Assessment of the pollutant elimination efficiency by gas chromatography/mass spectrometry, liquid chromatography–mass spectrometry and –tandem mass spectrometry
Comparison of conventional and membrane-assisted biological wastewater treatment processes

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Abstract

The elimination efficiency of advanced conventional biological wastewater treatment was compared to membrane-assisted biological wastewater treatment. The sum parameter analyses dissolved organic carbon (DOC) and chemical oxygen demand (COD) or substance-specific analyses such as gas chromatography combined with mass spectrometry, flow injection analysis (FIA–MS) and liquid chromatography (LC–MS) in combination with mass or tandem mass spectrometry (MS–MS) were applied to assess elimination of hardly eliminable compounds in both types of wastewater treatment plants (WWTP). Reduction of DOC and COD in wastewater treatment processes confirmed a favourable elimination efficiency. Substance-specific methods which were applied in addition permitted a qualitative and semi-quantitative assessment of elimination with a visual pattern recognition approach. In order to identify pollutants either the NIST library of electron impact mass spectra for unpolar compounds or the laboratory-made collision-induced dissociation spectra library for polar pollutants was used. To assess elimination efficiency FIA–MS in the selected ion monitoring mode (SIM) besides high selective substance-specific mass spectrometric techniques such as parent ion scans and neutral loss scans were used for quantification. Results proved that membrane-assisted treatment was more effective than advanced biological treatment. In both types of WWTPs predominantly unpolar pollutants were eliminated, while all effluents were dominated by polar compounds of anthropogenic and biogenic origin. These unpolar and polar compounds which had been identified as hardly eliminable are reported about. Quantitative results obtained by FIA–MS, LC–MS and MS–MS for the elimination of alkyl polyglycol ethers, nonylphenol ethoxylates and linear alkylbenzenesulfonic acids from wastewater are presented. © 2000 Elsevier Science B.V. All rights reserved.

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1. Introduction

Currently, under optimized conditions, more than 90–95% of pollutants can be eliminated by conventional biological wastewater treatment. Besides biodegradation, adsorption at the sludge is the most effective process. Polar compounds, however, often are hardly adsorbable and non-eliminable because of

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short retention times. So they are only slightly reduced in wastewater treatment process and are discharged into surface waters, where they are degraded slowly. Their properties make them also reach drinking water treatment in spite of soil filtration [1,2]. Despite great efforts, these pollutants consisting of, e.g., surfactants [1,2], pharmaceuticals [3], plasticisers, etc., and their metabolites, could be eliminated only incompletely from drinking water by activated carbon filtration. Despite this potential the knowledge about these hardly degradable pollutants in the effluents of wastewater treatment plants is very limited.

In order to reduce these pollutants in the wastewater effluents, various, quite different new biochemical and physical procedures are applied today. Yet it turns out that only very few and expensive procedures can eliminate these polar compounds efficiently. Some of the most successful procedures are membrane-assisted treatment methods such as reverse osmosis. In accordance with the exclusion limit of the membranes that have been applied, even molecules of low molecular mass ($\geq$200–300 D) can easily be separated from raw water that is to be used as drinking water [4]. Yet due to this efficiency only small water throughputs are possible, and high costs arise. Another, more promising and cost effective approach would be to use membranes with much larger pore sizes (and therefore larger throughputs) in combination with biological treatment which is estimated as a powerful tool to improve the reduction of the pollutant loads in wastewater effluents. Currently, this so-called microfiltration is successfully being applied in advanced biological treatment of municipal wastewater resulting in a removal of particles and germs from the effluents. This water can even be used as drinking water [5]. The procedure is advantageous, since during the treatment process the concentration of biologically active organisms, responsible for wastewater treatment in that they effect degradation and elimination, can be increased up to 20 g l^{-1} without discharge of particulate compounds into the effluent. Besides a higher degradation rate, due to much higher biomass concentration, there will grow "specialists" for the elimination of problematic compounds. I.e., microorganisms, which will be adapted to the degradation of these wastewater constituents, will grow in the biocoenosis because of a very low excess sludge amount and, therefore, a long mean cell residence time.

Analyses by sum parameters, such as chemical oxygen demand (COD) or total organic carbon (TOC), proved that membrane-assisted processes are quite efficient, but there is a striking lack of knowledge about the compounds which are hardly eliminable under these conditions. If these compounds were known, elimination could be improved by adaptation and optimization of membranes according to these types of molecules. Our objectives were to assess the efficiency of conventional and membrane-assisted wastewater treatment and to discover and identify several of the relevant non-eliminable pollutants. We therefore checked these methods not only by means of sum parameter analyses but also by substance-specific and reliable analytical methods. The pollutants were pursued within wastewater treatment by substance-specific gas chromatography–mass spectrometry (GC–MS), flow injection analysis (FIA) and liquid chromatography (LC), both coupled with mass (MS) and tandem mass (MS–MS) spectrometric detection. The comprehensive examinations were targeted to wastewaters containing problematic compounds e.g. hardly eliminable, polar and/or toxic compounds. If possible these pollutants should be identified by MS–MS [6] which would help to optimize membrane materials and treatment conditions.

2. Experimental

2.1. Materials

Wastewater for membrane-assisted treatment was taken from the Aachen-Soers wastewater treatment plant (WWTP). Polypropylene glycol (PPG) for calibration purposes and sodium azide for preservation were of analytical reagent grade (Merck, Germany). The ammonium acetate used in the atmospheric pressure chemical ionization (APCI) process was of analytical-reagent grade from Merck. Dichloromethane was used for liquid–liquid extraction. Hexane, diethyl ether and methanol used for selective desorption of water pollutants from the solid-

phase materials as well as acetone and methanol for solid-phase extraction (SPE)-conditioning purposes were Nanograde solvents. Methanol for LC applications was of HPLC grade (Promochem).

Ultra-pure water was prepared by a Milli-Q-system (Millipore). SPE cartridges filled with C_{18} Polar Plus material from Baker were used to concentrate pollutants from wastewater. Nitrogen to dry SPE cartridges was of 99.999% purity, nitrogen used as sheath gas in APCI ionization was of 5.0 purity, and argon used as collision gas was of technical grade (Linde).

Glass-fibre filters used for the pretreatment of the water samples were obtained from Schleicher and Schüll (Dassel). Before use, the glass-fibre filters were heated to 400°C.

The membrane-assisted pilot plants were equipped with hydrophilic polyvinylidenefluoride membranes of various pore sizes. Table 1.

2.2. Operation conditions in wastewater treatment

Experimental and operational conditions of the WWTPs are listed in Table 1. The inflows of the membrane plants varied in retention time because of the tank volumes. The biocoenosis of the membrane-assisted biological WWTPs was grown from organisms contained in the sewage and cultivated over a period of 21 days to reach steady state conditions.

2.3. Sampling and sample preparation

In a sampling period which covered several weeks all wastewater samples were taken as time-proportional grab samples. Sampling points were the effluent of the pre-settling tank as inflow to the conventional treatment plant and to the membrane-assisted treatment plants. Effluent samples were taken from the biological filter of Aachen is municipal WWTP and from permeates of three membrane treatment plants equipped with different types of membranes and operated under varying conditions.

The samples were stored in glass bottles at a temperature of 4°C prior to extraction using dichloromethane in liquid–liquid or C_{18} materials in SPE. Unless extraction was performed immediately the samples were preserved by adding sodium azide bringing about a concentration of 100 mg l^{-1}.

For GC–MS analysis, dichloromethane extracts of wastewater were prepared by liquid–liquid extraction. The organic layer was separated and dried over anhydrous sodium sulphate. After reduction of the organic solvent by evaporation to 2 ml, these concentrates could be used for injection in GC–MS examinations.

In order to avoid an overload of SPE cartridges, the degree of pollution of the wastewater was monitored by measuring the chemical oxygen demand (COD) or dissolved organic carbon (DOC) content (as described in Section 2.4) prior to SPE concentration of polar compounds.

For FIA– and LC–MS polar wastewater pollutants were extracted by commercially available 500 mg SPE C_{18} Polar Plus cartridges after they had been conditioned as prescribed by the manufacturer. After SPE procedure the cartridges were eluted with ultra-

Table 1
Experimental and operational conditions of the WWTPs

	Membrane assisted WWTP 1	Membrane assisted WWTP 2	Membrane assisted WWTP 3	WWTP Aachen-Soers
Average inflow (m^3 day^{-1})	5.2	5.2	12.0	88 000
Retention time (h)	11	16	11	20
MLSS (g l^{-1})	18	17	16	2.9
Sludge load [kgBOD (kgMLSS)$^{-1}$ day^{-1}]	0.04	0.03	0.05	0.07
Type of filtration	Microfiltration	Microfiltration	Ultrafiltration	Biofiltration
Pore size (μm)	0.4	0.1	–	–
MWCO (Dalton)	–	–	200 000	–
Pressure Δp (bar)	− 0.12[a]	− 0.3[a]	+ 0.5[b]	–

[a] Pressure below atmospheric.
[b] Pressure above atmospheric.

pure water and dried in a gentle stream of nitrogen gas at 30°C. The adsorbed compounds were desorbed by applying organic solvents of different polarities (hexane–diethyl ether (8:2, v/v), diethyl ether, methanol–water (2:8, v/v) and methanol [7]). The sequential repeated addition of small quantities (5×1 ml) of eluent ensured a widely selective elution according to the polarities of the pollutants. With the exception of the water–methanol fractions, all eluates from the SPE were evaporated to dryness in a gentle stream of nitrogen. The residues were dissolved in 1 ml of methanol and then could be used for injection during FIA– and LC–MS analysis.

2.4. Sum parameter analysis

For evaluation of the elimination efficiency inflow and effluents of all treatment plants were monitored by the COD [8]. DOC content of the water samples was measured with a Ströhlein total carbon analyzer (C-MATT 5500). After the pretreated samples have passed a glass-fibre filter, we performed the combustion mineralization in combination with infrared detection.

2.5. Gas chromatographic system

A Varian (Darmstadt, Germany) Model 3400 GC system with a fused-silica capillary column was used. The conditions were as follows: carrier gas, helium; linear gas velocity, 15 cm s^{-1}; injector temperature, 250°C; transfer line temperature, 250°C; column, DB-35 ms (J&W), film thickness 0.25 μm (30 m×0.32 mm I.D.).

Combined with GC, electron impact (EI) ionization was applied with an ionization energy of 70 eV. Under these conditions the pressure in the ion source was 3·10^{-3} Torr (1 Torr=133.322 Pa). In the vacuum system of the mass spectrometer, it was 3·10^{-6} Torr. The electron multiplier was operated at 1200 V with a conversion dynode voltage at 15 kV. The temperature in the ion source was 150°C.

