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Anaerobic degradability of alcohol ethoxylates and related non-ionic surfactants

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Abstract

The anaerobic degradability of alcohol ethoxylates with various degrees of branching and several related substances was studied. Different inocula were employed in order to increase the probability of obtaining capable bacteria, and the degradation assays were fed with several small doses of the test substances in order to avoid inhibition by too high initial concentrations. Mineralization was quantified by monitoring the biogas production and inorganic carbon concentration in the liquid phase. Almost complete mineralization was achieved in the assays with linear alcohol ethoxylate, poly(ethylene glycol), dodecanol, 2-ethyl-hexanoic acid and 3-methyl-valeric acid. No significant degradation was detected in the assays with highly branched alcohol ethoxylate, 2-butyl-branched alcohol ethoxylate, alcohol alkoxylate, poly(propylene glycol) and iso-tridecanol. A 2-ethyl-branched alcohol ethoxylate was transformed to (2-ethyl-hexyloxy)-acetate, which was not further degraded. Apparently already the first step of anaerobic degradation of alcohol ethoxylates, the ethoxylate chain shortening, is sterically hindered by the alkyl branching. Alkyl branching in alcohol ethoxylates and the inclusion of propylene oxide units in alcohol alkoxylates seem to have a clearly more detrimental effect on anaerobic degradability than on aerobic degradability.

Abbreviations: AAO – alcohol alkoxylate; Ace – acetate; AEO – alcohol ethoxylate; C12OH – dodecanol; 2EH – 2-ethyl-hexanoic acid; EO – ethylene oxide; HBAEO – Highly branched AEO; i-C13OH – iso-tridecanol; LAEO – Linear AEO; 3MV – 3-methyl-valeric acid; PEG – poly(ethylene glycol); PO – propylene oxide; PPG – poly(propylene glycol); SBAEO – single branched AEO

Introduction

Non-ionic surfactants are discharged to industrial and domestic wastewaters in large amounts. Up to the present, degradation studies have concentrated on aerobic biodegradability. However, the biodegradability under anaerobic conditions is important for two reasons: (1) during aerobic wastewater treatment the surfactants adsorb to the sludge and, subsequently, reach the anaerobic environment of sludge digesters, and (2) there is a trend to the direct anaerobic treatment of domestic wastewaters and a steadily increasing range of industrial wastewaters, which, in many cases, contain surfactants. Especially in high-rate treatment of wastewater with elevated surfactant concentration severe reactor instabilities can be encountered, if the surfactants are not degraded (Mösche & Meyer 2001).

The most important non-ionic surfactants are alcohol ethoxylates (AEOs) and alkylphenol ethoxylates (APEOs). They consist of a long-chain

aliphatic alcohol or alkylphenol (lipophilic moiety) linked to a chain of several ethylene oxide (EO) units (hydrophilic moiety). Due to incomplete biodegradation the use of APEO in detergents and in many industrial cleaning products is being phased out in the European Community and will continue to decrease (ECE 2000). AEO are usually considered to be well biodegradable both under aerobic and anaerobic conditions (ERASM 1999). The assumption of good anaerobic biodegradability is mainly based on the results of Steber & Wierich (1987), Wagener & Schink (1988) and Salanitro & Diaz (1995). In these studies alcohol ethoxylates with linear alkyl chains (LAEO) were used. It was found that in a first step PEG degrading bacteria break down the hydrophilic EO-chain, leaving the hydrophobic alkyl residue in the form of the corresponding fatty acid (Wagener & Schink 1988). The fatty acid is then degraded by other bacteria via β-oxidation.

For many applications AEOs with branched alkyl chains are preferred due to their special characteristics, for example superior wetting properties (Varadaraj et al. 1991). A large fraction of branched AEOs is based on 'iso-tridecanol', which is the technical term for a mixture of thousands of isomers of highly branched C13-alcohol. In this study these surfactants are called highly branched alcohol ethoxylates (HBAEOs). As an alternative to the HBAEOs single branched alcohol ethoxylates (SBAEOs) have been introduced. They are based on primary aliphatic alcohols with a single branching in the alpha position, e.g. 2-ethyl-hexanol, 2-butyl-octanol and higher homologues. These surfactants display favourable detergent characteristics as well as a good biodegradability under aerobic conditions (Battersby et al. 2000). In the aerobic degradation of branched AEO the central cleavage mechanism and the ω-oxidation of alkyl chains are very important (Marcomini et al. 2000), but these mechanisms are dependent on the availability of molecular oxygen. Thus, it remains unclear, if branched alkyl chains can be degraded under anaerobic conditions by β-oxidation or other, yet unknown, mechanisms.

