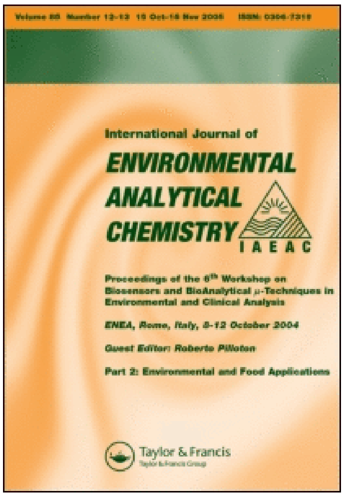


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Quantitative determination of toluene, ethylbenzene, and xylene degradation products in contaminated groundwater by solid-phase extraction and in-vial derivatization

Anna Ledin Lotte Ask Reitzel[a]; Poul L. Bjerg[a]
[a] Institute of Environment & Resources DTU, Bygningstorvet, Technical University of Denmark, DK-2800 Kgs, Lyngby, Denmark

To cite this Article Reitzel, Anna Ledin Lotte Ask and Bjerg, Poul L.(2005) 'Quantitative determination of toluene, ethylbenzene, and xylene degradation products in contaminated groundwater by solid-phase extraction and in-vial derivatization', International Journal of Environmental Analytical Chemistry, 85: 14, 1075 — 1087

To link to this Article: DOI: 10.1080/03067310500194920

URL: http://dx.doi.org/10.1080/03067310500194920

Intern. J. Environ. Anal. Chem.
Vol. 85, No. 14, 10 December 2005, 1075–1087

Quantitative determination of toluene, ethylbenzene, and xylene degradation products in contaminated groundwater by solid-phase extraction and in-vial derivatization

ANNA LEDIN, LOTTE ASK REITZEL* and POUL L. BJERG

Institute of Environment & Resources DTU, Technical University of Denmark,
Bygningstorvet, Building 115, DK-2800 Kgs. Lyngby, Denmark

(*Received 29 October 2004; in final form 12 May 2005*)

Benzylsuccinic acid (BSA) and methylbenzylsuccinic acids (mBSAs) are unambiguous indicators of anaerobic toluene and ethylbenzene/xylene degradation, and so the determination of these compounds in landfill leachates and contaminated groundwater is highly relevant. Samples were diluted to $<0.8\,\mathrm{mS\,cm^{-1}}$ in order to reduce their ionic strength, and subsequently extracted through strong anion exchange disks, followed by simultaneous in-vial elution and methylation. A detection limit of $0.1\,\mu\mathrm{g\,L^{-1}}$ was obtained for 100 mL samples. Using this method, $19.3\,\mu\mathrm{g\,L^{-1}}$ of BSA was measured in a landfill leachate, and low $\mu\mathrm{g\,L^{-1}}$ levels of all of the mBSAs were measured in gasoline-contaminated groundwater. The results were compared with the findings of BSAs at 16 other contaminated sites, and BSAs as indicators of biodegradation were evaluated. The estimation of biodegradation rates based on parent hydrocarbons and BSA concentrations or ratios is questionable. However, the degradation products serve as good qualitative *in situ* indicators for anaerobic biodegradation in contaminated groundwater.

Keywords: Benzylsuccinic acid; Solid-phase extraction; GC/MS; Landfill leachate; Groundwater; Biodegradation

1. Introduction

Groundwater contamination due to leaky underground storage fuel tanks is a common problem at civilian and military sites worldwide. Because benzene, toluene, ethylbenzene, and xylene (known collectively as BTEX) are the most water-soluble fraction in fuels, they are mobile in the subsurface and are recognized as ubiquitous contaminants in groundwater [1]. Leaching from landfills due to the deposition of different types of wastes, including fuel residuals, is another important source of BTEX-contaminated groundwater [2, 3].

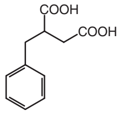
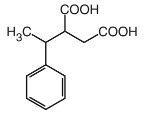
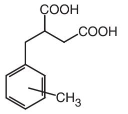
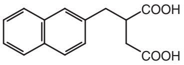
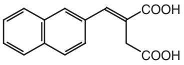
BTEX are biodegradable under aerobic conditions, while the anaerobic degradation of BTEXs shows a greater variation [4]. Toluene is readily degraded under all anaerobic

---

*Corresponding author. Fax: +45 45 25 15 91. Email: loa@er.dtu.dk

International Journal of Environmental Analytical Chemistry
ISSN 0306-7319 print: ISSN 1029-0397 online
DOI: 10.1080/03067310500194920

Table 1. Anaerobic degradation products of toluene, ethylbenzene, xylenes and 2-methylnaphthalene.

Metabolite	Parent compound	Abbreviation	Structure
Benzylsuccinic acid	Toluene	BSA	
α-Methylbenzylsuccinic acid	Ethylbenzene	α-mBSA	
2-, 3-, and 4-Methylsuccinic acids	*o*-Xylene *m*-Xylene *p*-Xylene	2-mBSA 3-mBSA 4-mBSA	
Naphthyl-2-methylsuccinic acid	2-Methylnaphthalene	NSA	
2-Naphthylitaconic acid	2-Methylnaphthalene	NIA	

redox conditions (reviewed by Heider *et al.* [5]), while benzene, ethylbenzene, and xylenes reveal a slower degradation and individual differences depending on the actual redox conditions [6–9]. Anaerobic degradation, however, is not a rule, and observations of recalcitrance have also been reported for one or more of the BTEX compounds in field scale studies of petroleum hydrocarbon plumes [10–12] or landfill leachate plumes [13–15].

