradation than is possible by any individual microorganism.

Upon reviewing the literature concerning the biodegradation of LAS, it becomes apparent that most researchers agree that LAS can be degraded aerobically but hardly anaerobically. There is extensive research on the aerobic biodegradation focusing mainly on the primary degradation (biotransformation) of mixtures of LAS homologues [14–17]. On the other hand, information on the ultimate biodegradation of LAS is rather limited. Only scarce studies are available on the kinetics of ultimate biodegradation (mineralization to carbon dioxide, water and sulphate) of the LAS benzene ring [11,13]. Jiménez et al. [13] observed that a four member consortia is necessary for the mineralization of LAS but did not give any information on the kinetics of LAS biodegradation and CO₂ evolution. Similarly, van Ginkel [11], through a theoretical analysis, concluded that the limited metabolic capacities of pure cultures of microorganisms utilizing surfactants, point to the requirement of consortia to degrade surfactants completely. The only kinetic study involving intermediates that came to our attention was that of Marcomini et al [17] who have detected intermediate products (sulphophenyl carboxylates, SPCs) and monitored their concentration during LAS degradation, concluding that some of them may be persistent, longer after the disappearance of the parent compound. However, the final products (such as CO₂ or sulphate) were not monitored to verify ultimate biodegradation.

The aim of this work was to examine the degree of primary and ultimate biodegradation of LAS by a consortium of aerobic microorganisms by applying a systematic methodology, based on LAS analysis, carbon mass balance, as well as determination of sulphate, the formation of which denotes the ultimate biodegradation of LAS.

2. Materials and methods

2.1. Chemicals

The LAS mixture used was purchased from Aldrich (28995-7). It had a mean molecular weight of 348.48 and contained the C₁₀ to C₁₃ homologues in the following proportion: C₁₀ (15.24%), C₁₁ (38.96%), C₁₂ (32.89%) and C₁₃ (12.91%), as determined through HPLC analysis. The C₈ homologue, used as an internal standard for the analysis of LAS, was also purchased from Aldrich (28748-2). All the other chemicals were obtained from MERCK. Organic solvents were of analytical grade or HPLC grade from MERCK and water was prepared on a Milli-Q purification system (Millipore). The solid phase extraction cartridges (C18-SPE) were purchased from Waters (HLB[®] cartridges of 50 mg). Filters used for filtration were obtained from Millipore (0.22 µm) and Macherey-Nagel (0.7 µm).

2.2. Analytical methods

Various parameters were measured during the conducted experiments. The total suspended solids (TSS) and the volatile suspended solids (VSS) concentrations were determined according to standard methods [18]. The pH was measured using a portable pH-meter (Hanna, HI 8224). Filtered samples were analyzed for sulphate by ion chromatography on a model DX300 gradient system (Dionex, Sunnyvale, CA) using an AS11 column and a CDM-3 conductivity detector. A TOC analyser (ANALYTIC JENA AG multi N/C 2000) was also used for the determination of total organic carbon (TOC) and inorganic carbon (IC). The organic carbon was determined in the samples prior (total organic carbon) and after (dissolved organic carbon) filtration through 0.22 µm membrane filters. The difference between TOC and DOC accounted for the biomass carbon content, since biomass was the only particulate substance present in the samples.

The analytical method for the analysis of LAS was based on microwave-assisted extraction (MAE) followed by solid phase extraction with liquid chromatography, coupled with a fluorescence detector (HPLC-FD). Briefly, after the primary filtration of the mixed liquor, the solid and aquatic phases were elaborated differently. The aquatic phase was filtered again and the filtrate was loaded to a C18 SPE-cartridge. The LAS were removed from the cartridge by passing 1.5 mL of methanol. In this solution, water, 1% v/v formaldehyde solution (to secure abiotic conditions) and 0.05 M sodium dodecyl sulphate (SDS) (to prevent adsorption of LAS on the surface of glass containers) were added to a final volume of 3 mL. About 20 μ L of the final solution were used for the analysis in the HPLC-FD. MAE used for the determination of LAS in the solid phase (dried at 40°C) was conducted in a microwave accelerated reaction system for extraction; model MARS 5 from CEM (North Carolina, USA). The solvent used for the extraction was methanol. All the information regarding LAS analysis in HPLC was given by A. Kouvarakis [19] in a personal communication and have been reported in Pakou et al. [20].

