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Inhibition of the anaerobic digestion process by linear alkylbenzene sulfonates

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Abstract

Linear Alkylbenzene Sulfonates (LAS) are the most widely used synthetic anionic surfactants. They are anthropogenic, toxic compounds and are found in the primary sludge generated in municipal wastewater treatment plants. Primary sludge is usually stabilized anaerobically and therefore it is important to investigate the effect of these xenobiotic compounds on an anaerobic environment. The inhibitory effect of Linear Alkylbenzene Sulfonates (LAS) on the acetogenic and methanogenic step of the anaerobic digestion process was studied. LAS inhibit both acetogenesis from propionate and methanogenesis from acetate and hydrogen and it is shown that the propionate-utilising bacteria are more sensitive to the presence of LAS than the acetoclastic methanogens. It has been proven that the inhibition intensity depends on the solids concentration and thus the term "biomass specific LAS concentration" has been introduced in order to describe the phenomenon better. Conclusively, it is believed that the inhibitory effect of LAS is the main reason that anaerobic microbial enrichments on LAS have not been succeeded yet. Also, the inhibition caused by LAS on the acetogenic and methanogenic step of the anaerobic digestion process should be seriously taken into consideration when wastewater from a surfactant producing industry is to be treated biologically or enter a municipal wastewater treatment plant that employs anaerobic technology. The upper allowable biomass specific LAS concentration should be 14 mg LAS $(\text{gVSS})^{-1}$.

Introduction

Linear Alkylbenzene Sulfonates (LAS) are the most common synthetic anionic surfactants used as domestic detergents. It has been reported that LAS are toxic to aquatic biota and acute toxicity may occur at concentrations as low as 0.1 ppm resulting in pathological changes of gills, decreased growth, impaired swimming activity and other abnormalities (Hofer et al. 1995; Lewis 1991; Takada et al. 1994). Their production amounts to 2,500,000 tonnes per year (Cook 1998; Schulze 1996). Although LAS have been reported to be readily biodegradable by aerobic processes, a considerable portion of the surfactant load into a sewage treatment facility (reportedly 20–50%) is associated with suspended solids thus escaping the aerobic treatment processes (Mackay et al. 1996; Van der Meeren & Verstraete 1996). The presence of LAS in the stabilised sludge has undesirable environmental effects due to LAS potential for acute toxicity (Hofer et al. 1995; Lewis 1991; Takada et al. 1994). Land application of the sludge is the most preferred disposal method while the presence of poorly biodegradable organic compounds, including detergents, negatively affects the sludge quality (Magoarou 2000). So far it was widely believed that LAS are not degraded during the anaerobic biological processes that are usually employed in sewage sludge stabilisation. Moreover no anaerobic microbial enrichment culture on LAS capable of utilising LAS carbon has been succeeded yet. LAS are easily biodegraded under aerobic conditions and the initiation of their biodegradation is similar to this of alkanes (ω-oxidation followed by β-oxidation) (Cavalli et al. 1996). Several recent studies (Wilson et al. 1986; Zeyer et al. 1986; Grbic-Galic & Vogel 1987; Federle & Schwab 1992) have shown that

benzene (sub unit of LAS molecule), some alkylbenzenes and even the extremely hydrophobic alkanes of C_{12}–C_{18} (Aeckersberg et al. 1991; Rueter et al. 1994; So & Young 1999) are biodegradable anaerobically and microbial enrichment cultures have already been established on them. Furthermore, recent studies showed that LAS could be anaerobically biodegradable (Angelidaki et al. 2000; Mogensen & Ahring 2002) but no bacterial strain or a microbial enrichment capable of degrading LAS by utilising LAS carbon under anaerobic conditions has been isolated. The study of the toxicity of LAS molecules on the anaerobic metabolism could be critical for the understanding of their recalcitrant nature and the difficulty in establishing a microbial enrichment capable of degrading them. So far the potential inhibitory effect that LAS have on the overall anaerobic process has not been studied in depth. The present study aims at the understanding of the inhibitory effect of LAS on the acetogenic and methanogenic step of the anaerobic digestion process. Batch experiments were performed with anaerobic sludge acclimated to linear dodecylbenzene sulfonate (LAS-12) for a period of more than one year. Furthermore, microbial enrichments on acetate and propionate were used for more fundamental study of the inhibition phenomenon.

