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Journal of Chromatography A, 889 (2000) 245–252

JOURNAL OF CHROMATOGRAPHY A

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Unknown bisethylisooctanollactone isomers in industrial waste water
Isolation, identification and occurrence in surface water

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Abstract

Unknown bisethylisooctanollactone isomers (BIOL isomers) which are chemical by-products of butyraldehyde synthesis, were isolated from industrial waste water applying various purification methods with subsequent semi-preparative high-performance liquid chromatography. Through interpretation of mass spectra after gas chromatographic separation the individual BIOL isomers were identified as stereoisomers of 2,4-diethyl-3-*n*-propyl-δ-valerolactone. Thus, it was possible for the first time to quantify the BIOL isomers in the river Rhine, Germany, with a mean sum concentration of 1.6 μg l^{-1}. A regular analysis performed over a period of almost two years of the river Rhine always gave the same ratio among the individual isomers. Drinking water production out of such water was studied, revealing that activated carbon filtration led to a 95% reduction of the BIOL concentration. Additional subsoil passage and a subsequent slowsand filtration led to a total elimination due to microbial degradation. Even if the BIOL isomers proved not to be relevant to drinking water, their behavior in the aquatic environment needs to be more thoroughly investigated since these compounds have been discharged for many years in high amounts into the river Rhine.

Keywords: Water analysis; Environmental analysis; Bisethylisooctanollactones; Diethylpropylvalerolactone; Lactones

1. Introduction

In the GC–MS chromatograms of an industrial waste water effluent, bisethylisooctanollactone isomers (BIOL isomers) were detected regularly as a characteristic peak cluster [1]. The mass spectra of these compounds were quite similar, showing significant fragments at *m*/*z* 127 and 170. The mass spectra were unknown and could not be found in the mass spectral databases or in chemical literature. The molecular ion was confirmed to be *m*/*z* 198 applying chemical ionization methods.

According to the manufacturer, these compounds are by-products from the synthesis of butyraldehyde, formed by aldol reaction [2]. As shown in Fig. 1, the first step in the reaction is the formation of 2-ethyl-2-hexenal. As described in previous literature [3,4] the base-catalyzed self-condensation of 2-ethyl-2-hexenal with butyraldehyde leads to the formation of 2,4-diethyl-3-*n*-propyl-δ-valerolactone. As can be seen in Fig. 1, theoretically, there are four possible configuration isomers of 2,4-diethyl-3-*n*-propyl-δ-valerolactone, differing in the axial and equatorial positions of the alkyl groups. However, the structural assignment and the stereochemistry of these BIOL isomers are only tentative. Since there was no

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0021-9673/00/$ – see front matter
PII: S0021-9673(00)00399-X

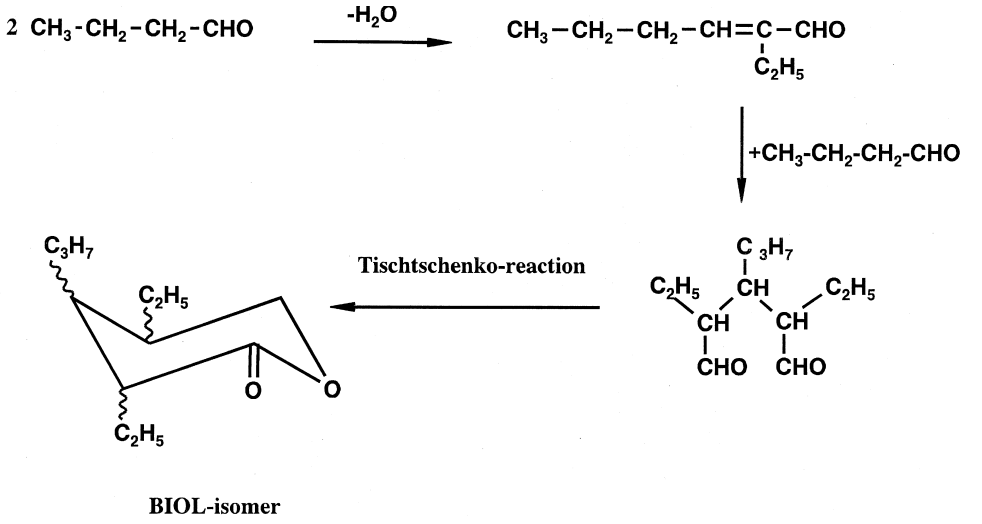

Fig. 1. Potential formation of the stereo isomers of 2,4-diethyl-3-*n*-propyl-δ-valerolactone (BIOL isomers) via self-condensation of butyraldehyde.