Metabolites have been suggested as a means for proving degradation in field studies. The anaerobic degradation product of toluene, benzylsuccinic acid (BSA) (table 1), was identified in anaerobic laboratory microcosm experiments under denitrifying [16, 17] and sulphate-reducing conditions [18, 19]. The pathway leading to this metabolite is referred to as the fumarate addition pathway, since the methyl carbon of toluene is added to the double bond of fumarate. The resulting degradation product does not have a commercial source and is produced only during the biodegradation of toluene, which is why the criterion as an unambiguous indicator of *in situ* toluene bioremediation is fulfilled [20].

Laboratory degradation studies have also shown that the degradation of xylenes and ethylbenzene can lead to similar metabolites [21–23]. Furthermore, Annweiler *et al.* [24] detected the metabolites naphthyl-2-methylsuccinic acid (NSA) and naphthylitaconic acid (NIA) during the degradation of 2-methylnaphthalene under sulphate-reducing conditions. Kropp *et al.* [25] found two dodecylsuccinic acids from the degradation of dodecane. These might be enantiomers or structural isomers. This study shows that fumarate addition, as the initial degradation step, is not limited to aromatic compounds.

Measurements of these indicators of anaerobic degradation have recently been included in a few field investigations at landfill sites [14, 26] and petroleum hydrocarbon-contaminated sites [27–31], but their use as *in situ* indicators of anaerobic degradation still needs to be evaluated at a broader range of field sites.

The analytical method used by Beller *et al.* [20] involves acidifying the sample to pH < 1 followed by liquid–liquid extraction and derivatization with diazomethane (detection limits in the order of $5\,\mu g\,L^{-1}$ for 1 L samples). Solid-phase extraction (SPE) is an attractive alternative to liquid–liquid extraction because it reduces the time, number of handling steps, and amount of organic solvent waste that is generated. Thus, Reusser and Field [29] used SPE cartridges for the extraction of BSA and mBSA (detection limit of $0.2\,\mu g\,L^{-1}$ for 1 L samples). The use of sophisticated hyphenated techniques can even make preconcentration unnecessary, as shown by Beller [32], who obtained a detection limit of about $0.3\,\mu g\,L^{-1}$ for sample volumes < 1 mL by LC/MS/MS analysis. However, such an instrument is still not standard laboratory equipment.

Compared with SPE cartridges where analytes must be eluted and physically separated from the cartridge, flexible extraction membranes or disks can be placed directly into small (2 mL) autosampler vials. This process, termed 'in-vial' elution, is rapid and simple, and uses less organic solvents. For analytes that require derivatization, such as BSA, strong anion exchange disks (SAX disks) are placed in an autosampler vial containing an alkylation reagent (methyl iodide). When heated, carboxylic acid analytes are simultaneously converted into their methyl esters and eluted from the SAX disk, which catalyses the derivatization. It has been shown that SAX disks coupled with in-vial elution can be employed for the extraction of a number of acidic compounds ranging from pesticides and metabolites to long-chain fatty acids [33, 34].

The main objective of this study was to adapt, optimize, and validate SPE using SAX disks with in-vial elution as a quantitative, rapid, simple, and cost-effective alternative analytical method for the determination of succinic acid derivatives of aromatic hydrocarbons in groundwater. The method was applied to landfill leachate samples and samples from an aquifer contaminated with petroleum hydrocarbons in order to validate the robustness of the method. Finally, the application of BSAs as *in situ* indicators was evaluated based on the analytical results from 18 contaminated sites including the sites investigated in this study.

2. Experimental

2.1 *Sandholt-Lyndelse Landfill, sampling and analyses*

Landfill leachate was collected in the drainage system at an engineered landfill: Sandholt-Lyndelse Landfill, Denmark. Sampling bottles were lowered into the leachate-collection well. Precautions were taken to avoid sampling leachate from the surface of the well. The leachate was characterized with respect to toluene and general water-quality parameters. Glass bottles were used for the sample collection and stored in the dark at 4°C until analysis. For detailed information concerning the analytical methods and instrumentation for all chemical analyses, except BSAs, see Baun *et al.* [26].

2.2 *Radsted field site, groundwater sampling, and analyses*

The Radsted site operated as a gasoline station from 1958 to 1975 (figure 1). The geology consists of a layer of moraine clay underlain by a layer of sand, where local heterogeneities have been identified [35]. In 1994, contamination with petroleum hydrocarbons was discovered, which led to the removal of the underground storage tanks along with contaminated soil and aquifer material in 1996. Subsequent monitoring

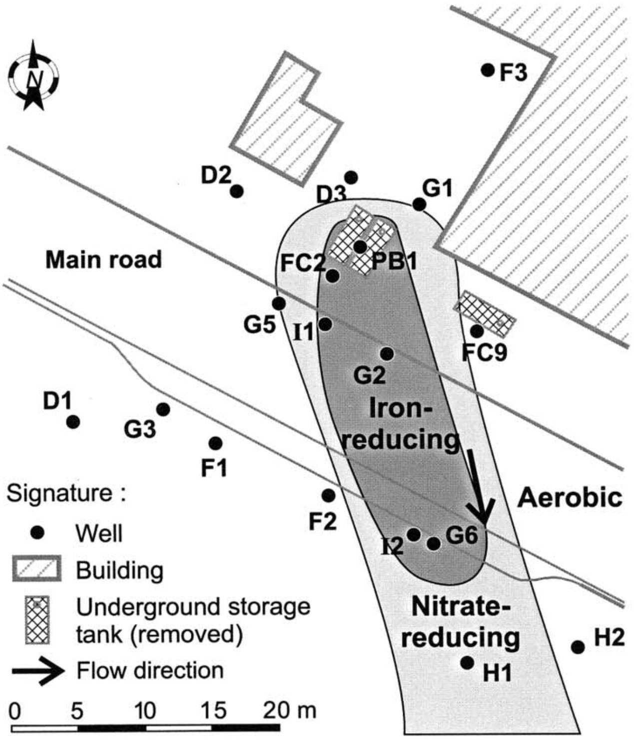


Figure 1. Map of the Radsted field site, showing the monitoring wells and the redox conditions developed downgradient of the removed underground storage tanks.

in 18 monitoring wells indicated biodegradation of BTEX compounds under anaerobic conditions. A decrease in hydrocarbon concentration with distance from the source, along with depletion of oxygen and nitrate and the generation of ferrous iron, was observed.