Another group of non-ionic surfactants closely related to alcohol ethoxylates are alcohol alkoxylates (AAOs), which contain both EO and propylene oxide (PO) groups in the hydrophilic moiety. These surfactants are used as low-foaming alternatives to AEOs. The PO groups are likely to present an obstacle to the biodegradation, since AEO and PEG degrading bacteria have been reported not to metabolize PPG (Dwyer & Tiedje 1986; Wagener & Schink 1988).

In addition to the surfactants other substances with similar chemical structures were tested for anaerobic degradability (branched aliphatic alcohols and carboxylic acids and PPG). This was made in order to gain information about which structures within the surfactant molecules limit their biodegradability.

Experimental design

Inocula

In order to increase the probability of including bacteria capable of degrading branched alkyl chains or PPG chains several different inocula were collected for this study from sources where similar substrates are present: (1) Anaerobically digested municipal sewage sludge – since household effluents contain a broad range of non-ionic surfactants. (2) Anaerobically digested horticultural waste – because the chlorophyll of green plants contains the phytyl moiety, which is a naturally occurring branched alkyl chain. (3) Biofilm from the aerobic rotating disk contactor treating the effluents from a surfactant producing factory – a source of bacteria adapted to the studied surfactants. Though taken from an aerobic plant, it was assumed that in the deeper layers of the biofilm at least facultative anaerobic bacteria were present. (4) Anaerobic sludge from an UASB reactor for the direct treatment of municipal sewage – because in contrast to the digested sewage sludge this sludge receives the surfactants directly (without a preceding aerobic treatment, during which they might be degraded). (5) A sediment from a forest pond containing much rotten foliage and showing evident gas production – since in such a natural ecosystem the solids retention time is extremely long, and thus, specialized bacteria have the opportunity to grow, even if their generation time is very long.

Dosage of test substances

In order to avoid an inactivation of the bacteria by a too high initial concentration of the surfactants, it

was decided to modify the usual protocol for anaerobic biodegradability tests: Instead of adding a single dose of the test substances at the beginning of the experiments, several small doses were added throughout the experiments. The aim was to acclimatise the bacteria to the relatively high amounts of surfactants that are necessary to obtain a sufficient gas production to be clearly distinguished from the background gas production. In one of the experimental series the sludge was incubated for almost 1 year, because very long adaptation times have been reported for the anaerobic degradation of certain substances (Field 2002).

Material and methods

Inocula and preculture

In the experimental series #1 and #3 (Table 1) the inoculum was obtained in the following way: Digested sewage sludge (WWTP Opfikon, Switzerland), digested organic waste ('Kompogas' plant Rümlang, Switzerland), and biofilm from a rotating disk contactor treating surfactant production effluents were sieved (mesh size 0.25 mm, for removal of coarse particles) and mixed at a ratio of 500 ml:500 ml:10 ml and filled into a stirred tank reactor. Subsequently, a highly concentrated substrate was dosed in fed-batch mode with very low volumetric flow (≤8 ml d^{-1}) during 6 months. The aim was to enrich bacteria capable of degrading alcohol ethoxylates, branched alkyl chains and polypropylene glycols and not to wash out slow growing bacteria.

For the experimental series #2 additional inocula were collected: sludge from an UASB reactor for the direct treatment of municipal wastewater (described in Álvarez et al. 2001), sediment from a forest pond, and normal digested sewage sludge (WWTP Opfikon, Switzerland). The first two inocula were homogenized, sieved and supplemented with digested sewage sludge (volumetric mixing ratio 1:1) in order to increase their low methanogenic activity. The three inocula were used directly (without preculture) for the tests in series #2.

In all series the biomass was centrifuged twice and re-suspended in washing solution before the experiments. The biomass concentrations in the degradation experiments were: 2.9 g SS l^{-1} (series #1), 12.2 g SS l^{-1} (series #2), and 3.2 g SS l^{-1} (series #3).

Table 1. Overview over the experimental series performed

Experimental series	Objective: Assessment of…
#1	Biodegradability of LAEO, HBAEO, AAO and related substances
#2	Long-term degradation behavior of HBAEO and PPG
#3	Biodegradability of SBAEO

Experimental setup

For each test a glass bottle (350 ml) was connected to two corresponding cylinders filled with sealing liquid (1 M H_2SO_4 saturated with NaCl) for volumetric measurement of the biogas production. At the beginning of the experiment the bottles were filled with 300 ml of biomass suspension and the gas phase was purged with nitrogen. Substrate was added and samples were taken with a syringe through a Teflon septum. The bottles were kept at 35 °C in a temperature controlled water bath.