2.3. Acclimation of activated sludge to LAS

The acclimation procedure involved the successive transfer of a small fraction of culture on a medium based on a mixture of LAS and acetate (easily degradable substrate). After a number of successive transfers had been performed, the culture was grown only on LAS. The acclimation procedure can be described as follows:

Initially, activated sludge obtained from the aeration tanks of the municipal sewage treatment plant (STP) of Patras (Greece) and the STP of University of Patras, was used as inoculum for the acclimation of aerobic biomass to LAS. The characteristics of the activated sludge taken from the two STPs were 4.36 g TSS/L, 3.00 g VSS/L and 3.33 g TSS/L, 2.21 g VSS/L, respectively.

The bioreactor, a 1000-mL Erlenmeyer flask, was operated in a draw-fill mode at ambient temperature, with mild aeration and stirring. The mixed liquor consisted of 400 mL of inoculum and 600 mL of the synthetic medium 1 (SM1). SM1 contained the following quantities of mineral salts and nutrients: 0.85 g/L $\text{CH}_3\text{COONa} \cdot 3\text{H}_2\text{O}$; 0.235 g/L $(\text{NH}_4)_2\text{SO}_4$; 0.2 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.026 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 5 g/L K_2HPO_4 ; 3 g/L KH_2PO_4 ; 0.7 g/L NaHCO_3 ; 0.1 mL/L of a stock solution of trace elements. The trace elements stock solution contained: 0.786 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$; 5 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$; 12.609 g/L $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$; 4.050 g/L $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$; 4.398 g/L $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$; 2.453 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$; 0.750 g/L KI; 3.000 g/L H_3BO_3 ; 5.000 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 5.000 g/L EDTA. LAS were also added to a final concentration of 10 mg/L. Every 2 days 50 mL were withdrawn from the bioreactor and were replaced with 50 mL of the SM1 containing also 10 mg LAS/L. The mean concentration of the volatile suspended solids in the bioreactor was about 800 mg/L and the pH was around 7.5. After a few days LAS reduction was observed.

In order to verify that the observed reduction of the LAS content was due to biodegradation, the following experiment was conducted: two similar cultures (A and B) were prepared by taking, for each one, a portion of the mixed liquor (200 mL) from the bioreactor (“mother” culture) that was diluted to a final volume of 1000 mL. The dilution medium was the SM1 and the LAS were added so that the final concentration was 50 mg/L. One of these cultures (B) was used as control experiment, which was run in parallel, where CHCl_3 had been added to secure abiotic conditions. All nutrient media were sterilized by autoclaving at 121°C for 20 min. LAS were added after the sterilization in order to avoid possible conversion or reaction due to the high temperature. Sterilization of the LAS containing solution was achieved by filtration through sterilized filters (0.22 μ m Millipore). When LAS biodegradation was observed, a new adaptation

procedure was followed. Hundred milliliters of the acclimated culture (A) were used as inoculum and transferred to a new batch reactor of 500 mL working volume. The SM1 no longer contained acetate and so the added amount of LAS (at 50 mg LAS/L) was tested as the sole organic carbon source for this culture. When LAS were completely degraded, this procedure of successive dilutions was continued so that several dilution cycles took place. In every cycle the mixed consortium was gradually acclimated to increasing concentrations of LAS (from 50 to 200 mg/L), so that an adequate amount of biomass could be attained while growing solely on LAS without being inhibited.

2.4. LAS mineralization experiments

In order to verify that LAS biodegradation was followed by complete mineralization, sulphate concentration was also monitored in a preliminary experiment, since sulphate is released during the benzene ring cleavage, which is the last step in the LAS mineralization. In the sequel, more experiments were conducted to study the LAS degradation rate in relation to the accumulation of intermediate products and CO₂ production.