Two multilevel samplers were installed in 2001 with sampling points over a depth of 6 m, with a distance of 25 cm between each sampling point (wells I1 and I2; figure 1). In this study, the concentrations of hydrocarbons and specific metabolites were measured for all of the wells within the nitrate- and iron-reducing areas (figure 1), including wells PB1, FC2, G5, G2, G6, and H1, and 24 sampling points on each of the multilevel samplers, I-1 and I-2. A background sample (well D1) was also analysed. Sampling was performed by suction through Teflon tubes. Three well volumes were pumped before the samples were taken. The samples were characterized with respect to BTEX, naphthalene, methyl-naphthalenes, corresponding BSAs (from TEX and 2-methylnaphthalene, table 1) and electrical conductivity.

2.3 *Reagents and standards*

Standards of benzylsuccinic acid (BSA; 99% purity) and 4-fluorobenzoic acid (4FBA; 98% purity; surrogate standard) were purchased from Sigma Chemical (St. Louis, MO). 2-, 3-, 4- and α-methylbenzylsuccinic acids (2-mBSA, 3-mBSA, 4-mBSA and

α-mBSA) were kindly donated by Dr Lisa Gieg and Dr Joseph Suflita, University of Oklahoma. Naphthyl-2-methylsuccinic acid (NSA) and 2-naphthylitaconic acid (NIA) were kindly donated by Dr Rainer Meckenstock, Eberhard-Karls-Universität Tübingen. A standard of 2-chlorolepidine (2CL; 2-chloro-4-methyl quinoline; 99% purity; internal standard) as well as methyl iodide was obtained from Aldrich Chemical (Milwaukee, WI). For this study, 4FBA was selected as the surrogate standard as introduced by Beller *et al.* [20]. Acetonitrile (HPLC-grade) was obtained from Fisher Scientific (Fairlawn, NJ).

2.4 *SPE and in-vial derivatization and elution*

Prior to extraction, the electrical conductivity of samples was measured using a WTW Multilab P4 system with a Tetracon 325 cell (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Samples with an electrical conductivity $> 0.8\,\mathrm{mS\,cm^{-1}}$ were diluted with Milli-Q water. Measurements of pH were conducted on the samples by a combination electrode. Samples with a pH of < 9 were adjusted to pH 9. If a particulate phase was visible, samples were centrifuged at 8000 *g* for 15 min.

All water samples (100 mL final volume) were spiked with the surrogate standard, 4FBA; to achieve a final concentration of $50\,\mu\mathrm{g\,L^{-1}}$. Samples were then extracted directly by SPE as described by Eriksson and Ledin [34]. Briefly, SAX disks 13 mm in diameter were cut from 47 mm disks (Varian, Sugarland, TX) with a cork-boring tool. The disks were soaked overnight in 12 mM HCl and acetonitrile to remove benzoic acid (BA), an impurity in the SAX disks. The disks were mounted in 13 mm filter holders (two disks in each holder) that were attached to a vacuum manifold (Supelco, Bellefonte, PA), and glass syringes were attached to the filter holders as reservoirs. Prior to use, the disks were flushed with 10 mL of acetonitrile and 10 mL of water to remove the excess HCl. The sample was then passed through the disk under vacuum (25 mmHg). For samples with a particulate phase, the supernatant was first passed through the disk, and then the centrifugate was resuspended and quantitatively loaded to the disk. The disks were dried under vacuum for 15 min and then transferred to a 2 mL autosampler vial followed by the addition of 0.5 mL acetonitrile containing $20\,\mathrm{mg\,L^{-1}}$ of 2CL and $200\,\mu\mathrm{L}$ of methyl iodide. The final volume of 0.7 mL was sufficient to cover the SAX disks completely. The GC-vial was capped and heated for 1 h at 80°C. After cooling the vials to room temperature, the samples were analysed by GC/MS. Samples as well as standards were extracted and analysed in duplicates.

2.5 *Detection and quantification*

The samples were analysed using a Hewlett Packard Model 6890 gas chromatograph equipped with a Model 5973 mass selective detector (MSD) and a Varian Autosampler 8200CX. The GC was equipped with a $30\,\mathrm{m} \times 0.25\,\mathrm{mm} \times 0.25\,\mu\mathrm{m}$ HP5-MS capillary column. The injector temperature was operated at 280°C in splitless mode with a $1\,\mu\mathrm{L}$ injection volume and helium as a carrier gas (flow $1.3\,\mathrm{mL\,min^{-1}}$). During the method development, the following temperature programme was used. The initial oven temperature of 65°C was held for 1 min and then increased by $5^{\circ}\mathrm{C\,min^{-1}}$ up to 140°C, after which the temperature was increased by $15^{\circ}\mathrm{C\,min^{-1}}$ to a final temperature of 280°C. The samples from the Radsted field site were run with

Table 2. GC/MS parameters used for the identification and quantification of BSAs.

			m/z		
Peak	RT[a] (min)	RT[b] (min)	Quantifier	Qualifiers	
4FBA	6.83	4.98	154	123	
BA	7.25		136	105	
2CL	18.21	10.15	177	179	
BSA	19.30	11.35	176	236	145[d]
α-mBSA[c]		12.19	190	250	145
α-mBSA[c]		12.35	190	250	145
3-mBSA		13.06	190	250	145
2-mBSA		13.20	190	250	145
4-mBSA		13.41	190	250	145
NSA		18.69	286	226	141
NIA		19.46	284	224	165

[a] Temperature programme used for method development. [b] Temperature programme used for samples from Radsted field site. [c] α-mBSA splits into two peaks in the chromatogram. [d] Only included for samples from Radsted field site.

another temperature programme: 65°C for 1 min, 25°C min^{-1} to 170°C, 2°C min^{-1} to 185°C, 15°C min^{-1} to 280°C, and held 4 min.