reference material available, quantitative determinations were not possible. Using single ion monitoring (SIM) of the characteristic fragments, the BIOL isomers were also detected in the river Rhine, Germany, which is the receiving stream of the industrial waste effluent of the manufacturing company [1]. Due to their presumably poor biodegradability, the BIOL isomers were also detected in bank-filtered waters [5].

To study the detailed behavior and fate of such compounds and to quantify the BIOL isomers in a monitoring program, reference compounds are necessary. If they are not commercially available, they have to be synthesized or isolated from sewage water. We preferred to do the latter since there were four isomers of which the stereochemistry was not known. A further advantage of isolation from sewage water was also the possibility of getting some information on the sum concentration of the four individual but chemically quite similar compounds.

2. Experimental

2.1. Extraction and purification from sewage water

Industrial sewage water (100 ml) and 35 g of sodium chloride were extracted with 5 ml methyl *tert*.-butyl ether (MTBE) in a liquid–liquid-extractor at 1200–1300 U min^{-1} for 20–30 min [6]. After cooling to 5°C for 45–60 min, the organic phase was removed and the water phase was discarded. During concentration of the MTBE phase under nitrogen gas, a brownish oily phase separated from the MTBE phase. The latter was removed and the oily phase was combined with the other oily phases obtained altogether from seven extraction procedures of 100 ml each. The enrichment of the BIOL isomers was followed up by GC–MS analysis.

2.1.1. Analytical HPLC

For a further clean-up, the obtained oily phase was dissolved in methanol–Milli-Q water (20:80, v/v) and diluted in the mobile HPLC-phase, which consisted of acetonitrile–Milli-Q water (60:40, v/v). The separation parameters were optimized on an analytical HPLC (Hewlett-Packard 1090) system equipped with a diode array detection (DAD) system run at a wavelength of 220 nm. A Chromasil RP C_{18} (5 μm) column (250×2.1 mm I.D.) was used at room temperature (25°C). The flow was isocratic at a rate of 0.2 ml min^{-1} while the volume of the injection was 10 μl.

2.1.2. Semi-preparative HPLC

After optimization of the separation parameters,

100 μl of the BIOL containing mixture were further cleaned-up by collecting the fraction between 6 and 7 min on a semi-preparative HPLC (Bischoff Lambda 1010) equipped with a ^{2}H lamp run at a wavelength of 220 nm. The separation was performed on a Chromasil 100 RP C_{18} (5 μm) column (250×8 mm I.D.) under the conditions worked out with the analytical HPLC.

The acetonitrile of the collected and pooled fractions was removed by evaporating at 40°C and 300 mbar in a rotary evaporator. The remaining extract was frozen at −23°C in a round-bottom flask, lyophilized for 12 h, dissolved in acetone, transferred into a 10 ml vial, evaporated under nitrogen gas to dryness and weighed out. The identity and purity of the isolated BIOL isomers were proven by HPLC-DAD, GC–MS and NMR analyses.

2.2. Solid-phase extraction (SPE) for river Rhine and drinking water samples

The extraction was followed in a SPE workstation (Autotrace, Tekmar). A 1-l sample (pH 7) was passed through glass cartridges filled with 0.1 g LiChrolut EN (Merck) and 0.25 g Isolute C18ec (IST) at 20 ml min^{-1}. The cartridges were conditioned with *n*-hexane, methanol and ground water. After drying the adsorbent material under a gentle stream of nitrogen gas for 60 min, the enriched BIOL isomers were eluted with 3 ml acetone. The extracts were concentrated under nitrogen gas to 1 ml and then 300 ng l^{-1} internal standard fluazifop-butyl was added.

