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Journal of Chromatography A, 770 (1997) 229–241

JOURNAL OF CHROMATOGRAPHY A

Analysis of aliphatic carboxylic acids and amino acids in effluents of landfills, composting plants and fermentation plants by ion-exclusion and ion-exchange chromatography

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Abstract

Short chain aliphatic acids, including volatile fatty acids (VFAs), di-/tricarboxylic acids, hydroxy- and keto-acids were analyzed in landfill leachates and related water samples by two independently operated ion-exclusion chromatographic systems, differing mainly in the retention characteristics of the separation columns (Merck Polyspher OA-HY, Dionex HPICE AS6), and in the detection mode (UV absorbance at 210 nm, conductivity). The amino acid content of the samples was determined by ion chromatography. Because methods for amino acids analysis are widely standardized, the main efforts were undertaken to optimize the determination of carboxylic acids. The VFAs (7 compounds) contributed between 33% and 89% to the sample's dissolved organic carbon (DOC) content. The DOC proportions of the multifunctional acids (9 compounds) ranged from 1.1–49%. Between 0.9% and 13% of the DOC content was apportioned to amino acids. Main components were alanine, valine and leucine. The analytical efficiencies of the ion-exclusion chromatography systems were compared and the specific application properties are discussed.

Keywords: Waste leachates; Water analysis; Carboxylic acids; Amino acids

1. Introduction

The amount and composition of the total organic carbon (TOC) content of hazardous waste landfill leachates and seepage waters of domestic refuse dumps are closely related to the inventory of dumped materials, the landfill design, the disposal conditions and to the age of the dump site. Usually the leachates generated under aerobic conditions during the acidification stage of a landfill contain higher amounts of low-molecular-mass organic compounds such as carboxylic acids, amino acids, amines, carbohydrates and alcohols [1–3]. Effluents of composting plants and process waters of fermentation plants are expected to have similar chemical compositions.

For several reasons, the analytical determination of volatile fatty acids (VFAs), polyfunctional aliphatic acids and amino acids in such waters is very important because of ecological and environmental technological considerations, as the following questioned topics clarify: (1) condition, kinetics and products of the degradation of organic wastes, (2) control and direction of biological processes in landfills and fermentation plants, (3) mobilization of

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PII S0021-9673(96)01087-4

heavy metals by released organic complexing and chelating agents, (4) coelution of organic xenobiotics with organic acids and 'humin-like-substances' [4,5], (5) pollution of ground water and surface waters and (6) control of degradation efficiency of waste water treatment plants for specific organic sewage constituents.

Whereas data about the amino acid content of landfill leachates and related waste waters are scarce [2,6], more information is available about the occurrence of carboxylic acids. Most of the analysis was performed by means of GC (GC–flame ionization detection, GC–electron-capture detection, GC–MS) [2,3,7–16]. RP-HPLC is seldom applied for this purpose [17].

To date, no attempt which utilizes ion-exclusion chromatography (IEC) for this analytical task has been reported. This is surprising, because IEC has demonstrated in various areas of application (see [18] for a short overview) its suitability for the analysis of organic acids in complex aqueous matrices.

Consequently it is the main concern of the presented study to select and to check appropriate conditions for the ion-exclusion chromatographic analysis of landfill leachates and to investigate a series of leachates and waste waters from different origins for their carboxylic and amino acid content. Since GC methods are not optimal for the measurement of very hydrophilic, non-volatile hydroxy-, di- and tricarboxylic acids, emphasis was put on a satisfactory separation and quantification of these groups of compounds.

Therefore two analytical IEC systems, differing in the properties of the eluents, the separation columns and the detection mode, both operated under two sets of chromatographic conditions, were applied to enhance the chromatographic versatility and the certainty of substance identification.

2. Experimental

2.1. Materials

All materials were of puriss. or analytical-reagent quality (purity >99%) unless stated otherwise.

2.1.1. Carboxylic acids

Adipic acid, isobutyric acid, citric acid, D-glyceric acid (hemi-calcium salt, monohydrate, >98%), glycolic acid, glyoxylic acid, glutaric acid (98%), 2-hydroxybutyric acid, L-lactic acid (40% solution in water, purum), malonic acid, propionic acid, *n*-valeric acid, isovaleric acid, perfluorobutyric acid (PFBA, LC eluent) and tartaric acid were purchased from Fluka (Buchs, Switzerland). Acetic acid, *n*-butyric acid, formic acid, oxalic acid, pyruvic acid, succinic acid and potassium hydrogenphthalate were obtained from Merck (Darmstadt, Germany).

2.1.2. Amino acids

The hydrolysate standard E4 (Pierce, Rockford, IL, USA), containing the following 15 fluorescence detectable amino acids Ala, Arg, Asp, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Ser, Thr, Tyr, Val plus ammonia, served as reference material for calibrations. Individual amino acids were received from Fluka or Merck.

2.1.3. Inorganic chemicals

Nitric acid (conc.), hydrochloric acid (conc.), sulphuric acid (conc.), 0.005 *M* sulphuric acid (Titrisol), sodium hydroxide and various element standard solutions (matrix: diluted nitric acid) for inductively coupled plasma (ICP) calibration were received from Merck, tetrabutylammonium hydroxide (TBAOH, suppressor regenerant) from Riedel-de Haen (Seelze, Germany). Ready prepared sodium phosphate buffers for the chromatographic separation of amino acids were purchased from Pierce.