The MSD was operated in the SIM mode with transfer line, source, and quadrupole temperatures of 270, 230, and 150°C, respectively. The EI mass spectrum for methylated BSA was similar to that reported by Beller *et al.* [20], and the EI mass spectrum for methylated FBA showed a molecular ion at *m/z* 154, corresponding to $[C_8H_7O_2F]^+$. This indicates that the derivatization procedure produces the methylated forms of both BSA and 4FBA. The ions used to identify the analytes are shown in table 2. Standards were prepared in Milli-Q water. The relative peak areas instead of the absolute peak areas were used for quantification of BSAs, i.e. the area of the analyte peak/area of the surrogate standard peak. For the recovery determinations, the internal standard was used instead of the surrogate standard, while the surrogate standard was treated as an analyte. Calibration curves were linear in a range of 0.1–100 μg L^{-1}, with a method detection limit of 0.1 μg L^{-1}. Calibration curves were typically linear with $r^2 = 0.996$.

3. Results and discussion

3.1 *Development of the analytical method*

Part of the testing and optimization of the method was performed with uncontaminated groundwater, which was spiked with BSA, and part of it was performed with leachate samples from the Sandholt-Lyndelse landfill, which had an original content of BSA and therefore was not spiked with BSA. Finally, the method was tested with leachates from other landfills [26]. All samples were spiked with 4FBA as a surrogate standard. Changes in recoveries in response to changes in the analytical method are seen as a change in the ratio between the peak areas of BSA or 4FBA and the internal standard 2CL. The method optimization aimed at the maximization of the recovery, i.e. the BSA/2CL and 4FBA/2CL ratios.

The effect of centrifugation of the samples was evaluated by spiking uncontaminated groundwater samples with BSA (1 μg L^{-1}). No significant loss of BSA or 4FBA

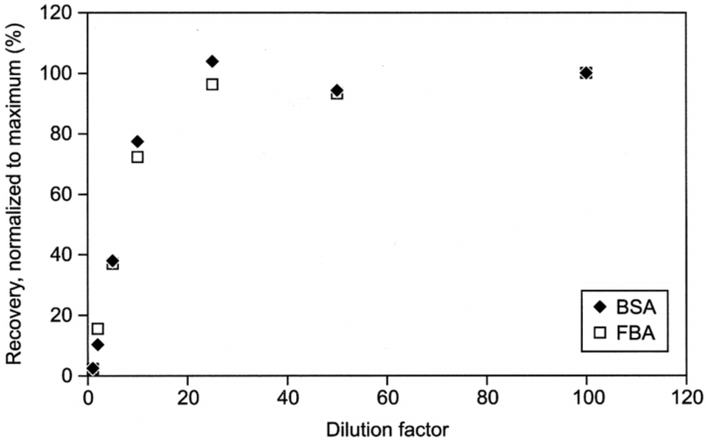


Figure 2. Increase in BSA and 4FBA recovery upon dilution of a sample of landfill leachate (Sandholt-Lyndelse). All diluted samples were spiked with 4FBA to give a final concentration of 50 μg L^{-1}. The naturally occurring BSA as well as 4FBA were normalized with respect to the maximum value.

occurred upon centrifugation (data not shown). The capacity of the disks to extract BSA was evaluated with respect to pH. To this end, Milli-Q water as well as leachate samples were spiked with BSA (10 μg L^{-1}) and the pH was adjusted to 5.5, 7.5, and 9.0 with NaOH ($n=3$ for each pH). The experiments showed that the extraction/derivatization/elution efficiency was greatest for samples adjusted to pH 9, while the recoveries decreased with at least 10% at the lower pH values. For this reason, all of the samples were adjusted with NaOH to obtain a pH of 9.

The extraction efficiency increased up to 30% when two disks were used instead of one. Adding a third disk did not significantly improve the extraction efficiency.

However, the recovery of BSA and 4FBA from spiked leachate samples was still low compared with the recovery from spiked Milli-Q water. To overcome the problem of poor recovery for complex, high-ionic-strength samples, the dilution of the samples with Milli-Q water was investigated. The recovery of the originally present BSA and the added 4FBA increased with increasing dilution up to a dilution factor of 25, after which the recovery remained constant. At this point, the recovery was increased by a factor of 50 (figure 2). In order to find the critical parameter, dilution series were performed in the same way for a number of other samples of leachate and leachate-contaminated groundwater (data not shown; the landfills have been described by Baun *et al.* [26]). The maximum recovery was reached at different dilution factors for the different leachates. The key parameter turned out to be the electrical conductivity, as the BSA and 4FBA recoveries reached their maxima at specific electrical conductance $\leq 0.8\,\mathrm{mS\,cm^{-1}}$, while no correlation was observed between 4FBA recovery and other geochemical parameters including alkalinity, chloride, and non-volatile organic carbon (NVOC; data not shown). Therefore, all of the samples prior to extraction were diluted to a point where the electrical conductivity was $\sim 0.8\,\mathrm{mS\,cm^{-1}}$.