2.4.1. Preliminary study via SO₄²⁻ monitoring

A new batch experiment was conducted in order to determine the type of LAS biodegradation (primary or ultimate) achieved by the enriched mixed microbial population. To this aim, the change of SO₄²⁻ concentration in the enriched culture was periodically monitored. Hundred milliliters of mixed liquor were taken from the bioreactor and centrifuged for 10 min. The supernatant was removed and 100 mL of the synthetic medium 2 (SM2) were added. SM2 contained the following quantities of mineral salts and nutrients: 0.059 g/L NH₄Cl; 0.169 g/L MgCl₂ · H₂O; 0.026 g/L CaCl₂ · 2H₂O; 0.250 g/L

K₂HPO₄; 0.150 g/L KH₂PO₄; 0.700 g/L NaHCO₃; 0.1 mL/L trace elements. SM2 was based on SM1 reported in 2.3 with all sulphate salts having been replaced by the equivalent chloride salts, so that the sulphate determined originated only from LAS mineralization. This procedure took place three times in order to remove the already existing SO₄²⁻ in the inoculum. Afterwards, the biomass was transferred to a 500-mL Erlenmeyer flask and diluted to a final volume of 500 mL with the SM2. LAS were also added to a final concentration of 50 mg/L. The batch bioreactor was operated at ambient temperature and with mild aeration and stirring. During the experiment, samples were taken periodically and the LAS biodegradation and the production SO₄²⁻ were estimated.

2.4.2. LAS mineralization kinetics

The kinetic experiment for LAS degradation was conducted using the mixed enriched culture grown solely on LAS (used as carbon source). For estimating the mineralization degree of LAS, both LAS degradation and CO₂ production was monitored. The experiment was conducted following the 301B protocol of OECD guideline for testing ready biodegradability [21]. According to this method, certain volume of inoculated mineral medium, containing a known concentration of the test substance as the nominal sole source of organic carbon, is aerated with carbon dioxide-free air at a controlled rate in the dark. Degradation is followed over 28 days by determining the carbon dioxide produced and relating it with the LAS removal. The CO₂ is trapped in barium or sodium hydroxide and is measured by titration of the residual hydroxide or as inorganic carbon. The degree of biodegradation may also be calculated from supplemental dissolved organic carbon (DOC) analysis made at the beginning and end of incubation.

Four experiments were conducted in parallel in batch reactors with an operating volume of 1 L; two replicates for studying LAS degradation

by the enriched culture (Experiments 1 and 2) and two control experiments [biomass-control (Experiment 3) in the absence of LAS and abiotic-control (Experiment 4) in the absence of biomass]. In Experiments 1 and 2, the amounts of inoculum and LAS added to the synthetic medium were calculated so that the mixed liquor would have a final TSS concentration of no more than 30 mg/L and a DOC or TOC (total organic carbon) concentration of no more than 20 mg/L respectively, according to the methodology applied. The composition of the synthetic medium 3 (SM3) used in these experiments was adopted from the OECD guideline and was 334.00 mg/L $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$; 5.00 mg/L NH_4Cl ; 36.00 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; 22.50 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.25 mg/L $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; 218.00 mg/L K_2HPO_4 ; and 85.00 mg/L KH_2PO_4 .

In the biomass control (Experiment 3) the same amount of inoculum (as in Experiments 1 and 2) was added in the synthetic medium in the absence of LAS, while in the abiotic control (Experiment 4), the same amount of LAS (as in Experiments 1 and 2) was added in the SM3 with simultaneous addition of CHCl_3 (1% v/v) that was used as a strong microbial activity inhibitor. All experiments were conducted in a shaking water bath (to maintain the temperature constant at 25°C) under sterilized conditions. Carbon dioxide-free air was provided at a rate of 4.5–5 L/h. Each bioreactor was connected with two CO_2 traps in series, each containing 30 mL of a 0.0125 M barium hydroxide solution. The produced CO_2 was trapped in barium hydroxide and was measured by titration of the residual hydroxide using HCl 0.05 M. Samples from the mixed liquor were taken from each bioreactor at regular intervals and analysed for LAS, DOC, TOC and pH.