Materials and methods

Analytical and computational methods

Determinations of the total (TSS) and volatile (VSS) suspended solids were carried out according to Standard Methods (APHA 1989). For the quantification of volatile fatty acids, the samples were analysed after acidification with 17% H_3PO_4 on a gas chromatograph (Hewlett Packard 5890 series II) with a flame ionisation detector and a capillary column (Hewlett Packard FFAP 30 m, inner diameter 0.53 mm, film 1 μm). The oven was programmed from 115 °C to 130 °C at a rate of 5 °C/min and nitrogen was used as carrier gas at 18 mL/min. The injector and detector temperature was set at 175 °C and 200 °C respectively. Methane production was quantified with a gas chromatograph (Shimadzu GC-8A) with a flame ionisation detector and a packed column (Porapak Q 2 m, outer/inner diameter 5/3mm, 80/100-mesh). The oven temperature was set at 120 °C and nitrogen was used as carrier gas. The injector and detector temperature was set at 130 °C. For the analysis of LAS-12, samples were dried at 60 °C, dissolved in alkaline methanol and then filtered through glass fibre filters. LAS-12 was quantified by HPLC equipped with an RP-C18 (250 by 4.6 mm, particle size 5 mm) column and a fluorescence detector set at 225 nm. The mobile phase had a flow of 1.5 ml/min and consisted of a mixture of methanol in Milli-Q water in a ratio of 1:0.28 and $NaClO_4$ 0.25 M. LAS-8 was used as internal standard. The medium (BA medium) used in enrichments and batch experiments was prepared from the following stock solutions (chemicals in g l^{-1} of distilled water): (A) NH_4Cl, 100; NaCl, 10; $MgCl_2{\cdot}6H_2O$, 10; $CaCl_2{\cdot}2H_2O$, 5; (B) $K_2HPO_4{\cdot}3H_2O$, 200; (C) resazurin, 0.5; (D) trace metals and selenite solution: $FeCl_2{\cdot}4H_2O$, 2; H_3BO_3, 0.05; $ZnCl_2$, 0.05; $CuCl_2{\cdot}2H_2O$, 0.038; $MnCl_2{\cdot}4H_2O$, 0.05; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 0.05; $AlCl_3$, 0.05; $CoCl_2{\cdot}6H_2O$, 0.05; $NiCl_2{\cdot}6H_2O$, 0.092; ethylene-diamine-tetra-acetate, 0.5; $Na_2SeO_3{\cdot}5H_2O$, 0.1; HCl 37%, 1 ml; (E) vitamin solution according to Wolin et al. (1963). The following volumes of stock solutions were added to 916 ml of distilled water: A, 10 ml; B, 2 ml; C, 1 ml; D, 1 ml; E, 10 ml. 50 ml of a 52 g l^{-1} $NaHCO_3$ solution were added as well. The medium was gassed with 80% N_2–20% CO_2, dispensed and autoclaved. Before inoculation the medium was reduced with a 25 g l^{-1} $Na_2S{\cdot}9H_2O$ solution to a ratio of 0.1 ml/10 ml of medium. Simulation of the results was made with a modified non-linear least squares fitting method. The least-squares objective was to select the best kinetic parameter values so as to minimise the squared residuals between the experimentally measured and the respective predicted concentrations.

Microbial enrichments on acetate and propionate

Enrichment cultures on propionate and acetate were established using 335 ml serum vials containing 100 ml of BA medium supplemented with propionate or acetate at a final concentration of approximately 2 g l^{-1} (27 mM propionate or 33 mM acetate). Yeast extract was also added in a concentration corresponding to a ratio of 0.2 mg/mg propionate and 0.15 mg/mg acetate. The vials were sealed with butyl rubber stoppers and aluminium crimps and autoclaved at 140 °C. Traditional enrichment method was applied, i.e. repeated transfers of approximately 10% of the enrichment culture to new medium and incubation at 37 °.