2.3. Calibration

A five-point calibration curve was used in ground water analysis in the range of 0.1–3.0 μg l^{-1}. For the measurements the ions m/z 127 for BIOL isomers and m/z 282 for the internal standard fluazifop-butyl were used for the calibration.

2.4. Samples

River Rhine water and processed drinking water samples were filtered through a glass fiber filter (0.45 μm) and 1 l samples were extracted following the previously described conditions for extraction.

2.5. GC–MS conditions

Analysis was done by ion trap GC–MS (Varian) consisting of an autosampler AS 8100, a gas chromatograph Varian 3400 and a detector Saturn II. A 30 m DB 17 (J&W) column (film thickness 0.15 μm, 0.25 mm I.D.) was used. Helium was the carrier gas. Injections (2 μl) were made in the splitless mode at 50°C injector temperature, which was held for 0.2 min, followed by a 100°C min^{-1} ramp to 300°C, held for 70 min The initial temperature of the column was 50°C which was held for 2 min, followed by a 10°C min^{-1} ramp to 120°C, a 6°C min^{-1} ramp to 235°C which was held for 2 min and finally a 30°C min^{-1} ramp to 290°C held for 10 min. The ion source of the mass spectrometer was held at 250°C.

3. Results and discussion

3.1. Isolation of the BIOL isomers

After extraction and a first clean-up step by liquid–liquid separation, an enrichment factor of about 20 was achieved leaving the pattern of the BIOL isomers unchanged. Thereby, GC–MS run in the scan mode proved to be a perfect tool for monitoring the progress of the purification.

The extracts obtained were further purified by HPLC and monitored by UV detection at a wavelength of 220 nm, which is the maximum absorbance of the BIOL isomers (UV spectrum not shown). The four isomers could not be separated and were always eluted as one peak with a retention time of 6.5 min. Variation of the mobile HPLC phase in terms of running different acetonitrile–Milli-Q water gradients as well as testing out different HPLC columns did not achieve a better peak separation. It was only possible to cut off the HPLC signal of the co-eluting BIOL isomers from other impurities still being present in the mixture.

The GC–MS chromatograms before and after semi-preparative HPLC separation are compared in the Fig. 2a and b. It can be clearly seen that despite a