3. Results and discussion

3.1. Acclimation of activated sludge to LAS

The enhanced culture exhibited an almost complete LAS elimination after a few days of

cultivation. In fact, the initial 16.50 ± 0.58 mg LAS/L decreased to 0.20 ± 0.02 mg LAS/L (98.79% decrease) in 7 days. The total initial concentration of LAS exceeded the 10 mg/L which was the added amount of LAS. This is attributed to the presence of an initial LAS content adsorbed in the activated sludge.

In order to verify that the observed LAS reduction was due to their biodegradation, a comparison experiment was conducted using almost the same total initial LAS concentration in two parallel batches (A and B), one of which (B) served as abiotic control by adding CHCl_3 in the control flask. As seen in Fig. 1, the abiotic control did not indicate any significant physicochemical LAS elimination taking place during the experiment. Consequently, any losses of LAS observed in batch A were attributed to biodegradation.

During the following successive dilutions the typical concentration profiles of LAS were similar to that presented in Fig. 1. Moreover, a complete biodegradation of LAS was observed when acetate was removed from the synthetic culture medium and different initial LAS concentrations (from 50 to 200 mg/L) were tested.

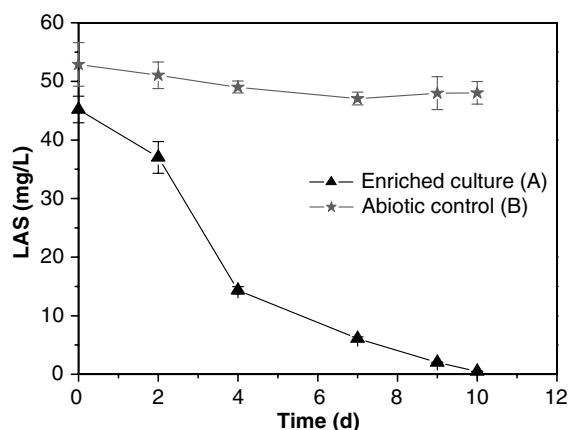


Fig. 1. LAS concentration profile in the enhanced culture and in the abiotic control.

3.2. Ultimate biodegradation of LAS by the enriched culture

The aim of this experiment was to determine the type of LAS biodegradation taking place in the presence of the enriched culture. In accordance with the proposed LAS degradation pathways, the last stage of LAS biodegradation is the cleavage of the carbon-sulphur bond and consequently the liberation of sulphate to the culture medium. Fig. 2 verifies the ability of this mixed culture to biodegrade LAS completely. The initial LAS concentration was about 44.50 ± 1.58 mg/L and after 12 days was reduced to 5.71 ± 0.40 mg LAS/L. This LAS reduction corresponded to a production of approximately 5 mg/L. As can be seen in Fig. 2, the measured SO_4^{2-} production was about 5.85 mg/L. This result was a satisfying indication that the enhanced culture had indeed the ability of complete LAS biodegradation and therefore further experiments were conducted to estimate the kinetics of LAS biodegradation.

3.3. LAS mineralization kinetics

Biomass concentration, expressed as mg TOC/L, was slightly varied throughout the course

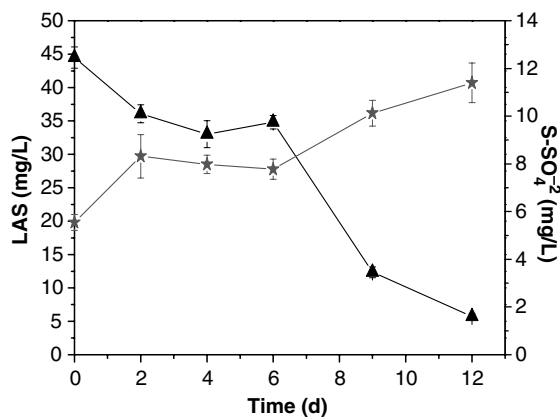


Fig. 2. LAS and SO_4^{2-} concentration profiles during the experiment.