Batch kinetic experiments with anaerobic sludge

Anaerobic sludge from a laboratory-scale (3.5 L useful volume) continuous stirred tank reactor acclimated to LAS-12 for a period of over one year was used as inoculum. The reactor was operated at the mesophilic temperature range (37 °C) at a hydraulic retention time of 15 days and the influent consisted of sewage sludge spiked with LAS-12 (Mogensen et al. 2002). Acetate and propionate degradation experiments were carried out in triplicates in 58 ml serum vials sealed with butyl rubber stoppers and aluminium crimps. The serum bottles contained 4 ml of BA medium, acetate or propionate (added in the form of a sodium acetate or sodium propionate solution) at a final concentration of approximately 0.5 g l^{-1} and were supplemented with yeast extract as described above. Different amounts of LAS were added in each triplicate in order to study the inhibition caused by LAS at various concentrations. Triplicates without LAS addition served as controls. Finally, the vials were inoculated with 20 ml of the LAS acclimated anaerobic sludge and incubated at 37 °C.

Batch kinetic experiments with enrichments on acetate and propionate

Acetate and propionate degradation experiments with microbial enrichments on acetate and propionate respectively were carried out in duplicates in 58 ml serum vials. The vials contained 10 ml of BA medium and acetate or propionate (added in the form of a sodium acetate or propionate solution) at a final concentration of 1 g l^{-1}. Different amounts of LAS were added in each duplicate in order to study the inhibition caused by LAS at various concentrations. Duplicates without LAS addition served as controls. The vials were inoculated with 8 ml of the enrichment on acetate or propionate and incubated at 37 °C. Propionate and/or acetate concentration was measured throughout the propionate and acetate degradation experiments respectively.

The study of the inhibition caused by the LAS molecules on the methanogenesis from hydrogen was carried out in 58 ml serum vials containing 10 ml of BA medium. The vials were inoculated with 8 ml of the enrichment on propionate and incubated at 37 °C. Different amounts of LAS were added in each triplicate and the gas phase was replaced by a mixture of H_2 and CO_2 (H_2/CO_2: 80/20) at a final pressure of 2 atm. Two kinds of controls were used in this case: a control triplicate without LAS addition and a control triplicate without LAS and hydrogen addition. Methane production in each vial was measured throughout the experiment.

Results and discussion

Experiments with anaerobic sludge

Experiments with acetate as substrate

Two sets (A and B) of acetate fed batch experiments were carried out with different LAS concentration added. LAS, TSS and VSS concentration of the inoculum used (control vials) are shown in Table 1. Acetate concentration was measured throughout the experiments and is shown in Figures 1a and 1b along with the experimental results for methane production in set B (Figure 1b). Acetate was found to be stoichiometrically converted into methane.

Despite the fact that the differences in acetate consumption rate were relatively small for LAS concentrations added up to 100 mg l^{-1} (190 mg l^{-1} total LAS), it is obvious that slight inhibition of acetate degradation occurred with even low concentrations of LAS added. Furthermore, neither acetate degradation nor methane production was observed in the experiment with 150 ppm LAS added (284 mg l^{-1} total LAS). The latter suggests that there is a critical LAS concentration between 100 and 150 mg l^{-1} LAS added (190 and 284 mg l^{-1} total LAS) where methanogenesis from acetate is completely inhibited.

Experiments with propionate as substrate

One set of propionate fed batch experiments was carried out with different LAS concentration added. LAS, TSS and VSS concentration of the inoculum used is shown in Table 1. Propionate and acetate concentration versus time is shown in Figures 2a and 2b respectively.

Inhibition of propionate degradation was also observed. In the experiment with 350 ppm LAS added (492 mg l^{-1} total LAS) propionate degradation did not proceed at all. Consequently, there is a critical LAS concentration between 150 and 350 mg l^{-1} LAS added (292 and 492 mg l^{-1} total LAS) where propionate consumption is completely inhibited. As it was anticipated acetate consumption did not proceed at all in the vials with 150 and 350 ppm LAS added. Furthermore and despite that no propionate degradation occurred in the vials with 350 ppm LAS added (Figure 2a) acetate accumulation was observed (Figure 2b). Probably, acetogenesis from other carbon

Table 1. LAS, TSS and VSS concentration of the inoculum used in the batch experiments with acetate and propionate fed anaerobic sludge

	LAS (mg l^{-1})	TSS (g l^{-1})	VSS (g l^{-1})
With acetate as substrate			
Experimental set A	90	25.8	12.7
Experimental set B	134	24.0	11.4
With propionate as substrate	142	31.1	13.6

