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Determination of the fate of alcohol ethoxylate homologues in a laboratory continuous activated-sludge unit study

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Abstract

Environmental monitoring indicates that the distribution of alcohol ethoxylate (AE) homologues in wastewater treatment plant (WWTP) effluents differs from the distribution in commercial AE products, with a relative higher proportion of fatty alcohol (AOH, which is AE with zero ethoxylation). To determine the contribution of AE-derived AOH to the total concentration of AE and AOH in WWTP effluents, we conducted a laboratory continuous activated-sludge study (CAS). This consisted of a test unit fed with AE-amended synthetic sewage and a control unit fed with only synthetic sewage to avoid AE contamination from the feed. The removal efficiencies of some 114 AE homologues were determined by the application of a specific and sensitive analytical method. The extent of the removal of AE ranged from 99.70% for C_{18} compounds to >99.98% for C_{12-16}. Relatively high-AOH concentrations were observed in the effluents from blank and test units. By building the concentration difference from the test minus the control unit, the AE in the CAS effluent originating from AE in the influent was determined. Thus, it could be shown that AOH represented only 19% of the total AE (EO_{0-18}) in the CAS, while monitoring in 29 WWTP effluents (European, Canadian, and US) revealed in total a mean AOH fraction of 55% (5–82%) of the total AE (EO_{0-18}). This shows that only a small fraction of AOH in WWTP effluents originates from AE entering the WWTP.

Keywords: Alcohol ethoxylate; CAS study; Ethoxymer distribution; Biodegradation; Simulation test

1. Introduction

Linear alcohol ethoxylates (AEs) represent the economically most important group of nonionic surfactants. Commercial AEs generally consist of a mixture of several homologues differing in alkyl chain length (C_x) and degree of ethoxylation (EO_y). AEs are widely used in domestic and commercial detergents, household cleaners, and personal care products. Thus, the major route of disposal of AE is down the drain, through sewage systems, and into municipal wastewater treatment plants (WWTP).

The biodegradability of AEs and, more decisively, their ultimate environmental fate is a key parameter for an accurate assessment of the potential risk to the environment posed by their release. A previous risk assessment carried out in the Netherlands (Feijtel and van de Plassche, 1995) was based on the measured average effluent AE structure (i.e., average C#, average EO#) determined by the analytical methods available at the time. These methods were only able to analyze C_{12-15} EO_{3-18}. Since then, a specific electrospray liquid chromatography/mass spectrometry (LC/MS) methodology following derivatization with 2-fluoro-*N*-methylpyridinium *p*-toluenesulphonate [pyr+] has been developed and can detect all 114 individual species in the range C_{12-18} EO_{0-18} at nanogram/liter levels in the

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form of an AE "fingerprint" (Dunphy et al., 2001). This methodology now enables more definitive environmental monitoring and risk assessment based on a single distribution of AE homologues. One finding of recent monitoring studies in Europe, Canada, and the USA using this method was high molar fractions of alcohol that accounted for up to 4.5 times greater the ethoxylated part (AE_{1-18}) in municipal WWTP effluents (Eadsforth et al., 2005; Morrall et al., 2005). Consideration of AE structure–activity relationships (Belanger et al., 2005) suggests that the alcohol in the fingerprint is likely to drive the risk characterization ratio (RCR). However, the origins of alcohols in a WWTP may be multiple. Alcohol is a component of many commercial and domestic products as well as natural sources (Leeming et al., 1994; Modler et al., 2002). It is also decomposed in various ways and can thus be a microbial degradation product of alcohol ether sulfates, alcohol sulfates, and other substances. Consequently, in the context of an AE risk assessment, the measure of alcohol in a WWTP effluent should not be attributed to one substance or family of substances.

Several studies have been performed to investigate the fate and degradation pathway of AE in more detail. However, currently little information is available on the fraction of alcohol originating from the degradation of AE under sewage treatment conditions. In a study with radiolabeled AE in microbial biocenosis, Steber and Wierich (1985) found two distinct primary degradation mechanisms acting simultaneously, the intramolecular scission of the surfactant, which leads to polyethylene glycol and the respective alcohol, and ω- and β-oxidation of the alkyl chain. Other studies indicate the oxidative central fission of linear AEs to be the primary degradation step, followed by a subsequent degradation of carboxylated poly-ethylene-glycole (PEG) (Marcomini et al., 2000; Szymanski et al., 2000). In all studies, it is concluded that alcohol has been formed during the degradation process of AE. However, due to its rapid biodegradation, alcohol levels in the liquor phase have generally been below the detection limit of previous analytical methods and could only be detected adsorbed on the sludge (Battersby et al., 2001; Szymanski et al., 2003).

Monitoring studies have provided valuable data on the removal of AEs and other surfactants during sewage treatment. To understand the amount of alcohol in effluent attributable specifically to AE, however, it is more practical to perform detailed studies using laboratory simulations of sewage treatment. The use of laboratory continuous activated-sludge (CAS) units with synthetic sewage may be the only way to ensure that the components of AE that are measured do not originate from other sources. Additional advantages are many and include the control of key operating parameters that affect treatability performance, such as sludge retention time (SRT), influent surfactant concentration and homologue distribution, hydraulic residence time (HRT) and temperature, and mass balancing of source substance and degradation products in the liquor and solid phase, enabling a distinction between degradation and removal. For example, Battersby et al. (2001) showed in a CAS study that $>98.7\%$ of C_{12-15} EO_7 AE is biodegraded, while total removal was $>99.9\%$. In the present study we investigated the relative contribution of AOH (AE with zero ethoxylation) in the aqueous and solid phases of a CAS study effluent as a component and degradation product of a mixture of AE. To prevent contamination by nondosed AE and alcohols, synthetic sewage (OECD, 1996) was used in the CAS study described herein. The pyr+ LC/MS technique was applied to analyze samples of influent, effluent, and solids from a model activated-sludge system treating a feed containing AEs. By applying this method, it was possible, for the first time, to account for the fraction of alcohol derived from the degradation of AEs and to calculate specific removal and biodegradation efficiencies for single AE homologues, enabling an accurate exposure assessment of AE under environmental conditions. This has generated data that can be compared directly with monitoring data from actual field samples analyzed by the same methodology.

2. Materials and methods

2.1. Dosing of the test substance

The test substance used in the study was a 2:1 (w/w) mixture of NEODOL 25-7 and GENAPOL T110. The homologue composition of this mixture, NEODOL 25-7 (Shell Chemicals, UK; CAS 68131-39-5), is an alcohol (C_{12}–C_{15}) ethoxylate with an average ethoxymer number of 7 and a range of 0–18. GENAPOL T110 (Clariant, Germany; CAS 61791-28-4) is a tallow alcohol (C_{16}, C_{18}) ethoxylate with an average ethoxylate number of 11 and a range of 0–22. The test substance was characterized by an analysis of its alkyl-carbon and ethoxymer mass distribution in the influent (Fig. 1). It had an average ethoxylate number of 8.6, while the alkyl chain distribution showed a ratio of C12:C13:C14:C15:C16:C18 of 1:2:2.3:1.8:1.1:2.9.

The two ethoxylates were melted together at 43 °C in a 2:1 (w/w) ratio, and the mixture was used to prepare stock solutions in deionized water. Initially a 1-g/L AE stock solution was prepared, but after day 9 of the study a 4-g/L stock solution was prepared for use throughout the remainder of the study. Both solutions proved to be stable while stored under refrigeration at ~5 °C.

The stability of the stock solutions was monitored by measurement of their chemical oxygen demand (COD) on preparation and also on a weekly basis when used to prepare feed concentrate for the test unit.

2.2. Test procedure

The test procedure was based on the ISO 11733 (1995) standard for the evaluation of the elimination and

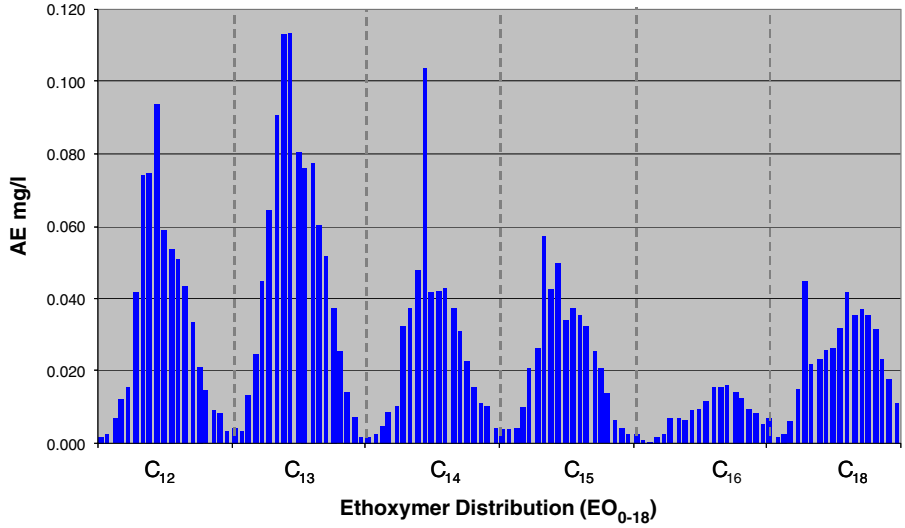

Fig. 1. Influent ethoxymer distribution (note that for each chain length a total of 18 ethoxymers (EO_{0-18}) were measured; average of analyses for days 20, 23, and 27).

biodegradability of organic compounds in aqueous medium, using laboratory-scale continuously fed activated-sludge units according to the OECD *Draft Test Guideline 303 A: Simulation Test—Aerobic Sewage Treatment: Activated Sludge Units* (OECD, 1996).

The test used a pair of "Husmann" CAS units fed with OECD synthetic sewage (OECD, 1996). The "test" unit feed contained 4 mg/L of the AE test substances mixture, which is within the range of typical environmental AE influent concentrations (Gledhill et al., 1989; Schmitt et al., 1990; Fendinger et al., 1995; Matthijs, 1996). The "control" unit did not receive any AE. The CAS units were operated at 20 °C with a HRT of 6 h and a SRT of about 10 days, which results in a loading rate that is typical of full-scale activated-sludge sewage treatment plants (STPs) (Verstraete and van Vaerenbergh, 1986).

Each of the Husmann CAS unit consisted of a 3.5-L cylindrical aeration vessel and a 2.5-L cylindrical clarifier/settler. The aeration vessels were fitted with 0.4-L precontactor zones in order to reduce the possibility of sludge "bulking" (poor settling), a factor present when using the OECD synthetic sewage feed. The precontactor consisted of a 48-mm-diameter vertical tube positioned centrally in the aeration vessel and fitted with its own central sintered-glass sparger; the feed and recycled-sludge input streams were supplied to its top and passed into the main aeration vessel via holes at its bottom. The annulus of the aeration vessel was fitted with two further spargers positioned opposite each other to provide efficient aeration and mixing of the activated sludge.

Each clarifier/settler was provided with a perforated horizontal disk that was normally positioned about 50 mm below the clarified effluent outflow to act as a flocculation filter and retain any floating sludge. Sludge from the settler section was returned to the aeration vessel precontact zone using a peristaltic pump.