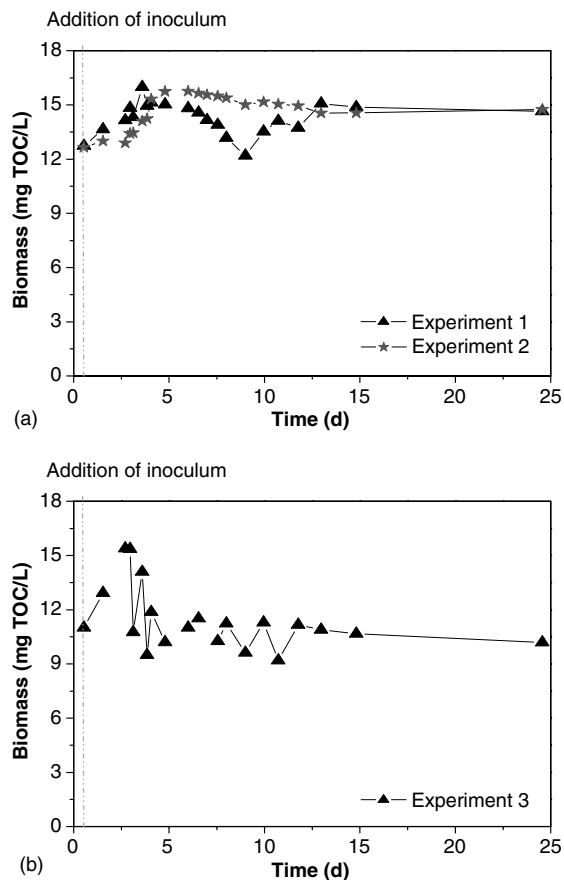


Fig. 3. Biomass concentration (expressed as mg TOC/L) during (a) LAS degradation and (b) biomass control experiments.

of the 3 parallel experiments (Fig. 3). The LAS concentration profile during the experiments is shown in Fig. 4 and, after expressed in mg DOC/L, is compared to the DOC concentration measured in the same samples (Fig. 5). The Experiments 1 and 2 that were conducted under the same conditions showed that the LAS degradation profile obtained is quite repeatable (Fig. 4a).

LAS contribution to the DOC (theoretical) was estimated based on the measured concentration of LAS in each sample, while the experimental

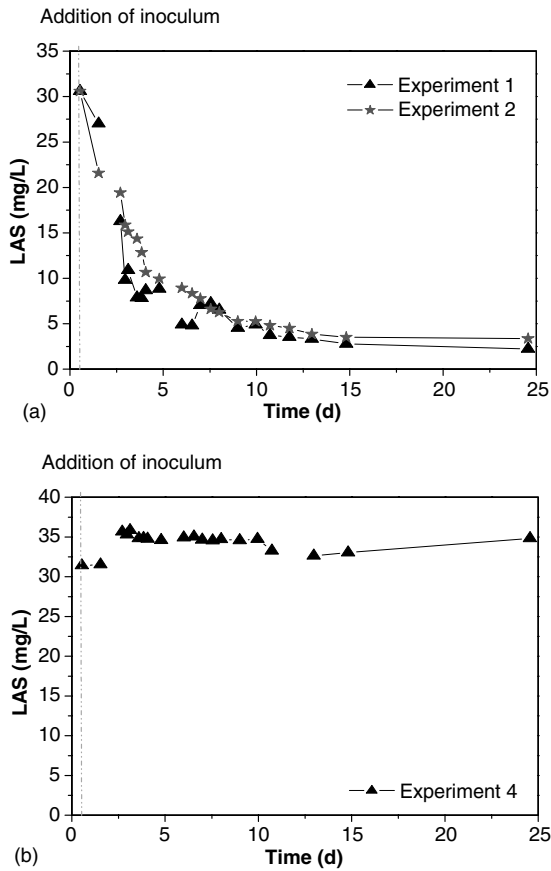


Fig. 4. LAS degradation profile during (a) LAS degradation and (b) abiotic control experiments.