2.6. Liquid chromatographic system

LC separations coupled with MS, MS–MS and UV detection were achieved with a Waters 600 MS system. A Waters 510 pump was used for post-

column addition of a 0.1 M ammonium acetate solution in the APCI mode. A Waters 996 photodiode array detector system in combination with a Millenium 2010 data system (Millipore) was connected in-line with the APCI or electrospray ionization (ESI) interface. The conditions in FIA bypassing the analytical column were as follows: mobile phase methanol–water (30:70) containing 0.05 M ammonium acetate. The overall flow-rate was 0.5 ml min^{-1} in the APCI mode and 0.2 ml min^{-1} in the ESI mode.

The chromatographic separations in both, the non-ion-pairing mode and the ion-pairing mode on the analytical column were carried out after an optimization of the conditions by standardized method had taken place. The flow rate for both column separations was 1.0 ml min^{-1}. Identical conditions for gradient elution were programmed starting with A–B (10:90) while the concentration was increased linearly to 90% A within 15 min. In the non-ion-pairing mode the mobile phase was composed as follows: solvent A: methanol; solvent B: water. Under ion-pairing conditions the mobile phase was composed as follows: solvent A: acetonitrile–0.5 mM ammonium acetate in water (80:20); solvent B: acetonitrile–0.5 mM ammonium acetate in water (20:80). After passing the UV detector, 0.5 ml min^{-1} of 0.1 M ammonium acetate was added, which resulted in an overall flow rate of 1.5 ml min^{-1}. The post-column flow split ratio was 1:2 in favour of the MS in APCI and ESI mode or waste, respectively.

2.7. MS and MS–MS systems

A TSQ 700 mass spectrometer (Finnigan MAT) combined with a DEC 5000/33 data station was used for research work. The APCI and the ESI interfaces were obtained from Finnigan.

For coupling the LC system with the mass spectrometer, the conditions for APCI and ESI were chosen as shown in Table 2.

FIA and LC analyses were applied, recording APCI or ES mass spectra scanning from 100 to 1200 u at 1 or 3 s, respectively. FIA bypassing the analytical column with MS or MS–MS [daughter- and parent-ion or neutral loss (NL) mode] detection was performed while accumulating a maximum of 50 scans after injection. The mass spectrum averaging

Table 2
MS and MS–MS parameters using APCI or ES interface

Parameter	MS mode		MS–MS mode: CID	
	APCI	ESI	APCI	ESI
Vaporizer temperature (°C)	400	–	400	–
Capillary temperature (°C)	200	200	200	200
Spray voltage (kV)	–	4.5	–	4.5
Corona current (μA)	4	–	4	–
Capillary lens voltage (V)	10	10	10	10
Tube lens voltage (V)	40	40	40	40
Octapole voltage (V)	−3	−3	−3	−3
Electron multiplier (V)	1200	1200	1200–1700	1200–1700
Conversion dynode (kV)	15	15	15	15
Collision energy (eV)	–	–	−10 to −50	−10 to −50
Sheath gas pressure (p.s.i.)[a]	40	40	40	40
Ion source pressure (Torr)[a]	0.3	0.3	0.5	0.5
Collision gas	–	–	Ar	Ar
Collision cell pressure (mTorr)[a]	–	–	1.0	1.0

[a] 1 p.s.i.=6894.76 Pa; 1 Torr=133.322 Pa.

the total ion current from the beginning of the signal up to the end in FIA–MS mode is termed "overview spectrum".

APCI and ESI were carried out in positive and negative modes.

2.8. Quantification by MS and MS–MS

FIA–MS and LC–MS quantifications in the selected ion monitoring mode (SIM) were performed in the positive and negative mode according to the literature [9,10]. Defined mixtures of alkyl polyglycol ethers (AEOs), nonylphenol ethoxylates (NPEOs) and linear alkylbenzenesulfonic acids (LASs) were used for calibrations and standard addition. Besides recovery experiments with selective eluates of C_{18} concentrated spiked wastewater samples presented in Table 3 recoveries in inflow and effluent samples of Aachen's WWTP were also determined after standard addition and concentration applying SPE in combination with RP-C_{18} (cf. Table 4). Calibration curves were calculated by MS data of sequential selective eluats or by their mixtures, respectively.

The quantitative determinations during membrane treatment were performed in the FIA–MS, FIA–MS–MS, LC–MS and LC–MS–MS modes. For AEO and NPEO quantifications the hexane–diethyl ether, diethyl ether and methanol eluates of the same cartridge were mixed. For LAS determination only the methanol eluates were applied.

In the FIA mode a minimum of 5 injections were performed and the selected mass traces of the target compounds [AEO (FIA–MS(+): m/z 336, 380 (C_{12}), 306, 350 (C_{13}) and 364, 408 (C_{14}); NPEO (FIA–MS(+): m/z: 370, 414, 458; LAS (FIA–MS(−): m/z 311, 325, 339] were accumulated for calculation. LC–MS quantifications were performed by calculation of the area under the selected mass traces. For quantification with increased selectivity reaction monitoring in the FIA–MS–MS and LC–MS–MS modes was applied using characteristic parent ion mass traces of the pollutants [AEO (FIA–MS–MS(+): parent ion m/z 89 or 151 (C_{12}–C_{14}); NPEO (FIA–MS–MS(+): parent ion m/z 291; LAS (FIA–MS–MS(−): parent ion m/z 183] [9,10].

For the recovery examinations using spiked effluent samples the concentrations of the target pollutants (AEOs, NPEOs and LASs) in the different sequential eluats of SPE cartridges (hexane–diethyl ether, diethyl ether, methanol–water and methanol) as well as in their mixtures were determined (cf. Table 3). In these comprehensive recovery examinations applying FIA–MS the following mass traces were quantified [AEO (FIA–MS(+): m/z 292, 336, 380, 424, 468, 512, 556, 600, 644, 688, 732 and 776

Table 3
Recoveries for hardly degradable polar target pollutants in fractionated elutates after C_{18}-SPE. Material: spiked effluent samples. Sequential selective eluents applied: hexane–diethyl ether, diethyl ether, methanol–water and methanol

Pollutants	Mode	Eluents				Mixture of eluates
		Hexane–diethyl ether (%)	Diethyl ether (%)	Methanol–water (%)	Methanol (%)	
AEOs	FIA–MS	37[d]	36[d]	« 1[d]	34[d]	112[d]
(C_{12}–C_{15})	FIA–MS–MS[a]	29[d]	34[d]	« 1[e]	33[e]	102[d]
	LC–MS	32[f]	36[f]	n.d.	33[f]	n.d.
NPEO	FIA–MS	7[d]	31[d]	« 1[f]	66[d]	109[d]
	FIA–MS–MS[b]	10[d]	19[d]	« 1[f]	70[d]	99[d]
	LC–MS	9[f]	22[f]	n.d.[g]	66[e]	n.d.
LAS	FIA–MS	« 1[d]	« 1[d]	« 1[d]	97[d]	103[d]
	FIA–MS–MS[c]	« 1[e]	« 1[e]	« 1[f]	98[e]	99[d]
	LC–MS	n.d.	n.d.	« 1[f]	101[e]	n.d.

[a] Parents(+) m/z 89 or 151.
[b] Parents(+) m/z 291.
[c] Parents(−) m/z 183.
[d] Average of four measurements.
[e] Average of three measurements.
[f] Average of two measurements.
[g] n.d.=not determined.

for C_{12} homologues; 306, 350, 394, 438, 482, 526, 570, 614, 658, 702, 746 and 790 for C_{13} homologues; 320, 364, 408, 452, 496, 540, 584, 628, 672 and 716 for C_{14} homologues; NPEO (FIA–MS(+): m/z: 326, 370, 414, 458, 502, 546, 590, 634, 678, 722, 766, 810 and 854 for $NP(EO)_{2-14}$ homologues; LAS (FIA–MS(−): m/z 297, 311, 325, 339 for C_{10-13} homologues].

3. Results and discussion

Our aim was to assess the elimination efficiency of membrane-assisted biological wastewater treatment and to compare it to conventional advanced biological WWT. For this purpose, either sum parameter analyses or substance-specific analyses were performed. Substance-specific quantitative determinations of selected relevant pollutants were performed, while we monitored their concentrations in inflow and effluents in order to calculate elimination rates and also, in order to compare reliability of the different MS and MS–MS techniques we applied for quantification. The information about elimination rates this extensive analytical approach provided should help engineers, biologists and chemists to find out weak points in the processes and materials applied.

First we determined the sum parameters DOC (Fig. 1) and COD in different wastewaters. When the results of the inflow samples were compared to the analyses of the different effluents, both parameters gave evidence of a quite favourable elimination of ≥90%. Determination of COD and DOC in the inflow resulted, during the week, in average values of about 680 mg l^{-1} or 216 mg l^{-1}, respectively. For all effluents, including the conventional WWTP effluent, average COD results could be found <20 mg l^{-1}, whereas the DOC concentrations varied between 8 and 12 mg l^{-1} during the whole sampling campaign. Some characteristic features could be observed, e.g., that the pollution load increased from Monday to Friday. During the weekend less wastewater was discharged, while the pollution load decreased. The results presented in Fig. 1 are the only information about elimination efficiency that can be obtained by the sum parameter DOC over a 4-week sampling period.

Table 4
Recoveries. concentrations (Conc.) and elimination rates (Elim.) for hardly degradable polar target pollutants in inflow (Infl.) and effluents (Effl.) of membrane-assisted (MA) and conventional (Conv.) wastewater treatment plants (WWTP) under different substance-specific determination techniques applying FIA–, LC–MS and –MS–MS. Recoveries in inflow and effluents were determined using mixtures of selective eluates after RP-C_{18} SPE [AEO ($M_r < 600$): hexane–diethyl ether and diethyl ether, ($M_r > 600$) diethyl ether and methanol–water; NPEOs: diethyl ether; LASs: methanol]