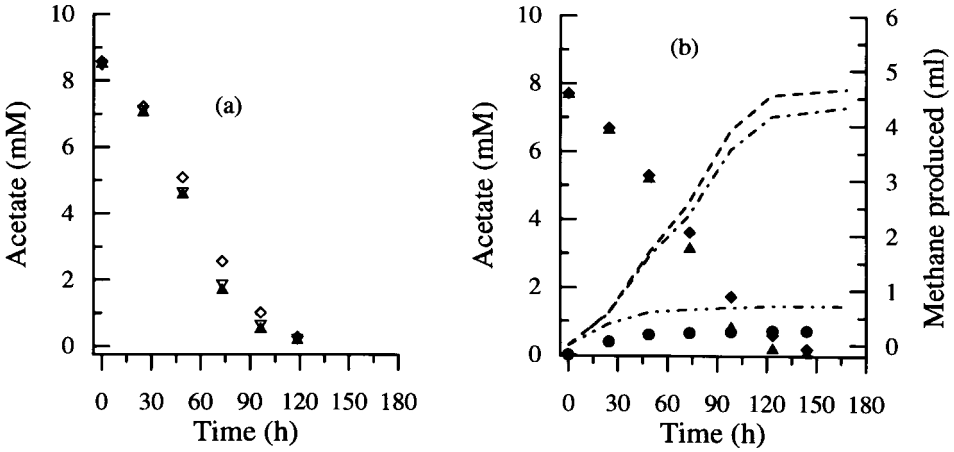

Figure 1. Acetate concentration profile (a) during set A and acetate concentration and methane production profile (b) during set B of batch experiments with acetate fed anaerobic sludge. Acetate concentration in control vials (▲) (with 90 and 134 ppm LAS for set A and B respectively), vials with 10 mg l^{-1} LAS added (▽) (100 ppm total LAS), vials with 50 mg l^{-1} LAS added (♦) (184 ppm total LAS), vials with 100 mg l^{-1} added (◊) (190 ppm total LAS) and vials with 150 mg l^{-1} LAS added (●) (284 ppm total LAS). Methane production in control vials (- - - -),vials with 50 mg l^{-1} LAS added (·-·-·-·-·) and vials with 150 mg l^{-1} LAS added (·-·-·-·-·-·-·). The standard deviation for the acetate and methane experimental data was lying between 0.1–8.5% and 0.9–9.6% respectively.

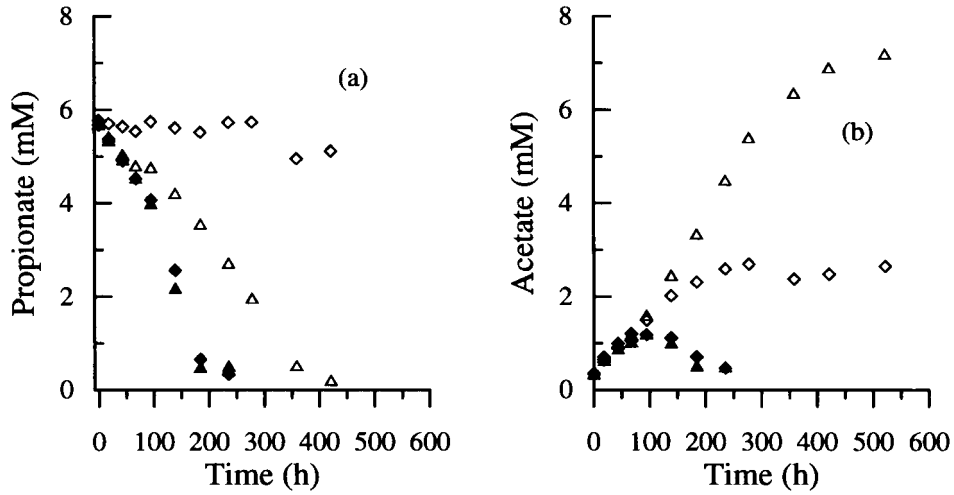

Figure 2. Propionate (a) and acetate (b) concentration versus time in the batch experiments with the propionate fed anaerobic sludge. Control vials (▲) (with 142 ppm LAS), vials with 50 mg l^{-1} LAS added (♦) (192 ppm total LAS), vials with 150 mg l^{-1} LAS added (△) (292 ppm total LAS) and vials with 350 mg l^{-1} LAS added (◊) (492 ppm total LAS). The standard deviation for the propionate and acetate experimental data was lying between 0.2–10.8% and 0.2–13.4% respectively.