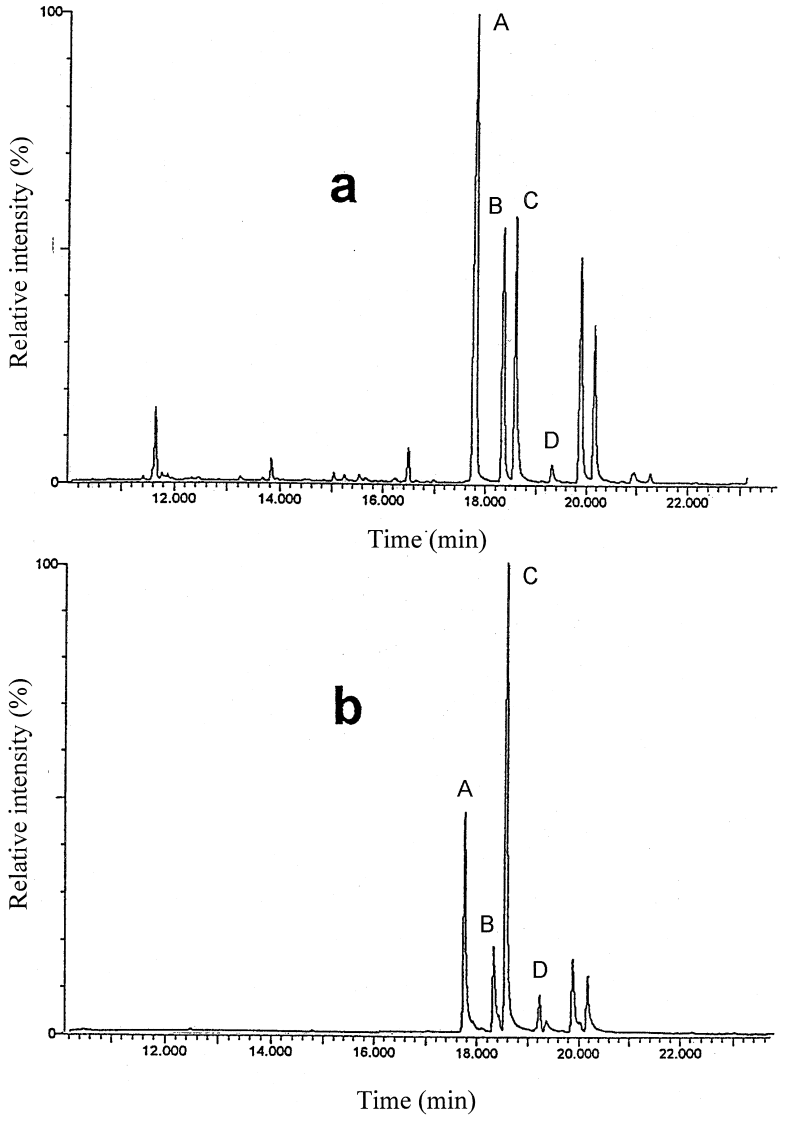

Fig. 2. GC–MS chromatograms (total ion current; TIC) of the BIOL isomers before (a) and after (b) semi-preparative HPLC separation.

clean-up procedure not all interfering signals could be separated due to co-elution. Besides, the ratio of the four isomers was also changed. Several attempts to achieve purity through distillation or recrystallization in methanol–water failed due to the quite similar chemical behavior of presumably structure-related by-products (t_R=19.9 and 20.1 min) of butyraldehyde synthesis.

In the GC–MS-chromatograms, the separated BIOL isomers (BIOL *A–D*) could be assigned at the following retention times: t_R=17.8 min for BIOL *A*, 18.4 min for BIOL *B*, 18.6 min for BIOL *C* and 19.3 min for BIOL *D*. The acquired mass spectra of all the four isomers were identical. The 100% ion was always *m/z* 127, whereas *m/z* 170 was always the highest mass observed (Fig. 3). The molecular radical cation of the BIOL isomers ($C_{12}H_{22}O_2$; *m/z* 198) was never obtained under these experimental conditions, only under chemical ionization (CI) MS [5]. Due to the characteristic fragmentation pattern of

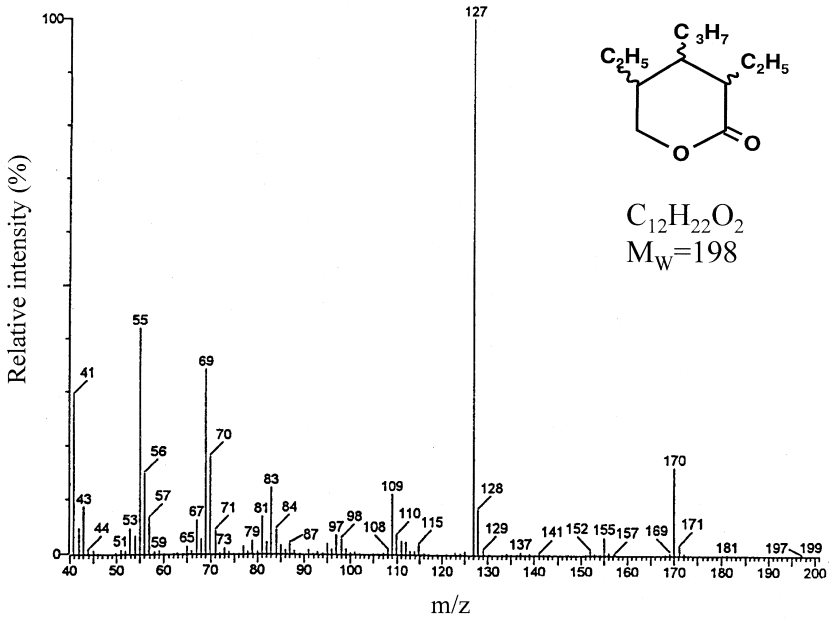

Fig. 3. EI-mass spectrum of the BIOL isomer *A*.

the electron impact ionization (EI) mass spectra, it was possible to come up with tentative assignment of fragment structures as shown in Fig. 4.