Pollutants	Mode	Sampling date/period	Recoveries (%)		Conv. WWTP, Infl. Conc. ($\mu g\ l^{-1}$)	MA WWTP 1		MA WWTP 2		MA WWTP 3		Conv. WWTP	
			Infl.	Effl.		Effl. Conc. ($\mu g\ l^{-1}$)	Elim. (%)	Effl. Conc. ($\mu g\ l^{-1}$)	Elim. (%)	Effl. Conc. ($\mu g\ l^{-1}$)	Elim. (%)	Effl. Conc. ($\mu g\ l^{-1}$)	Elim. (%)
AEOs (C_{12}–C_{15})	FIA–MS	03.05.99–	114	108	2.150–3.800	80–150	96	65–230	97	70–205	97	190–380	91
		01.06.99	($n=3$)	($n=4$)	($n=25$)	($n=23$)	($n=21$)	($n=29$)	($n=24$)	($n=29$)	($n=22$)	($n=30$)	($n=25$)
	FIA–MS	07.05.99			2.650	80	97	65	98	65	98	220	92
		27.05.99			3.050	92	97	85	97	70	98	265	91
	FIA–MS–MS	07.05.99			1.100	44	96	40	96	36	97	92	92
		16.05.99			1.280	35	97	42	97	38	97	108	92
		27.05.99			1.190	40	97	44	96	30	97	104	91
	LC–MS	27.05.99			1.120	22	98	26	98	20	98	65	94
NPEOs	FIA–MS	03.05.99–	105	103	120–270	12–22	91	10–19	94	7–15	96	24–48	85
		03.06.99	($n=4$)	($n=4$)	($n=28$)	($n=27$)	($n=24$)	($n=25$)	($n=21$)	($n=30$)	($n=22$)	($n=31$)	($n=27$)
	FIA–MS	07.05.99			245	19	92	19	92	8	97	35	86
		27.05.99			220	18	92	12	95	9	96	43	80
	FIA–MS–MS	07.05.99			225	20	91	19	92	7	97	32	86
		16.05.99			185	17	91	14	92	7	96	30	84
		27.05.99			218	17	92	10	95	10	95	33	85
	LC–MS	27.05.99			213	14	93	10	95	10	95	29	86
	LC–MS–MS	27.05.99			208	14	93	11	95	8	96	29	86
LASs	FIA–MS	03.05.99–	101	98	1.900–3.120	5–13	99	7–16	99	4–15	99	65–115	96
		02.06.99	($n=4$)	($n=4$)	($n=23$)	($n=28$)	($n=23$)	($n=25$)	($n=21$)	($n=28$)	($n=18$)	($n=29$)	($n=23$)
	FIA–MS	07.05.99			2.990	13	>99	13	>99	11	>99	90	97
		16.05.99			2.250	10	>99	12	>99	6	>99	68	97
		27.05.99			1.980	8	>99	15	>99	15	>99	85	96
	FIA–MS–MS	07.05.99			2.830	10	>99	11	>99	11	>99	79	97
		27.05.99			1.820	7	>99	15	>99	13	>99	93	95

We had started to assess the efficiency of membrane-assisted WWT and compared it to conventional WWT by substance-specific analytical techniques, since we expected much more information from this procedure. As this information should also be obtained as quickly as possible, we had to apply screening methods before the more time-consuming identification of compounds could be performed [6]. In order to monitor the unpolar pollutants, we chose the GC–MS screening approach in the EI mode. This procedure provided both the total ion current traces (TIC) for screening and the EI spectra for identification. In a first step, the polar pollutants were determined by applying APCI or ESI in the FIA–MS

mode for screening purposes. Application of these soft ionizing interfaces for polar compounds resulted in overview spectra which could be used to monitor elimination in the different WWT processes by means of visual pattern recognition. If compounds were present in the effluent or permeates — i.e., hardly eliminable ones — we tried to identify the unpolar ones by means of library search with EI spectra provided by GC–MS screening. Prior to identification of the polar pollutants a second step, generating daughter ions by collision induced dissociation (CID) in the MS–MS mode, was essential. Then their identification by means of a search in our laboratory-made daughter ion library could take
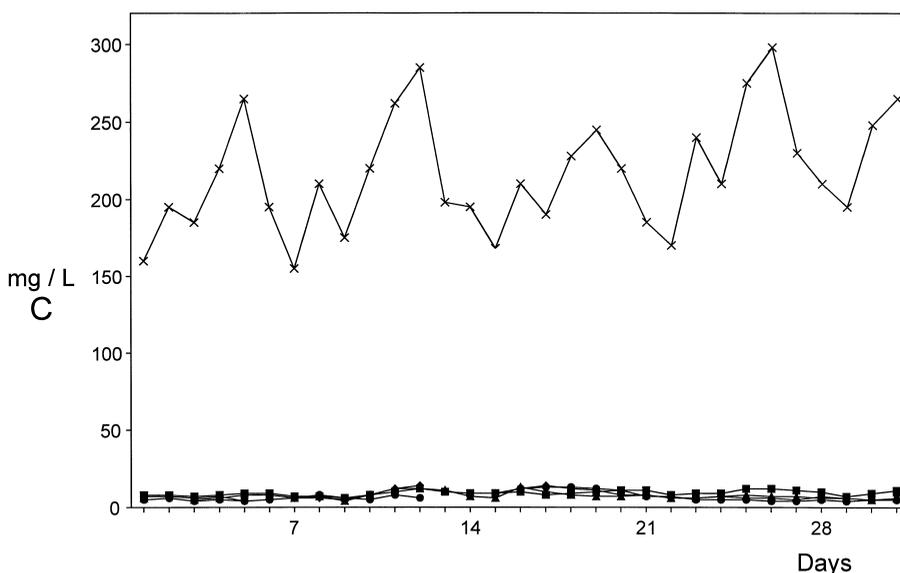
Fig. 1. Concentrations of dissolved organic carbon (DOC) in the different wastewaters examined: (×) inflow into the conventional and three membrane-assisted wastewater treatment plants (WWTPs), (■) effluent of conventional WWTP; effluents of membrane-assisted WWTPs: (▲) permeate 1, (♦) permeate 2 and (●) permeate 3.

place. In order to identify unpolar, volatile compounds by their EI spectra, a NIST library with 67 000 EI-MS spectra was available. The laboratory-made library, however, contained only about 1500 CID-MS–MS spectra of different polar organic pollutants of anthropogenic or biogenic origin.

In the GC–MS screening [6] approach, the TIC of the dichloromethane extracts of the pre-settling tank effluent was the 100% scale for judging the elimination rates, since this effluent was used as inflow for all membrane-assisted WWTPs as well as for the conventional WWTP. As the samples taken were time-proportional, visual comparison of the inflow TIC with the effluent TICs standardized in their signal intensities to the inflow TIC permitted assessment of the elimination efficiencies. This is demonstrated in Fig. 2 which shows the GC–MS TICs of the effluent extracts of conventional WWTP and of membrane-assisted WWTP 1 (Fig. 2b and c) and compares them to the inflow TIC (Fig. 2a). Such visual pattern recognition approach comparing the TICs of GC–MS analyses of inflow extract and effluent extracts of time-proportional samples gave evidence of a high elimination rate for unpolar

compounds with all these different treatment techniques.

Identification of volatile compounds by NIST library showed that unpolar pollutants in the inflow were, besides phenols, alkylaryl ethers and phthalic esters, predominantly fatty acids. The effluents of all treatment plants contained mainly phthalic esters as hardly eliminable pollutants. Some organic acids were eliminated to an extent even below the detection limit (LOD).

The elimination efficiency of the different treatment techniques for polar compounds contained in the wastewaters had been examined by FIA–MS in the positive and negative mode using the selective eluates [6,7] of the C_{18}-SPE cartridges. The combination of soft-ionizing APCI or ESI interface and the addition of an excess of ammonium acetate for ionization support so produced either molecular ions or ammonium adduct ions ($[M+H]^+$ or $[M+NH_4]^+$) in the positive mode. In parallel the generation of complex mixtures of adduct ions containing protons and other cations like sodium and potassium was avoided. Under negative ionization $[M-H]^-$ ions could be observed. The overview spectra obtained in
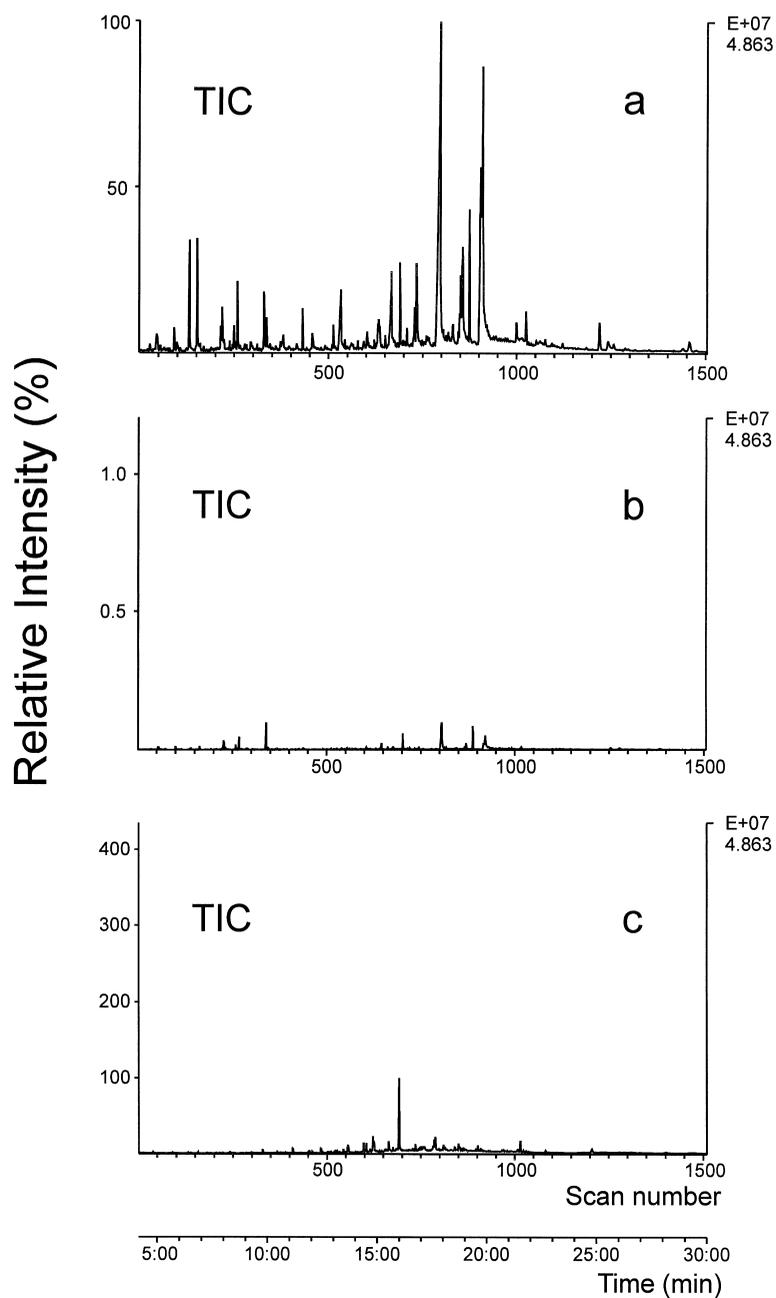
Fig. 2. (a) GC–EI-MS total-ion current tracing for wastewater sample of WWTP inflow. Standardized GC–EI-MS total-ion current tracings for wastewater sample (b) of effluent of conventional WWTP and (c) of effluent of membrane-assisted WWTP 1. Positive EI. For GC and MS conditions, see Section 2.

this way were used for qualitative check-up of the spectrum of pollutants in the eluates by visual comparison of the ion patterns. Since no fragment ions were generated under these conditions disturbing pattern recognition, comparison with the overview spectrum of the inflow (Fig. 3a) allowed to recognize those ions in the effluents which could not be eliminated by the different treatment methods (Fig. 3b–e). So dominant ions observed in the inflow (Fig. 3a) had disappeared whereas other ions with low intensity in the inflow now were dominating the overview spectra as shown in Fig. 3b–e. Applying qualitative pattern recognition the overview spectra in Fig. 3 proved that differences in the pollutant spectra could be observed when the effluent of conventional biological treatment was compared to the permeates of membrane-assisted biological treatment (cf. Fig. 3b with Fig. 3c–e). The membrane treated effluents (Fig. 3c–e), however, showed a good conformity in their pollutant spectrum.

