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Inhibition of biogas production by alkyl benzene sulfonates (LAS) in a screening test for anaerobic biodegradability

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Abstract

The effect of the inoculum source on the digestion of linear alkylbenzene sulfonates (LAS) under anaerobic conditions has been investigated. The potential for primary and ultimate LAS biodegradation of anaerobic sludge samples obtained from wastewater treatment plants (WWTPs) of different geographical locations was studied applying a batch test system. It was found that only 4–22% of the LAS added to the batch anaerobic digesters was primarily transformed suggesting a poor primary degradation of the LAS molecule in anaerobic discontinuous systems. Regarding ultimate biodegradation, the addition of LAS to the batch anaerobic digesters caused a reduction on the extent of biogas production. Significant differences in the inhibition extent of the biogas production were observed (4–26%) depending on the sludge used as inoculum. Effect of the surfactant on the anaerobic microorganisms was correlated with its concentration in the aqueous phase. Sorption of LAS on anaerobic sludge affects its toxicity by depletion of the available fraction of the surfactant. LAS content on sludge was related to the total amount of calcium and magnesium extractable ions. The presence of divalent cations promote the association of LAS with anaerobic sludge reducing its bioavailability and the extent of its inhibitory effect on the biogas production.

Abbreviations: ECETOC – European Centre for Ecotoxicological and Toxicological Safety Assessment of Chemicals; LAS – Linear alkyl benzene sulfonate; WWTP – Waste water treatment plant

Introduction

Linear alkylbenzene sulfonate (LAS) is the most important anionic surfactant used in household and industrial cleaning agents. LAS with other surface active agents form a group of chemicals with a high overall environmental relevance, due to a combination of their inherent environmental properties and their very large production volume. They are typically discharged into the environment through the sewage treatment infrastructure or directly in situations where no treatment is available.

Biodegradation is by far the most important mechanism for the irreversible removal of chemicals from the aquatic and terrestrial environments. Primary biodegradation defined as initial or partial breakdown of surfactant molecules that destroys their amphiphilic properties is usually measured by a substance specific analytical method. The subsequent complete breakdown of residues formed by primary biodegradation is termed ultimate biodegradation. Many studies about the biodegradation of LAS have shown that the surfactant is well biodegraded under a wide variety of aerobic conditions (Painter & Zabel 1989; Sanchez-Leal et al. 1994; Swisher 1987;). Surfactants in domestic and industrial waste are most likely to receive some form of biological treatment. Most treatment systems are aerobic, but sludge arising

from such treatments is often subjected to anaerobic digestion. Due to the sorption of LAS on suspended solids and/or precipitation depending on the water hardness (Garcia et al. 2002), up to 20% of the surfactant escapes aerobic treatment (Prats et al. 1997). This effect is most likely to occur with all anionic surfactants due to similar physical chemical properties as LAS, and in fact substantial soap concentrations have been reported in sludge (Prats et al. 1999). Thus, the effect of undegraded surfactant on the anaerobic digester functioning, and its biodegradation by methanogenic microorganisms, are important considerations. However, it has been reported that, even at the highest LAS concentrations found in anaerobic digesters, no inhibition was detected in the digester functioning (Berna et al. 1989).

From most of the earlier published investigations about the anaerobic biodegradability of sulfonated surfactants, it appears that they are readily degraded aerobically either solely or mainly by pathways involving molecular oxygen, being recalcitrant under anaerobic conditions. Anaerobic recalcitrance of LAS has been reported by Mc Evoy & Giger (1986) as well as by Federle & Schwab (1992). These earlier conclusions were supported by the findings than in anaerobically treated sludge relatively high concentrations of LAS can be found (5–10 g/kg dry solids) whereas activated sludge and air-dried digester-sludge, i.e. aerobically treated sludge, contain low LAS concentrations (0.1–0.8 g LAS/kg dry matter) (Berna et al.1989; McAvoy et al. 1993; Painter & Zabel 1989; Waters & Feijtel 1995). However, some recent studies have shown that LAS degradation is possible under anaerobic conditions. Thus, evidence of LAS biodegradation without oxygen has been found when anaerobic treatment is preceded by aerobic exposure (Larson et al. 1993). Mogensen et al. (2003), have reported the anaerobic biodegradation of LAS by granular sludge using UASB reactors. In addition, desulfonation of LAS has been reported to occur by anaerobic bacteria in the laboratory (Denge & Cook 1999). Recently, Angelidaki et al. (2000) have assessed the influence of the source of the environmental sample used as inoculum on the biotransformation of xenobiotics.