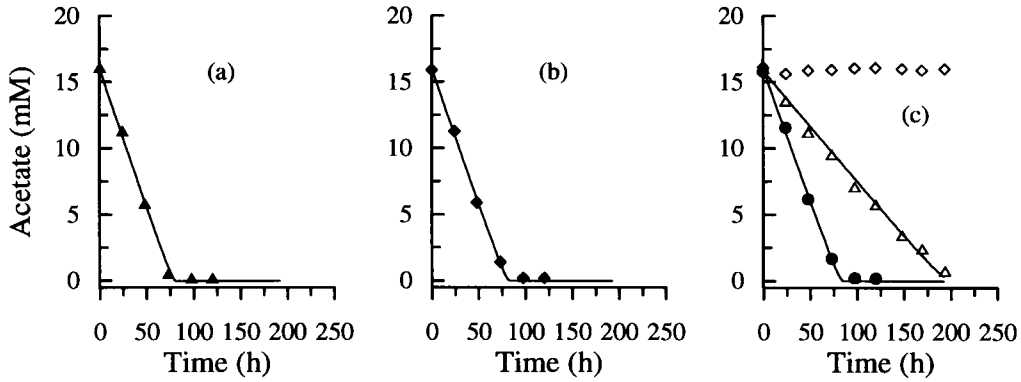

Figure 3. Experimental and calculated (solid lines) acetate concentration profiles during the acetate fed batch experiments with the enrichment culture on acetate. Control vials (▲), vials with 1 mg l^{-1} LAS (♦), vials with 2 mg l^{-1} LAS (●), vials with 5 mg l^{-1} LAS (△) and vials with 10 mg l^{-1} LAS (◇). The standard deviation for the experimental data was lying between 0.7–8.5%.