3.2 *Discussion of the analytical method*

It has been shown that BSA and similar compounds can be analysed by a SAX-disk extraction method modified after Eriksson and Ledin [34]. Highly complex samples such as landfill leachates always constitute an analytical challenge. In this study, it was found that the measurement of electrical conductivity and the inclusion of a simple dilution step in the sample preparation, along with the use of two disks instead of one, solved the problem of low recovery from such samples.

It is interesting to note that dilution does not change the ratio between the concentrations of ions and BSA, but rather the ionic strength of the sample. The fact that some samples have to be diluted will unfortunately increase the detection limit. How much the samples need to be diluted is obviously related to the sample matrix. The detection limit is, as mentioned previously, about $0.1\,\mu g\,L^{-1}$ when extracting 100 mL of the sample. In a worst-case scenario where the sample has to be diluted e.g. 50 times, this would correspond to a detection limit of $5\,\mu g\,L^{-1}$. The worst-case scenario corresponds to the highest dilution that would be necessary in order to analyse for BSA in landfill leachates according to data for electrical conductivity in landfill leachates compiled by Kjeldsen *et al.* [36]. However, if samples of high electrical conductivity are not diluted, the detection limit might be even higher due to low recovery. As shown above, BSA was below detection in some of the undiluted samples but could be detected upon dilution.

The detection limit for BSA obtained in this study is comparable with the detections limit obtained by solid-phase cartridge extraction ($0.2\,\mu g\,L^{-1}$, 1 L sample [29]). The smaller sample volume (100 mL) used in the present SAX disk extraction method is advantageous for different reasons. For instance, it is not always possible to sample a volume of 1 L of groundwater from a well, since it depends on the hydraulic properties and the specific type of well. Another aspect is that some SPE systems fail to extract a sample volume of 1 L in the case of landfill leachate, due to clogging/high resistance to sample throughput [37]. Alternatively, liquid–liquid extraction may provide detection limits in the order of $5\,\mu g\,L^{-1}$ for 1 L samples [20], or direct analysis by LC/MS/MS without preconcentration may provide a detection limit of $0.3\,\mu g\,L^{-1}$ for sample volumes <1 mL [32]. The latter suggests that detection limits can be significantly improved through the combination of SPE and a sophisticated technique like LC/MS/MS, which will probably become standard laboratory practice in the future.

3.3 *Application of the method to samples from contaminated sites*

The method was applied to one leachate sample from the Sandholt-Lyndelse landfill, and 55 groundwater samples from Radsted. Specific metabolites were detected in the leachate sample and in two samples from Radsted. The results from these samples are shown in table 3 [38]. In the leachate from the Sandholt-Lyndelse landfill, the measured BSA concentration ($19.3\,\mu g\,L^{-1}$) was in the same order of magnitude as the mother compound, toluene ($16.6\,\mu g\,L^{-1}$).

The hydrocarbon contamination at the Radsted site is limited to a small area represented by wells G5 and I1 (figure 1, table 3), where the hydrocarbons are present in high concentrations (sum of measured hydrocarbons: $5–15\,mg\,L^{-1}$; maximum single compound concentration: $2.7\,mg\,L^{-1}$ ethylbenzene). Only benzene was found outside this area in low concentrations (data not shown). The contamination was also limited in depth. The upper screen in I1 (I1–1), which is located close to the groundwater table, contains high concentrations of hydrocarbons (table 3), while the concentrations in the next screen (I1–2) are 2–3 orders of magnitude lower (data not shown). One metre below the groundwater table and deeper (screens I1–5 to I1–24), no hydrocarbons

Table 3. Concentrations of identified metabolites and corresponding hydrocarbon concentrations at the Sandholt-Lyndelse landfill and the Radsted field site.

Compound/Parameter	Sandholt-Lyndelse Landfill	Radsted G5	Radsted I1, 1
Toluene (μg L^{-1})	16.6	1283	860
BSA (μg L^{-1})	19.3	nd	nd
Ethylbenzene (μg L^{-1})	10.4	686	2688
α-mBSA (μg L^{-1})[a]	na	2.1	3.0
o-Xylene (μg L^{-1})	18.5	672	1587
2-mBSA (μg L^{-1})	na	nd	0.9
m/*p*-Xylene (μg L^{-1})	51.4	1390	5041
3-mBSA (μg L^{-1})	na	0.5	2.3
4-mBSA (μg L^{-1})	na	nd	2.9
2-Methylnaphthalene (μg L^{-1})	nd	1.4	7.5
NSA (μg L^{-1})	na	nd	nd
NIA (μg L^{-1})	na	nd	nd
Electrical conductivity (mS cm^{-1})	15.3	2.3	0.5
pH	8	6.8–6.9[b]	na
Alkalinity (meq L^{-1})	79.4	440–510[b]	na
Chloride (mg L^{-1})	2730	na	na
Ammonium (mgN L^{-1})	546	0.08–0.26[b]	na
NVOC (mgC L^{-1})	218	na	na

[a] α-mBSA is the sum of two peaks in the chromatogram. [b] Data from earlier samplings [38]. na: not analysed. nd: not detected.

were present in concentrations above the detection limit. The extension of the contamination is significantly reduced compared with earlier measurements at the site [35], indicating that the plume is controlled by anaerobic degradation.

The matrix of the samples from the Radsted field site was less complicated than the matrix associated with leachate and leachate-contaminated groundwater. Most of the samples had electrical conductivities close to 0.8 mS cm^{-1} (0.4–0.9 mS cm^{-1}). Only the samples from the upper 15 sampling points of I-2 (data not shown) and from boring G5 in the high contamination area had a higher electrical conductivity (1.0–2.3 mS cm^{-1}) (figure 1, table 3). It was therefore decided to treat all of the samples from the site identically, since the general differences were relatively small, and thus no samples were diluted.