The feed to each CAS unit was supplied as two streams: a sterile synthetic sewage concentrate, at 25 times final concentration (SSC25), and a nonsterile diluent stream of tap water. The SSC25 delivered to the test CAS unit had 100 mg/L of AE test substance added to it. Fresh SSC25 batches were prepared weekly by dissolving the following in 5 L of deionized water: Peptone (20.0 g), Lab-Lemco powder (Oxoid, Basingstoke, UK) (11.0 g), urea (3.75 g), K_2HPO_4 (3.5 g), $MgSO_4 \cdot 7H_2O$ (0.25 g), NaCI (0.875 g), $CaCl_2 \cdot 2H_2O$ (0.5 g). Clear solutions, free of precipitate, were obtained in all cases. The feed streams were delivered with a peristaltic pump from a glass reservoir via a glass flow-measurement burette system, to a 10-mL glass mixing vessel from which the diluted feed dripped into the precontactor zone. The SSC25 feed line was small-bore PTFE tube, with a short section of silicone-rubber (Marprene) pump tubing; the diluent line was silicone rubber throughout. Both feed-line systems were sterilized by autoclaving at 121 °C for 15 min at the beginning of the test. The complete units were housed in a temperature-controlled room that was maintained at 20 ± 1 °C.

2.3. Operation of CAS units

The test run consisted of a 19-day sludge acclimation or "run-in" phase, followed by a further 19-day "evaluation" phase. The duration of the run-in phase was driven by the achievement of a quasi-steady state of the CAS units as monitored by COD removal, sludge concentration, and nitrification activity. At the start of the test (day 0), both CAS units were filled with 6 L of activated sludge taken from a sewage treatment works that received predominantly (~90%) domestic sewage (Chester Sewage Treatment Works, Welsh Water, Sealand Road, Chester, UK). The activated sludge was aerated for 24 h, without feed, before use and had a total suspended-solids (TSS) content of 3.4 g/L. The sludge recycle pumps were adjusted to give a recycle ratio of ~1.0, and this resulted in an initial mixed-liquor suspended-solids (MLSS) concentration of ~5.5 g/L in the aeration vessels. The contents of the aeration vessels were vigorously aerated (1.2 L/min) to keep the sludge in suspension and maintain dissolved O_2 levels >1 mg/L.

The synthetic sewage feed was adjusted to give a HRT of ~6 h (based on the aeration vessel volume); ramping of the feed loading rate was not adopted. Flow rates of synthetic sewage concentrate (SSC25) and tap water were adjusted to provide the required 25-fold dilution of the SSC25 and the total input flow rate. Since the test and control units' aeration vessel volumes were not exactly identical, the total feed flow rates and thus the individual feed stream flows were set up appropriately to deliver the same HRTs.

The test unit received an input of 4 mg/L of the AE test substance mixture throughout the run. After day 7, a SRT of ~10 days was maintained in the units by the daily removal of 350 mL activated sludge from the aeration vessel. The test and control units were not "coupled" (OECD, 1996), as this procedure gives at best only a marginal improvement in the precision of the test and can give a lower extent of biodegradation (Painter and Bealing, 1989; AISE/CESIO, 1991). Reinoculation with fresh activated sludge was not necessary.

2.4. Monitoring of the CAS unit performance

The basic operational performance of the test and control units was monitored routinely by sampling and measurement several times per week in the reactor (influent and effluent). All analyses were performed on the day of sampling. Monitoring was undertaken on both units in a particular sequence designed to minimize interference between the measurements. Samples for AE analyses were preserved with 8% (v/v) formalin (40% w/v formaldehyde solution) and stored at 5 °C; previous stability studies (Williams, 2003) have confirmed that samples are stable for up to 3 months when employing this procedure. All glassware was acid-washed and segregated for use either in the test or in the control unit. All items involved in AE determinations were thoroughly prerinsed with methanol.

Treated effluent samples were collected from the clarifier overflow. For the specific AE analyses, a minimum of 4 L of effluent was first collected into a preweighed glass container containing 320 mL of formalin (40% w/v formaldehyde solution) and then made up to 8% v/v concentration. For the other analyses, a minimum of 1.1 L of effluent was collected, of which 30 mL was centrifuged at 15,000 rpm for 15 min to prepare a supernatant for COD and N-ions analyses.

The reactor temperature and dissolved oxygen were measured in situ. The units were cleaned 3–5 times a week. The aerator and clarifier vessels were mixed and cleaned by brushing, and the system was left undisturbed for about an hour to allow the clarifier sludge to return to the reactor. Mixed-liquor wastage (350 mL) was drained from the reactor and used for pH, MLSS, and MLVSS (volatile portion of the MLSS) determinations. This mixed-liquor sample was either made up to 8% (v/v) formalin for AE analyses or discarded.

Influent samples were collected directly at the feed point to the reactor, mixed, and used directly for COD and total N analyses. For AE analysis, a minimum of 0.5 L test influent and 4 L control influent samples was collected into formalin.

The pH of the activated-sludge mixed liquor was measured daily using a Mettler Toledo 340 pH meter calibrated with pH 4.0 and 7.0 buffers. The dissolved oxygen concentration in the aeration vessel was determined in situ using a Jenway 9071 DO_2 meter while the feed was being supplied. The meter was calibrated prior to use by immersion of the probe in air-saturated and nitrogen-saturated tap water at 20 °C to provide the 100% and 0% settings, respectively. The MLSS contents of the activated sludges were determined by filtering 25-mL samples through tared Whatman GF/C filters and drying to constant weight (Standard Method 209D, APHA, 1981). The volatile portion of the mixed-liquor suspended solids was determined by ashing the dry sludge in a furnace at 600 °C (Standard Method 209G, APHA, 1981). The TSS content of the biotreated effluent was determined by filtration of a 1-L of sample through tared Whatman GF/C filters, which were then dried to constant weight (Standard Method 209D, APHA 1981). The COD concentrations of the influent and treated effluent supernatant were determined [Dr. Lange Cuvette Test LCK 414, (5–60 mg/L)]. The colorimetric reactions were measured using a Dr. Lange CADAS 50S spectrophotometer. Influent and treated effluent nitrification parameters were also measured using the Dr. Lange Cuvette Test [NH_4^+–N, LCK 304 (0.015–2 mg/L); NO_2^-–N, LCK 303 (2–47 mg/L); NO_3^-–N, LCK 341 (0.015–0.6 mg/L) and LCK 342 (0.6–6 mg/L); total N, LCK 338 (20–100 mg/L)]. The flow rates of the influent SSC25 concentrate and of the diluent were measured by timing the delivery of 2- and 25-mL volumes, respectively, using in-line flow burettes.

2.5. Specific chemical analyses of alcohols and alcohol ethoxylates

During the run-in period of 19 days, the removal of total AE in the CAS units was monitored using a relatively fast, but less specific, high-performance liquid chromatography (HPLC-FI) method with fluorescence detection (Battersby et al., 2001). The HPLC-Fl method covers C_{12-18} chain lengths but fails to recover some (i.e., EO_{0-2}) of the low EO components during the clean-up stage. It has a limit of quantitation (LOQ; defined as the sample analyte concentration producing a 10:1 signal-to-noise ratio on the analytical instrument) for total AEs of 5–10 μg/L.

In the evaluation phase the pyr+ LC/MS method was used for the determination of AE fingerprints for influents and effluents based on the range of C_{12-18} chain lengths and EO_{0-18} oligomers (Dunphy et al., 2001). This method was also used to confirm the AE level in one of the samples during the run-in phase. An example of an average influent AE fingerprint during the evaluation phase is shown in Fig. 1.

The pyr+ LC/MS method has a LOQ for individual AEs in the range of 0.2–7.1 ng/L and for total AEs (all 114 ethoxymers) of 250 ng/L. The variable LOQ across the range of ethoxymers results from the different absolute MS detector sensitivities and the background signal (noise) at each ethoxymer ion mass. Since the LOQ varies by at least an order of magnitude across the ethoxymers, the individual LOQ for each ethoxymer is used, rather than the highest single figure for all, which would limit the overall sensitivity. When analytical data for each ethoxymer were less than the respective LOQ, an estimated contribution of 50% of the LOQ was used in totaling the results. In typical effluent samples, the precision of the analytical data for individual ethoxymers varies widely, since the normal mass distribution

of the ethoxymers leads to very small concentrations of low- and high-EO-number components relative to the background noise. In terms of percentage standard deviation relative to the mean (% RSD), the precision ranged from 10% to 150%. For each carbon chain length, the precision ranged from about 10% to 50% RSD, while for total alcohols and AEs it was about 100% and 15%, respectively.

The pyr+ LC/MS method for effluents was also adapted for use in analyzing AEs in MLSS samples from the control and test CAS units during the evaluation phase. The sludge samples were extracted with hot acetonitrile and the extract was dissolved in water and then treated in the same way as an effluent sample. This method has a LOQ for total AEs (all 114 ethoxymers) of ~10 μg/g dry wt sludge. The LOQ values for the sludge analysis are unique to each sample determination because of the differing quantities extracted. As for the effluent samples for which values were less than the respective LOQ, an estimated contribution of 50% LOQ was used in totaling the results.

The analytical recovery efficiencies for AEs in the effluent and sludge samples were measured by spiking control CAS unit samples with a standard solution of the test substance, since only a very limited range of the pure individual ethoxymers is available commercially.

The recovery data of the individual AE components through the pyr+ LC/MS method were determined by spiking control effluent samples with 2.5 and 5.0 μg/L of the test AE mixture. The variability of the individual species data was high (% RSD range of 10–120%) because of the small spike concentrations of low- and high-EO-number compounds relative to background levels. Recoveries tended to reduce with increasing EO number; however, they were more constant by C number. Mean recoveries ($n = 4$) for the respective C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, and C_{18} ethoxymers in the effluent were 87%, 71%, 60%, 66%, 76%, and 90%, respectively, with an overall mean of 75%. The mean recovery for the alcohols was 95%.

The recovery of sludge AEs through the pyr+ LC/MS method was carried out using two control sludge samples spiked with 190 and 454 μg/g of the test AE mixture. Mean recoveries for the C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, and C_{18} ethoxymers were 85%, 74%, 74%, 64%, 63%, and 15%, respectively, with an overall mean of 62%. Recoveries for which the spiking concentration of an ethoxymer was less than the amount found in the control were excluded from the data.

The degree of uncertainty of recovery data for the low- and high-EO-number components was expected to be high because of the low spike concentration of these species relative to background and also because of the limited precision of the analysis at these low levels. Consequently, it was considered to be inappropriate to adjust the sample pyr+ LC/MS analytical data reported here either for individual ethoxymer or for total AE recoveries, except in cases in which mass balance conversion efficiencies were being calculated (see Section 4).

2.6. Calculation procedures

2.6.1. Calculation of percentage removal of influent analytes

The percentage removals of the measured AE analytes from the influent of the test and control units were calculated according to the OECD (1996) as

$$R_{\%} = \frac{M_{\mathrm{f}} - M_{\mathrm{e}}}{M_{\mathrm{f}}} \times 100, \tag{1}$$

where, $R_{\%}$ is the percentage removal of the analyte fed to the unit (%) and M_{f} and M_{e} are the mass flow rate of the analyte in the stream in the feed and in the effluent (including SS) from the unit, respectively.

Note that for simplicity of the calculation of percentage removals, we have taken the effluent flow rate to be equal to the influent flow rate, since the sludge-wastage flow rate was relatively low. Hence, for the calculations in Table 4 we used the measured concentrations (test minus control) instead of the mass flow.

2.6.2. Calculation of percentage biodegradation of influent analytes

The calculation of the percentage of biodegraded AE analyte from the synthetic sewage influent of the CAS units was calculated according to

$$R_{\%\mathrm{Bio}} = \frac{M_{\mathrm{f}} - (M_{\mathrm{e}} + M_{\mathrm{w}})}{M_{\mathrm{f}}} \times 100, \tag{2}$$

where M_{w} is the mass flow rate of the analyte in the stream via sludge wastage and is given by the flow rate of the stream × total concentration of the analyte. The total concentration refers to the analyte present in solution and in solid material (test minus control) and $R_{\%\mathrm{bio}}$ is the percentage biodegradation of analyte fed to the unit (%).