The same overview spectra of the effluents, now normed in their intensities according to the inflow as 100% scale (Fig. 4a), allowed a semi-quantitative assessment about the elimination efficiency by visual pattern recognition as demonstrated with the Fig. 4b–e in comparison with Fig. 4a.

The overview spectra of the inflow (Fig. 5a) and a selected effluent sample (Fig. 5b) obtained by ESI in negative mode confirmed the presence of the hardly degradable LASs, anionic surfactants. This finding would also be possible using APCI, but if ESI is applied the sensitivity for the detection of LASs increased by the factor 10.

The visual semi-quantitative assessment of the elimination efficiency of pollutants as presented in the Fig. 4a–e could be improved for LASs, AEOs and NPEOs in the multiple ion detection (MID) mode resulting in real quantitative determinations (Table 4). To quantify LASs the characteristic mass traces m/z 311, 325 and 339 of LASs in the negative FIA–ESI-MS-MID mode were recorded. These FIA examinations with inflow and permeate samples for monitoring relevant polar pollutants had for result LAS concentrations of 3.120–1.900 μg l^{-1} in the feed. The effluent of the municipal WWTP contained 115–65 μg l^{-1}, whereas the more effective membrane-assisted biological wastewater treatment processes led to LAS concentrations of 4–16 μg l^{-1}. I.e., elimination efficiency reached about 97% in the

conventional treatment whereas membrane treatment even eliminated more than 99% (cf. Table 4). LAS pollution was diminished almost reaching LAS detection limit of the FIA–MS method applied to this matrix (Fig. 5b). 91–97% of the non-ionic surfactant NPEOs, precursors of the endocrine active nonylphenol (NP) were eliminated under membrane treatment with different types of membranes. The elimination rate for NPEOs in the conventional WWTP, however, reached a maximum of only 86% (cf. Table 4).

When these qualitative and semi-quantitative FIA–MS screening results were compared with the examinations by GC–MS screening, without any knowledge of the identity of the pollutants, it could be registered immediately that polar compounds in all different types of treatment plants were less successfully eliminated than unpolar pollutants. Compared to that of conventional WWT the elimination rates of membrane-assisted biological treatment for both types of pollutants, polar and unpolar, was higher (cf. Table 4).

Because of the information in the results of substance-specific determination contained there again arose the question which type of polar compounds could not be eliminated to the desired extent. The FIA–MS method for determination of polar compounds provided qualitative and semi-quantitative information, when the results were compared by means of visual pattern recognition and by use of standardized spectra (cf. Figs. 3 and 4). But this information has to be judged critically if identity estimations have to be done. In contrast to GC–MS screening, where the excellent separation efficiency of GC provided good resolutions of the signals in the TIC and therefore library search allowed quick identification, the results obtained by FIA–MS only gave some preliminary hints about the spectrum of pollutants. A problem arising out of this analytical approach was that when isomeric compounds with the same distinct molar mass, which were all present in the same wastewater sample, were ionized by low resolution FIA–MS, only one signal without any structural information was the result. It is therefore impossible to characterize any of these compounds. As a result the compounds in the inflow and all hardly eliminable pollutants, that could be detected in the effluents, remain unknown.

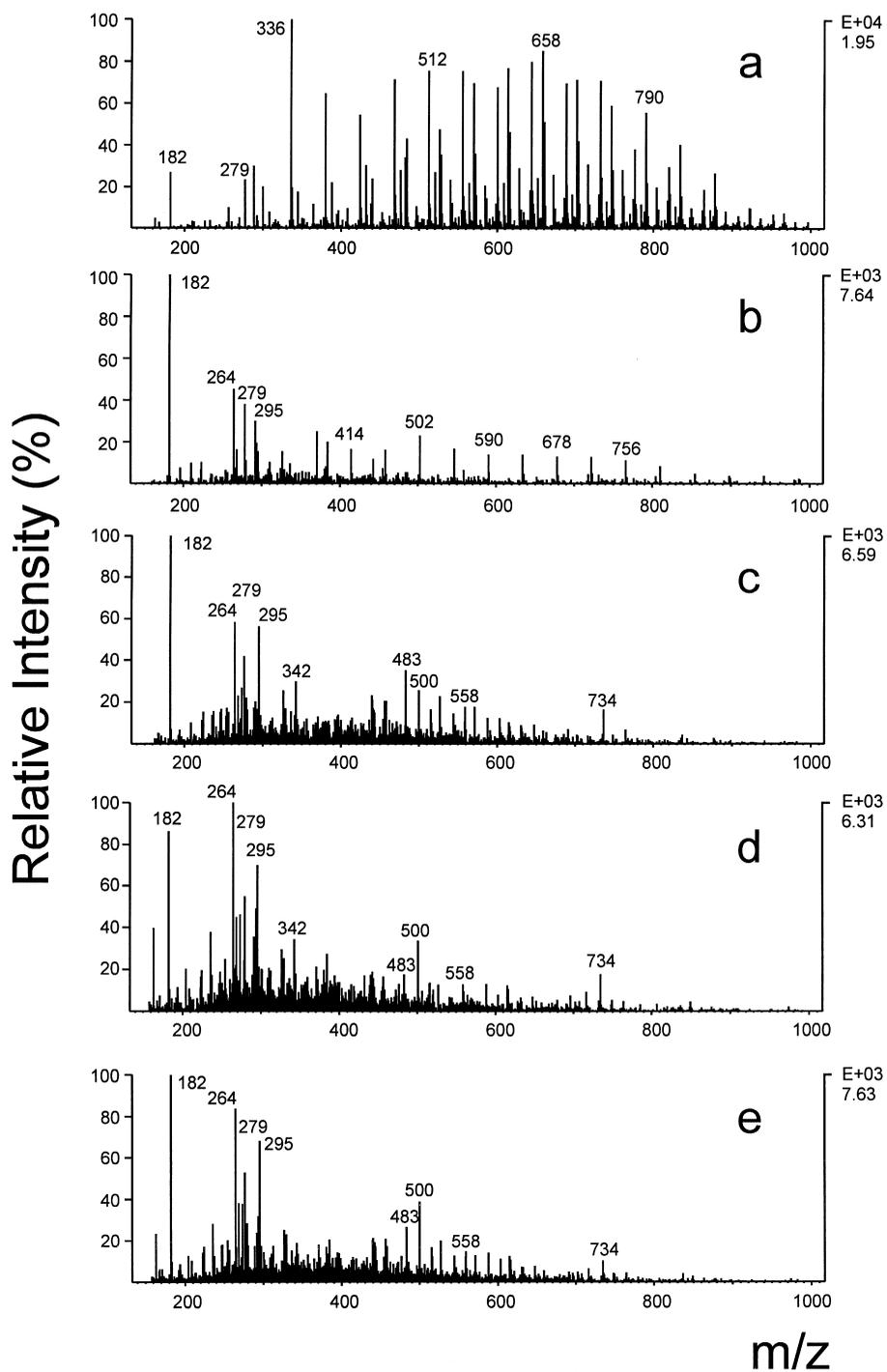In this case, the generation of daughter ions in the