Dosage of test substances and sampling

The test substances were added in several small doses throughout the experiments. In series #1 and #3 liquid samples were taken at regular intervals and the volume lost was replaced by feed solution (series #1:3 feedings per week, loading rate = 12 mg C l^{-1} d^{-1}; series #3:2 feedings per week, loading rate = 6.86 mg C l^{-1} d^{-1}). The blank tests received feed solution without carbon source (only minerals).

In series #2 0.65 ml of a 10 g l^{-1} HBAEO stock solution and 1.3 ml of a 20 g l^{-1} PPG stock solution were dosed at the beginning of the experiments. Further doses of 1.5 ml of stock solutions were added on days 87 and 139. The blank tests did not receive any feed. No liquid samples were taken from these experiments.

Chemicals

The following surfactants of technical quality were kindly supplied by Dr W. Kolb AG, Switzerland:

LAEO (dodecanol with an average of 9 EO units), HBAEO (iso-tridecanol with an average of 9 EO units), SBAEO1 (2-ethyl hexanol with an average of 8 EO units), SBAEO2 (2-butyl octanol with an average of 6 EO units), AAO (C12/C14/C16 fatty alcohol with an average of 5 EO and 3 PO units, PO units in the terminal position), and iso-tridecanol.

Acetic acid (≥99.8%), 2-ethyl-hexanoic acid (≥98%), 3-methyl-valeric acid (≥97%), PEG 400 (purum), PPG 400 (purum), dodecanol (≥99.5%) and nutrient salts ('pro analysi') were purchased from Fluka AG, Switzerland.

Substrate solutions

Highly concentrated substrate for inoculum preculture (in mg l^{-1})
LAEO: 2300, PPG: 1600, 2EH: 7200, 3MV: 5800, propionic acid: 3700, NaOH: 2500.

Feed solution used in series one and three (in mg l^{-1})
Test substance (mg C l^{-1}): 2400, NaOH: 1200, NH_4Cl: 150, K_2HPO_4: 15, KH_2PO_4: 11, $Na_2S \cdot 8H_2O$: 36, $FeCl_2 \cdot 4H_2O$: 5, Na_2EDTA: 5, $CaCl_2 \cdot 2H_2O$: 6, $MgCl_2 \cdot 6H_2O$: 15, trace element solution: 0.3 ml l^{-1}, vitamin solution: 0.3 ml l^{-1}.

Dodecanol and iso-tridecanol are water insoluble alcohols. For this reason 90% of the substrate (2160 mg C l^{-1}) was added in the form of the alcohol and 10% (240 mg C l^{-1}) in the form of the degradable surfactant LAEO. Upon vigorous mixing a homogeneous emulsion was obtained which could easily be dosed.

Alternative feed solutions used in series one
Apart from the fully concentrated feed solution several less concentrated feed solutions of the surfactants LAEO, HBAEO and AAO were prepared and used in additional experiments. They contained 70%, 40%, 20%, and 10% of the test substance and of the NaOH, but equal amounts of minerals as in the recipe given above.

Washing solution (in mg l^{-1})
$NaHCO_3$: 2500, HCl 37%: 0.57 ml l^{-1}, NH_4Cl: 150, K_2HPO_4: 15, KH_2PO_4: 11, $Na_2S \cdot 8H_2O$: 10, $FeCl_2 \cdot 4H_2O$: 5, EDTA: 5, $CaCl_2 \cdot 2H_2O$: 6, $MgCl_2 \cdot 6H_2O$: 15, trace element solution: 0.3 ml l^{-1}, vitamin solution: 0.3 ml l^{-1}, pH fine adjustment to 7.0 with HCl.

Trace element solution (in mg l^{-1})
$NiCl_2 \cdot 6H_2O$: 1200, $CoCl_2 \cdot 6H_2O$: 600, $ZnCl_2$: 1200, $MnCl_2$: 30, $CuCl_2 \cdot 2H_2O$: 80, $Na_2MoO_4 \cdot 2H_2O$: 40, Na_2SeO_3: 60, H_3BO_3: 10.

Vitamin solution (in mg l^{-1})
Thiamin-HCl: 10, riboflavin: 10, pyridoxine-HCl: 20, cobalamin: 1, folic acid: 5, biotin: 5, 4-amino-benzoic acid: 10.