2.6.3. Calculation of the alcohol ratio and fraction

The degradation of AE leads to a mixture of 114 different AE homologues, including a portion of AE measured as 0 ethoxylate, the fatty alcohol. In order to calculate the contribution of free alcohol for each chain length (C_x) to the total amount of AE (C_xEO_y) as measured in the effluent, a molar alcohol ratio (f_x) can be calculated as follows:

$$f_x = \frac{(c_{x\,\mathrm{AOH}}/\mathrm{MW_{AOH}})}{(\sum_{y=1}^{y=18} c_{x\,\mathrm{AE}}/\mathrm{MW_{AE}})}, \tag{3}$$

where $c_{x\,\mathrm{AOH}}$ is the calculated difference concentration of alcohol in the effluent (test minus control) (μg/L). $\mathrm{MW_{AOH}}$ is the molecular weight of the alcohol homologue (g/mol), while $c_{x\,\mathrm{AE}}$ is referring to the calculated difference concentration of AE in the effluent (test minus control) (μg/L) and $\mathrm{MW_{AE}}$ is the molecular weight of the AE homologue (g/mol). The indicator for the chain length and the degree of ethoxylation EO_{1-18} is described by x and y, respectively. The molar AOH fraction is calculated accordingly but for EO_{0-18} (i.e., $y = 0 - 18$).

The calculation was carried out for each single sample date during the evaluation phase on days 20, 23, 27, and 30. When $c_{x\,\mathrm{AOH}}$ or any value of $c_{x\,\mathrm{AE}}$ of the control exceeded the test values, a concentration of zero was assumed, as negative results are not interpretable.

3. Results

3.1. Operational performance of the CAS units

A run-in phase of 19 days was allowed for the CAS units to approach steady-state operating conditions, during which both units quickly established good performance characteristics. The evaluation period covered days 20–38. There was very little difference between the performance trends of the test and control units. The aeration vessel temperature, pH, and dissolved oxygen values remained within normal test operating limits (19–21 °C, pH of 6.6–7.7, dissolved oxygen >1 mg/L). During the evaluation period, the mean feed COD concentrations entering the test and control units were, respectively, 264 ± 6 (SD) and 249 ± 5 mg/L. The accuracy of matching these values was limited by the available resolution of the feed pump controls. The total nitrogen content in the test and control feeds was 64 ± 4 and 61 ± 3 mg/L, respectively. Single determination gave values of NH_4^+–N (4 mg/L), NO_2^-–N (0.01 mg/L), and NO_3^-–N (9 mg/L), which reflects the fact that the bulk of the feed N is in the form of urea, amino acids, and protein. The nitrification activity was maintained throughout the test. In the test unit, 66–83% of the feed total N was recovered in the effluent as inorganic N, mainly as nitrate, while in the control the range was 33–76%.

Generally, the operability of the systems was stable throughout the test, with good COD removals, sludge settleability, and effluent clarification; thus, there was no need to reinoculate with fresh activated sludge. Nevertheless, the reactor sludge (MLSS) concentrations did diminish significantly once sludge wastage was started on day 7 to achieve a 10-day SRT. A MLSS steady state was not achieved, since the average value decreased from about 4 to 3 g/L during the beginning of the 3-week evaluation period. It was noticeable that the control sludge tended to develop wall growth more strongly than the test sludge, possibly because of the absence of surfactant in the feed. This phenomenon resulted in apparently lower MLSS concentrations ($\sim$2.3 vs. $\sim$3.0 g/L) during days 28–31, after which a more rigorous precleaning of tubes and sampling procedure was used. During the evaluation period, the test feed mean specific loading rate of the analyte (F/M) increased from 0.28 to 0.37 g COD/g MLSS/day as a result of the decreasing biomass concentrations; the control F/M was a little lower (i.e., 0.26–0.31 g COD/g MLSS/day), mainly due to a slightly lower feed COD concentration. Despite this difference, the percentage COD removals in both units remained relatively constant at 91.6–91.7%, and while the test unit effluent COD (22.2 mg/L) was slightly greater than the control effluent (20.7 mg/L), the difference was not considered significant. The sludge settleability of both the test and control units was good throughout the study, and at the end of the evaluation phase sludge volume indices of 71 and 85 mL/g, respectively, were measured. Microscopic examination of the sludges showed them to consist of nonfilamentous bacterial flocs with ciliated protozoa, rotifera, and some nematodes, which is typical of a good-quality, nonbulking activated sludge. Clarification of the treated effluent was also relatively good, with a median TSS of 6–7 mg/L in both units.

The results show that the CAS units effectively modeled the operation of typical full-scale STPs. The treated effluent quality of 22 mg/L COD would roughly equate to a biological oxygen demand of about 15 mg/L, and this together with TSS values of mostly less than 10 mg/L amounts to an excellent biotreatment performance typical of well-operated activated-sludge systems.

3.2. Monitoring AE levels in influent and effluent during the run-in phase

During the 19-day run-in phase, analysis of the influent and effluent samples from both test and control CAS units was carried out using the HPLC-fluorescence method. Within the first day after the start of the test, the test unit gave concentrations $<$LOQ, indicating that the test biomass was removing more than 99.6% of the feed AE. In the control unit, both the influent and effluent levels remained at the LOQ for the method (5–10 μg/L). Subsequent analysis (day 14) by the more specific pyr+ LC/MS method confirmed that, during the run-in phase of the study, the actual AE levels in effluent from the test unit were much lower than the LOQ of the HPLC-fluorescence method.

3.3. Monitoring AE levels in influent and effluent during the evaluation phase

The evaluation phase spanned from days 20 to 38. However, since the samples from day 38 were not analyzed, only the results from days 20 to 30 are reported here. The AE analyses carried out during this period were undertaken using the pyr+ LC/MS method. For the reasons discussed below all of the results for these analyses are presented as values that have not been adjusted for analytical recoveries.

The mean values for total AE and alcohol in the test influent during the evaluation phase ($n = 3$) were 3.048 and 0.021 mg/L, respectively. Assuming a mean recovery value of 75% (as shown in the effluent) would result in a mean influent total AE concentration of 4.06 mg/L, which agrees well with the design feed concentration of 4 mg/L.

Total AE levels in the test effluents during the evaluation period ranged from 1000 to 2710 ng/L (mean of 1790 ng/L), compared with 170–460 ng/L (mean of 330 ng/L) in the control effluents. Differences between

test and control units during the evaluation phase ranged from 640 to 2540 ng/L (mean of 1460 ng/L). The differences between the test and control concentrations in the effluents represent the net unremoved fraction of AE contributed solely to the degradation processes of AEs (Fig. 2). When concentration differences (test minus control) were calculated to be negative, a difference of 0 ng/L was assumed (Fig. 2). It is notable that a high contribution (81%) of the total AE in the effluent comes from C_{18} AE components and that alcohol concentrations were high relative to EO_{1-18} at all chain lengths. A summary of the alcohol levels in the effluent samples during the evaluation phase is shown in Fig. 3. Alcohol levels in the test effluent ranged from 190 to 350 ng/L (mean of 290 ng/L) compared with 20–270 ng/L (mean of 160 ng/L) in the control effluent. Alcohol differences (test minus control) during the evaluation phase ranged from 20 to 280 ng/L (mean of 130 ng/L), with the highest contribution (46%) to the total coming from C_{18} alcohol.

The medians and ranges of measured concentrations on days 20, 23, 27, and 30 are shown in Table 1. The median values shown for the control, test, and test minus control are taken from the raw data. Calculations of the difference (test minus control) from these data may therefore slightly differ from the median values shown in the table. For the control effluents, the majority of the AE homologues (EO_{1-18}) across all of the carbon chain lengths were close to their respective LOQs (often <2 ng/L per ethoxymer), while corresponding alcohol levels were noticeably higher across the carbon chain lengths (at least an order of magnitude higher). Analytical measurements of alcohols in control influents/effluents as well as blank samples indicate that the alcohol levels were caused by low-level contamination from a range of potential sources during the sample preparation. Further investigation of these sources is ongoing.

3.4. Monitoring AE levels in sludge during the evaluation phase

Monitoring of AE and alcohol levels in sludges from the bioreactor took place on four occasions (days 21, 23, 27, and 30) during the evaluation phase. The analysis was carried out by the pyr+ LC/MS method and results are given on a dry-weight basis. When values were less than the respective LOQ an estimated contribution of 50% of the LOQ was used in totaling the results.

Total AE levels in the test unit ranged from 30 to 160 μg/g (mean of 86 μg/g) compared with 29–69 μg/g (mean of 42 μg/g) in the control unit. Alcohol levels in the test sludges ranged from 4.8 to 28.4 μg/g (mean of 18.2 μg/g) compared with 8.7–27.7 μg/g (mean of 16.1 μg/g) in the control sludges. The differences between the test and control AE levels for each of these sampling days ranged from 8.7 to 100 μg/g (mean of 50 μg/g) and are shown in Fig. 4. Similar to the AE

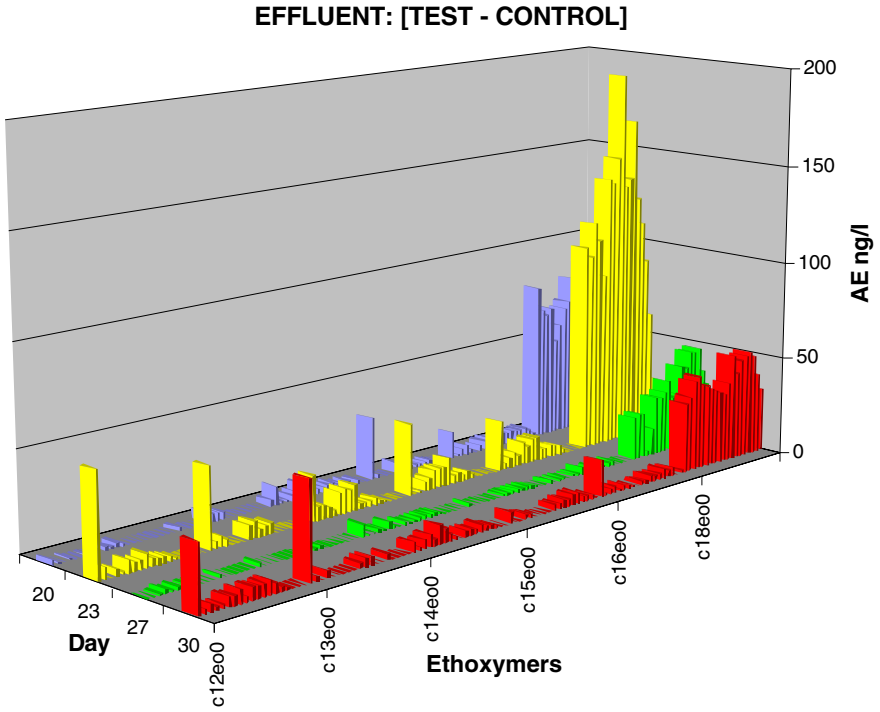

Fig. 2. Differences of AEs in CAS test effluents, relative to the control, during the evaluation phase (days 20–30). Note that for each chain length a total of 18 ethoxymers (EO_{0-18}) is shown as a single bar starting with EO_0.