Fig. 3. FIA–APCI-MS(+) loop injection spectra for wastewater samples. (a) WWTP inflow, (b) conventional WWTP effluent, (c–e) membrane-assisted WWTP permeates. C_{18} SPE; eluent, methanol. Positive APCI. For FIA and MS conditions, see Section 2.
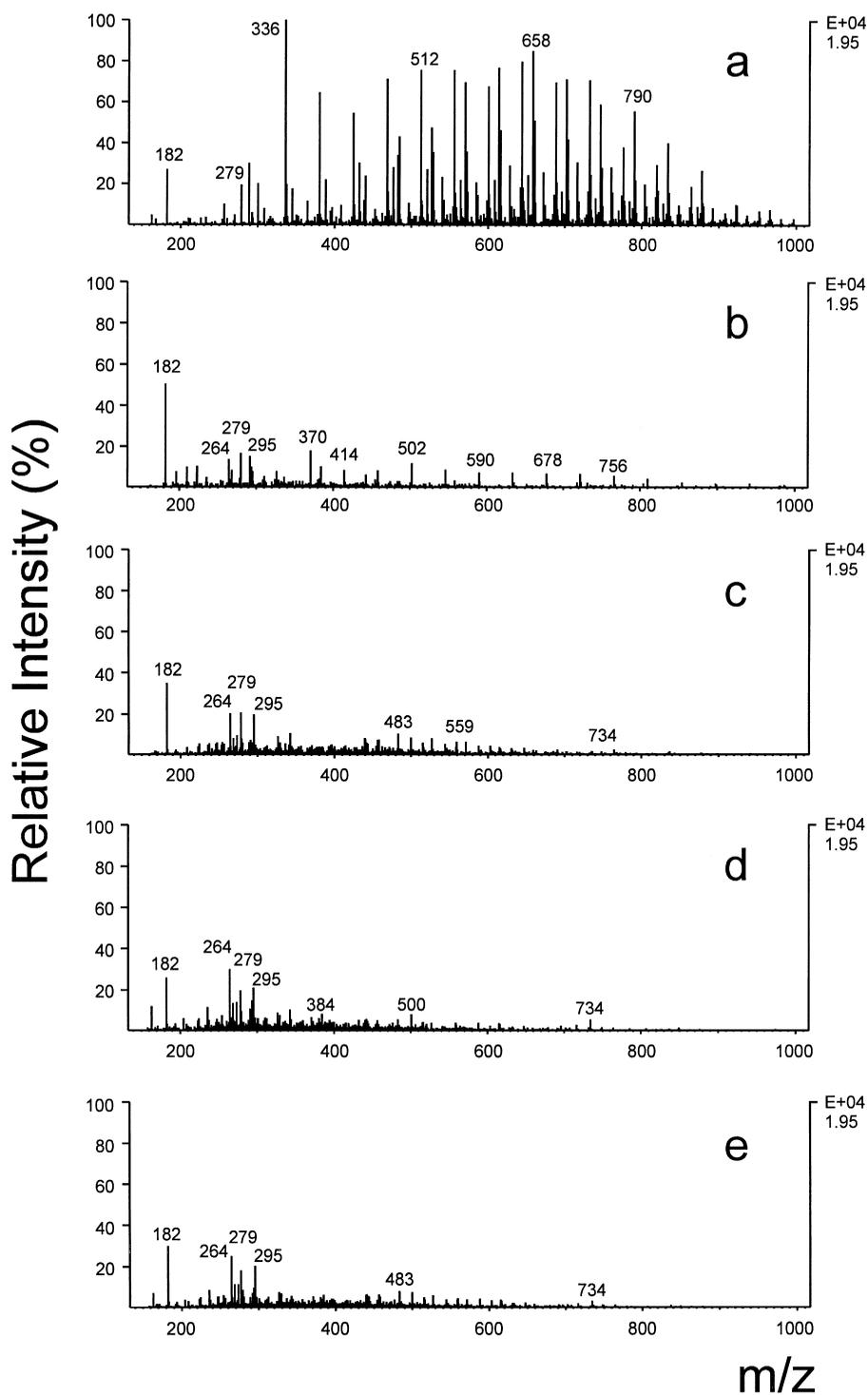
Fig. 4. FIA–APCI-MS(+) spectra for wastewater samples as in Fig. 3. (a) WWTP inflow (=100%), (b–e) standardized to concentration and dilution factors for elimination assessment by visual pattern recognition.
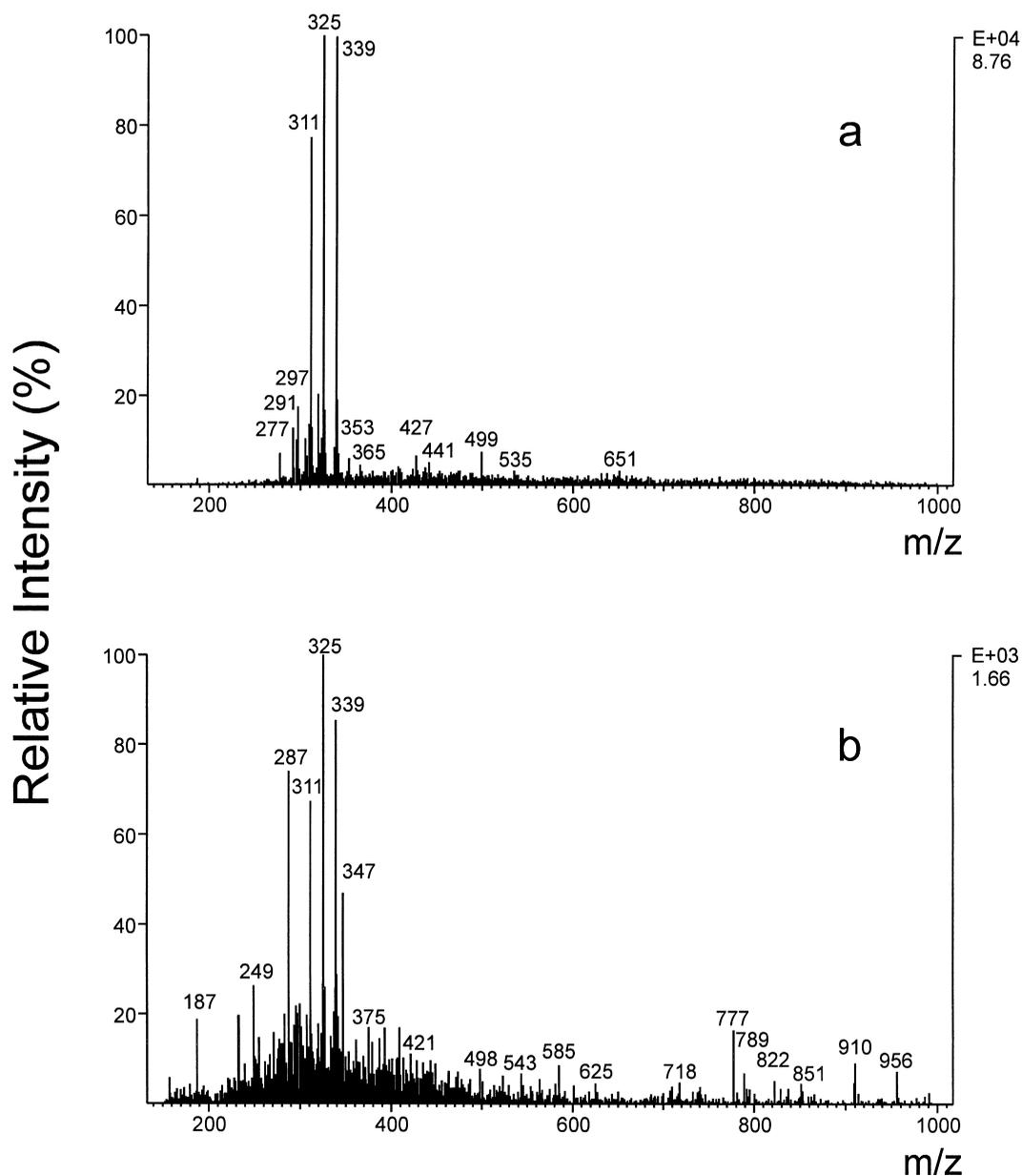
Fig. 5. FIA–ESI-MS(−) overview spectra for wastewater samples (a) WWTP inflow, (b) membrane-assisted WWTP effluent. C_{18} SPE; eluent, methanol. Negative ESI. For FIA and MS conditions, see Section 2.

FIA–MS–MS mode is essential and may help to identify these pollutants by means of their structure-characteristic fragment ions. Sequential selective elution with organic solvents of different polarities should be applied prior to FIA–MS–MS in order to improve the chance of identification [6,11]. A suc-

cessful fractionation during elution should minimize the generation of a mixture of daughter ions from isomeric compounds. A successful fractionation, with which mixed daughter ion spectra could be avoided, was achieved for the target LAS homo-logues, whereas the quantification of AEOs and

NPEOs was performed using mixtures of selected eluates. The reason why the whole number of homologues of AEOs and NPEOs were not eluted with only one eluent could be found in the dominating influence of the hydrophilic polyethylene glycol (PEG) chains, i.e., the number of PEG chains was determinative for the elution efficiency from C_{18}-SPE cartridges (cf. Fig. 11a–d and Table 3).

Nevertheless here the examinations by FIA–MS–MS for identification with ''mixture analysis'' [12] first of all were performed with APCI and ESI in positive or negative mode. With the help of the laboratory-made library, we could characterize several dominating ions in the inflow and effluents. Besides aliphatic (AEOs) and aromatic (NPEOs) non-ionic and anionic (LASs) surfactants, phthalic esters could be immediately identified by their characteristic patterns of daughter or parent ions. Obviously the content of pollutants in inflow and effluents were quite different. The inflow, e.g., contained anthropogenic compounds such as AEOs. The same compounds, though in reduced concentrations, were found in the effluents. Parallelly, however, polyethers like polyethylene glycol (PEG) as biochemical degradation products (metabolites) of these compounds could be observed.

The prerequisite for successful application of the FIA–MS–MS approach is, as mentioned above, a preceding sequential selective elution. Yet if this very quick screening procedure — consisting of an application of FIA–MS for a survey, followed by FIA–MS–MS for the identification — fails, the application of LC–MS analysis in order to separate the complex mixtures and LC–MS–MS for identification are essential to obtain reliable information. The verification of two doubtful results obtained by FIA–MS–MS is presented as example of the different analytical pathways that we applied.

In our examination in the effluents of the conventional and membrane-assisted treatment plants, parent ions at m/z 279 and 370, 414 and 458 were examined by means of FIA–MS–MS(+). Prior to this procedure, we had inferred from the FIA–MS spectra that the compound with the ion at m/z 279 had to be dibutyl phtalate, ubiquitious in the environment. The mixtures of homologue ions with equally spaced signals with Δ m/z 44 and ions at m/z 370, 414 and 458, however, could be part of a mixture of NPEO homologues.

The daughter-ion spectra we obtained by CID were complex but their resemblance to the CID-MS spectra of the pure compounds (dibutyl phtalate or NPEOs) was small. Daughter-ion spectra of these ions performed by FIA–MS–MS in the mixture analysis approach had, in both cases, resulted in a mixture of daughter ions, since the spectra had been generated from a mixture of isomeric compounds with identical m/z ratios.

This had already been observed in samples from the German Elbe river [13] where we could verify, besides dibutyl phtalate with the ion at m/z 279, the drinking-water-relevant triphenyl phosphine oxide of the same parent ion. The orders of magnitude of dibutyl phtalate and triphenylphosphine oxide in inflow and effluent samples — a high dibutyl phtalate concentration compared to only very small concentrations of triphenyl phosphine oxide — prevented a successful fractionation by sequential selective elution. Therefore we had to solve this problem with RP-C_{18} LC–MS and LC-MS–MS as reported in the literature [13].

The elucidation of the mixture of daughter ions that was found in the spectrum of the pretended NPEO homologues was, however, more difficult and therefore has to be described in further detail. Recurrently during our sampling campaign, a mixture of homologues with ions at m/z 370, 414 and 458 (Δ m/z 44) was observed in high concentration in the inflow (Fig. 6a). The same ions could be observed in reduced concentration in the effluents of the various treatment plants (cf. Figs 3b–3e). Yet it was only in the FIA–MS–MS(+) spectra of the inflow, that a mixture of daughter ions, obviously from differently structured isomeric parent compounds, could be observed (Fig. 6b). Additionally, the compounds with positive ions at m/z 370, 414, 458, etc. ionizabale in the FIA–MS mode in the inflow extracts, were also active in the negative mode. They resulted in ions at m/z 351, 395 and 439. At first we assumed that besides the NPEOs, an alkyl ethoxylate surfactant mixture with isomeric positive ions at m/z 370, 414, 458, etc. was simultaneously present in the inflow. But the possibility to ionize these compounds in the negative mode, too, let us discard this assumption.