All solutions were prepared with deionized, degassed water.

Analyses

The liquid samples were filtered (0.45 μm membrane filters, cellulose acetate, Sartorius, Germany) and analyzed for: TOC and TIC (Shimadzu TOC 5050 TOC-analyzer), organic acids including 2EH and 3MV (HPLC: Wescan Anion Exclusion Column, Alltech, Deerfield, IL, USA; eluant: 2 mM H_2SO_4; UV detection at 210 nm) and pH.

Mass-spectrometric analyses were performed with a Hewlett Packard Series 1100 LC-MS with electrospray ionization and a quadrupole mass analyzer, operated in FIA modus with 5 μl injection volume and 50/50 water/methanol as the eluant. The fragmentor voltage was 100 V, the capillary voltage was 4000 V in positive ionization mode and 5000 V in negative ionization mode. Under these operating conditions no fragmentation of the surfactants was observed. The filtered liquid samples were analyzed without any further pretreatment. Because of the sample matrix, Na^+ adducts were detected instead of molecular ions. In order to provide comparable conditions, the mass spectra of the original test substances were recorded in positive ionization mode with solutions of 50 mg l^{-1} test substance in a 20 mM $NaHCO_3$ buffer (pH 7.0).

Measurement and correction of biogas production

The cumulative amount of biogas produced was read from the cylinders containing the sealing liquid. The biogas volume was normalized to standard conditions (273.15 K 101,333 Pa) and then converted to the unit 'mg C l^{-1}':

$$\text{gas production}\,[\text{mg C l}^{-1}] = \frac{V_{\text{gas}} \cdot 12{,}000\,\text{mg C mole}^{-1}}{22.411\,\text{gas mole}^{-1}} \cdot \frac{1}{V_{\text{liq}}} + \text{CO}_{2,\text{loss+accum}} \quad (1)$$

In this calculation, also a correction term for the dissolution of CO_2 in the liquid phase is considered. This is necessary, because the loss of inorganic carbon with the samples removed and the accumulation of inorganic carbon in the liquid phase during the experiments make a significant contribution to the carbon balance. The correction term is calculated from the measured concentration of inorganic carbon in the liquid phase (TIC):

$$\text{CO}_{2,\text{loss+accum}} = \sum_{i=1}^{n} \text{TIC}_i - \left(1 - \frac{V_{\text{smp},i-1}}{V_{\text{liq}}}\right) \cdot \text{TIC}_{i-1} \quad (2)$$

With $CO_{2,\text{loss}+\text{accum}}$ = Correction for CO_2 lost with samples and accumulation of dissolved CO_2 in the liquid phase (in mg C l^{-1}); n = sample number; V_{smp} = sample volume; V_{liq} = volume of the liquid phase in the test bottle.

Since no liquid samples were taken in series #2 and, thus, no TIC data were available, no correction for CO_2 dissolution was applied in this series.

The gas production in series #1 and #3 is given as the net biogas production, meaning the gas production of the test assay minus the average gas production of the blank tests.

Results and discussion

Preculture

At the beginning of the preculture a pulse of 5 ml substrate solution was administered. During the following days the concentrations of the branched fatty acids 2EH and 3MV were monitored. After 12 (19) days 2EH (3MV) had disappeared. After a second pulse these substances were degraded within 5 days. Then the continuous feeding was started and gradually increased up to a loading rate of 100 mg C l^{-1} d^{-1}. No accumulation of fatty acids was observed during this period. Apparently the enrichment of branched fatty acid degrading bacteria was successful. Anaerobic degradation of such branched fatty acids had already been reported by Sin & Chua (2000). Particularly interesting is the degradation of 3MV. Since this fatty acid has an alkyl branching in the beta position, it cannot be degraded by β-oxidation. An alternative degradation mechanism could involve the carboxylation of the methyl-branching and a subsequent ketal elimination as in the case of anaerobic isovalerate degradation (Stieb & Schink 1986).