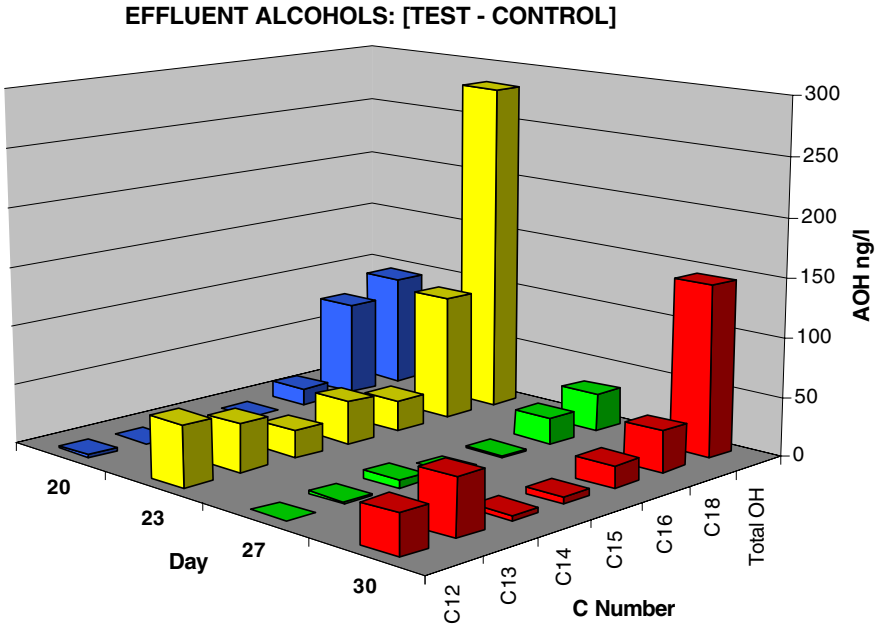

Fig. 3. Summary of differences of C_{12-18} alcohols in CAS test effluents, relative to the control (days 20–30).

distribution in effluents, the mean contribution of C_{18} AE components to the total was comparatively high at 58% (especially on days 21 and 23). AE homologue concentrations (median and range) in sludge are shown in Table 2. In general, the sample analysis shows a matrix with unique LOQ values for single homologues. As for effluents, the median of the difference (test minus control) as reported is based on raw data and thus may slightly differ from the calculation of the median shown in Table 2.

Alcohol values (test minus control) ranged from 0 to 11.6 μg/g (mean of 2.1 μg/g). As with the presentation of the AE results, for which the test minus control was negative, a concentration of zero is depicted in Fig. 5.

3.5. Calculation of the alcohol fraction relative to total AE

Alcohols of all chain lengths were observed in both the test and the control CAS units. In order to calculate the fraction and ratio of alcohol that arises from AE (EO_{0-18} and EO_{1-18}), calculations were made of the difference between concentrations in the test and control units based on the molar concentrations of each chain length using Eq. (3) (Table 3).

The results indicate a high variation of the alcohol ratio to the EO_{1-18}, differing by a span of 0.12–2.6 depending on the chain length. Converted to the AOH fraction, the span translates to 10–56% of the total AE being AOH. A trend can be seen with alcohol forming a smaller and less variable percentage of the total fingerprint at longer than at shorter chain lengths.

3.6. Percentage removal of AEs as the difference between the test and control units

The percentage removals of AEs by the test CAS unit were calculated using test effluent analyses relative to the mean test influent analyses averaged over days 20, 23, and 27 (no influent analysis was performed on day 30). Calculations of the removals, relative to the control, were made using test minus control data to allow for background levels of AEs determined in the control effluents (Table 4). Table 4 gives the mean percentage removals in the test CAS unit for each individual ethoxymer during the evaluation period. Removals of total AEs were consistently high during the evaluation period relative to the control. Generally, the mean removals of the C_{12-16} (EO_{0-18}) homologues were greater than 99.95%, whereas C_{18} compounds showed slightly lower removals of about 99.73% (range, 96.2–99.8%). Removals of total alcohols were high during the evaluation period, with a mean of 99.34% relative to the control. There was no clear trend of alcohol removal as a function of C number, and the mean individual alcohol removals were in the range of 98.62–99.57% relative to the control.

3.7. Conversion efficiencies during the evaluation phase

Table 5 presents the mass balance results of calculating the rate-conversion efficiencies of total AE (EO_{0-18}) and alcohols in the CAS units based on input data averaged over the evaluation period. In contrast to the reporting of the raw analytical data, which were not

Table 1
Measured AE homologue concentration (C_{12-18}; EO_{0-18}) in the CAS effluent of test and control units (in ng/L)

	C_{12}	$C_{12}EO_0$	$C_{12}EO_1$	$C_{12}EO_2$	$C_{12}EO_3$	$C_{12}EO_4$	$C_{12}EO_5$	$C_{12}EO_6$	$C_{12}EO_7$	$C_{12}EO_8$	$C_{12}EO_9$	$C_{12}EO_{10}$	$C_{12}EO_{11}$	$C_{12}EO_{12}$	$C_{12}EO_{13}$	$C_{12}EO_{14}$	$C_{12}EO_{15}$	$C_{12}EO_{16}$	$C_{12}EO_{17}$	$C_{12}EO_{18}$
Control	Median (ng/L)	21	3.1	3.8	2.5	0.68	1.9	1.8	2.2	0.70[a]	0.85[a]	0.90[a]	0.95[a]	0.90[a]	0.85[a]	0.75[a]	0.65[a]	1.1[a]	2.3[a]	1.2[a]
	Range	1–34	0.6[a]–4.9	2.7–5.9	1.7–3.1	0.51–0.91	0.65[a]–2.6	1.1–3.1	0.6[a]–3.1	—	—	—	—	—	—	—	—	—	—	—
Test	Median (ng/L)	38	5.2	5.1	4.5	1.8	4.6	4.0	5	2.5	3.8	3.1	3.8	2.9	1.5	0.75[a]	0.65[a]	1.1[a]	2.3[a]	1.2[a]
	Range	16–67	3.1–7.4	2.8–7.9	3.2–6.2	0.60–3.1	1.8–8.9	0.97–7.5	1.7–8.1	0.70[a]–7.6	0.85[a]–7.2	0.90[a]–6.9	0.95[a]–7.2	2.3–7.8	0.85[a]–5.7	0.75[a]–3.4	0.65[a]–1.9	—	—	—
Test minus control	Median (ng/L)	18	1.7	0.46	2.1	1.1	3.4	2.4	3.0	1.8	2.9	2.2	2.8	2.1	0.67	0	0	0	0	0
	Range	0–51	0–33	0–4.1	1.1–4.1	0–2.4	0–6.3	0–5.3	0–6.7	0–6.9	0–6.3	0–6.3	0–6.3	1.4–6.9	0–6.9	0–4.9	0–2.7	0–1.3	—	—
	C_{13}	$C_{13}EO_0$	$C_{13}EO_1$	$C_{13}EO_2$	$C_{13}EO_3$	$C_{13}EO_4$	$C_{13}EO_5$	$C_{13}EO_6$	$C_{13}EO_7$	$C_{13}EO_8$	$C_{13}EO_9$	$C_{13}EO_{10}$	$C_{13}EO_{11}$	$C_{13}EO_{12}$	$C_{13}EO_{13}$	$C_{13}EO_{14}$	$C_{13}EO_{15}$	$C_{13}EO_{16}$	$C_{13}EO_{17}$	$C_{13}EO_{18}$
Control	Median (ng/L)	30	2.5[a]	5.8	1.4[a]	3.6[a]	3.6[a]	4.35[a]	2.4[a]	2.4[a]	2.4[a]	1.1[a]	1.1[a]	3.4[a]	2.8[a]	2.1[a]	1.5[a]	2.0[a]	1.3[a]	0.40[a]
	Range	15–40	–	1.8[a]–24	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Test	Median (ng/L)	49	3.7	7.1	3.9	3.6[a]	3.6[a]	4.35[a]	2.4[a]	5.3	5.5	5.1	5.0	3.4[a]	2.8[a]	2.1[a]	1.5[a]	2.0[a]	1.3[a]	0.40[a]
	Range	18–83	2.5[a]–5.9	1.8[a]–20	1.4[a]–4.9	—	—	—	2.4[a]–9.6	2.4[a]–11	2.4[a]–6.6	2.3–6.0	2.6–6.6	—	—	2.1[a]–5.3	—	—	—	—
Test minus control	Median (ng/L)	21	1.3	0	2.5	0	0	0	0	2.9	3.1	4.0	3.9	0	0	0	0	0	0	0
	Range	0–48	0–48	0–5.4	0–3.5	0–3.3	—	—	0–7.2	0–8.3	0–4.2	1.2–4.9	1.6–5.6	0–4.19	—	0–3.2	0–3.2	—	—	—
	C_{14}	$C_{14}EO_0$	$C_{14}EO_1$	$C_{14}EO_2$	$C_{14}EO_3$	$C_{14}EO_4$	$C_{14}EO_5$	$C_{14}EO_6$	$C_{14}EO_7$	$C_{14}EO_8$	$C_{14}EO_9$	$C_{14}EO_{10}$	$C_{14}EO_{11}$	$C_{14}EO_{12}$	$C_{14}EO_{13}$	$C_{14}EO_{14}$	$C_{14}EO_{15}$	$C_{14}EO_{16}$	$C_{14}EO_{17}$	$C_{14}EO_{18}$
Control	Median (ng/L)	9	1.3	1.3	0.84	0.57	1.3	2.2	1.1	0.60	1.6	0.97	0.63	0.40[a]	0.75[a]	0.65[a]	0.55[a]	0.90[a]	2.0	1.0[a]
	Range	0.8–13	0.15–1.9	0.68–3.5	0.3[a]–1.5	0.2[a]–0.89	0.55[a]–2.6	0.35[a]–3.2	0.5[a]–6.3	0.6[a]–2.9	0.7[a]–3.5	0.4[a]–1.9	0.4[a]–1.7	0.4[a]–1.2	—	—	—	—	—	—
Test	Median (ng/L)	15	4.2	6.6	4.6	2.9	7.6	7.6	8.3	4.1	3.8	3.4	3.7	3.3	3.7	2.6	2.2	1.6	2.0[a]	1.0[a]
	Range	12–24	1.8–7.3	1.9–13	1.8–6.9	1.2–3.9	4.2–13	1.5–15	0.5–13.6	2.2–14	2.2–4.5	1.9–4.4	2.1–4.4	2.2–4.8	2.1–4.7	1.9–3.6	0.55–2.8	0.90[a]–3.4	—	1–2.7
Test minus control	Median (ng/L)	5.5	2.6	5.7	3.4	2.2	6.6	5.4	5.5	2.3	1.7	2.5	2.8	2.9	2.9	1.9	1.65	0.72	0	0
	Range	0–23	0.6–7.1	0.51–9.1	1.4–6.2	0.96–6.2	2.6–11	1.2–12	0–11	1.6–13	0.38–3.0	0.9–3.1	1.7–3.2	1.8–3.6	1.4–4.0	1.2–4.0	0–2.9	0–2.5	0–1.5	0–1.7
	C_{15}	$C_{15}EO_0$	$C_{15}EO_1$	$C_{15}EO_2$	$C_{15}EO_3$	$C_{15}EO_4$	$C_{15}EO_5$	$C_{15}EO_6$	$C_{15}EO_7$	$C_{15}EO_8$	$C_{15}EO_9$	$C_{15}EO_{10}$	$C_{15}EO_{11}$	$C_{15}EO_{12}$	$C_{15}EO_{13}$	$C_{15}EO_{14}$	$C_{15}EO_{15}$	$C_{15}EO_{16}$	$C_{15}EO_{17}$	$C_{15}EO_{18}$
Control	Median (ng/L)	23	1.1[a]	0.80[a]	1.2[a]	1.6[a]	1.6[a]	1.9[a]	2.0[a]	1.1[a]	1.0[a]	0.95[a]	0.90[a]	0.70[a]	0.60[a]	0.45[a]	0.30[a]	0.20[a]	0.25[a]	0.15[a]
	Range	2.9–37	1.1[a]–3.1	0.80[a]–9.3	—	—	—	—	—	—	—	0.95[a]–3.2	—	—	—	—	—	—	—	0.15[a]–1.5