In the present paper the effect of the inoculum source on the anaerobic digestion of LAS has been studied. The potential for primary and ultimate LAS transformation of anaerobic sludge obtained from various waste water treatment plants (WWTPs) has been examined applying a batch test system. The effect of the LAS sorption on the sludge on the surfactant availability has been investigated.

Materials and methods

Linear sodium alkylbenzene sulfonate (LAS)

LAS was synthesized by Petresa (Spain). Its alkyl chain distribution (C_{10}–C_{14}) obtained by gas chromatography after desulfonation of LAS was: 12.8% C_{10}LAS, 33.7% C_{11}LAS, 29.8% C_{12}LAS, 23.0% C_{13}LAS and 0.7% C_{14}LAS and its mean molecular mass 342.4.

Inoculum

As inoculum, sludge samples collected from the anaerobic digesters of four municipal wastewater treatment plants (Manresa, Gavà, Vilanova and Abrera) near Barcelona were used. The temperature and the hydraulic retention time of the anaerobic digesters of these WWTPs ranged between 35–36 °C and 20–30 days, respectively. Total and volatile solids of anaerobic sludge samples were determined according to Standard Methods (APHA 1995a, 1995b) obtaining values between 30–50 g/l and 45–60%, respectively. After collection, sludge was washed with the mineral salt solution as described in the ECETOC-test (ECETOC 1988) to reduce the amount of inorganic carbon to a value $\leq$ 10 mg/l. A final re-suspension step allowed adjusting the dried solids concentration between 3–4.5 g/l.

Anaerobic batch test system

The potential of different anaerobic sludge for LAS biodegradation was studied using a batch test system based on the method proposed by Birch et al. (1989) and the European Centre for Ecotoxicology and Toxicity of Chemicals (ECETOC 1988) . This method evaluates the extent of ultimate anaerobic biodegradation of a chemical based on the production of biogas (methane and carbon dioxide) as compared to a blank without the addition of the test substance.

Batch anaerobic digesters were inoculated with anaerobic sludge samples from the anaerobic digesters of the following WWTPs: Manresa I (November, 01), Vilanova (November, 01), Gavà (December, 01), Manresa II (May, 02), Abrera (May, 02). The sludge concentration ranged from 3.1 to 4.3 g dry solids/L. To minimize potential toxic effects of the surfactant on the methanogenic microorganisms (Sanz et al. 1999), LAS degradation was tested at 20 mg C/L (32 mg/l as active matter). Bottles with only anaerobic sludge were tested to determine the endogenous biogas production. The anaerobic sludge and the test compound were incubated in 250 ml pressure-resistant glass bottles contained 175 ml of liquid volume leaving a 75 ml headspace volume. The temperature was set at 36 °C. The bottles were fitted with gas tight septa and aluminium crimp seals. After sealing the vessels and incubating them for about 1 h, excess gas was released to the atmosphere and the incubation proceeded in the dark. The evolved pressure was measured with a digital manometer connected to a syringe needle which was inserted through the septum. The incubation time was 40–50 days. Eight replicates of each experiment (control and LAS spiked digesters) were performed. The increase in headspace pressure in the closed bottles was used to follow the mineralization process. At the end of the test, after allowing the sludge to settle, vessels were opened and the dissolved part of carbon dioxide was determined as the concentration of inorganic carbon (IC) in the clear supernatant. For the measurements of inorganic carbon (IC), a carbon analyzer was used (Shimadzu TOC-5050). At the end of the test period, specific analysis of anionic surfactant both in the supernatant liquor and in the settled sludge was carried out to determine the extent of primary biodegradation.