It is highly likely that the molecular radical cation lost C_2H_4 (*m*/*z* 28) after a McLafferty rearrangement, which led to a fragment ion at *m*/*z* 170. A subsequent allyl cleavage and loss of the C_3H_7 group (*m*/*z* 43) should lead to the most stable fragment ion at *m*/*z* 127. Calculation of the mean C_{12}/C_{13} ratio ($n=12$) revealed that the molecular sum of this fragment ion is $C_7H_{11}O_2$.

The further fragments can be assigned through additional losses of H_2O (*m*/*z* 109), C_2H_6 (*m*/*z* 97) or C_2H_5 (*m*/*z* 98). The ions at *m*/*z* 83, 69, 55 and 41 can be assigned to alkenes and alkene ketones being formed during the fragmentation process.

This fragmentation pattern leads to the assumption that the mass spectra obtained from the four BIOL isomers *A*–*D* all belong to stereoisomers of 2,4-diethyl-3-*n*-propyl-δ-valerolacton.

For further structure elucidation, a ^{1}H-NMR spectrum (Bruker, 500 MHz) was acquired in $C^2H_2Cl_2$. Because of four possible stereoisomers, an assignment of the ethyl and propyl groups was impossible. A bunch of signals was obtained for the range of 0.7 up to 2.2 ppm. For an exact assignment of the individual stereoisomers, a separation on a chiral HPLC column would be necessary. This was not the primary aim of this work since the environmental behavior of the sum of the BIOL isomers was the matter of interest.

Out of 700 ml of extracted sewage water, almost 800 mg of the BIOL isomers were obtained which should be considered as reference mixture. Since a 100% rectification was not possible, the purity and the distribution of the single BIOL isomers have been investigated by GC–MS. Therefore, the area of each BIOL isomer was divided by the sum of the areas of all the peaks in the chromatogram. This was only possible since the ratios among the signals were constant and increased linearly with the same slope over the entire calibration range.

According to this assumption, the approximate content of BIOL *A*, *B* and *C* (BIOL sum) in the isolated substance was determined as 77% (mean value $n=3$). The distribution of the single isomers was 31% BIOL *A*, 15% BIOL *B*, 50% BIOL *C* and 4% BIOL *D.*

Including BIOL *D* the purity would even be 80%, but since the concentration of BIOL *D* in the waste

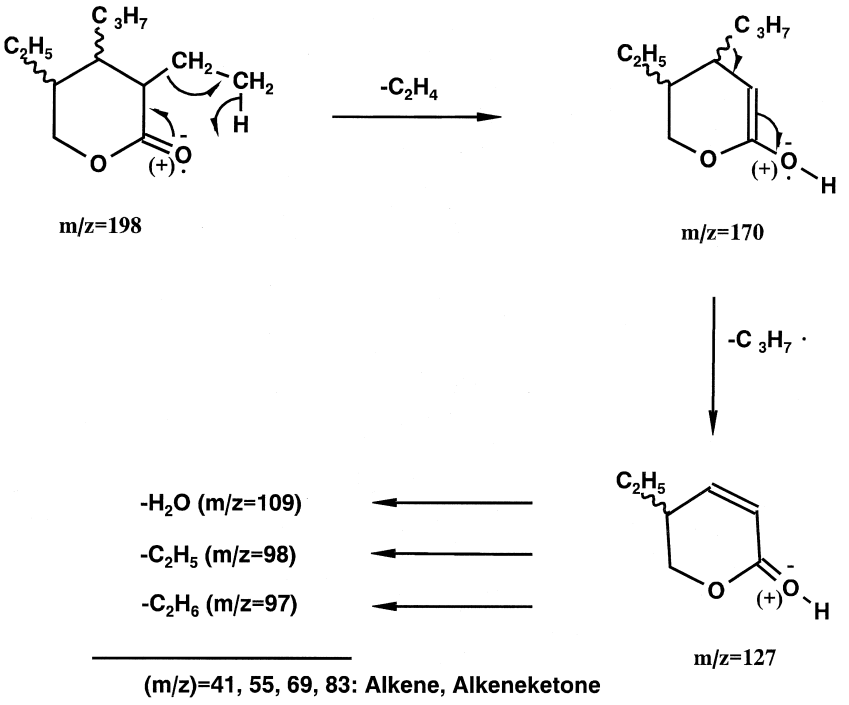

Fig. 4. Tentative fragmentation pattern and assignment of characteristic m/z values of the BIOL isomers.