Test	Median (ng/L)	25	1.1[a]	2.0	3.4	2.6	1.6[a]	1.9[a]	4.7	2.4	3.5	4.2	3.4	3.2	2.6	2.3	1.32	0.70	0.25[a]	0.15[a]
	Range	18–39	1.1[a]–2.6	0.80[a]–32	1.2[a]–6.3	1.6[a]–7.8	1.6[a]–11	1.9[a]–12	2.0[a]–12	1.1[a]–14	2.0–5.0	2.4–6.0	2.2–5.0	2.1–4.9	2.3–5.8	1.2–5.9	0.3–3.5	0.2–2.8	0.25–2.9	0.15[a]–0.57
Test minus control	Median (ng/L)	2.9	0	1.2	2.2	1.1	0	0	2.7	1.3	2.5	2.5	2.5	2.5	2.0	1.8	1.02	0.50	0	0
	Range	0–36	0–5.8	0–32	0–5.1	0–6.2	0–9.2	0–10	0–9.5	0–14	1.0–4.0	1.5–4.5	1.3–4.1	1.4–4.2	1.7–5.1	0.76–5.5	0–5.5	0–3.2	0–2.6	0–2.6
	C_{16}	$C_{16}EO_0$	$C_{16}EO_1$	$C_{16}EO_2$	$C_{16}EO_3$	$C_{16}EO_4$	$C_{16}EO_5$	$C_{16}EO_6$	$C_{16}EO_7$	$C_{16}EO_8$	$C_{16}EO_9$	$C_{16}EO_{10}$	$C_{16}EO_{11}$	$C_{16}EO_{12}$	$C_{16}EO_{13}$	$C_{16}EO_{14}$	$C_{16}EO_{15}$	$C_{16}EO_{16}$	$C_{16}EO_{17}$	$C_{16}EO_{18}$
Control	Median (ng/L)	25	0.5	0.55[a]	0.25[a]	1.9	0.84	0.30[a]	0.40[a]	0.50[a]	0.55[a]	1.2[a]	1.6[a]	1.3[a]	1.3[a]	1.2[a]	2.0[a]	1.7[a]	1.4[a]	0.90[a]
	Range	0.3–27	0.1[a]–0.9	0.55[a]–1.6	0.25[a]–0.51	0.81–3.9	0.50[a]–3	0.30[a]–0.84	—	—	—	—	—	—	—	—	—	—	—	—
Test	Median (ng/L)	34	1.4	3.8	4.1	3.9	2.9	2.6	4.4	3.6	3.6	4.1	6.0	5.3	4.9	4.7	3.8	3.7	1.4[a]	1.6
	Range	26–42	0.9–3.2	1.2–5.8	1.5–7.8	2.5–6.1	2.2–12	1.3–9.0	1.2–12	0.5–13	1.5–5.6	2.6–6.2	1.6[a]–7.7	2.8–7.1	3.1–7.2	3.2–6.4	2.0[a]–7.4	1.7[a]–7.2	1.4[a]–5.3	0.90[a]–2.5
Test minus control	Median (ng/L)	15.9	0.97	2.7	3.7	1.5	1.8	2.0	4.0	3.09	3.0	2.9	4.4	4.0	3.6	3.6	1.8	2.0	0	0.68
	Range	1.5–25	0.05–18	0.63–5.3	1.2–7.6	0–5.3	0–11	0.97–8.7	0.84–12	0–12.136	0.99–5.0	1.4–5.0	0–6.1	1.5–5.8	1.8–6.0	2.1–5.3	0–5.4	0–5.6	0–4.0	0–1.6
	C_{18}	$C_{18}EO_0$	$C_{18}EO_1$	$C_{18}EO_2$	$C_{18}EO_3$	$C_{18}EO_4$	$C_{18}EO_5$	$C_{18}EO_6$	$C_{18}EO_7$	$C_{18}EO_8$	$C_{18}EO_9$	$C_{18}EO_{10}$	$C_{18}EO_{11}$	$C_{18}EO_{12}$	$C_{18}EO_{13}$	$C_{18}EO_{14}$	$C_{18}EO_{15}$	$C_{18}EO_{16}$	$C_{18}EO_{17}$	$C_{18}EO_{18}$
Control	Median (ng/L)	54	0.97	1.0	2.3	1.5[a]	0.55[a]	0.70[a]	0.85[a]	1.2[a]	1.2[a]	1.3[a]	1.7[a]	1.5[a]	1.4[a]	2.6[a]	2.3[a]	1.9[a]	3.0[a]	2.0[a]
	Range	0.76–157	0.25[a]–1.8	0.6[a]–1.9	1.3–33	1.5[a]–11	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Test	Median (ng/L)	102	54	56	60	47	50	53	54	60	48	54	73	65	68	72	61	46	38	29
	Range	66–236	25–101	24–118	35–112	24–91	30–140	29–138	32–151	37–194	28–136	30–139	44–170	43–128	50–115	51–95	52–90	30–72	15–64	5.1–51
Test minus control	Median (ng/L)	58	53	55	58	46	49	53	53	59	47	52	71	63	67	69	58	44	35	27
	Range	22–106	23–101	22–118	2.1–108	14–89	29–140	28–137	31–150	36–193	27–135	28–138	43–168	41–127	49–113	48–93	50–88	28–70	12–61	3.1–49
	C_{12-18}	EO_0	EO_1	EO_2	EO_3	EO_4	EO_5	EO_6	EO_7	EO_8	EO_9	EO_{10}	EO_{11}	EO_{12}	EO_{13}	EO_{14}	EO_{15}	EO_{16}	EO_{17}	EO_{18}
Control	Median (ng/L)	161.0	9.3	13.3	8.5	9.8	9.7	11.3	8.8	6.3	7.6	6.4	6.8	8.1	7.7	7.7	7.3	7.7	10.2	5.6
Test	Median (ng/L)	263.4	69.6	80.5	79.1	62.0	70.1	73.7	79.0	77.7	68.1	73.4	94.3	82.7	83.8	84.2	70.1	55.0	45.2	33.7
Test minus control	median (ng/L)	102.5	60.2	67.3	70.6	52.3	60.4	62.5	70.2	71.4	60.5	67.0	87.5	74.6	76.2	76.5	62.9	47.3	35.1	28.1

Indicated are the median and the concentration range of four measurements on days 20, 23, 27, and 30 during the evaluation period of the study.

[a]Half the limit of quantification (LOQ/2).

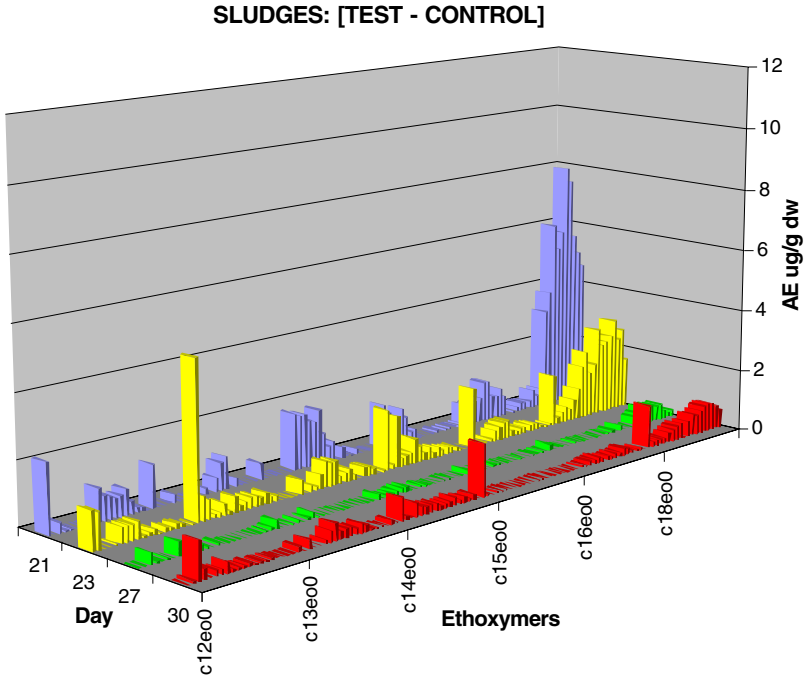

Fig. 4. Differences of AEs in CAS test sludges, relative to the control, during the evaluation phase (days 20–30). Note that for each chain length a total of 18 ethoxymers (EO_{0-18}) is shown as a single bar starting with EO_0.

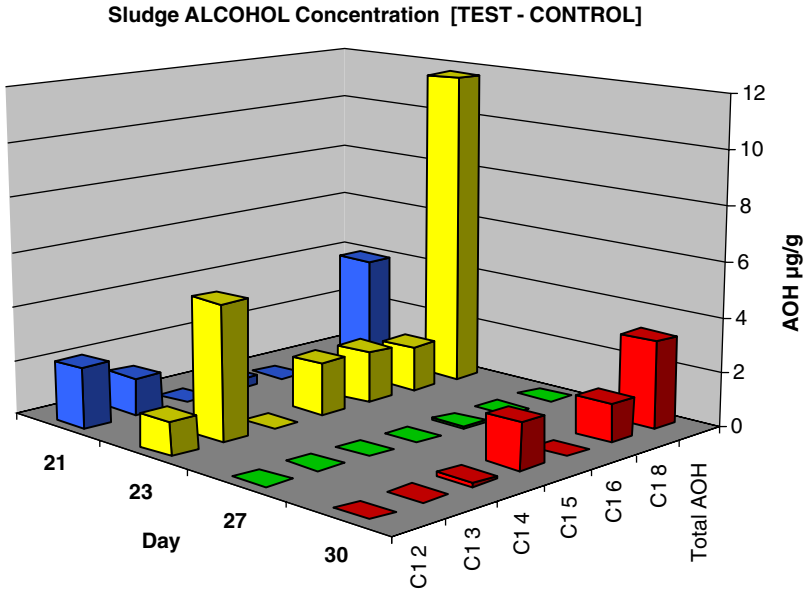

Fig. 5. Summary of differences of C_{12-18} alcohols in CAS test sludges, relative to the control, during the evaluation phase (days 20–30).

corrected for recovery, concentrations of total AEs and alcohols have been adjusted using mean analytical recoveries to account for differences in effluent and sludge recoveries. It was assumed that influent and effluent samples would have the same recovery levels. The output in Table 5 represents the total mass outflow of AE of the respective unit during the run described as mass flow rates in terms of unit reactor volume (mg/L/day) to account for the small differences in volumes of the test and control CAS units. Thus, the table shows the split of the AEs and alcohols between the solids (wastage sludge) and dissolved phases (treated effluent and suspended solids). The "removal" efficiencies were calculated by comparing the mass flow in the total effluent (solids plus dissolved) with that of the influent [cf. Eq. (1)]. The biodegradation efficiencies, on the other hand, are the difference between all remaining AE material in the CAS unit and the influent mass flow.