Calculation of ultimate biodegradation and inhibition on biogas production

For each sample the extent of ultimate biodegradation was given by

$$\%\text{Biodegradation} = \frac{C_{\text{T(test)}} - C_{\text{T(control)}}}{\text{test compound carbon added}} \times 100$$

and for each vessel the total mineralised carbon (C_T) was given by

$$C_{\text{T}} = C_{\text{H}} + C_{\text{L}}$$

where C_{H} (inorganic carbon in the headspace) was obtained from biogas pressure according to the gas laws and C_{L} (inorganic carbon in the liquid phase) was given by:

$$C_{\text{L}} = \text{IC} \times V_{\text{L}}$$

where V_{L} was the volume of the digesting liquor and IC was the concentration of inorganic carbon in the liquor. Inhibition of biogas production was calculated from the measurement of final biogas production in test samples (V_{T}) and controls (V_{c}), applying the following equation:

$$\%\text{Inhibition} = \left(1 - \frac{V_{\text{T}}}{V_{\text{C}}}\right) \times 100$$

Analytical procedures

Total and volatile solids were determined according to Standard Methods (1985a, b). LAS was determined by high performance liquid chromatography (HPLC) using a Waters liquid chromatograph equipped with a variable wavelength UV detector and a reversed-phase column (μ-Bondapack C_{18}, 10 μm, 300 mm length, 4.6 mm i.d., Waters Associates). The mobile phase consisted of 20% of solvent A (water) and 80% of solvent B (0.15 M $NaClO_4$ in acetonitrile/water 80/20). The flow rate was maintained at 1 ml/min. The column effluent was monitored at 223 nm. LAS quantification was conducted on the basis of an external standard. The LAS extraction procedure was based on the method already described by Matthijs and De Henau (1987). At the end of the biodegradation assays, aqueous and solid phases were separated by centrifugation at 4000 rpm for 15 min. *Liquid samples*: dissolved LAS was concentrated by solid phase extraction using octadecyl (C_{18}) reversed phase silica columns. The eluted solutions were then analysed by HPLC. *Solid samples*: sludge from anaerobic digesters, after being washed with the inorganic salt described in the ECETOC method, and suspended solids at the end of the anaerobic assays after being centrifuged, were dried at 105 °C and extracted using methanol Sohxlet extraction for 8 h. These extracts were passed through a strong

anion exchange column, eluted with methanol/HCl solution, neutralized and then passed over a C_{18} reversed phase silica column. The eluted solutions were then analysed by HPLC.

The extractable cations Ca^{2+}, Mg^{2+}, K^+ and Na^+ from anaerobic sludge samples were determined by extraction with ammonium acetate at pH 7 as described by Grant (1986). The water hardness was analyzed by the EDTA titrimetric method (APHA 1995c).

Results and discussion

Association of LAS with anaerobic sludge

Although all the anaerobic tests were performed adding the same initial concentration of soluble LAS, i.e. 32 mg/l as sodium salt, the surfactant was distributed between the aqueous and the solid phases resulting in a variable depletion of the remaining surfactant in the aqueous phase. The extent of LAS removal from the aqueous phase will depend on the LAS molecules that can be hydrophobically sorbed on to the sludge and/or precipitated as calcium salts (Garcia et al. 2002).

Analytical data of LAS content in aqueous and solid phases can be used to obtain the adsorption isotherms of LAS on anaerobic sludge. Sorption data of LAS on sludge can be described by the Freundlich equation:

$$A = k \cdot D^{1/n}$$

where A is the amount of LAS adsorbed per unit of adsorbent (mg/g), D is the equilibrium concentration of LAS in solution (mg/l), k is the sorption coefficient and $1/n$ is a measure for the adsorption intensity. The results of applying the Freundlich equation to the adsorption data are represented in Figure 1 and gave a straight line derived from regression analysis. The Freundlich constants, k and $1/n$, were determined from the intercept and the slope, respectively, being 3.24 and 0.92 with a correlation coefficient $r = 0.94$ ($p < 0.0001$). The values of the Freundlich constants obtained in this study are in good agreement with those reported for aerobic sludge (Garcia et al. 2002; Swisher 1987). This fact suggests that adsorption intensity of LAS on anaerobic sludge was very similar to that on aerobic sludge.