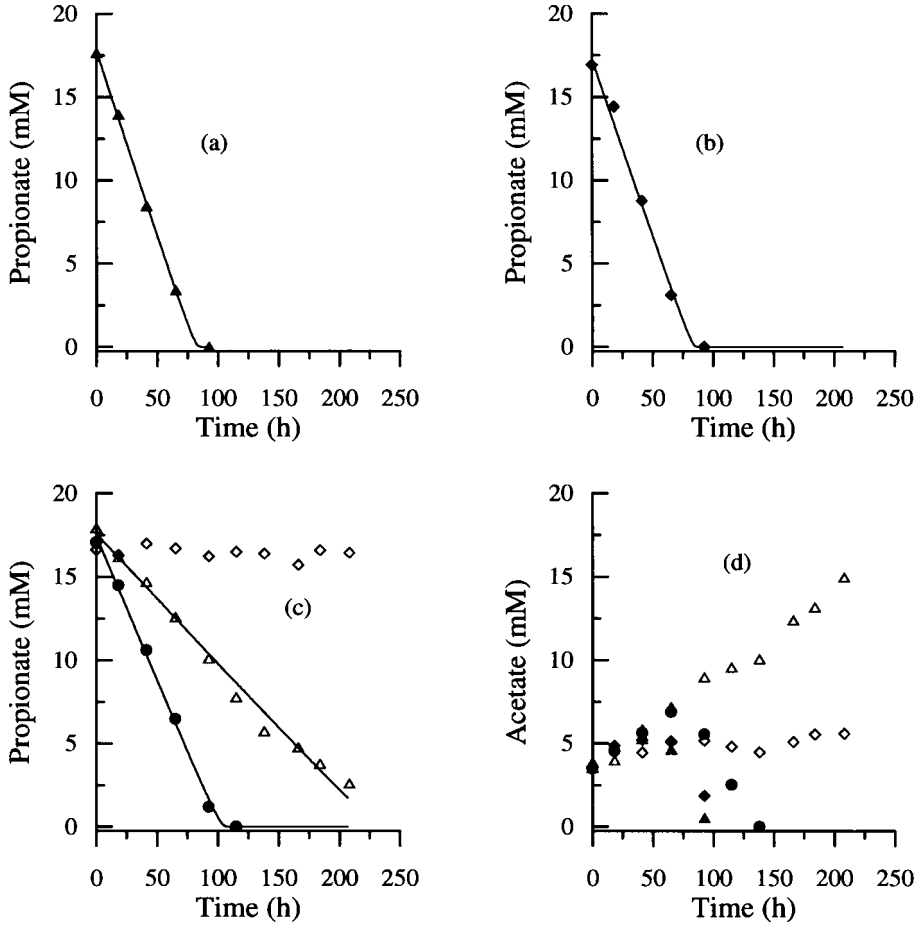

Figure 4. Experimental and calculated (solid lines) propionate concentration profiles during the propionate fed batch experiments with the enrichment culture on propionate. Also the acetate profile during these experiments is shown (4d). Control vials (▲), vials with 2 mg l^{-1} LAS (♦), vials with 5 mg l^{-1} LAS (●), vials with 10 mg l^{-1} LAS (△) and vials with 20 mg l^{-1} LAS (◇). The standard deviation for the experimental propionate and acetate data was lying between 0.1–8.6% and 0.4–9.4% respectively.

sources (e.g. carbohydrates, proteins etc.) than propionate proceeded to some extent despite the elevated concentration of LAS.

Experiments with enrichments on acetate and propionate

Experiments with acetate as substrate

Five duplicates of acetate fed batch experiments were carried out with LAS concentration of 0 (control), 1, 2, 5 and 10 mg l^{-1} using enrichments on acetate as inoculum (Figures 3a, b, c). The initial VSS concentration in all vials equalled the TSS concentration and was 58 mg l^{-1}. Acetate degradation did not proceed at all in the experiment with 10 ppm LAS (Figure 3c).

Experiments with propionate as substrate

Five duplicates of propionate fed batch experiments were carried out with LAS concentration of 0 (control), 2, 5, 10 and 20 mg l^{-1} using enrichments on propionate as inoculum (Figures 4a, b, c). The initial VSS concentration in all vials equalled the TSS concentration and was 105 mg l^{-1}. Propionate degradation did not proceed at all in the experiment with 20 ppm LAS (Figure 4c). The acetate profile during these experiments is shown as well (Figure 4d).

Experiments with hydrogen as substrate

Five triplicates of hydrogen supplied batch experiments were carried out with LAS concentration of 0 (two control triplicates), 2, 5 and 10 mg l^{-1} using enrichment cultures on propionate as inoculum. The initial VSS concentration in all vials equalled the TSS concentration and was 72 mg l^{-1}. As one can see in Figure 5, LAS are inhibitory to the methanogenesis from hydrogen as well; however the inhibition follows a different pattern in this case. The differences in methane production rate are very small among the different experiments even in the ones with relatively high concentration of LAS (i.e. 10 ppm of LAS were completely inhibitory for acetoclastic methanogens and inhibited considerably the propionate consumption).

Analysis of the results

The inhibition types most commonly used in anaerobic models are the Haldane inhibition first used by Andrews (1969) and the non-competitive inhibition first introduced by Ierusalimsky (1967), respectively. Several researchers have used the Haldane inhibition for describing the inhibition caused by either the

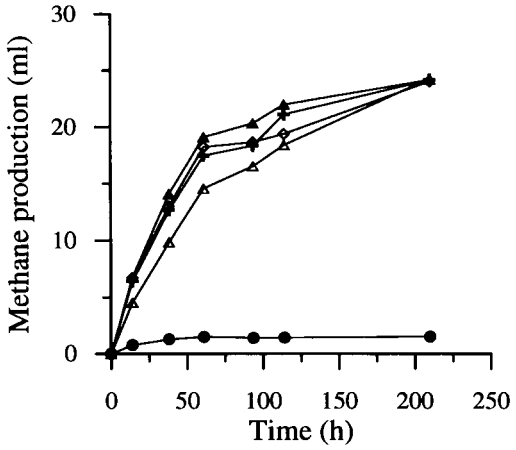

Figure 5. Methane production versus time in the batch experiments with hydrogen supplied enrichment culture on propionate. Control vials without LAS and hydrogen addition (●), control vials without LAS addition (▲), vials with 2 mg l^{-1} LAS (◊), vials with 5 mg l^{-1} LAS (+) and vials with 10 mg l^{-1} LAS (△). The standard deviation for the experimental data was lying between 0.1–14.2%.