In order to elucidate the phenomenon that the FIA–MS–MS(+) daughter ion spectrum of m/z 414 appeared to contain NPEO and non-NPEO daughters
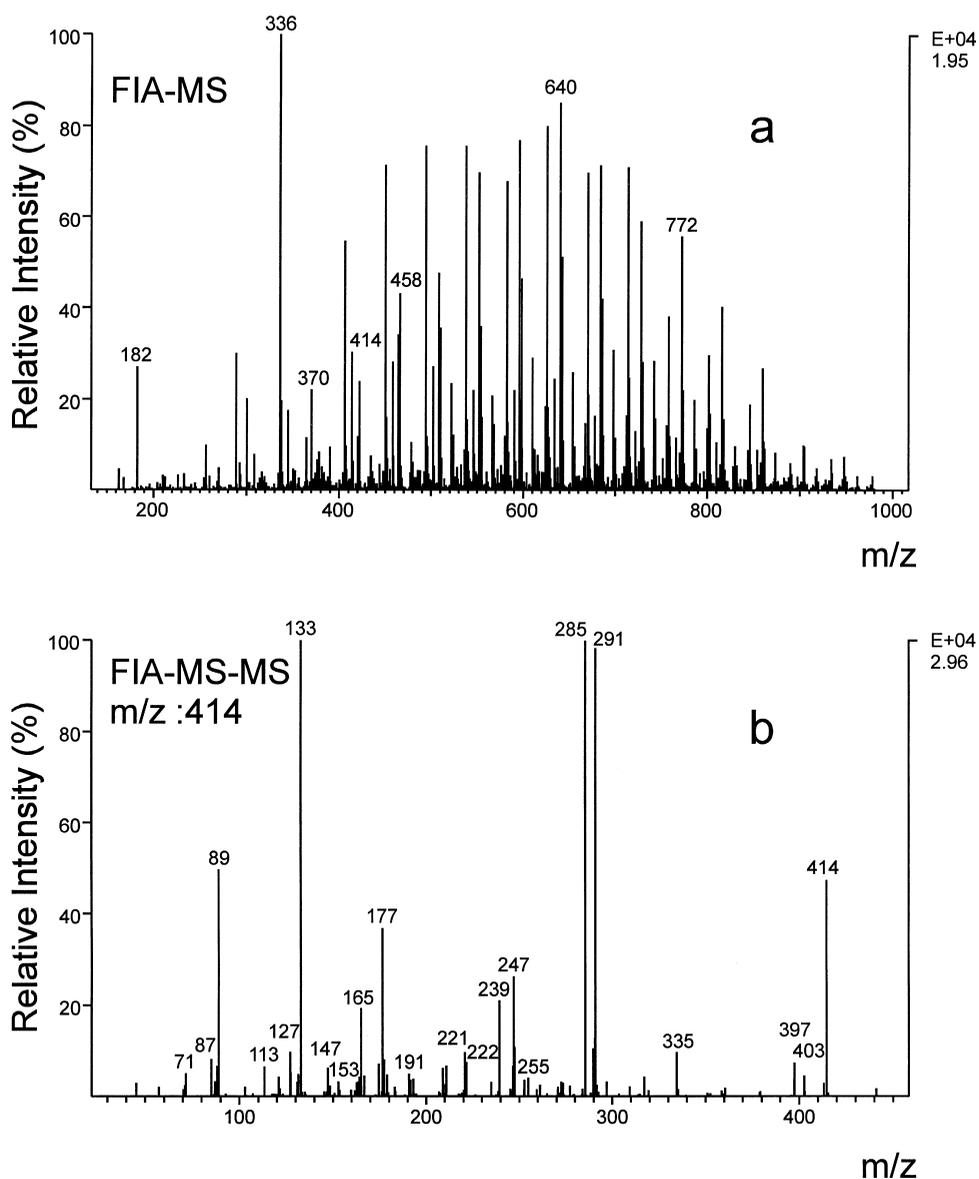
Fig. 6. (a) FIA–APCI-MS(+) overview spectrum for WWTP inflow and (b) FIA–APCI-MS–MS(+) daughter ion spectrum of selected ion m/z 414 as in (a). C_{18} SPE; eluent, ether. Positive APCI. For FIA, MS and MS–MS conditions, see Section 2.

(Fig. 6b), we chose the more tiresome way of LC–MS(+) (Fig. 7a). But RP-C_{18} separation of the inflow extract on an analytical column failed. Under the LC conditions that had been applied, the isomeric compounds had comparable retention times, and because of the identical m/z ratios of the isomeric ions, mass trace analysis was not possible (Fig. 7c). We therefore applied LC–MS–MS in the parent ion mode, generating the parents of m/z 291, characteris-

tic for the NPEOs, in the first run (Fig. 7d). As parents the NPEO ions at m/z 370, 414 and 458 were found (cf. inset in Fig. 7d).

From the CID-MS spectrum in Fig. 6b the dominant daughter ion of the non-NPEO compounds with m/z 285 was selected as diagnostic ion. It could not be observed in NPEO daughter ion spectra. Then in the second separation parent ions of this diagnostic ion with m/z 285 were generated (Fig. 7e). This
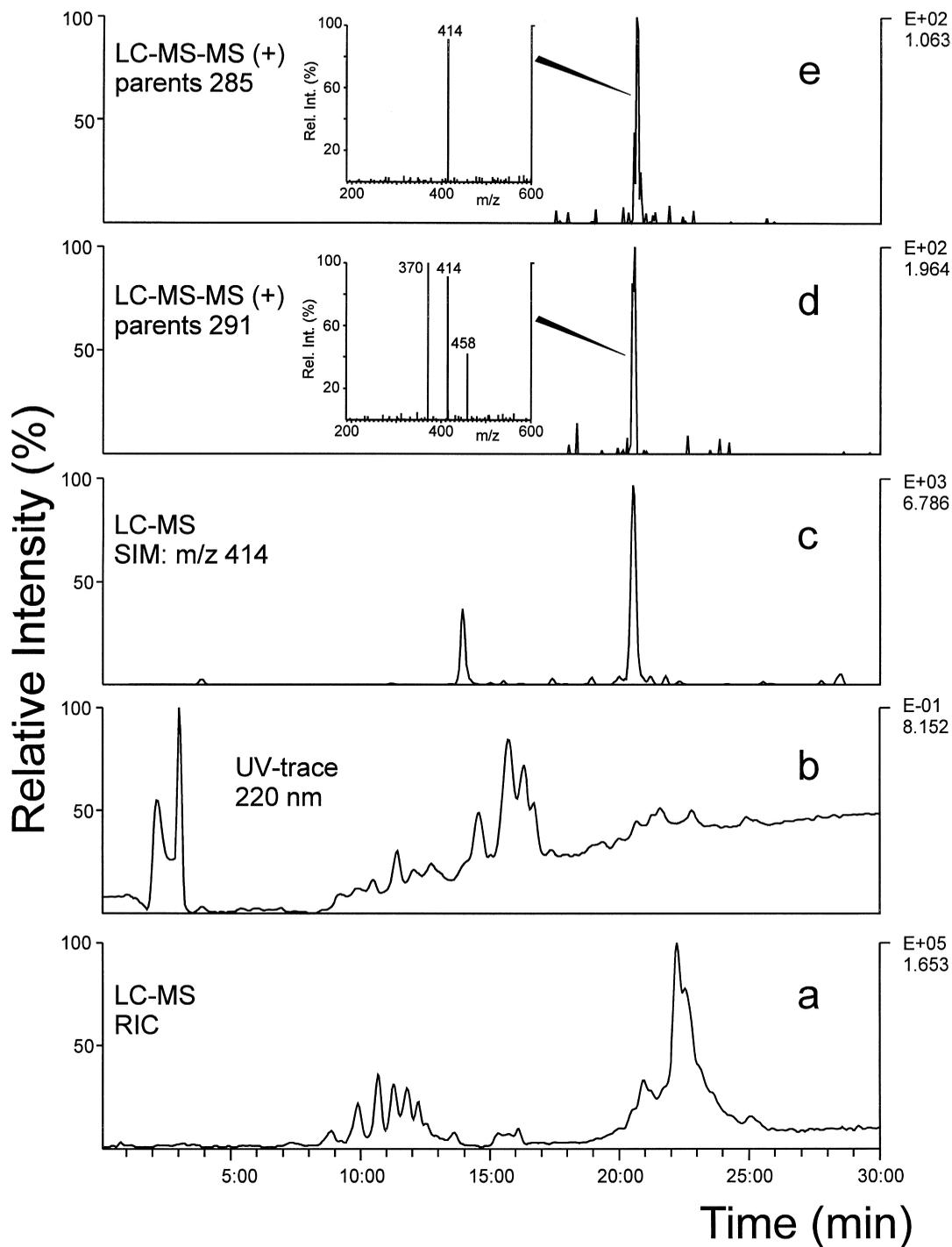
Fig. 7. (a) LC–APCI-MS(+) total-ion current tracing for waste water sample of WWTP inflow as in Fig. 6a. (b) UV trace (220 nm) of (a). (c) LC–MS(+)-SIM mass traces *m/z* 414. (d–e) LC–APCI–MS–MS(+) parent-ion mass traces of daughter-ions (d) *m/z* 291 and *m/z* 285 *as* in (a). For LC and MS–MS conditions, see Section 2.

brought about just one parent ion at m/z 414 (inset in Fig. 7e). Both TICs of these two LC–MS–MS separations in the parent ion mode confirmed our assumption that two isomeric compounds had been present in the inflow. The LC–APCI-MS examinations with the ion-pairing reagent ammonium acetate performed in the positive (Fig. 8a) and negative (Fig. 8b) mode resulted in a favourable separation. Under these conditions, the unknown compounds which could be ionized in the positive as well as negative mode were eluted very rapidly, within 11 min. They could be characterized in the inset of Fig. 8b by their equally spaced negative ions (Δ m/z 44), differing by Δ m/z -19 ($-[NH_4]^+, -[H]^+$) from the positive NPEO ion masses. LC–MS analysis in the positive mode under ion-pairing conditions resulted in a delayed elution of the NPEO homologues when compared to the separation under non-ion-pairing conditions [14]. This separation permitted interpretation of the UV trace 220 nm (Fig. 8c) and UV spectra in the Fig. 8d and e, which were recorded by diode array detection. The situation clarified with the information that both types of compounds, the unknown one as well as the NPEOs, were UV-active and had nearly identical UV spectra. The fact that the non-NPEO compound could be ionized in both positive and negative mode with comparable efficiency, and the circumstance that fragments with Δ m/z 60 have been generated led to the assumption that the unknown compound had to be an alkylphenol ether carboxylate (APEC) with the general formula $C_nH_{2n+1}-C_6H_4-O-(CH_2-CH_2-O)_m-CH_2-COOH$ ($n=8$; $m=1-5$; m/z ($+$) 326–502 or m/z ($-$) 307–483). In order to obtain an ultimate confirmation of this assumption, neutral loss scans of 60 were performed with the SPE eluate of the inflow in the negative LC–ESI-MS–MS mode (Fig. 9). The negative ions with m/z 307, 351, 395, 439 and 483 (Fig. 9, inset) which could be observed turned out to be ions which can loose acetic acid (CH_3-COOH) under neutral loss conditions whenever LC–MS–MS is applied.

FIA–MS–MS examinations of the effluents via the NL 60 scan confirmed that the APEC compounds had been eliminated by biodegradation in all WWTPs. NPEOs, however, were still present in all effluent extracts and could be verified by mass trace analysis in full scan LC–MS mode (cf. Fig. 10).

The results of recovery experiments of target compounds in Table 3 had proved that AEOs, NPEOs and LASs were found in several or one sequential eluate of SPE cartridges, respectively. Fig. 11a–d present the FIA–MS spectra of AEO homologues contained in the different sequential selective eluates of C_{18}-SPE cartridges. According to their polarities, determined by the PEG chains, mixtures of homologues were eluted. With the exception of the eluent mixture methanol–water, the other eluents applied contained the short (eluent: hexane–diethyl ether), medium (eluent: diethyl ether) or long chain (eluent: methanol) AEOs.