At Radsted, no BSA was detected, although the toluene concentration was almost two orders of magnitude higher than at Sandholt-Lyndelse. In the analysis of the samples from Radsted, a number of other anaerobic hydrocarbon metabolites were included (mBSAs, NSA, and NIA, table 1). Relatively low concentrations of mBSAs were detected in wells G5 and I1–1 (up to 3 μg L^{-1}) as shown in table 3, which is the area with high concentrations of their parent hydrocarbons, while no metabolites were detected outside this area. The concentration of 2-methylnaphthalene was significantly lower than the concentrations of ethylbenzene and the xylenes, and consistently NSA and NIA were not found.

3.4 *Discussion of BSAs measured in field investigations*

Measurements of the possible indicators of anaerobic degradation, BSAs, have been included in some field investigations at hydrocarbon-polluted sites during recent years. Reinhard *et al.* [10] detected BSA, 2-mBSA, and 3-mBSA in a push-and-pull test, where BTEX-amended groundwater was released into a gasoline-contaminated aquifer. Relatively high concentrations of BSA and 2-mBSA were found in fuel-contaminated groundwater by Beller [27] ($\sim 220\,\mu g\,L^{-1}$ 2-mBSA and $> 18\,mg\,L^{-1}$ total BTEX). Elshahed *et al.* [28] detected 3-mBSA, 4-mBSA and α-mBSA in a gas condensate-contaminated aquifer, which, from other lines of evidence, is believed to undergo natural attenuation [39]. Furthermore, Reusser and Field [29] found BSA and mBSA in groundwater from a bulk-fuel terminal, and mBSA in groundwater from a former petroleum refinery. At least two mBSAs were detected, but they were not compared with authentic standards, which is why the exact identity could not be evaluated. Push-and-pull tests at the same sites with deuterated toluene and *o*-xylene demonstrated the formation of deuterated BSA and 2-mBSA [40]. Martus and Püttmann [31] detected several BSA homologues corresponding to different alkylbenzenes from C2-benzenes (xylene isomers or ethylbenzene) to C6-benzenes in groundwater from a jet-fuel-contaminated site. Concentrations were in the low and sub-$\mu g\,L^{-1}$ level. Finally, Griebler *et al.* [41] found naphthyl-2-methylsuccinic acid (NSA) in 4–13 $\mu g\,L^{-1}$ at a former gasworks site, and Gieg and Suflita [30] detected low $\mu g\,L^{-1}$ levels ($\sim$ 10–170 nM) of the succinate derivatives of different *n*-alkanes in groundwater from different petroleum-impacted aquifers.

In addition to the identification of BSAs and other succinate derivatives in hydrocarbon-contaminated groundwater, BSA was also found in the leachate plume downstream of the Vejen landfill [14], as well as in leachate and leachate-contaminated groundwater from 10 different Danish landfills [26].

Figure 3 shows the corresponding metabolite and mother compound concentrations reported in different field investigations. For hydrocarbon-contaminated sites, the

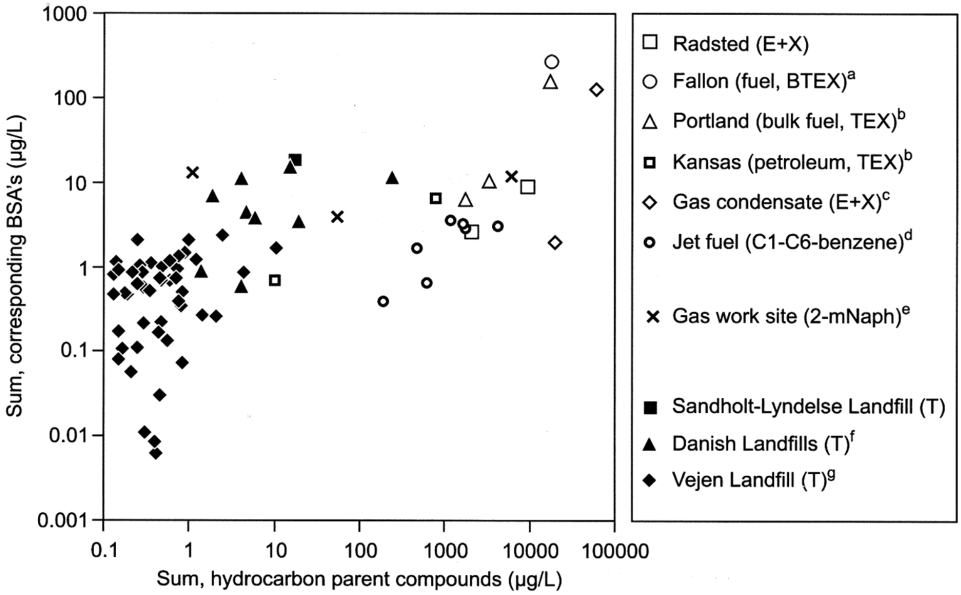


Figure 3. Corresponding concentrations of BSAs and parent hydrocarbons in field investigations. (a) Beller [27], (b) Reusser and Field [29], (c) Elshahed *et al.* [28], (d) Martus and Puttmann [31], (e) Griebler *et al.* [41], (f) Baun *et al.* [26], (g) Baun *et al.* [14].

metabolite concentrations seem to be approximately 3 orders of magnitude lower and correlated to the concentrations of their parent compounds, as long as the parent compounds are mono-aromatic hydrocarbons. The findings at Radsted contribute to this picture. The concentrations of NSA *versus* 2-methylnaphthalene measured by Griebler *et al.* [41] do not seem to follow the same trend. This could be due to a different molecular structure or could be related to the origin of the sample (creosote contamination at a gas-works site).