Degradation experiments with LAEO, HBAEO, AAO and related substances

Figure 1A and B show the stepwise dosage of test substances to the assays of series #1. The assays shown in Figure 1A were regularly fed, whereas the assays shown in Figure 1B did not receive substrate between days 8 and 15. The CH_4 + CO_2 production in the various assays is shown as mg carbon per litre reactor volume. In this way the carbon recovery can be seen directly. Most of the substrate (82–89%) was finally converted to CH_4 and CO_2 in the experiments with acetate, 2-ethyl-hexanoic acid, 3-methyl-valeric acid, PEG, dodecanol and linear alcohol ethoxylate. The incomplete conversion to biogas is partly due to the formation of biomass (≈5%). The remaining difference to 100% is

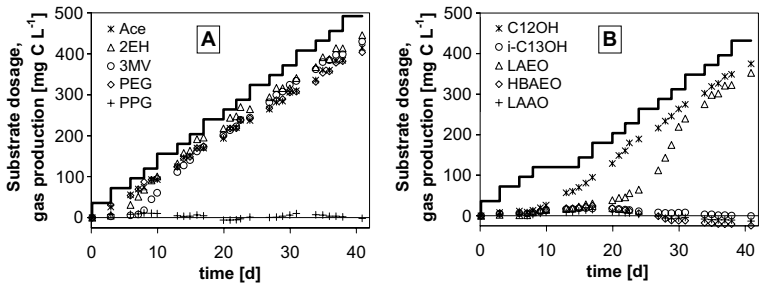

Figure 1. Substrate dosage (solid lines) and net biogas production (symbols) in the assays of series #1.

probably caused by gas leakage and/or solubilization of CO_2 in the sealing liquid.

In the assays with iso-tridecanol, the highly branched alcohol ethoxylate, PPG, and the alcohol alkoxylate no significant gas production could be observed at all. The negative values in some assays result from the higher raw biogas production in the blank assays compared to the test assays and indicate an inhibition of the bacteria by the surfactants.

In the assays with dodecanol and linear alcohol ethoxylate the gas production started with a delay of about 10 or 20 days, respectively. However, this does not mean that there was no degradation of LAEO before day 20. As can be seen in Figure 2, most of the LAEO added to the assay was rapidly converted to acetate, which accumulated strongly during the days 6–31. During this phase the methanogenesis was inhibited by the surfactant. Only after regrowth of the methanogenic bacteria the formation of biogas was possible. A similar, but less pronounced, effect is revealed by the assay with dodecanol.

In the assay with HBAEO a continuous increase of the acetate concentration was observed (Figure 2). However, the amount of acetate produced only corresponds to about 16% of the carbon added as substrate. Apparently, the methanogenesis was inhibited and the acetate originated either from the hydrolysis of dead biomass or from a partial degradation of the ethoxylate chain of the surfactant. In the other assays no significant accumulation of acetate was observed. No significant amounts (>5 mg C l^{-1}) of other short-chain fatty acids were detected in any of the assays.

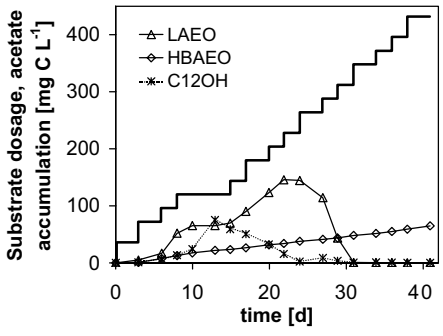

Figure 2. Substrate dosage (solid line) and accumulation of acetate during the assays with LAEO, HBAEO and C12OH.

In the assays with the surfactants that were not degraded an accumulation of surfactant occurred and the bacteria were inhibited. Thus, it was investigated, if this inhibition could be avoided by a reduced dosage of the surfactants. The experiments shown in Figure 1 were run with an average substrate loading rate of 12 mg C l^{-1} d^{-1}. Additional experiments were performed with LAEO, HBAEO and AAO and loading rates of 8.4, 4.8, 2.4 and 1.2 mg C l^{-1} d^{-1} (by reducing the concentration of the feed solution). In none of the assays a significant conversion of HBAEO or AAO to biogas could be observed (data not shown). On the other hand, in the assays with LAEO the degree of mineralization at the end of the experiments dropped from 82% (at 12 mg C l^{-1} d^{-1}) to 38% (at 1.2 mg C l^{-1} d^{-1}). One explanation could be that, when only a small amount of LAEO was dosed, a large fraction adsorbed to the organic matter and, thus, was not bioavailable.

The results of series #1 show a clear distinction between biodegradable and non-biodegradable substances under the conditions employed: As expected, Ace, LAEO, C12OH, and PEG were degraded. The mineralization of 2EH and 3MV confirmed the enrichment of branched fatty acid degrading bacteria during the preculture of the inoculum. However, the presence of these bacteria did not result in a degradation of the highly branched surfactant (HBAEO) or alcohol (i-C13OH). One reason for the recalcitrance of the iso-tridecyl moiety is probably the occurrence of quarternary carbons in most of the isomers (Battersby et al. 2000). The branched fatty acid degrading anaerobic enrichment culture of Sin & Chua (2000) was not able to degrade fatty acids with a quarternary carbon either.