The overall recoveries summed up from the recoveries of the target compounds in the fractions, which were determined by FIA–MS, FIA–MS–MS and LC–MS, were excellent. Even the quantification of the target pollutants by FIA–MS the mixture of eluates of the spiked WWTP effluent provided good results (cf. Table 3).

In the membrane-assisted treatment plants, the application of FIA–MS, LC–MS and –MS–MS for quantitative calculations of the elimination rates of all relevant pollutants brought about elimination rates which were more effective than those which occurred within the conventional advanced treatment process. Besides the results of systematical screening by FIA–MS, concentrations of polar pollutants were also determined in selected sample series, applying all techniques, to compare the results of various MS quantification techniques such as LC–MS, FIA and LC–MS–MS to FIA–MS (cf. Table 4). The results of the MS quantification techniques we applied may differ widely, if one is to use unspecific method FIA–MS instead of the highly specific and selective MS–MS techniques.

4. Conclusions

The high elimination potential of membrane-assisted WWT as used for municipal and industrial purposes was already known from sum parameter analytical results. Up to now, however, no examinations which characterize the non-eliminable pollutants and permit their identification existed. With our extensive examinations by sum parameter analysis as well as substance-specific analysis, the information
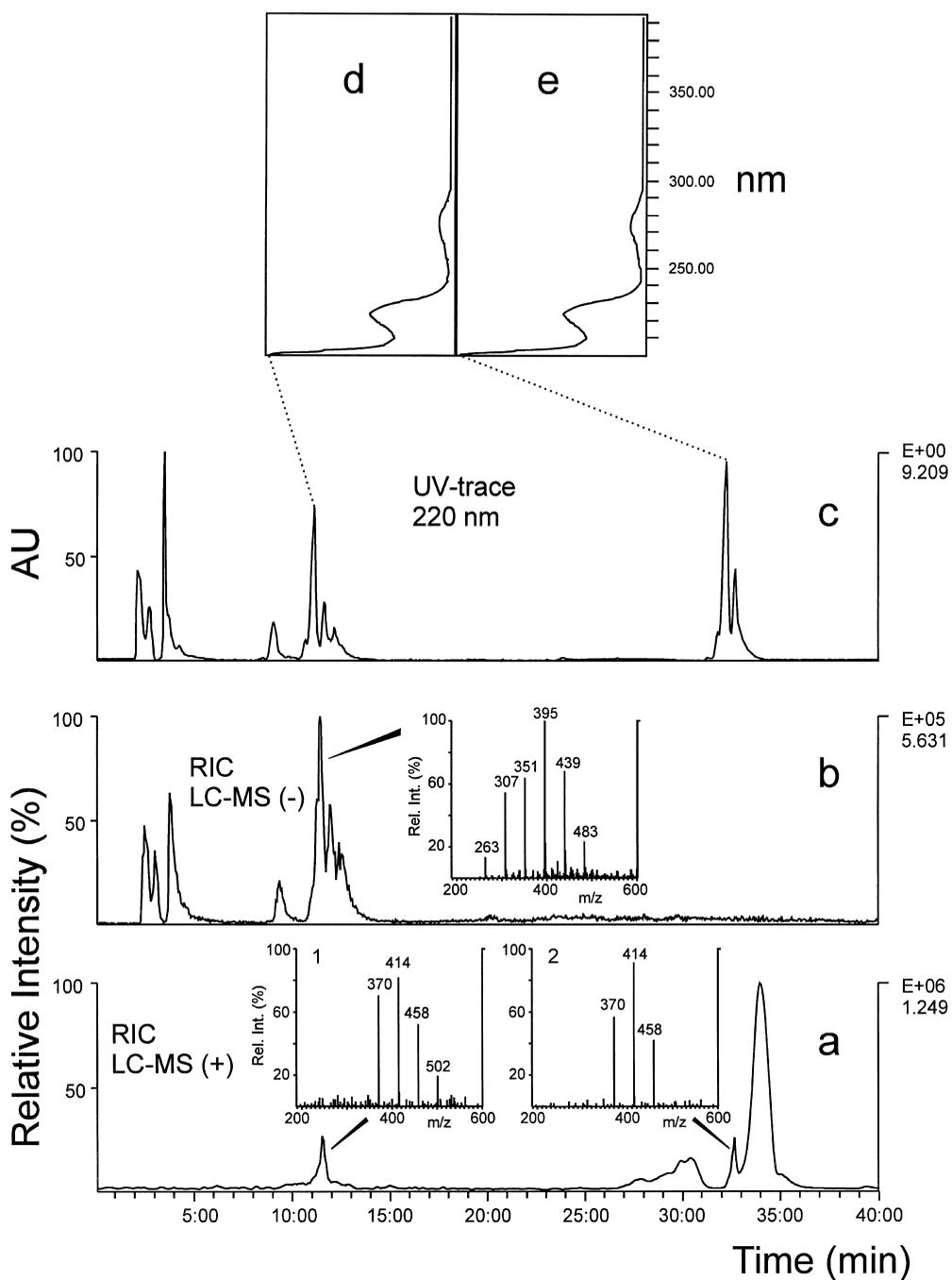
Fig. 8. (a) LC–APCI-MS(+) total-ion current tracing for wastewater sample of WWTP inflow as in Fig. 6a under ion-pairing separation conditions. Ions of LC separated isomeric compounds are contained in mass spectra in insets 1 and 2 in (a). (b) LC–APCI-MS(−) total-ion current tracing for wastewater sample as in (a) under negative ionization. Inset in (b): mass spectrum of negative ionizable compounds as in inset 1 of (a). (c) UV trace (220 nm) of (a) and (b). (d) and (e) UV-diode array spectra of isomeric compounds separated by LC under ion-pairing conditions. For LC, UV and MS conditions, see Section 2.
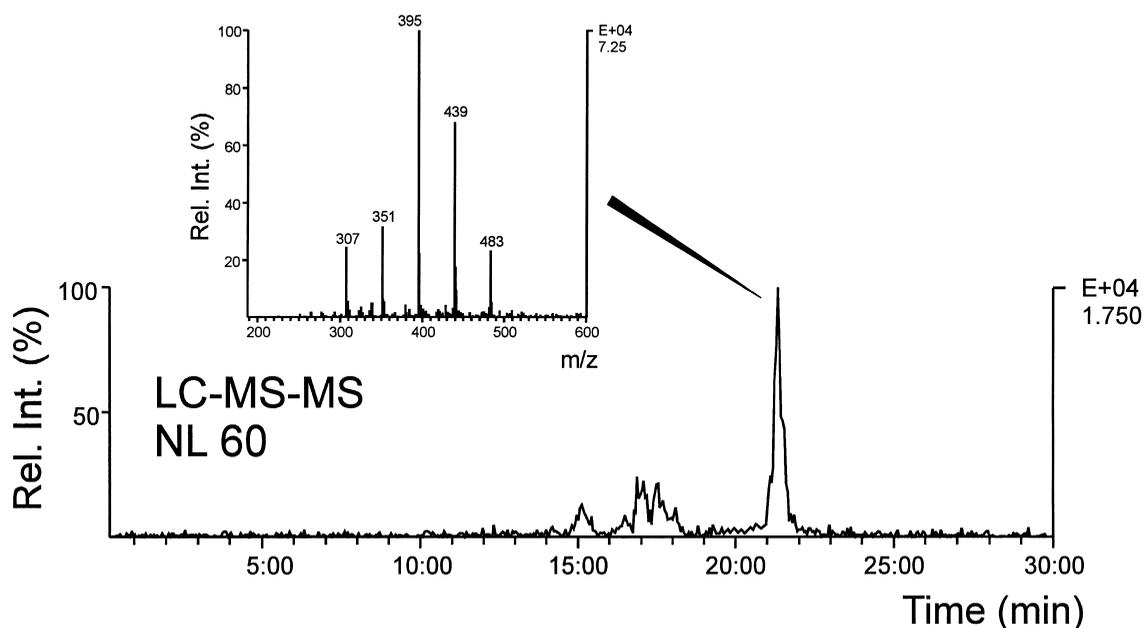
Fig. 9. LC–APCI-MS–MS(−) NL 60-tracing for wastewater sample of WWTP inflow as in Fig. 7a. Inset: mass spectrum shows ions NL 60-active (characteristic for the loss of acetic acid).

about the elimination efficiency of conventional and membrane-assisted wastewater treatment could be obtained. Further research showed a favourable elimination rate in all systems, and the substance-specific determination provided information about non-eliminable compounds. These findings should be used for the development of modified methods with improved elimination rates.

The application of the substance-specific analytical approach proved that semi-quantitative estimations are possible by means of visual pattern recognition provided by the TICs of GC–MS analysis and FIA–MS overview spectra. Identification of unpolar compounds by their EI-MS spectra is easy with the help of NIST library. To identify polar compounds, daughter ions generated by CID with FIA–MS–MS are essential. Only if complex mixtures are discovered in FIA–MS overview spectra, owing to the fact that selective elution after SPE, combined with FIA–MS, has failed, the analytical approach to reliable identification must be broadened in accordance with the objectives desired. Sometimes LC–MS may solve the problems. If all these methods fail, the various time-consuming procedures such as parent

ion scan and neutral loss (NL) in combination with LC–MS–MS have to be applied.

The results of all our analytical examinations confirmed quite favourable elimination rates. Yet membrane-assisted biological wastewater treatment eliminates pollutants still more efficiently. However, no differences became observable, when we applied microfiltration or ultrafiltration with MWCO (molecular mass cut-off) values of 200 000.

Non-eliminable pollutants were phthalates observable by GC–MS and FIA–MS and identifiable by GC–EI-MS, FIA–MS–MS as well as by LC–MS–MS in the effluents. But predominantly polar, involatile polyethers and their precursors, the aryl- and alkylpolyglycol ethers, were observed under positive ionization in the effluents. In the negative ESI mode, the hardly degradable LASs could be verified in all effluents.