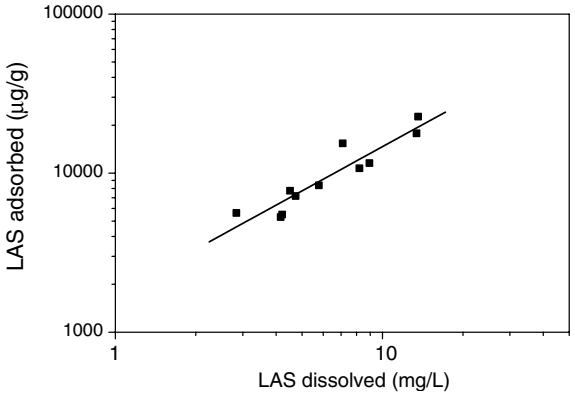

Figure 1. Freundlich isotherm for the adsorption of LAS on anaerobic sludge.

LAS homologue distribution

The individual LAS homologue distribution was determined by HPLC analysis. Similar homologue distributions were found for the supernatant liquors of the anaerobic digesters inoculated with different sludge samples. Likewise, alkyl chain length distributions in the sludge of the anaerobic digesters were very similar between them. In Figure 2, LAS homologue distribution for digesters inoculated with sludge from the Waste water treatment plant (WWTP) of Gavà and spiked with surfactant is given. The alkyl chain length average in the liquid and solid phases of the anaerobic digesters is reported in Table 1.

There are significant differences between the homologue distribution of the LAS associated with the sludge and the homologue distribution of the LAS remaining in the liquid phase (Figure 2).

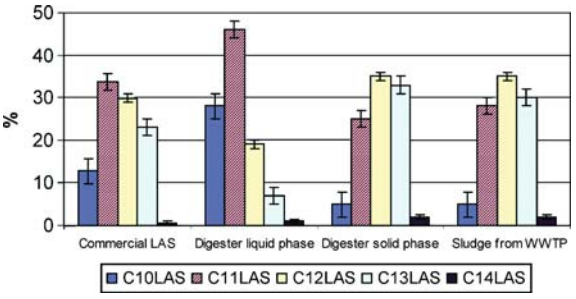

Figure 2. LAS homologue distribution of the commercial LAS, anaerobic sludge from Gavà WWTP and the solid and liquid phases of the batch anaerobic digesters inoculated with sludge from Gavà WWTP and spiked with LAS.

Table 1. Average of the LAS alkyl chain length in anaerobic digesters inoculated with different sludge source

Inoculum source	Digester liquid phase		Digester solid phase	
	(control)	(LAS spiked)	(control)	(LAS spiked)
Manresa (I)	11.0	10.4	12.8	12.6
Vilanova	10.7	10.2	12.5	12.5
Gavà	11.2	11.2	12.1	12.0
Manresa (II)	11.3	11.0	12.1	12.0
Abrera	11.3	11.1	11.9	11.9
Mean value (95% C.I.)	11.1 ± 0.2	10.8 ± 0.4	12.3 ± 0.3	12.2 ± 0.3

Solid phases, i.e., sludge from the anaerobic digesters, were enriched in longer chain LAS homologues while the overlaying water was depleted of longer chain homologues (Table 1) as compared to the parent chemical distribution (average of 11.7 alkyl chain length). Sorption increases with increasing alkyl chain length in the LAS molecule which would be consistent with a bonding mechanism mainly due to hydrophobic interactions as previously reported for association of LAS with aerobic sludge (Garcia et al. 2002). The increased sorption with increasing LAS chain length is also consistent with monitoring studies (Prats et al. 1993) reporting that anaerobic sludge and sediments are enriched in longer chain LAS homologues while the water overlaying the solids was depleted of longer chain homologues compared to the parent chemical distribution.

Sludge parameters

In order to investigate potential parameters related to LAS associated with anaerobic sludge, extractable bases on the cation exchange sites of sludge as well as water hardness of secondary effluents of the WWTPs were determined (Table 2). Exchangeable cations (i.e. those that are exchanged by a cation of an added salt solution) were determined by extraction with ammonium acetate at pH 7.