For landfill sites, the BSA concentration is correlated and generally in the same order of magnitude as the toluene concentration. The toluene concentrations measured at the landfill sites are, however, relatively low compared with the toluene concentrations at hydrocarbon-contaminated sites.

A t-test [42] was used to determine whether the correlation between the logarithmic concentrations of BSAs and parent compounds was significant. This was done for hydrocarbon sites with mono-aromatic hydrocarbons, for landfill sites, and finally for all of the sites. The correlation coefficients in each case are statistically significant at the 95% significance level as well as the 99% level, as the calculated t-value is greater than the tabulated value (t-distribution, two-tailed test, $n-2$ degrees of freedom; table 4).

A decrease in the ratio between total aromatic hydrocarbon and metabolites with distance from a source can indicate degradation, but the field observations in the literature are inconsistent. Some studies showed no pattern in the findings of BSAs within the contamination plume [14, 28, 41], while Martus and Püttmann [31] observed a decline in ratio at a jet-fuel-contaminated site. This was actually caused by a decreasing hydrocarbon concentration and a more or less constant concentration of BSAs along the flowline. Downgradient of the source, a decrease in BSAs was observed, thus indicating that the BSAs are rather persistent within the plume, but degrade further downgradient under undefined redox conditions. In the leachate plume at Vejen Landfill, the data also indicate that BSA is more persistent than toluene [14]. While toluene degradation was observed downgradient the landfill, BSA was not removed within the investigated part of the plume.

Thus, persistence of BSA within a contamination plume has been observed at a landfill site as well as at a hydrocarbon site. On the other hand, the fact that the metabolites are detected only within (or just downgradient [31]) the contaminated areas [28, 31] in the field investigations described so far indicates that the compounds are being further degraded.

Table 4. Significance test for the correlation between the logarithm of the sum of BSA concentrations ($\log y$) and the logarithm of the sum of parent compound concentrations ($\log x$).

	Hydrocarbon sites (mono-aromates)	Landfills (toluene)	All sites
Line of regression	$\log y = 0.68 \times \log x - 1.53$	$\log y = 0.63 \times \log x - 0.13$	$\log y = 0.34 \times \log x - 0.20$
Correlation coefficient, r	0.7469	0.5666	0.6619
Number of samples, n	17	57	77
Calculated t-value, $t = (\lvert r\rvert\sqrt{n-2})/(\sqrt{1-r^2})$	4.35	5.10	7.65
Critical value of t (95% level)	2.13	2.01	1.96
Critical value of t (99% level)	2.95	2.68	2.58

4. Conclusions

A SAX-disk extraction method has been developed to analyse BSA and similar compounds in highly complex samples such as landfill leachates. It was found that the measurement of electrical conductivity and the inclusion of a simple dilution step in the sample preparation, along with the use of two disks instead of one, solved the problem of low recovery from such samples. The robustness of the method was tested on samples from a landfill and a petroleum hydrocarbon plume.

The detection of succinic acid metabolites of ethylbenzene and the xylenes is a good supplement to the documentation of natural attenuation at the Radsted field site. Since the metabolites have only been detected (and looked for) in relatively few contaminated aquifers, the findings at Radsted are an important contribution when the broader applicability of using such indicators for natural attenuation is to be evaluated.

BSAs are useful as qualitative indicators of *in situ* biodegradation, but based on the current knowledge, it is not possible to use BSAs to quantify the degradation, e.g. by estimating degradation rates in the field.

The analytical method developed in this study is simple and cost-effective, and thus well suited for inclusion in field investigations associated with the monitored natural attenuation of contaminant plumes containing monoaromatic hydrocarbons.

Acknowledgements

Assistance provided in the laboratory from Ms Anja Foverskov and Mrs. Lena Troelsen is gratefully acknowledged. The authors would also like to express their gratitude to Dr Jennifer Field and Dr Torben Nilsson for their valuable comments on the experimental procedure. Funding for this study was provided by the Groundwater Research Center/Environment & Resources DTU and the Technical University of Denmark. The Danish Petroleum Industry's Association for Remediation of Retail Sites (OM) and the County of Storstroem kindly provided access to the Radsted field site.

References

[1] T.H. Wiedemeier, J.T. Wilson, D.H. Kampbell, R.N. Miller, J.E. Hansen. In *Technical Protocol for Implementing Intrinsic Remediation with Long-term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Groundwater*, Air Force Center for Environmental Excellence Technology Transfer Division, Brooks Air Force Base, San Antonio, TX (1995).

[2] T.H. Christensen, P. Kjeldsen, H.J. Albrechtsen, G. Heron, P.H. Nielsen, P.L. Bjerg, P.E. Holm. *Crit. Rev. Environ. Sci. Technol.*, **24**, 119 (1994).

[3] T.H. Christensen, P. Kjeldsen, P.L. Bjerg, D.L. Jensen, J.B. Christensen, A. Baun, H.J. Albrechtsen, C. Heron. *Appl. Geochem.*, **16**, 659 (2001).

[4] D. Aronson, P.H. Howard. In *Anaerobic Biodegradation of Organic Chemicals in Groundwater: A Summary of Field and Laboratory Studies*, Environmental Science Center, North Syracuse, NY (1997).

[5] J. Heider, A.M. Spormann, H.R. Beller, F. Widdel. *FEMS Microbiol. Rev.*, **22**, 459 (1999).

[6] H.A. Ball, M. Reinhard. *Environ. Toxicol. Chem.*, **15**, 114 (1996).

[7] J.D. Coates, R. Chakraborty, M.J. McInerney. *Res. Microbiol.*, **153**, 621 (2002).