and surface water samples was too low, further quantification was only done for the sum of BIOL *A*, *B* and *C*.

3.2. Quantification method

The recoveries of the investigated BIOL isomers were determined through three solid-phase extractions. Ground water was spiked with 0.5 μg l^{-1} BIOL sum. Measurements were compared with a 0.5 μg l^{-1} sample analyzed without extraction. The obtained mean recovery for the BIOL sum was 42% (Table 1) and the standard deviation was less than 4%.

The calibration curve had a correlation coefficient of 0.999 for the BIOL sum (Table 1) using a five-point calibration curve in the range from 0.1 to 3.0 μg l^{-1}. The limit of quantification (LOQ) of the BIOL sum was calculated to be 0.05 μg l^{-1} in drinking and surface waters.

With the available standards isolated and the calibration methods established, it was possible for the first time to study the concentrations of the BIOL isomers in the river Rhine, Germany upon their entry in sewage water at a point source and to follow their behavior during drinking water purification of river Rhine water.

3.3. BIOL isomers in the river Rhine

The occurrence and distribution patterns of the BIOL isomers were monitored in the river Rhine, Germany (km 507) over a period of almost two years yielding a nearly constant ratio between the individual isomers (Fig. 5a and b). The determined

Table 1
Recoveries and correlation coefficients of BIOL sum (BIOL *A*, *B* and *C*) by (SPE) ($n=3$)

Compound	Recovery (%)	Correlation coefficient
BIOL sum	42±4	0.999
BIOL *A*	37±4	0.999
BIOL *B*	59±3	1.000
BIOL *C*	41±4	0.999

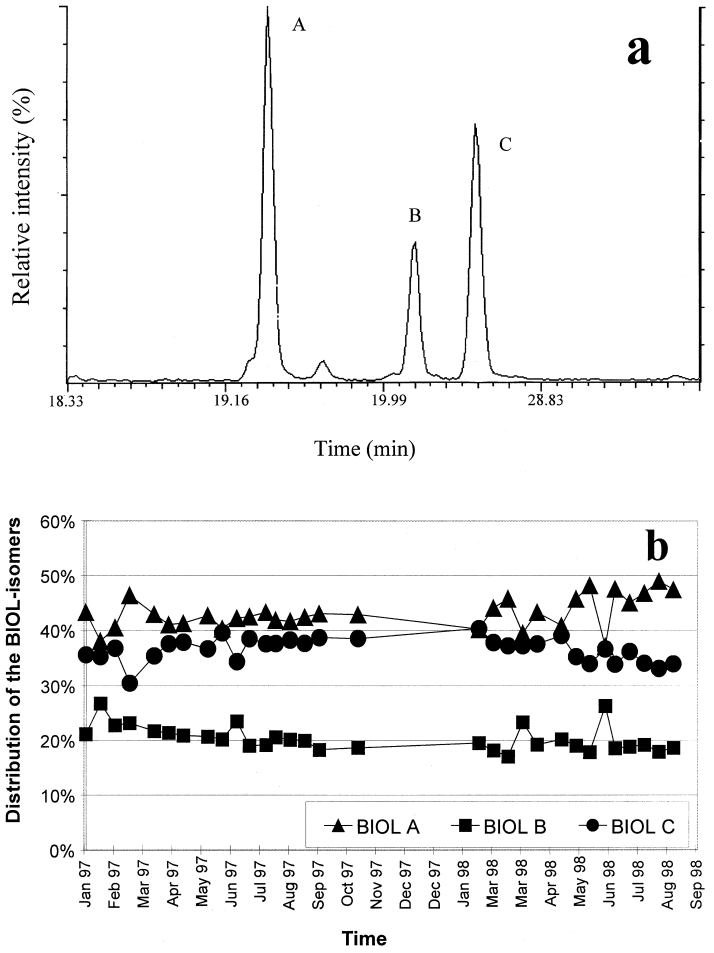

Fig. 5. GC–MS-chromatogram (a) and relative distribution of the individual BIOL isomers (b) for almost two years in the river Rhine, Germany.