As expected, water hardness of the treated effluents (Table 2) was closely related to the LAS content in the sludge from the various WWTP anaerobic digesters (Table 3 footnote (a)). Thus, the surfactant amount in sludge increased with increasing water hardness. On the other hand, the LAS content on the sludge at the end of the anaerobic tests (Table 3 footnote (c)) was correlated with the extractable calcium and magnesium ions from the sludge (Table 2). Enhanced LAS adsorption occured with increasing the amount of extractable divalent cations. The dependence on divalent cations concentration can be attributed either to electrostatic interactions or to specific chemical interactions with surface functional groups. Thus, LAS sorption on sludge particles can be facilitated by divalent extractable cations owing to the decrease of the electrostatic repulsion between the ionic heads of LAS. Furthermore, divalent cations could adsorb directly to the sludge particle, yielding positively-charged sites onto

Table 2. Extractable cations of the anaerobic sludge used as inoculum in the biodegradation tests

Sludge source	Water hardness (mg/l $CaCO_3$)	meq/100 g dry sludge				
		Ca^{2+}	Mg^{2+}	K^+	Na^+	total
Manresa (I)	300	22	4	7	11	45
Vilanova	475	31	13	12	82	138
Gavà	600	43	2	21	50	116
Manresa (II)	320	26	7	15	22	70
Abrera	340	31	16	15	30	92

Table 3. LAS mass balance in the anaerobic digesters spiked with LAS

Inoculum source	Initial LAS (μg)		Final LAS (μg)		LAS removal (%)
	LAS in solids[a] mean ± SD	LAS spiked[b]	LAS in solids[c] mean ± SD	LAS in liquid phase[d] mean ± SD	mean ± SD
Manresa (I)	3789 ± 334	5000	6092 ± 530	1040 ± 121	19 ± 10
Vilanova	7223 ± 497	5000	9251 ± 428	2448 ± 311	4 ± 7
Gavà	11981 ± 544	5796	13965 ± 584	2414 ± 360	8 ± 13
Manresa (II)	7372 ± 657	5635	10243 ± 804	812 ± 126	15 ± 15
Abrera	4319 ± 383	5635	6185 ± 463	1609 ± 76	22 ± 13

[a]LAS contribution of the anaerobic sludge used as inoculum.
[b]LAS added to the anaerobic digesters.
[c]LAS content of the anaerobic sludge at the end of the assays.
[d]LAS content in the liquid phase at the end of the assays.

which negatively charged LAS molecule can adsorb. As a result, these cations promote LAS association with anaerobic sludge reducing the surfactant concentration in the aqueous phase.

Primary biodegradation

Primary biodegradation of LAS in anaerobic tests was calculated from the overall mass balance of the surfactant in the anaerobic digesters. For this purpose, LAS contents in anaerobic sludge from the WWTPs, after washing with the ECETOC anaerobic solution as well as in the supernatants and solids at the end of the tests were determined. A distribution of LAS between aqueous and solid phases occurs when LAS is added to digesters containing anaerobic sludge. At the end of the experiments, in all the digesters where LAS was initially added as sodium salt, an increase in the concentration of LAS in the solid phase was found in relation to the LAS content in sludge without spiked LAS (Table 3). The percentages of LAS removal were calculated from the mass balance of the surfactant (Table 3).

In digesters spiked with LAS, the extent of LAS removal was in the range of 4–22%. The percentages of LAS removal are in good agreement with the results of the LAS mass balance over full scale anaerobic digesters (0–35%) reported in monitoring studies (Berna et al. 1989). The low degree of LAS removal measured both in our laboratory screening tests (4–22%) as well as in the monitoring studies (0–35%) suggests that the microbial transformation of LAS under these anaerobic conditions is not significant. These findings are consistent with an oxidation of the alkyl chain of the LAS molecule as the main initial degradative pathway of this molecule (Cain 1987). In field systems, it is not entirely clear to which process the small degree of removal observed can be ascribed (Larson et al. 1993). In anaerobic digesters, the surfactant depletion observed could be due to some oxygen diffusion that occurred in the handling of sludge samples in the initial steps of the experiments. So, under oxygen-limited conditions, sulfonates could mineralize even if the rate is not as rapid as that observed under aerobic conditions, once the sulfonate biodegradation has been initiated the intermediates might continue to biodegrade anaerobically (Larson et al. 1993). No significant correlation was found between the surfactant removal (Table 3) and the characteristics of the sludge (Table 2) at the 95% probability level. This would give support to the oxygen diffusion in the earlier steps of the degradation tests as the main reason for the small extent of primary degradation observed in the anaerobic digesters.