value was determined by the DOC analysis (experimental) of the same sample. The difference between the “theoretical” from the “experimental” DOC depicts the DOC of compounds other than LAS that have been produced during LAS degradation. It is clear that the high difference (approximately 10 mg/L) is observed during the first 3 days of the experiments while after that, it is negligible and lies within the error of DOC measurement. The same was observed in both experiments where LAS degradation was studied. This means that eventually LAS is practically mineralized completely as there are

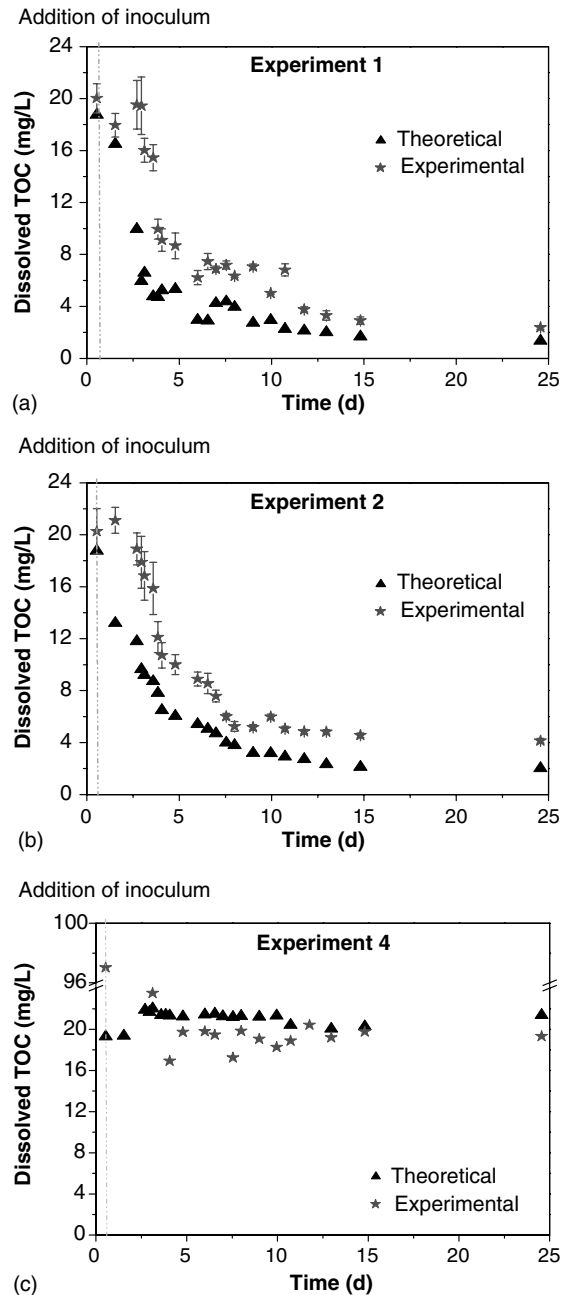


Fig. 5. Comparison of the estimated LAS contribution to DOC (theoretical) and measured DOC (experimental) during (a) and (b) LAS degradation and (c) abiotic control experiments.

no persistent metabolites. LAS did not decrease in abiotic conditions (Figs. 4b and 5c) and the DOC can be attributed solely to the presence of LAS. The initial concentration of DOC in the abiotic control was higher than expected due to the presence of CHCl_3 added as a strong microbial activity inhibitor, which evaporated in the sequel. It should also be noted the LAS adsorbed on the biomass was in negligible concentrations since the solids present were in very low concentrations. Therefore, the comparison was made upon the dissolved concentrations of the organic carbon and LAS.