They also account for the mass flow of undegraded AE that was adsorbed—thus not biodegraded—to the wasted sludge solids and removed via sludge disposal.

In the CAS test unit, only 11.5% of the residual AE, and 2.8% of the alcohols, were in the dissolved phase. The mean removal of AEs was 99.94%, with a biodegradation efficiency of 99.67%, while alcohol removal was 98.54% and biodegradation 84.52%. Specific removal rates for the AEs and alcohols were 5.060 and 0.026 mg/g MLSS/day, respectively.

In the control CAS unit, there was an apparent net production of AEs_{1-18} and alcohols, on the order of 10 times that fed into this unit (cf. Table 5; input of total effluent) and equivalent to about 40% of the AEs_{0-18} and 80% of the alcohols found in the test unit output. Again, most of the measured AE and alcohol appeared in the solid fraction.

To compensate for the levels of AEs and alcohols seen in the control CAS unit, Table 5 also shows the difference (test minus control) values for the conversions. The results show that 20.1% of the residual AE, and 26.6% of the alcohols, were in the dissolved phase. A fraction of about 20% of the AE_{0-18} in the effluent remained adsorbed to the suspended matter. The mean removal of AEs was 99.95%, with a biodegradation efficiency of 99.81%, while alcohols removal was 99.34% and biodegradation 97.36%.

4. Discussion

Measured concentrations of AEs in influents to activated-sludge plants in Europe and the USA are usually in the range of 1–4 mg/L (Gledhill et al., 1989; Schmitt et al., 1990; Fendinger et al., 1995; Matthijs, 1996). Influent monitoring of nine different municipal WWTPs in Germany between 1993 and 2001 confirmed this finding with mean AE concentrations of 2.6 mg/L (AE_{12-18}, EO_{2-20}; $n = 68$; Henkel KGaA, internal data). In contrast to earlier studies on the elimination of AE in WWTPs, the AE influent concentration used in this study (4 mg/L as linear C_{12-18} EO_{0-18}) was within this real-world range. It was possible to achieve such realistic, low-dosing conditions because the pyr+ LC/MS analytical method is sufficiently sensitive and selective to be able to detect the full range of ethoxymers in effluents and sludges even after high removal rates.

4.1. Applicability of the pyr+ method

In the case of AEs analysis from effluents or sludges, the analyte is not a discrete defined chemical entity, but rather a complex mixture of 114 ethoxymers of varying physicochemical properties. This mixture is extracted from environmental matrices (i.e., effluent and sludge) that contain competing coextractives that vary in concentration from sample to sample, resulting in different recoveries for each ethoxymer and for each sample. Specifically, analytical recoveries showed a high variability for the low- and high-EO-number compounds, reflecting the fact that spiking concentrations were close to background levels for these species. Overall recoveries for total AE and alcohol were estimated to be 75% and 95% for effluent and 62% and 50% for sludge samples. Although analytical recoveries have been quoted throughout this paper, results have not been corrected for recoveries for several technical and analytical reasons identified by IUPAC. Among these, recoveries based on spiking and allied methods may be higher than the true recovery of the native analyte (Szymanski et al., 2003). In addition, estimates of recovery close to the LOQ have a high relative uncertainty. The limit of quantitation or lowest limit of quantitation is the level above which quantitative results may be obtained with a specific degree of confidence. The LOQ is mathematically defined as equal to 10 times the standard deviation of the results for a series of replicates used to determine a justifiable limit of detection. This may cause a recovery-corrected result that has a higher relative uncertainty than an uncorrected result, which may in turn undermine the credibility of the analysis. Furthermore, the recoveries from effluent samples are a function of the relative proportions of the AEs in the soluble and solid fractions and thus are dependant on the suspended-solids content of each sample (van Compernolle et al., 2005). To address these concerns one would need to perform a recovery determination on every sample, which is impractical for analyses using the pyr+ LC/MS method. The combination of the factors given above lead us to the conclusion that estimates of recoveries for individual ethoxymers, and thus total AEs, could have a high degree of uncertainty, which if used to adjust the analytical data could cause the recovery-corrected results to have a much higher relative uncertainty than the uncorrected results. Consequently, the analytical data reported here were not adjusted for recoveries. Bearing this explanation in mind, an exception was made for the calculation of rate-conversion results of the CAS units because significant differences between sludge and effluent recoveries were observed. In order to conduct a plausible conversion analysis these differences could only be accounted for by normalizing the data with the different average recoveries of sludge and effluent.

4.2. Generation of a molar alcohol cap

The aim of this study was to investigate the specific removal efficiencies of all the AE homologues in order to determine the fraction of alcohol in a STP following AE degradation. To control for other sources of alcohol and

Table 2
Measured AE homologue concentrations (C_{12-18}; EO_{0-18}) in CAS sludges of the test and control units (in μg/g)

	C_{12}	$C_{12}EO_0$	$C_{12}EO_1$	$C_{12}EO_2$	$C_{12}EO_3$	$C_{12}EO_4$	$C_{12}EO_5$	$C_{12}EO_6$	$C_{12}EO_7$	$C_{12}EO_8$	$C_{12}EO_9$	$C_{12}EO_{10}$	$C_{12}EO_{11}$	$C_{12}EO_{12}$	$C_{12}EO_{13}$	$C_{12}EO_{14}$	$C_{12}EO_{15}$	$C_{12}EO_{16}$	$C_{12}EO_{17}$	$C_{12}EO_{18}$
Control	Median (μg/g)	4.71	0.59	0.68	0.62	0.16	0.46	0.37	0.31	0.27	0.35	0.27	0.21	0.19	0.13	0.08	0.05[a]	0.08[a]	0.17	0.04[a]
	Range	2.23–6.72	0.28–1.98	0.4–0.92	0.25–0.88	0.12–0.30	0.33–1.00	0.29–0.86	0.18–0.78	0.15–0.41	0.26–0.54	0.18–0.40	0.13–0.27	0.08–0.25	0.10–0.17	0.03[a]–0.15	0.04[a]–0.05[a]	0.07[a]–0.09[a]	0.14–0.19	0.04[a]–0.05[a]
Test	Median (μg/g)	4.51	0.94	1.06	0.63	0.20	0.73	0.56	0.62	0.19	0.41	0.37	0.35	0.23	0.25	0.19	0.15	0.13	0.17	0.04[a]
	Range	0.97–8.92	0.20–2.02	0.83–1.58	0.56–0.92	0.16–0.34	0.52–1.00	0.39–0.83	0.42–0.85	0.006–0.70	0.31–1.58	0.24–1.19	0.28–1.00	0.15–0.83	0.08–0.80	0.15–0.86	0.05[a]–0.45	0.09[a]–0.24	0.8[a]–0.29	0.04[a]–0.05[a]
Test minus control	Median (μg/g)	0.61	0.04	0.33	0.05	0.04	0.12	0.09	0.40	0.04	0.08	0.09	0.17	0.05	0.11	0.10	0.11	0.10	0.00	0.00
	Range	0–2.19	0–1.14	0–1.18	0–1.18	0.01–0.33	0–0.49	0–0.52	0–0.49	0–0.55	0.02–1.04	0.07–0.78	0.11–0.73	0.01–0.62	0–0.65	0.06–0.83	0–0.42	0–0.20	0–0.21	0–0.002
	C_{13}	$C_{13}EO_0$	$C_{13}EO_1$	$C_{13}EO_2$	$C_{13}EO_3$	$C_{13}EO_4$	$C_{13}EO_5$	$C_{13}EO_6$	$C_{13}EO_7$	$C_{13}EO_8$	$C_{13}EO_9$	$C_{13}EO_{10}$	$C_{13}EO_{11}$	$C_{13}EO_{12}$	$C_{13}EO_{13}$	$C_{13}EO_{14}$	$C_{13}EO_{15}$	$C_{13}EO_{16}$	$C_{13}EO_{17}$	$C_{13}EO_{18}$
Control	Median (μg/g)	4.06	0.46	1.40	0.33	0.24	0.20	0.31	0.41	0.39	0.44	0.38	0.38	0.30	0.20	0.18	0.12	0.08[a]	0.05[a]	0.03[a]
	Range	3.45–9.44	0.24–0.95	0.90–1.67	0.21–0.39	0.09[a]–0.34	0.10[a]–0.70	0.18–0.545	0.23–0.81	0.08[a]–0.77	0.30–1.39	0.26–1.27	0.23–1.23	0.12–0.72	0.10–0.64	0.65[a]–0.42	0.05[a]–0.52	0.06[a]–0.08[a]	0.04[a]–0.05[a]	0.01[a]–0.03[a]
Test	Median (μg/g)	5.90	0.55	0.73	0.44	0.32	0.41	0.38	0.64	0.34	0.69	0.68	0.68	0.53	0.51	0.37	0.23	0.17	0.05[a]	0.03
	Range	1.40–10.78	0.22–1.43	0.68–2.08	0.31–0.49	0.18–0.58	0.19–0.57	0.29–0.67	0.33–0.73	0.18–0.7	0.32–1.23	0.35–1.47	0.22–1.23	0.20–1.14	0.29–1.51	0.20–1.43	0.12–0.36	0.07[a]–0.39	0.04[a]–0.07[a]	0.01[a]–0.03
Test minus control	Median (μg/g)	0.67	0.09	0.00	0.08	0.07	0.00	0.12	0.18	0.08	0.13	0.24	0.14	0.23	0.31	0.24	0.04	0.06	0.00	0.00
	Range	0–4.94	0–0.76	0–0.61	0–0.28	0–0.28	0–0.37	0–0.25	0–0.50	0–0.62	0–0.62	0.092–0.33	0–0.33	0.08–0.41	0.19–0.87	0.11–1.01	0–0.22	0–0.32	0–0.125	0–0.09
	C_{14}	$C_{14}EO_0$	$C_{14}EO_1$	$C_{14}EO_2$	$C_{14}EO_3$	$C_{14}EO_4$	$C_{14}EO_5$	$C_{14}EO_6$	$C_{14}EO_7$	$C_{14}EO_8$	$C_{14}EO_9$	$C_{14}EO_{10}$	$C_{14}EO_{11}$	$C_{14}EO_{12}$	$C_{14}EO_{13}$	$C_{14}EO_{14}$	$C_{14}EO_{15}$	$C_{14}EO_{16}$	$C_{14}EO_{17}$	$C_{14}EO_{18}$
Control	Median (μg/g)	2.26	0.16	0.37	0.31	0.18	0.48	0.62	0.67	0.45	0.45	0.42	0.37	0.34	0.38	0.23	0.18	0.07	0.14	0.04[a]
	Range	0.52–3.86	0.12–0.37	0.33–0.67	0.25–0.53	0.15–0.29	0.34–1.14	0.45–1.46	0.27–1.35	0.33–1.03	0.38–0.90	0.36–0.82	0.25–0.60	0.19–0.54	0.21–0.60	0.159–0.49	0.13–0.29	0.04[a]–0.09	0.04[a]–0.20	0.03[a]–0.04[a]
Test	Median (μg/g)	1.90	0.26	0.63	0.41	0.24	0.79	0.89	0.96	0.68	0.68	0.63	0.63	0.50	0.52	0.40	0.41	0.22	0.24	0.07
	Range	0.35–3.80	0.08–0.63	0.27–1.1	0.22–0.47	0.156–0.32	0.49–0.86	0.55–1.26	0.81–1.34	0.16–1.08	0.44–2.71	0.43–2.52	0.35–2.30	0.33–2.02	0.37–1.91	0.23–2.24	0.11–1.25	0.14–0.55	0.14–0.76	0.03[a]–0.12
Test minus control	Median (μg/g)	0.00	0.02	0.25	0.06	0.18	0.48	0.62	0.19	0.12	0.22	0.21	0.26	0.16	0.20	0.23	0.24	0.15	0.12	0.01
	Range	0–0.12	0–0.45	0–0.72	0–0.72	0.15–0.29	0.35–1.14	0.45–1.46	0–0.74	0–0.76	0.06–1.81	0.07–1.69	0.10–1.71	0.14–1.48	0–1.40	0–1.75	0–0.95	0.09–0.52	0.05–0.69	0–0.16
	C_{15}	$C_{15}EO_0$	$C_{15}EO_1$	$C_{15}EO_2$	$C_{15}EO_3$	$C_{15}EO_4$	$C_{15}EO_5$	$C_{15}EO_6$	$C_{15}EO_7$	$C_{15}EO_8$	$C_{15}EO_9$	$C_{15}EO_{10}$	$C_{15}EO_{11}$	$C_{15}EO_{12}$	$C_{15}EO_{13}$	$C_{15}EO_{14}$	$C_{15}EO_{15}$	$C_{15}EO_{16}$	$C_{15}EO_{17}$	$C_{15}EO_{18}$
Control	Median (μg/g)	1.47	0.08	0.57	0.18	0.21	0.24	0.45	0.31	0.22	0.29	0.27	0.22	0.18	0.12	0.17	0.05	0.02	0.01[a]	0.01
	Range	1.05–2.41	0.04[a]–0.05[a]	0.12–0.86	0.13–0.39	0.14–0.48	0.12–0.62	0.10–0.98	0.23–1.26	0.09–0.45	0.18–0.52	0.22–0.54	0.12–0.44	0.15–0.27	0.11–0.213	0.11–0.36	0.01[a]–0.16	0.01[a]–0.07	0.01[a]–0.01[a]	0.01[a]–0.07