PPG was not degraded in the assay, although it had been fed as a substrate during the 6 months of the preculture. From a mere chemical point of view the tertiary carbons present in PPG theoretically should not impede the pathway of anaerobic PEG degradation as proposed by Frings et al. (1992), but apparently no bacteria could be enriched performing this pathway. This is in accordance with the observation that up to the present the degradation of PPG has only been observed under aerobic conditions (Kawai 2002). Consequently, the recalcitrance of AAO observed in this series can be explained with its terminal

PPG chain, which cannot be attacked anaerobically.

Long-term incubation experiments

Since no degradation of HBAEO, AAO and PPG was observed in series #1 with the inoculum from the preculture, long-term incubation experiments (series #2) were performed with HBAEO and PPG utilizing three other inocula: digested sewage sludge, sludge from the direct treatment of municipal wastewater, and sediment from a forest pond (three assays for each inoculum: HBAEO, PPG, and a blank without addition of test substance). The incubation time was 322 days and doses of the test substances were fed on days 0, 87 and 139. In none of the test assays an increased gas production compared to the blank could be observed. On the contrary, in some assays the autolytic gas production of the sludges was inhibited after the additions on days 87 or 139. The pond sediment was the inoculum most sensitive to this inhibition (data not shown).

Degradation experiments with single-branched surfactants

The results of series #1 – degradation of single branched fatty acids, but no degradation of highly branched AEO – led to the conclusion that single branched AEO (SBAEO) might be degradable under anaerobic conditions. Two SBAEO were investigated: SBAEO1 contained the same alkyl rest (2-ethyl-hexyl) as the fatty acid 2EH, which had been shown to be degradable. SBAEO2 was based on a larger alkyl rest (2-butyl-octyl), thus being more representative for detergent range surfactants.

Figure 3 shows the substrate dosage and the net gas production in the assays with the linear and the two single-branched alcohol ethoxylates. Like in series #1, a temporary accumulation of acetate was observed in the assay with LAEO (days 7–24) and the substrate dosage was suspended (day 14–24). Subsequently, a good mineralization of LAEO was observed (93% after 109 days, Figure 3A). In the assays with the single-branched alcohol ethoxylates (SBAEO1 and especially SBAEO2) the biogas production was clearly lower than the substrate dosage (Figure 3B and C). As a consequence in order to avoid surfactant inhibition the substrate dosage was reduced and later completely suspended.

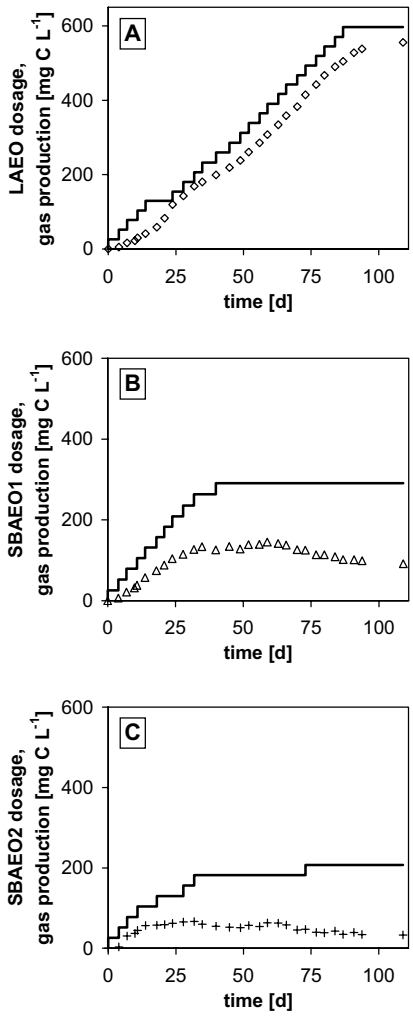

Figure 3. Substrate dosage (solid lines) and net biogas production (symbols) in series #3: assays with LAEO (A), SBAEO1 (B) and SBAEO2 (C).