If complex mixtures disturb FIA–MS–MS, more powerful analytical approaches, such as LC–MS–MS in the daughter and parent ion mode or in the neutral loss mode, have to be applied. So the information that NPEOs, precursors of the metabolite nonylphenol with endocrine disruptor activity, had
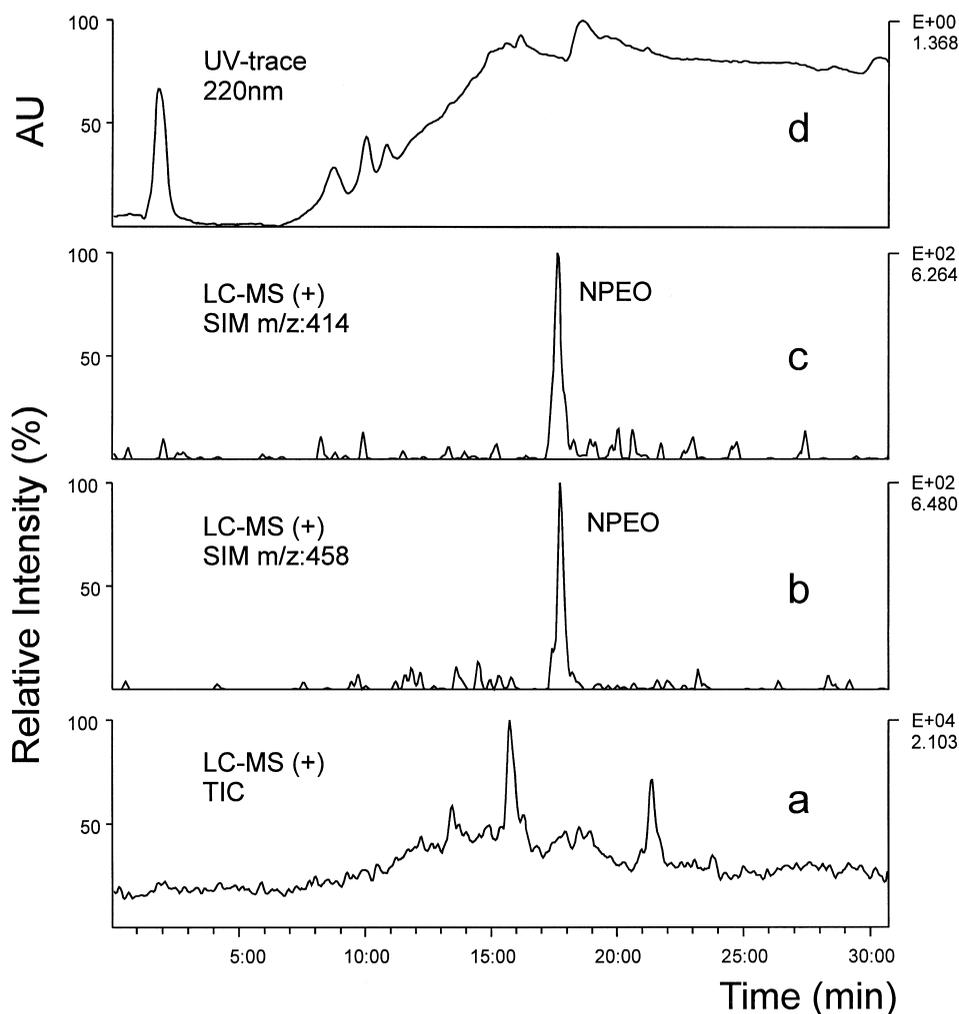
Fig. 10. (a) LC–APCI-MS total-ion current tracing for wastewater sample of conventional WWTP effluent. LC–APCI-MS mass traces (b) m/z 458 and (c) m/z 414 of wastewater extract in (a). (d) UV trace (220 nm) of (a). C_{18} SPE; eluent, ether. Positive APCI ionization. For LC, UV and MS conditions, see Section 2.

been present in the inflow as well as in the effluents, was indeed provided by FIA–MS–MS and LC–MS. Knowledge about the presence of APECs in the inflow could only be gained by means of different MS–MS techniques. We applied LC–MS–MS in the daughter and parent ion mode and for the ultimate confirmation of APECs we had to record the neutral loss scan NL 60.

Quantification of target AEO, NPEO and LAS compounds showed quite good results. A comparison of the different MS-quantification techniques proved that the concentrations determined by FIA–MS were slightly higher than those recorded by FIA–MS–MS, LC–MS or –MS–MS. The reason for these incorrect positive results can be estimated in the interferences of matrix compounds with the pollutants to be determined. This especially could be observed in the FIA–MS determination of the AEO compounds whereas the negative FIA–MS quantification of LASs could be estimated as very selective and quite reliable (cf. Table 4). The highest specificity was gained under FIA–MS–MS and LC–MS–MS conditions when we quantified the pollutants in the selected reaction monitoring (SRM) mode.
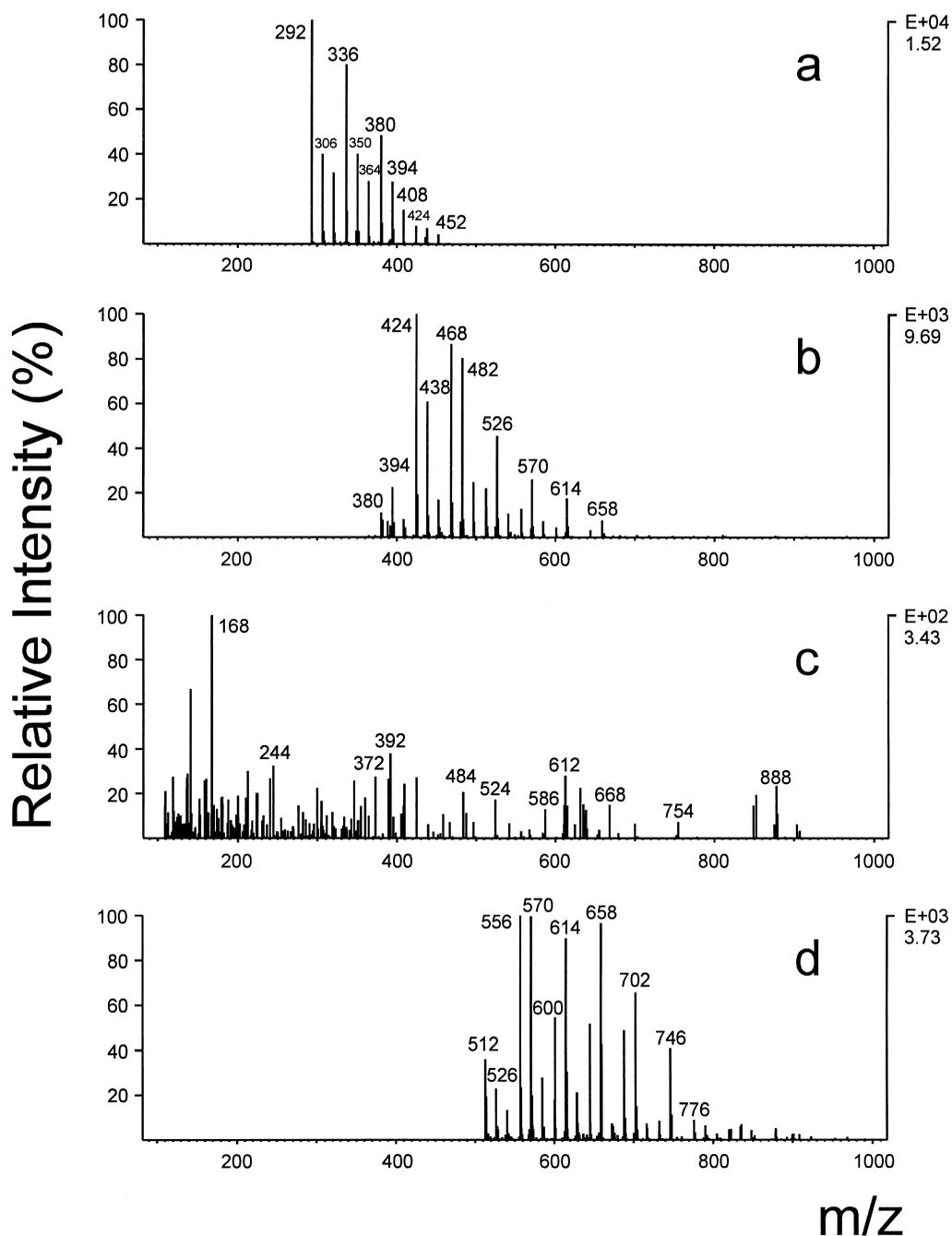
Fig. 11. FIA–APCI-MS(+) loop injection spectra for sequentially eluted WWTP effluent sample spiked with standard mixture of C_{12}, C_{13} and C_{14} AEOs. Concentration by C_{18} SPE, elution conditions: (a) eluent, hexane–diethyl ether, (b) eluent, diethyl ether, (c) eluent, methanol–water (d) eluent, methanol. Positive APCI. For FIA and MS conditions, see Section 2.

Compared to conventional WWT membrane-assisted biological WWT with biomass concentrations of about 20 g l^{-1} could only improve the elimination efficiency of pollutants but could not stop entirely the discharge of mainly polar pollutants with the permeates. Therefore the treatment process for elimination of hardly degradable polar pollutants which are of environmental relevance, i.e. toxic or mutagenic, can be optimized only by a modification of the membranes and/or by a modification of the treatment process. The variation of materials and/or the reduction of molecular mass cut-off limits and/or the inoculation of the special microorganisms may help to overcome these problems, which present a great challenge for chemists, biologists and engineers. The substance specific analytical methods that have been presented proved to be the most powerful tool for monitor the development of various WWT processes and for assessing any intended optimization. Analytical approaches using unspecific analytical methods like COD, DOC or biochemical oxygen demand (BOD) to assess elimination efficiency of problematic pollutants, can not solve problems arising from toxic or mutagenic polar pollutants in the WWT process.

References

[1] H.Fr. Schröder, J. Chromatogr. 554 (1991) 251.
[2] H.Fr. Schröder, Wat. Sci. Tech. 25 (1992) 241.
[3] T.A. Ternes, Wat. Res. 32 (1998) 3245.
[4] R. Rautenbach, K. Vossenkaul, in: Rautenbach, Melin and Dohmann, Proceedings of Possibilities and perspectives of the membrane technique in the municipal waste water treatment and drinking water treatment, 1. Aachener Tagung Siedlungswasserwirtschaft und Verfahrens-Technik, Aachen, 1997, p. A1.
[5] L. Vera, R. Villarroel, S. Delgado, S. Elmaleh, Proceedings of Water Quality International 1998, IAWQ 19th Biennial International Conference, Vancouver, Conference Preprint Book 4, p. 152.
[6] H.Fr. Schröder, Waste Manage. 19 (1999) 111.
[7] H.Fr. Schröder, J. Chromatogr. 647 (1993) 219.
[8] ASTM, Standard Methods for the Examination of Water and Wastewater, 5–10, 5220 C (Chemical Oxygen Demand, COD), American Public Health Association, Washington, DC, 17th ed., 1989.
[9] R.B. Geerdink, P.G.M. Kienhuis, U.A.Th. Brinkman, J. Chromatogr. 647 (1993) 329.
[10] H.Fr. Schröder, Wasser 81 (1993) 299.
[11] H.Fr. Schröder, Water Sci. Tech. 38 (1998) 151.
[12] D.F. Hunt, J. Shabanowitz, T.M. Harvy, M. Coates, Anal. Chem. 57 (1985) 525.
[13] H.Fr. Schröder, J. Chromatogr. A 777 (1997) 127.
[14] H.Fr. Schröder, F. Ventura, in: D. Barceló (Ed.), Sample Handling and Trace Analysis of Pollutants, Elsevier, Amsterdam, 2000, p. 823.