Test	Median (μg/g)	2.94	0.13	0.75	0.31	0.35	0.37	0.60	0.46	0.27	0.42	0.33	0.32	0.27	0.25	0.24	0.14	0.04	0.02	0.01
	Range	0.82–3.41	0.03–0.22	0.15–2.16	0.22–0.53	0.20–0.49	0.20–0.50	0.29–0.94	0.23–0.78	0.11–0.46	0.28–1.89	0.23–1.60	0.19–1.38	0.16–1.05	0.18–1.28	0.14–1.14	0.04–0.42	0.02–0.33	0.01[a]–0.04	0.01[a]–0.03
Test minus control	Median (μg/g)	1.00	0.07	0.03	0.10	0.01	0.02	0.00	0.07	0.01	0.19	0.09	0.15	0.10	0.14	0.11	0.10	0.02	0.00	0.00
	Range	0–1.93	0–1.67	0–1.66	0.04–0.27	0–0.32	0–0.38	0–0.82	0–0.48	0–0.26	0–1.37	0–1.06	0–0.94	0–0.78	0.05–1.08	0–0.78	0.03–0.27	0–0.32	0–0.03	0–0.03
	C_{16}	$C_{16}EO_0$	$C_{16}EO_1$	$C_{16}EO_2$	$C_{16}EO_3$	$C_{16}EO_4$	$C_{16}EO_5$	$C_{16}EO_6$	$C_{16}EO_7$	$C_{16}EO_8$	$C_{16}EO_9$	$C_{16}EO_{10}$	$C_{16}EO_{11}$	$C_{16}EO_{12}$	$C_{16}EO_{13}$	$C_{16}EO_{14}$	$C_{16}EO_{15}$	$C_{16}EO_{16}$	$C_{16}EO_{17}$	$C_{16}EO_{18}$
Control	Median (μg/g)	0.91	0.02	0.07	0.04	0.03	0.03	0.04	0.03	0.02	0.04	0.04[a]	0.11[a]	0.04[a]	0.05[a]	0.03[a]	0.06[a]	0.05[a]	0.04[a]	0.04[a]
	Range	0.30–3.07	0.02–0.14	0.02[a]–0.17	0.01–0.08	0.01–0.04	0.01[a]–0.04	0.01[a]–0.05	0.02–0.09	0.01[a]–0.02[a]	0.02[a]–0.03[a]	0.04[a]–0.05[a]	0.10[a]–0.12[a]	0.04[a]–0.06[a]	0.04[a]–0.05[a]	0.04[a]–0.05[a]	0.65[a]–0.08[a]	0.06[a]–0.07[a]	0.09[a]–0.11[a]	0.03[a]–0.04[a]
Test	Median (μg/g)	1.07	0.02	0.09	0.08	0.07	0.13	0.12	0.21	0.12	0.18	0.18	0.26	0.23	0.30	0.28	0.26	0.18	0.16	0.09
	Range	0.32–3.23	0.014–0.04	0.02–0.14	0.04–0.11	0.02–0.10	0.06–0.35	0.05–0.48	0.14–0.55	0.04–0.56	0.09–0.70	0.09–0.73	0.122–0.83	0.10–0.89	0.15–1.38	0.11–1.37	0.16–0.89	0.07[a]–0.83	0.12–1.02	0.03[a]–0.68
Test minus control	Median (μg/g)	0.01	0.00	0.00	0.03	0.03	0.12	0.09	0.20	0.10	0.15	0.14	0.21	0.19	0.26	0.25	0.20	0.13	0.12	0.05
	Range	0–1.91	0–0.002	0–0.10	0.02–0.07	0.0006–0.09	0.04–0.31	0.03–0.42	0.05–0.52	0.016–0.54	0.07–0.68	0–0.68	0–0.77	0–0.84	0.10–1.33	0.06–1.33	0–0.81	0–0.76	0.07–0.97	0–0.65
	C_{18}	$C_{18}EO_0$	$C_{18}EO_1$	$C_{18}EO_2$	$C_{18}EO_3$	$C_{18}EO_4$	$C_{18}EO_5$	$C_{18}EO_6$	$C_{18}EO_7$	$C_{18}EO_8$	$C_{18}EO_9$	$C_{18}EO_{10}$	$C_{18}EO_{11}$	$C_{18}EO_{12}$	$C_{18}EO_{13}$	$C_{18}EO_{14}$	$C_{18}EO_{15}$	$C_{18}EO_{16}$	$C_{18}EO_{17}$	$C_{18}EO_{18}$
Control	Median (μg/g)	0.89	0.01	0.06	0.08	0.10	0.05	0.06	0.08	0.09	0.10	0.14	0.11	0.11	0.20	0.20	0.18	0.15	0.21	0.06[a]
	Range	0.41–2.32	0.002–0.10	0.02[a]–0.18	0.02[a]–0.14	0.04[a]–0.12	0.02[a]–0.16	0.02[a]–0.15	0.02[a]–0.14	0.04[a]–0.16	0.04[a]–0.17	0.06[a]–0.24	0.09[a]–0.22	0.05[a]–0.3	0.08[a]–0.26	0.08[a]–0.26	0.07[a]–0.22	0.06[a]–0.17	0.09[a]–0.12[a]	0.06[a]–0.16[a]
Test	Median (μg/g)	1.44	0.17	0.27	0.34	0.30	0.37	0.57	0.54	0.61	0.95	1.22	1.95	1.60	1.74	1.88	2.15	1.75	1.90	1.20
	Range	0.9813–2.26	0.048–0.21	0.06–0.36	0.12–0.52	0.12–0.49	0.18–0.67	0.06–1.02	0.08–2.25	0.08–3.32	0.47–3.93	0.70–6.26	0.49–5.42	0.64–5.97	0.67–8.20	0.67–8.20	0.68–7.73	0.37–5.77	0.36–5.27	0.36–4.74
Test minus control	Median (μg/g)	0.69	0.11	0.15	0.22	0.23	0.26	0.46	0.53	0.91	1.11	1.80	1.47	1.57	1.72	1.72	2.01	1.64	1.76	1.16
	Range	0–1.66	0.05–1.38	0.04–0.34	0.10–0.50	0–0.44	0.16–0.66	0–1.0	0–2.22	0–3.27	0.42–3.89	0.63–6.19	0.43–5.37	0.58–5.91	0.57–8.10	0.57–8.10	0.59–7.64	0.29–5.70	0.24–5.16	0.28–4.67
	C_{12-18}	EO_0	EO_1	EO_2	EO_3	EO_4	EO_5	EO_6	EO_7	EO_8	EO_9	EO_{10}	EO_{11}	EO_{12}	EO_{13}	EO_{14}	EO_{15}	EO_{16}	EO_{17}	EO_{18}
Control	Median (μg/g)	14.30	1.32	3.16	1.54	0.92	1.47	1.84	1.81	1.43	1.66	1.53	1.42	1.22	1.03	0.95	0.72	0.57	0.72	0.40
Test	Median (μg/g)	17.76	2.07	3.54	2.23	1.47	2.80	3.11	3.43	2.20	3.32	3.41	4.20	3.36	3.57	3.37	3.35	2.50	2.58	1.49
Test minus control	Median (μg/g)	3.5	0.8	0.4	0.7	0.6	1.3	1.3	1.6	0.8	1.7	1.9	2.8	2.1	2.5	2.4	2.6	1.9	1.9	1.1

Indicated are the median and the concentration range of four measurements on days 21, 23, 27, and 30 during the evaluation period of the study.

[a]Half the limit of quantification (LOQ/2).

AE we used a defined synthetic sewage and set up a second CAS unit without AE in its feed. Major advantages of the synthetic feed, besides that it was surfactant free, were that it was consistent in its preparation from batch to batch and that it permitted low-level analysis of AE components in the effluents. This enabled an accurate measurement of the biodegradation and removal efficiencies of the commercial AE mixture during the treatment of AE. Because of differences in nutrient composition, the synthetic sewage may encourage the growth of filamentous microorganisms and the quality of the sludge (e.g. settleability, nitrifying activity) may deteriorate after being run for more than a week or two (Painter, 1971; Verstraete and van Vaerenbergh, 1986). In this study, however, the use of a "precontactor" section in the aeration vessel allowed us to avoid any operational problems and thus prevented the need for further inoculation with fresh activated sludge.

The AE and alcohol levels in the effluent and sludge samples taken during the evaluation period of this study were low but were successfully quantified using the pyridinium method. As shown in Table 5, the most significant fraction of remaining AE (ca. 75%) was associated with the sludge leading to low concentrations of single homologues in the effluent. Reflecting the low suspended-solids concentration of 6–7 mg/L, more than 80% of the total AE and alcohol in the effluent was in the dissolved phase.

The inclusion of a control unit (not dosed with AE) was fundamentally important because the AE and alcohol concentrations in the control unit were 18.8% of AE_{0-18} and 54.8% of the alcohol compared to what was seen in the test unit effluent. It is not clear how much of the relatively high alcohol fraction is due to the actual production of alcohols within the control CAS unit and how much represents background noise close to the limit of detection. Background levels could also be due to artifacts associated with the analytical procedure

Table 3
Mean molar ratios and fractions of alcohol (test minus control) per each chain length relative to EO_{1-18} and EO_{0-18}, respectively

	Ratio (EO_0:EO_{1-18})		% fraction (EO_0:EO_{0-18})	
	Mean	SD	Mean (%)	SD (%)
C12	1.14	1.0	42.4	31
C13	2.6	5.9	56.0	38
C14	0.38	0.1	23.9	20
C15	0.40	0.3	21.2	26
C16	0.63	0.1	35.8	15
C18	0.12	0.002	10.3	2
Weighted mean	0.25	0.03	19.1	8

SD, standard deviation of single data points of days 20–30; $n = 4$. The weighted mean represents the total average of four single data points between days 20 and 30.