Biogas production and ultimate biodegradation

The evolution of the net pressure, i.e., the mean pressure in the digesters spiked with surfactant substracting the mean endogenous biogas pressure is shown in Figure 3. In all the tests, excepting those performed using anaerobic sludge from the WWTPs of Vilanova, biogas formation was detected. The biogas production for digesters spiked with LAS was similar or lower than the endogenous biogas production. In tests performed with sludge from the WWTP of Vilanova, a complete

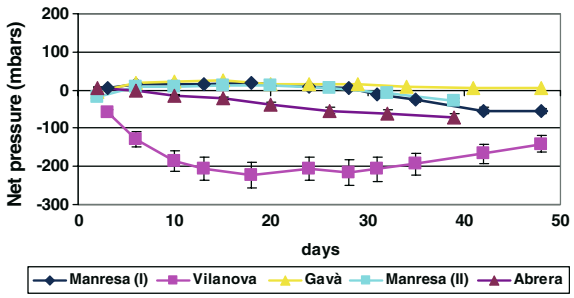

Figure 3. Evolution of the net pressure of biogas in the anaerobic digesters.

inhibition on biogas production was initially observed. However, after a period of 20 days, the methanogenic population started to produce biogas. The recovery could be attributed to acclimation of the microbial community to the surfactant.

In most of the digesters, the addition of surfactant caused a decrease in the rate of biogas production. Sensitivity of methanogenic microorganisms to LAS has been previously described (Alexander 1994). However, the anaerobic test conditions used in our study, as those generally used in screening tests, were more stringent than the real conditions prevailing in anaerobic digesters where LAS/sludge ratio is at least one order of magnitude lower.

The extent of the biogas inhibition was calculated comparing the gas production in digesters spiked with LAS and those containing only anaerobic sludge (Table 4). Significant differences in the inhibition extent were observed for the different sludge source. Assuming that the inoculum source is the main difference between the anaerobic tests carried out, reasons for the observed variability on the inhibition extent should be related with the characteristics of the sludge. Due to the background concentration of LAS in the anaerobic sludge, a fraction of the LAS sorbed on the sludge is dissolved in the aqueous phase according to its partition coefficient. Thus, the effect of surfactant addition to the anaerobic digesters on the biogas production should be correlated with the increase of LAS concentration in relation to control. A significant positive correlation was found between the increase in LAS concentration in the supernatant liquor and the extent of the inhibition of the biogas production at the 95% probability level (Table 4). These results suggest that the toxicity of LAS to anaerobic microorganisms is closely related to the LAS fraction in the aqueous medium. Since the adsorption of LAS on sludge determines the distribution of the surfactant between solid and liquid phases (Garcia et al. 2002), sludge parameters affecting LAS sorption will affect the toxicity of the surfactant to the methanogenic consortia. Furthermore, as it has been already discussed in a previous section, extractable calcium and magnesium cations seem to promote LAS association with anaerobic sludge. This phenomenon reduces LAS availability and, consequently, diminishes its toxic action.

Table 4. Increment of LAS concentration in the supernatant liquor and inhibition of the biogas production in the anaerobic digesters spiked with LAS compared to control digesters

Sludge source	△LAS concentration (mg/l)	mean inhibition (95% C.I.) (%)
Manresa (I)	2.7	16 ± 5
Vilanova	6.8	26 ± 10
Gavà	4.6	13 ± 4
Manresa (II)	1.7	4 ± 1
Abrera	4.3	15 ± 4

Conclusions

The study showed that only 4–22% of LAS added to the batch anaerobic digesters was primarily transformed. These results provide evidence of a poor primary degradation of the LAS molecule in anaerobic discontinuous systems. Regarding ultimate biodegradation, the addition of LAS to the batch anaerobic digesters caused a reduction on the extent of biogas production. Significant differences on the extent of biogas inhibition were observed (4–26%) depending on the source of the sludge used as inoculum. The effect of LAS on anaerobic populations was related to the surfactant fraction in the aqueous phase. Thus, the sorption of LAS with anaerobic sludge reduces its toxicity to the anaerobic microorganisms by depletion of the available fraction of surfactant. The presence of calcium and magnesium extractable cations seems to promote the association of LAS with suspended solids, reducing its availability and inhibitory effect on biogas production.

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