unionised volatile fatty acids (butyrate, propionate, acetate) or the total volatile acids concentration. Other investigators have used the non-competitive inhibition type in order to describe the inhibition caused by either the volatile fatty acids or other toxic substances, e.g. ammonia. In general, both inhibition types have been used to mathematically simulate the inhibition on the microbial metabolism caused by soluble, hydrophilic substances. None of these two inhibition types are appropriate for describing the inhibition caused by LAS. As surfactants, LAS are surface-active compounds containing both hydrophobic and hydrophilic groups and having the tendency to migrate to surfaces and interfaces (Hamilton 1971). Probably, the inhibition caused by the LAS molecules is due to their interaction with the microbial membrane and thus preventing the transport of nutrients and/or substrate into the bacterial cells. This is in accordance with the mechanism proposed by Henderson (1973) and Angelidaki & Ahring (1992) in order to explain the inhibition caused by long chain fatty acids (LCFA). In this case, the inhibition depends rather on the relative concentration of LAS and solids present in the medium than on the concentration of LAS. Therefore it is decided to define the "biomass specific LAS concentration, BSC_{LAS}" expressed as the mass of LAS per mass of volatile suspended solids, mg LAS $(gVSS)^{-1}$. In Table 2 the critical BCS_{LAS} where the microbial activity is completely inhibited in batch experiments with anaerobic sludge and enrichments are presented.

Table 2. Critical Biomass specific LAS concentration (BSC_{LAS}) where microbial activity is completely inhibited in batch experiments with anaerobic sludge and enrichments

	Critical BSC_{LAS} (mg LAS/gVSS) where microbial activity is completely inhibited in:	
	Anaerobic sludge	Microbial enrichment
Acetogenesis from propionate	21.5-36.2	116-191
Methanogenesis from acetate	15.0-24.9	86.0-172
Methanogenesis from hydrogen	–	$\gg$139

It is obvious that the critical biomass specific LAS concentration, where methanogenesis from acetate (and propionate consumption) is completely inhibited, was much higher during the experiments with microbial enrichment on acetate (and propionate) compared with this obtained from the experiments with the anaerobic sludge (86–172 and 15.0–24.9 mg LAS (g VSS)$^{-1}$ respectively in the case of acetate based experiments and 116–191 and 21.5–36.2 mg LAS (g VSS)$^{-1}$ respectively in the case of propionate based experiments). This could be attributed to the higher number of methanogens and acetogens in the enrichment than in the anaerobic sludge and/or to the preferable adsorption of LAS on bacterial cell surfaces than on other organic solids present in the sludge. Examining the acetate-case and assuming that the growth of acetoclastic methanogens follows Monod kinetics, the consumption of the substrate S (acetate) is:

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}} \cdot \frac{\mu_{max} \cdot S}{K_S + S} \cdot X_m \qquad (1)$$

where $Y_{X/S}$ is the biomass yield factor and X_m is the concentration of methanogens.

Supposing that the kinetic constants are the same for both the enrichment culture and the anaerobic sludge (the origin of the enrichment culture is the anaerobic sludge), the division of the acetate consumption rate in the enrichment with that in the anaerobic sludge (control vials in Figures 3a and 1a, respectively) gives the ratio of methanogens in the different experiments.

It was found that $X_m^{enrich}/X_m^{sludge} = 1.84$ where X_m^{enrich} and X_m^{sludge} denotes the acetate utilizing methanogens concentration in the enrichment and in the anaerobic sludge, respectively. Despite the fact that the concentration of methanogens in the enrichment is approximately double of that in the anaerobic sludge, the threshold of the totally inhibitory mass of LAS per mass of VSS, BSC_{LAS}, is still lower in the case of the anaerobic sludge. This supports the hypothesis that there is a preferable adsorption of LAS on microbial surfaces.

The proposed inhibition mechanism, namely the interaction of LAS with the microbial membrane and the subsequent prevention of the nutrients and/or substrate transport into the bacterial cells could be expressed as a decrease in the number of active microorganisms in the medium and thus as a decrease in the maximum substrate utilisation rate, u_{max}:

$$-\frac{dS}{dt} = \frac{u_{max} \cdot S}{K_S + S} \qquad (2)$$

where

$$u_{max} = \frac{\mu_{max}}{Y_{X/S}} \cdot X_m.$$

Based on the aforementioned hypothesis and in order to estimate the maximum substrate utilisation rate, u_{max}, during the experiments with the enrichment cultures on acetate and propionate the following procedure took place: the kinetic constants, u_{max} and K_s, characterizing the microbial enrichment were calculated by fitting the equation 2 to the results of the control vials. Subsequently, the u_{max} was estimated in each experiment with different concentrations of LAS using the same kinetic expression and K_s values as found for the control vials. The results are summarised in Table 3. The calculated acetate and propionate concentration profiles during the batch experiments with the enrichment cultures on acetate and propionate are shown in Figures 3 and 4 respectively (solid lines). The reduction of the maximum utilisation rate, u_{max}, as a function of the BSC_{LAS} for both acetate and propionate is shown in Figure 6. It seems that the pronionate-utilising bacteria are more sensitive to the presence of LAS than the acetoclastic methanogens.