After stopping the feeding in all assays, samples for mass-spectrometric analyses were taken on the days 91 and 109 in order to detect possible residues of surfactants or degradation products. In Figure 4 the mass spectra of the degradation samples (right side) are compared to the mass spectra of the corresponding original surfactants (left side). The broad distribution of different ethoxylate chain lengths can be seen in the reference spectra A, C and E. The detected masses matched the calculated masses of the sodium adducts of the surfactant ethoxymers with a precision of ≤0.2 mass units. In addition to the principal peaks distributions of minor peaks corresponding

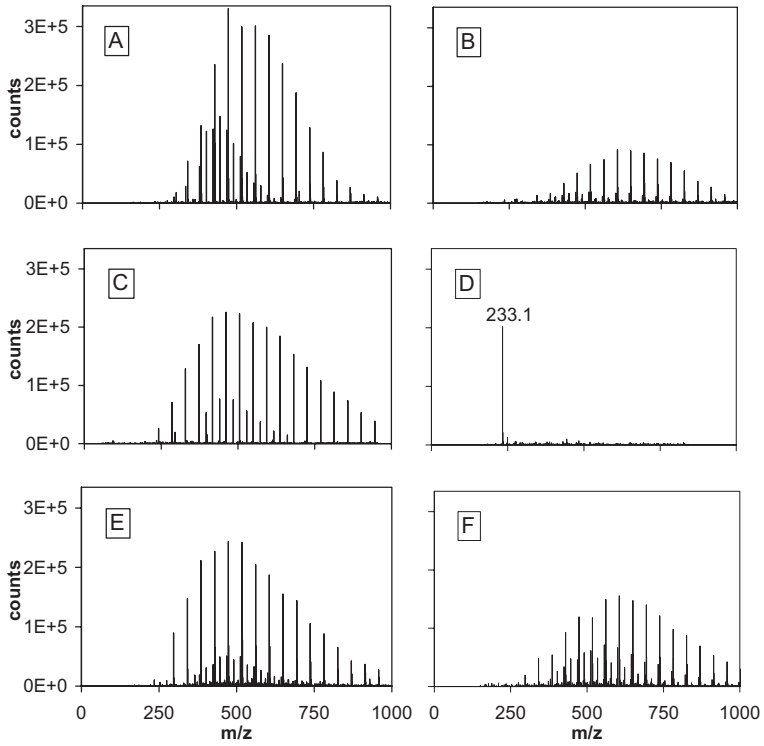

Figure 4. Electrospray mass spectra (positive ion mode) of the surfactants LAEO (A), SBAEO1 (C) and SBAEO2 (E) and of the liquid samples taken from the assays with LAEO (B), SBAEO1 (D) and SBAEO2 (F) on the 91st day.

to impurities in the technical surfactants can be seen.

The mass spectrum of the first liquid sample from the assay with LAEO (Figure 4B) still shows a distribution of various homologues of ethoxylated dodecanol, however the centre of the distribution is shifted to longer ethoxylate chains. This effect is due to the adsorption of the more hydrophobic short-chained ethoxylates to the biomass, whereas the more hydrophilic long-chained homologues are enriched in the liquid phase. The assay had been fed with LAEO 4 days before this sample was taken. The second sample, which was taken on day 109 without any feeding in the meantime, did not show peaks corresponding to the surfactant anymore (not shown). This confirmed the anaerobic degradability of LAEO.

The mass spectrum of the liquid sample from the assay with SBAEO2 (Figure 4F) also shows the homologue distribution of the original surfactant with a shift to longer ethoxylate chains. The explanation is the same as in the case of LAEO. However, this assay with SBAEO2 had not received any surfactant feed for a long time. The second sample taken 18 days later was not significantly different from the one shown in Figure 4F. These results indicate that SBAEO2 was not degraded.

The assay with SBAEO1 revealed a completely different mass spectrum: All peaks corresponding to the original surfactant had disappeared. Instead, a single main peak at $m/z = 233.1$ was found in positive ion modus (Figure 4D). Among several potential degradation intermediates only one corresponded to this mass: an adduct of (2-ethyl-hexyloxy)-acetate with two Na^+ ions (to obtain the positive charge needed for detection). The structural formula of this hypothetical degradation product is shown in Figure 5. In order to verify this hypothesis the liquid samples were analyzed once more in negative ion modus. Again, in the assay with SBAEO1 one clearly dominating peak was found, and the mass was $m/z = 187.1$. This corresponds exactly to (2-ethyl-hexyloxy)-acetate without Na^+ adducts.