The mineralization of LAS during aerobic degradation was also verified through carbon

mass balances that were carried out in each experiment (Fig. 6). The organic carbon contained in solution (due to LAS or other compounds) and the inorganic carbon (carbonate or gaseous CO_2) produced were summed at each gaseous CO_2 sampling and, as it can be seen, the sum was almost equal to the initial amount of carbon present in the synthetic medium. This means that the carbon balance holds throughout the course of each experiment. Nevertheless, degradation did not reach 100%. Probably it happened because of limited bioavailability due to adsorption on biomass.

Marcomini et al. [17] conducted a LAS biodegradation study using inoculum taken from the effluent of a STP and lower LAS concentration,

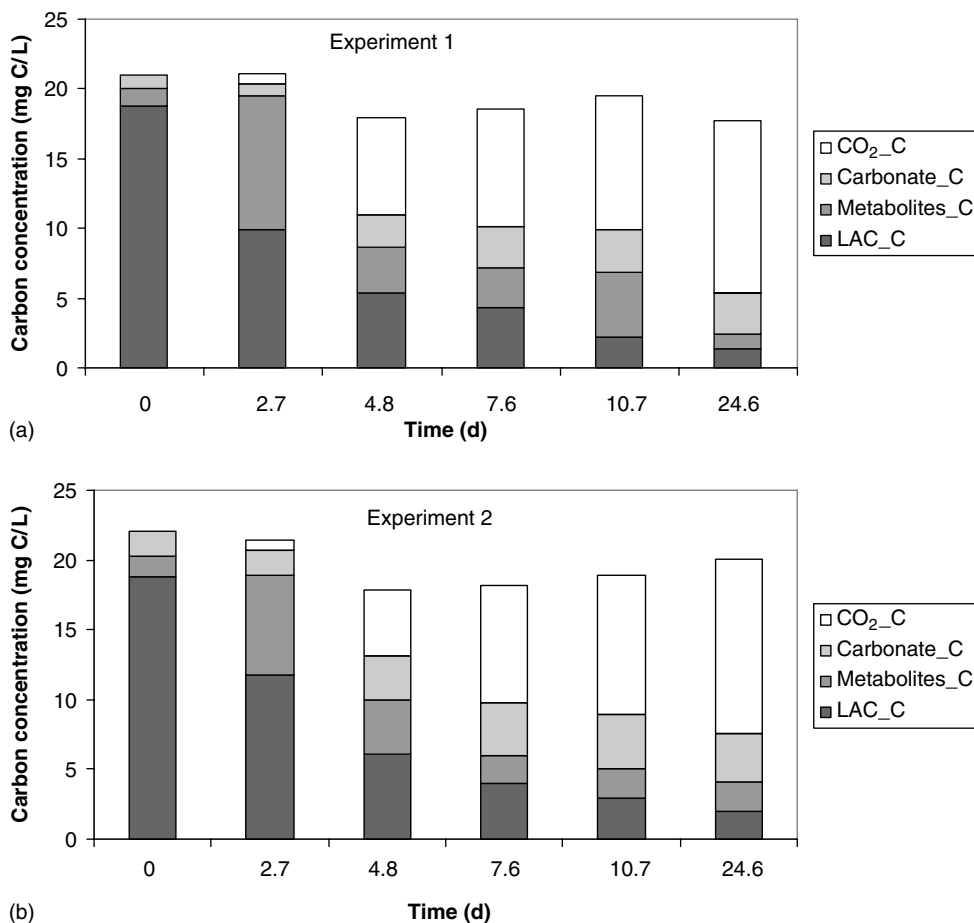


Fig. 6. Carbon balance estimated for (a) Experiment 1 and (b) Experiment 2 during LAS degradation.

and observed an almost total primary LAS biodegradation but not an ultimate biodegradation. When the primary biodegradation of LAS was completed, the metabolic product concentration leveled off and small reduction of this concentration observed only after a long period (20 days). This comes in contrast to our results which clearly show that accumulation of any intermediate lasts less than 5 days and in the end LAS are almost completely mineralized (Fig. 5).