Table 4
Mean percentage removals of AEs during the evaluation period by the test CAS unit relative to the control

Carbon number EO number	Mean % removal						
	C12	C13	C14	C15	C16	C18	Total
0	98.622	99.515	99.573	99.835	99.464	99.117	99.338
1	99.911	>99.859	99.877	>99.941	99.834	96.280	99.541
2	99.983	>99.973	99.891	99.857	99.371	97.523	99.761
3	99.982	>99.989	99.958	99.969	99.790	99.026	99.886
4	99.993	>99.984	99.979	>99.985	99.927	99.667	99.945
5	99.993	>99.989	99.979	99.987	99.951	99.850	99.960
6	99.997	>99.990	99.984	99.993	99.947	99.688	99.970
7	99.996	>99.996	99.988	99.987	99.914	99.683	99.969
8	99.997	>99.996	99.995	99.991	99.947	99.660	99.973
9	99.995	>99.994	99.996	99.992	99.966	99.758	99.969
10	99.995	99.996	99.995	99.993	99.967	99.783	99.967
11	99.994	99.995	99.994	99.992	99.969	99.788	99.960
12	99.993	>99.989	99.993	99.991	99.969	99.790	99.959
13	99.993	>99.989	99.990	99.988	99.970	99.798	99.953
14	>99.993	>99.989	99.989	99.986	99.968	99.797	99.943
15	>99.991	>99.988	99.987	99.989	99.966	99.791	99.933
16	>99.977	>99.971	>99.984	99.984	99.955	99.794	99.922
17	>99.944	>99.967	>99.962	99.983	>99.966	99.778	99.922
18	>99.930	>99.952	>99.957	>99.989	>99.964	99.745	99.890
Total	99.991	>99.991	99.987	99.987	99.948	99.725	
Overall	99.950						

Influent data taken as mean of days 20, 23, and 27; effluent data taken as mean of raw analyses for days 20, 23, 27, and 30.

Table 5
Mass rate conversions based on averaged data over the evaluation period (days 20–30)

	SRT: ~10 days, HRT: 6 h	Total AE				Alcohols			
		Conc. μg/L or μg/g	Rate mg/L day	% of Output	% of Input	Conc. μg/L or μg/g	Rate mg/L day	% of Output	% of Input
Test unit									
Input		4064.27	16.1487			20.84	0.0828		
Output	In total effluent	2.39				0.31			
	Dissolved	1.56	0.0062	11.5	0.04	0.09	0.0004	2.8 6	0.43
	In suspended solids	139.03	0.0032	6.0	0.02	36.40	0.0008	.6 9	1.02
	In waste sludge solids	139.03	0.0444	82.5	0.27	36.40	0.0116	0.6	14.02
	Total		0.0538	100.0	0.33		0.0128	100.0	15.48
Elimination			16.0949		**99.67**		0.0700		**84.52**
Removal based on effluent output only			16.1393		**99.94**		0.0816		**98.54**
Specific removal rate	mg/g MLSS day		**5.0604**				**0.0258**		
Control unit									
Input		0.47	0.0019			0.21	0.0008		
Output	In total effluent	0.45				0.17			
	Dissolved	0.00	0.0000	0.0	0.00	0.00	0.0000	0.0	0.00
	In suspended solids	67.10	0.0018	8.1	96.65	32.20	0.0009	8.3	104.99
	In waste sludge solids	67.10	0.0208	92.3	1096.37	32.20	0.0100	93.8	1190.92
	Total		0.0226	100.0	1188.10		0.0106	100.0	1269.98
Elimination			0.0000		**0.00**		0.0000		**0.00**
Removal based on effluent output only			0.0002		**8.27**		0.0002		
Specific removal rate	mg/g MLSS day		**0.0006**				**0.0003**		**20.94**
Difference Test—control									
Input		4063.79	16.1468			20.63	0.0820		
Output	In total effluent	1.95				0.14			
	Dissolved	1.58	0.0063	20.1	0.04	0.15	0.0006	26.6	0.70
	In suspended solids	71.94	0.0014	4.5	0.01	4.20	0.0000	0.0	0.00
	In waste sludge solids	71.94	0.0235	75.4	0.15	4.20	0.0016	75.0	1.98
	Total		0.0312	100.0	0.19		0.0022	100.0	2.64
Elimination			16.1156		**99.81**		0.0798		**97.36**
Removal based on effluent output only			16.1391		**99.95**		0.0814		**99.34**
Specific removal rate	mg/g MLSS day		**5.0597**				**0.0255**		

AE and AOH concentrations shown are corrected for recoveries. Dissolved concentrations of AE and AOH are estimated on the assumption that the effluent SS have the same concentrations as the MLSS. Aerator volumes: Test, 3.5 L; Control, 3.6 L.

Table 6
Summary of AE concentrations for the evaluation period (days 20–30)

	Test CAS unit		Control CAS unit		Test minus control	
	Range	Mean	Range	Mean	Range	Mean
Effluent total AE (μg/L)	1.00–2.71	1.79	0.17–0.46	0.33	0.64–2.54	1.46
Effluent alcohols (μg/L)	0.19–0.35	0.29	0.02–0.27	0.16	0.02–0.28	0.13
Sludge total, AE (μg/g)	30–160	86	29–69	42	0–91	45
Sludge alcohols (μg/g)	5–28	18	9–28	16	0–12	2

(e.g., due to contamination from a range of potential sources during sample preparation). However, all discussed factors were leveled out in the difference calculation between the test and control unit because they potentially occurred in both units.

Subtracting control from test unit measurements shows that dosing with 4 mg/L of a 2:1 NEODOL 25-7:GENAPOL T110 AE mixture results in an effluent with mean levels of 1.46 μg/L total AE and 0.13 μg/L alcohol and sludge with 45 μg/g total AE and 2 μg/g alcohol (Table 6).

The results in Table 3 indicate that the longest chain alcohols (C_{18}) measured in effluents represented the smallest molar ratio (0.12) of alcohol compared to EO_{1-18}. This observation is noteworthy in the context of AE risk assessment because ecotoxicity increases as carbon chain length increases and the degree of ethoxylation decreases. Consequently, the long-chain alcohols are the main drive of the overall toxicity of an AE mixture (Boeije et al., 2005) and the main contributor of the RCR (Belanger et al., 2005). Therefore, this CAS study's determination of an AOH:EO_{1-18} ratio is important because it enables one to attribute to AE a fraction of the alcohol measured in an effluent fingerprint, and this enables one to refine a monitoring-based risk assessment of AE. As indicated in Figs. 3 and 5 (test minus control), alcohol may not necessarily be attributed to the degradation process of AE (e.g., when test minus control is $\leqslant 0$ mg/L). It can therefore be concluded that a large proportion of the high alcohol concentrations frequently measured in STP effluents (Eadsforth et al., 2005) may be due to sources other than AE.

4.3. Fate of alcohol ethoxylates

AEs based on linear alcohols and ethoxylated with fewer than ~20 EO units have a high biodegradability in the aquatic environment (e.g., Swisher, 1987; Holt et al., 1992). NEODOL ethoxylates with alkyl chain lengths from C_{9-11} to C_{14-15} and an average of EO_{1-12} are "readily" biodegradable, undergoing rapid and extensive "ultimate" biodegradation (Battersby, 1993). Previous monitoring studies in Europe and the USA have demonstrated that the average removal of linear AEs during activated-sludge treatment is high at >99% (e.g., Matthijs, 1996; Fendiger et al., 1995; Gledhill et al., 1989). These percentage removal values are based on levels of AEs, usually as C_{12-15} alcohol with >2–18 ethylene oxide (EO) units per mole, measured in the influent sewage to, and the treated effluent from, municipal activated-sludge plants. This is a measure of removal, as opposed to biodegradation, as no distinction is made between the loss of AEs by biodegradation and their abiotic removal through adsorption to sludge solids. Further model CAS studies of NEODOL 25–7 and NEODOL 25–3 (Battersby et al., 2001) showed biodegradation was >98.7% for both compounds at 20 °C and >97.2% for NEODOL 25–7 at 10 °C. In the same study, the levels of polyethylene glycols in the effluents from CAS plants dosed with NEODOL 25–7 indicated that biodegradation occurred through a central cleavage mechanism. Central cleavage results in rapid "primary" biodegradation and is thought to be the principle means by which sewage treatment bacteria break down linear (and 2-alkyl branched) AEs (Balson and Felix, 1995). An assessment of the mass flow rates over the evaluation period showed that the biodegradation of total AE and alcohol from the test feed was 99.8% and 97.4%, respectively; hence, removal by passive adsorption to sludge solids in the effluent was not significant. Nevertheless, the majority (>75%) of the AE and alcohols output from the CAS units was found to be adsorbed and removed via the waste sludge solids (Table 5, cf. Test-Control); consequently, this indicated that the treated effluent quality was partly dependant on the effluent TSS levels; future monitoring studies on full-scale STPs would benefit from taking this factor into account. In fact, recent activated-sludge die-away studies with distinct radiolabeled AE and alcohol ethoxymers have shown that biodegradation is a very fast process with half-lives in the range of minutes (Itrich and Federle, 2005). Desorption processes from particulate matter appear to be rate limiting to biodegradation. Over the evaluation period of the current study, the mean removals of total C_{12-18} AE (EO_{0-18}) and alcohols were >99.9% and 99.4%, respectively. In general, the removals of ethoxymers in the range $C_{12}EO_{2-18}$–C_{16} EO_{5-18} were all very high, i.e.,

greater than 99.9%, whereas removals of C_{18} ethoxymers were consistently lower (range of 96.2–99.8%); removals increased significantly as a function of EO number over the approximate range EO_{0-3}. While these figures serve to demonstrate the ready biodegradability of the test compound, they may also reflect the different concentrations of the individual ethoxymers fed into the CAS unit. Under normal steady-state operating conditions, it is expected that CAS systems can be described by Monod kinetics such that the residual concentration of a substrate in the treated effluent is determined by the prevailing SRT and is independent of the feed concentration. Consequently, the lower percentage removal figures seen here for the low-EO-number ethoxymers might be due to their low feed concentration, or to less favorable kinetics for their biodegradation, relative to the higher EO number compounds. However, since the observed removals for the EO_{16-18} species were about 99.9% despite having similar feed concentrations to the EO_{0-3} species, it must be concluded that the central cleavage to alcohol is fast and that the biodegradation of the latter was kinetically limited relative to the higher EO number species. The test effluent samples showed the residual ethoxylate to be predominantly C_{18} compounds, although the levels appeared to follow a decreasing trend with time. There is no clear explanation for this observation, and one can only suggest that adaptation of the sludge for the efficient removal of the C_{18} species may be a slower process than that for the C_{12-16} compounds.

5. Conclusion

In this study, the removals of the 114 AE species ($C_{12-18}EO_{0-18}$) from the test feed of 4 mg/L of the 2:1 NEODOL 25-7:GENAPOL T110 AE mixture were measured for a CAS unit operated at 20 °C. For the first time, it was determined quantitatively how AE removal processes contribute to AOH levels in STP effluents. The outcome of the present work enables us to refine the risk assessment of AE in STP effluents based on the entire AE fingerprint taking the realistic contribution of AE_xEO_0 (AOH) relative to the remaining AE_xE_{1-18} into account.

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