mean concentration of the BIOL sum was about 1.6 μg l^{-1}. Additional monitoring of the BIOL isomers in the river Rhine, Germany further downstream towards the Dutch border gave a similar pattern among the individual isomers; but since there were by then no reference compounds available, a quantification has not been done yet [1].

The observed ratio among the individual isomers in the river Rhine is very similar to the pattern measured in the industrial waste water. It appears that the formation of the individual BIOL isomers during synthesis process of butyraldehyde is almost constant and consequently diluted when discharged in the river Rhine.

3.4. BIOL concentration during different steps of water treatment out of river Rhine water

The occurrence and behavior of BIOL isomers during the different steps of drinking water production from the river Rhine, Germany (km 507) was investigated over a period of three months in the Summer of 1998. During this period of time, the concentrations of the BIOL sum (BIOL *A*, *B* and *C*) in the river Rhine ranged between 0.6 and 2.5 μg l^{-1} (data not shown). After granular activated carbon (GAC) filtration, the BIOL sum concentration was reduced to an average value of about 0.08±0.03 μg l^{-1} making this purification step very effective with

a removal of about 90%. Complete removal out of the raw water was achieved through microbial degradation. In particular a subsoil passage had no measurable effect on the sum of the BIOL isomers, whereas a subsequent slowsand filtration led to a total elimination of the BIOL isomers. This biodegradation was supported by the results of degradation studies in a laboratory-scale biodegradation set-up [7] resulting in a half-life of eight days for the sum of the BIOL isomers (data not shown).

As already observed for other drinking water relevant compounds such as e.g. phenylsulfonamides [8], it seems that medium to poorly degradable compounds are not much affected during the subsoil passage. On the other hand the microbial degradation during a slowsand filtration step seems to be very effective for the elimination of such compounds.

4. Conclusion

The BIOL isomers seemed to have been discharged into the river Rhine, Germany over several years. This was documented through acquired GC–MS chromatograms in which the characteristic peak-pattern was found. But it is only now, after the isolation of the BIOL isomers, that it was possible to assess their concentration levels and get a better insight into their fate. Since this has been done mainly at two drinking water facilities along the river Rhine, those compounds should be included into a more widespread monitoring program.

It proved again that activated carbon filtration is beside the nonpolar persistent compounds also an efficient removal step for the elimination of medium polar, poorly biodegradable xenobiotics. Even if the BIOL isomers were not totally removed after GAC filtration, they could not be detected in drinking water due to microbial degradation during the subsequent subsoil passage and slowsand filtration. This could be confirmed through standardized degradation assays utilizing a testfilter device with immobilized microorganisms. Since this technology is state-of-the-art at the drinking water facilities located at the river Rhine, these compounds should not be present in the finished drinking water, which could be proven at the so far investigated drinking waters. However, it would be much better, if such compounds could be more effectively treated directly at their source of production.

In order to assess the relevance of the BIOL isomers different toxicological tests still have to be performed since nothing is known about their risk to population and environment.

Overall, the enormous investigative effort of the isolation as well as characterization of such by-products of chemical synthesis is more than worthwhile, because only with available reference compounds it will be possible to study their impact on the aquatic environment as well as their fate.

Acknowledgements

We express our thanks to Mr. Johannes Driemler for his skillful help during the purification of the sewage water samples and Dr. Manfred Wagner, Max-Planck-Institut für Polymerforschung, Mainz, Germany, for doing the NMR analyses. We are indebted for the financial support of the ‘‘Arbeitsgemeinschaft Rheinwasserwerke e. V. (ARW)’’ and the ‘‘Verband der Chemischen Industrie (VCI)’’.

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