As it has already been reported, the establishment of a microbial enrichment culture on LAS capable of

Table 3. The calculated maximum substrate utilisation rate (u_{max}) and saturation constant (K_s) coming from the batch experiments with the enrichment cultures on acetate and propionate

Experiments with acetate fed enrichments on acetate				
	Control vials	Vials with 1 ppm LAS	Vials with 2 ppm LAS	Vials with 5 ppm LAS
Maximum substrate utilisation rate (mg l^{-1} h^{-1})	12.8	12.5	12.2	5.10
Saturation constant (mg l^{-1})	13	13	13	13
Experiments with propionate fed enrichments on propionate				
	Control vials	Vials with 2 ppm LAS	Vials with 5 ppm LAS	Vials with 10 ppm LAS
Maximum substrate utilisation rate (mg l^{-1} h^{-1})	16.9	16.0	13.0	5.86
Saturation constant (mg l^{-1})	17	17	17	17

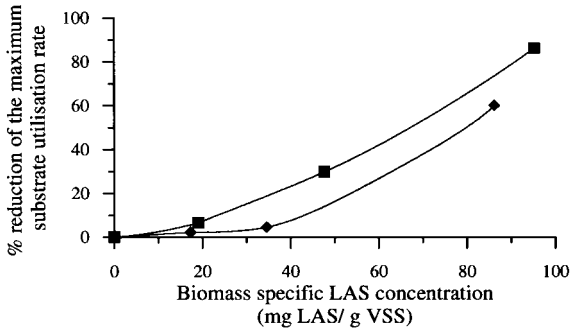

Figure 6. Dependency of acetate (♦) and propionate (■) maximum utilisation rate on the biomass specific LAS concentration.

utilising LAS carbon has not been achieved yet, despite the fact that several researchers have reported that LAS could be biodegradable under anaerobic conditions (Angelidaki et al. 2000; Mogensen & Ahring 2002). One possible explanation could be that the establishment of a microbial enrichment on LAS by applying traditional enrichment techniques meets insurmountable obstacles due to the inhibitory effect that LAS have on the bacterial metabolism. Therefore it is suggested that other techniques than the traditional ones should be developed and applied for a successful microbial enrichment on LAS. Furthermore, precautions should be taken when wastewater from a surfactant producing industry is to enter a municipal wastewater treatment plant that employs anaerobic technology for the treatment of the solid part of the waste (primary sludge); in this case a considerable amount of the surfactant load will be found mainly in the primary sludge and potentially cause instability problems to the anaerobic treatment step. Applying a raw estimation, the biomass specific LAS concentration, BSC_{LAS}, in the digester must be lower than 14 mg LAS $(gVSS)^{-1}$ in order to maintain a stable operation of the anaerobic system.

Conclusions

The inhibitory effect of Linear Alkylbenzene Sulfonates (LAS) on the acetogenic and methanogenic step of the anaerobic digestion process was studied. LAS inhibit all both acetogenesis from propionate and methanogenesis from acetate and hydrogen. The pronionate-utilising bacteria are more sensitive to the presence of LAS than the acetoclastic methanogens. Since the inhibition intensity depends on the solids concentration, the term "biomass specific LAS concentration" (BSC_{LAS}) has been introduced in order to describe the phenomenon better. It is suggested that the inhibitory effect of LAS is the main reason that anaerobic microbial enrichments on LAS have not been succeeded yet. Also, the inhibition caused by LAS on the acetogenic and methanogenic step of the anaerobic digestion process should be seriously taken into consideration when wastewater from a surfactant producing industry is to be treated biologically or enter a municipal wastewater treatment plant that

employs anaerobic technology. The upper allowable biomass specific LAS concentration should be 14 mg LAS $(gVSS)^{-1}$.

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