[8] D.R. Lovley. *J. Ind. Microbiol. Biotechnol.*, **18**, 75 (1997).

[9] R.T. Anderson, D.R. Lovley. *Environ. Sci. Technol.*, **34**, 2261 (2000).

[10] M. Reinhard, S. Shang, P.K. Kitanidis, E. Orwin, G.D. Hopkins, C.A. Lebron. *Environ. Sci. Technol.*, **31**, 1580 (1997).

[11] A.P. Borole, K.L. Sublette, K.T. Raterman, M. Javanmardian, J.B. Fisher. *Appl. Biochem. Biotechnol.*, **63–65**, 719 (1997).

[12] G.B. Davis, C. Barber, T.R. Power, J. Thierrin, B.M. Patterson, J.L. Rayner, Q.L. Wu. *J. Contam. Hydrol.*, **36**, 265 (1999).

[13] K. Rugge, P.L. Bjerg, J.K. Pedersen, H. Mosbaek, T.H. Christensen. *Wat. Resour. Res.*, **35**, 1231 (1999).

[14] A. Baun, L.A. Reitzel, A. Ledin, P.L. Bjerg, T.H. Christensen. *J. Contam. Hydrol.*, **65**, 269 (2003).

[15] J.J. Johnston, R.C. Borden, M.A. Barlaz. *J. Contam. Hydrol.*, **23**, 263 (1996).

[16] B. Seyfried, G. Glod, R. Schocher, A. Tschech, J. Zeyer. *Appl. Environ. Microbiol.*, **60**, 4047 (1994).

[17] H.R. Beller, A.M. Spormann. *Appl. Environ. Microbiol.*, **63**, 3729 (1997).

[18] H.R. Beller, M. Reinhard, D. Grbicgalic. *Appl. Environ. Microbiol.*, **58**, 3192 (1992).

[19] H.R. Beller, A.M. Spormann, P.K. Sharma, J.R. Cole, M. Reinhard. *Appl. Environ. Microbiol.*, **62**, 1188 (1996).

[20] H.R. Beller, W.H. Ding, M. Reinhard. *Environ. Sci. Technol.*, **29**, 2864 (1995).

[21] H.R. Beller, A.M. Spormann. *J. Bacteriol.*, **179**, 670 (1997).

[22] C.J. Krieger, H.R. Beller, M. Reinhard, A.M. Spormann. *J. Bacteriol.*, **181**, 6403 (1999).

[23] O. Kniemeyer, T. Fischer, H. Wilkes, F.O. Glockner, F. Widdel. *Appl. Environ. Microbiol.*, **69**, 760 (2003).

[24] E. Annweiler, A. Materna, M. Safinowski, A. Kappler, H.H. Richnow, W. Michaelis, R.U. Meckenstock. *Appl. Environ. Microbiol.*, **66**, 5329 (2000).

[25] K.G. Kropp, I.A. Davidova, J.M. Suflita. *Appl. Environ. Microbiol.*, **66**, 5393 (2000).

[26] A. Baun, A. Ledin, L.A. Reitzel, P.L. Bjerg, T.H. Christensen. *Wat. Res.*, **38**, 3845 (2004).

[27] H.R. Beller. *Biodegradation*, **11**, 125 (2000).

[28] M.S. Elshahed, L.M. Gieg, M.J. McInerney, J.M. Suflita. *Environ. Sci. Technol.*, **35**, 682 (2001).

[29] D.E. Reusser, J.A. Field. *J. Chromatogr. A*, **953**, 215 (2002).

[30] L.M. Gieg, J.M. Suflita. *Environ. Sci. Technol.*, **36**, 3755 (2002).

[31] P. Martus, W. Püttmann. *Sci. Tot. Environ.*, **307**, 19 (2003).

[32] H.R. Beller. *Environ. Sci. Technol.*, **36**, 2724 (2002).

[33] J.A. Field, K. Monohan. *Anal. Chem.*, **67**, 3357 (1995).

[34] E. Eriksson, A. Ledin. *Intern. J. Environ. Anal. Chem.*, **83**, 987 (2003).

[35] C. Mossing, L.C. Larsen, H.C.L. Hansen, D. Seifert, P.L. Bjerg. Monitored natural attenuation (MNA) of petroleum hydrocarbons in a heterogenous aquifer affected by transient flow, paper presented at *Natural Attenuation of Environmental Contaminants. The Sixth International in situ and on-site Bioremediation Symposium*, 4–7 June, 2001, San Diego, CA, Batelle Press, Columbus, OH (2001).

[36] P. Kjeldsen, M.A. Barlaz, A.P. Rooker, A. Baun, A. Ledin, T.H. Christensen. *Crit. Rev. Environ. Sci. Tech.*, **32**, 297 (2002).

[37] L.A. Reitzel, A. Ledin. *J. Chromatogr. A*, **972**, 175 (2002).

[38] C. Mossing, L.C. Larsen. *Miljøprojekt 752*, Miljøstyrelsen, Copenhagen, Denmark (2003) (in Danish).

[39] L.M. Gieg, R.V. Kolhatkar, M.J. McInerney, R.S. Tanner, S.H. Harris, K.L. Sublette, J.M. Suflita. *Environ. Sci. Technol.*, **33**, 2550 (1999).

[40] D.E. Reusser, J.D. Istok, H.R. Beller, J.A. Field. *Environ. Sci. Technol.*, **36**, 4127 (2002).

[41] C. Griebler, M. Safinowski, A. Vieth, H.H. Richnow, R.U. Meckenstock. *Environ. Sci. Technol.*, **38**, 617 (2004).

[42] J.C. Miller, J.N. Miller. In *Statistics for Analytical Chemistry*, 2nd Edn, Ellis Horwood, Chichester, UK (1988).