4. Conclusions

Based on the results of this investigation, it can be concluded that the established enriched mixed microbial population, originating from activated sludge, revealed a great ability to degrade the aromatic surfactant LAS. The comparison with the respective reduction of LAS in an abiotic control verified that the LAS elimination was exclusively due to their biodegradation. The results from the LAS mineralization kinetic experiments showed that the LAS degradation profile was repeatable. The abiotic control did not indicate that any significant physicochemical LAS reduction occurred during the experiment. By monitoring the DOC in the mixed liquor during the experiment, it was proved that LAS were mineralized completely and there were no persistent metabolites. The mineralization of LAS during aerobic degradation was also verified through carbon mass balances, as well as sulfate production.

Therefore, native microorganisms proliferating in typical wastewater treatment plants are able to completely degrade and mineralize LAS present in raw wastewater under the provision that adequate hydraulic retention time (HRT) is provided for aerobic biological treatment. It can also be concluded that under the typical conditions of short HRTs (in the range of few hours) applied in most typical WWTPs, LAS ultimate biodegradation may be hard to succeed and therefore high concentrations of undegraded LAS or

their metabolites may be found in the treated effluents in case that high LAS concentration levels are present in the raw wastewater and depending, of course, on the amount of acclimatized biomass available for LAS mineralization.

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References

- [1] G.G. Ying, *Environment International*, 32 (2006) 417–431.
- [2] M.J. Scott and M.N. Jones, *Biochimica et Biophysica Acta*, 1508 (2000) 235–251.
- [3] E. Matthijs and H. De Henau, *Tenside Surfactants Detergents*, 24 (1987) 193–199.
- [4] J. Jensen, *Sci. Total Environ.*, 226 (1999) 93–111.
- [5] A. Marcomini and W. Giger, *Tenside Surfactants Detergents*, 25 (1988) 226–229.
- [6] M. Holysh, S. Paterson and D. Mackay, *Chemosphere*, 15 (1986) 3–20.
- [7] P.H. Brunner, S. Capri, A. Marcomini and W. Giger, *Water Res.*, 22 (1988) 1465–1472.
- [8] H. De Henau and E. Matthijs, *Intern. J. Environ. Anal. Chem.*, 26 (1986) 279–293.
- [9] M.T. García, E. Campos, I. Ribosa, A. Latorre and J. Sánchez-Leal, *Chemosphere*, 60 (2005) 1636–1643.
- [10] P. Schöberl, *Tenside Surfactants Detergents*, 26 (1989) 86–94.
- [11] C.D. van Ginkel, *Biodegradation*, 7 (1996) 151–164.
- [12] J.C. Sigoillot and M.H. Nguyen, *FEMS Microbiol. Ecol.*, 73 (1990) 59–68.
- [13] L. Jiménez, A. Breen, N. Thomas, T.W. Federle and G.S. Saylor, *Appl. Environ. Microbiol.*, 57 (1991) 1566–1569.
- [14] J.S. Yadav, D.L. Lawrence, B.A. Nuck, T.W. Federle and C.A. Reddy, *Biodegradation*, 12 (2002) 443–453.

- [15] P. Eichhorn, S.V. Rodrigues, W. Baumann and T.P. Knepper, *Sci. Total Environ.*, 284 (2002) 123–134.
- [16] S. Terzić, D. Hršak and M. Ahel, *Water Res.*, 26 (1992) 585–591.
- [17] A. Marcomini, A. Giacometti and G. Pojana, *Ann. Chim.*, 92 (2002) 27–39.
- [18] APHA, *Standard Methods for the Examination of Water and Wastewater*, 19th edn., United States of America, 1995.
- [19] A. Kouvarakis, Department of Chemistry, University of Crete, GR-71409, Iraklion, Greece, personal communication.
- [20] C. Pakou, M. Kornaros, K. Stamatelidou and G. Lyberatos, *Proc. WasteEng 2005*, Albi, France, 2005.
- [21] Organization for Economic Cooperation and Development (OECD). 1992, Ready Biodegradability, CO₂ evolution (Modified Sturm Test), Method 301-B, Paris, France.