With these MS results in mind, it is possible to interpret other data obtained from the analysis of

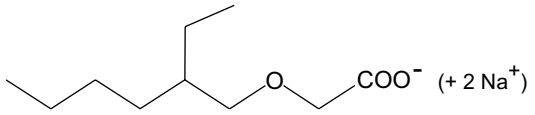

Figure 5. Hypothetical structure of the degradation product from SBAEO1.

the liquid samples. In all assays (blank, LAEO, SBAEO1, SBAEO2) nearly constant levels of TOC and acetate were observed between the days 45 and 91. The average concentrations during this period are shown in Figure 6. The slightly higher TOC of the assay with LAEO compared to the blank test is probably caused by a residue of not degraded surfactant during the period of constant feeding, as this was detected by mass spectrometry.

In the assay with SBAEO2 the elevated TOC concentration is not only due to undegraded surfactant, but also to acetate, which was found in significant concentrations and indicated an inhibition of methanogenesis. Compared to the 207 mg C l^{-1} of surfactant that had been added to the assay, and most of which had not been converted to biogas, the TOC concentration in the liquid phase (60 mg C l^{-1}) appears small. It is assumed that most of the surfactant, especially the more hydrophobic fraction with shorter EO chains, adsorbed to the biomass.

A clearly higher TOC concentration was found in the assay with SBAEO1. This can be explained by the formation of (2-ethyl-hexyloxy)-acetate. Because of its ionic charge and the rather small alkyl moiety probably this product was better water-soluble and had a reduced tendency to adsorb to the biomass. This also had a positive influence on the methanogenic bacteria, which were not inhibited. In contrast to the other assays with non-biodegradable surfactants no accumulation of acetate was observed.

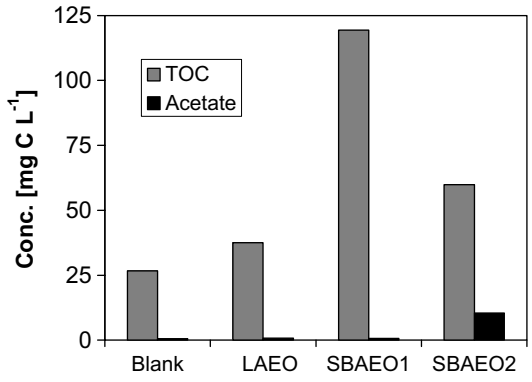

Figure 6. Average TOC and acetate concentrations during days 45–91 in the assays of series #3.

The formation of (2-ethyl-hexyloxy)-acetate implies that most of the EO chain has been degraded. This is in accordance with the partial conversion of SBAEO1 to biogas (Figure 3B). However, the absence of further degradation suggests that the enzymes involved in the degradation of the EO chain were sterically hindered by the ethyl branching of SBAEO1 and, thus, could not remove the last EO unit. Probably, the monoethoxylate was oxidized to the observed product instead of an oxidation the fatty alcohol to the corresponding fatty acid, which would be the normal pathway (Wagener & Schink 1988).

In the assay with SBAEO2, no analogous transformation product to (2-ethyl-hexyloxy)-acetate was observed. Possible reasons are: (a) the more severe sterical hindrance by the butyl-branching impeded the formation of the carboxylic acid, or (b) a carboxylic acid was formed, but the larger alkyl-moiety rendered it hydrophobic, so that it was not released to the liquid phase. In both cases a rapid accumulation of hydrophobic products would have occurred in the EO chain degrading bacteria, leading to an inactivation of these and the cessation of any surfactant degradation.

Conclusions

It was not possible to enrich anaerobic mixed cultures capable of degrading branched alcohol ethoxylates, alcohol alkoxylate or poly(propylene glycol). If the anaerobic degradation of these substances is possible at all, competent bacterial consortia must be extremely rare, since various different inocula and rather permissive degradation conditions were employed in this study.

These findings are contrary to results from aerobic degradability tests, in which branched AEOs were degraded, though more slowly than linear AEOs (Kravetz et al. 1991; Marcomini et al. 2000). PPG (Kawai 2002) and AAO (Naylor et al. 1988) have also been shown to be degradable under aerobic conditions. The reduced spectrum of anaerobically degradable surfactants is probably due to the fact that the shortening of the ethoxylate chain is the only primary degradation

mechanism under methanogenic conditions (Huber et al. 2000), and PO units in the hydrophilic moiety or alkyl branching of the hydrophobic moiety apparently impede this mechanism.

On the other hand, the single-branched carboxylic acids 3-methyl-valeric acid and 2-ethylhexanoic acid were confirmed to be biodegradable under anaerobic conditions. The reason for the non-biodegradability of single-branched AEOs is probably a sterical hindrance during the removal of the last EO-unit of the hydrophilic moiety.

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