All aqueous solutions and dilutions were prepared with ultrapure Milli-Q water (Millipore, Eschborn, Germany). Calibration standards were made by mixing aliquots of aqueous stock solutions and subsequent dilution to the desired concentration by addition either of ultrapure water or diluted nitric acid or of the starting LC eluent, respectively.

2.2. Apparatus

Measurements of pH values were conducted using a digital pH meter (pH-DIGI 520; WTW, Weilheim, Germany), combined with an U-455-S7 electrode (Ingold, Germany). Calibration was performed with NBS buffers. Conductivity values were recorded by

Table 1
Instrumentation and operating conditions of the ion-chromatography/ion-exclusion chromatography systems

	Analytes				
	Carboxylic acids		Carboxylic acids		Amino acids
System unit	Gynkotek/Shimadzu modules		Dionex DX-500		Dionex DX-500
Separation column	Merck Polyspher OA-HY (with guard column)		Dionex IonPac HPICE-AS6		Type no. 4315, Pickering (with guard column)
Detection mode	Spectrophotometry (λ_{abs}=210 nm)		Conductivity		Fluorescence (λ_{abs}: 340 nm/λ_{em}: 455 nm)
Injection volume (μl)	20		25		50
Eluent	Sulphuric acid		PFBA		Sodium phosphate buffer gradient, NaOH
	Condition I	Condition II	Condition I	Condition II	
Eluent concentration (m*M*)	5	50	0.4	1.6	Variable (gradient)
Eluent pH value	2.2	1.1	3.3	2.75	Variable (3.15–≅13.7)
Column temperature (°C)	45	10	60	10	50
Flow-rate (ml min^{-1})	0.5	0.5	1.0	1.0	0.4
Suppressor/regenerant and flow (ml min^{-1})	–		AMMS–ICE (Dionex)/ 5 m*M* TBAOH/2.5–3.0		
Post-column derivatization reagent/eluent:reagent mixing ratio	–		–		OPA/1:0.8
Chromatography software	GYNKOSOFT (Gynkotek)		PEAKNET (Dionex)		PEAKNET (Dionex)

means of a measuring unit (WTW), consisting in the conductivity meter LF 521, the conductivity cell LTA 1 and in the temperature sensor TFK 530 for an automatical compensation of temperature influences.

The DOC content was analyzed by Rosemount–Dohrmann (Oberpfaffenhofen, Germany) DC 180 carbon analyzer using a combined UV-persulfate sample digestion method. The released carbon dioxide was purged with oxygen and measured by an IR detector. Calibrations were performed with solutions of potassium hydrogenphthalate. Leachates were filtered through 0.1 μm cellulose nitrate membrane filters (Sartorius, Göttingen, Germany) to separate dissolved and undissolved organic carbon fractions.

Metal concentrations were determined by a Spectroflame ICP atomic emission spectroscopy (AES) instrument (Spectro Analytical Instruments, Kleve, Germany). Concentrated nitric acid was added to all samples to minimize matrix influences on the detection sensitivity.

2.2.1. Ion chromatographic/ion-exclusion chromatographic analytical systems

Instrumentation and chromatographic conditions. The main features of the LC systems and the chosen operating conditions are summarized in Table 1. The properties of the separation columns are listed in Table 2.

The details of the analytical equipment are as follows:

(a) Gynkotek/Shimadzu system:

The device consisted of a Gynkotek (Germering, Germany) 600–200 dual piston high-pressure pump with a Gynkotek 250-B ternary gradient former and an Erma ERC-3520 eluent degasser unit, a Rheodyne (Cotati, CA, USA) 8125 injector for manual and automatic injection, a Gynkotek Gina 50 auto-sampler and an SPD-10AV Shimadzu (Duisburg, Germany) dual beam UV–Vis detector. Normal detection sensitivity was 0.01 AUFS. The columns were enclosed in a thermostat (Industrial Electronics, Langenzersdorf, Austria) for temperature regulation.

(b) Dionex DX-500 chromatographic unit, determination of carboxylic acids:

The analytical system comprised a dual piston high-pressure pump (GP-40), a quarternary gradient forming module, a helium degassing/purge unit for eluents and suppressor regenerant, a Dionex ASM autosampler, a LC-20 chromatography module with sample injection port and sample loop, and an ED-40 electrochemical detector, operated in the conductivity mode. The temperature of the separation column was maintained as described above.

(c) Different from the instrumentation under (b), amino acids were analyzed using a Thermo Separation Products SpectraSYSTEM AS 3000 (Fremont, USA) autosampler with sample cooling facility, a vacuum eluent degassing unit and a Shimadzu RF 551 fluorescence detector. To transform the amino acids to fluorescence detectable derivatives, the

Table 2
Column characteristics

Separation column	Merck Polyspher OA-HY	Dionex HPICE-AS6	Pickering (type-no.: 4315)
Resin	Merck (46-67A)	n.d.	n.d.
Analytes	Carboxylic acids	Carboxylic acids	Amino acids
Length (mm)	300	250	150
Inner diameter (mm)	6.5	9.0	4.0
Core material	PS–DVB[a]	PS–methacrylate–DVB	PS–DVB[a]
Functional group(s)	$-SO_3^-$	$-SO_3^-/-CO_2^-$	$-SO_3^-$
Cross-linking (%)	8	8	8–9
Particle size (μm)	8.0	8.0	5.0
Ion-exchange capacity ($mmol_c\ g^{-1}$)	1.08	n.d.	n.d.
Guard column	20×3 mm I.D., Same resin as separation column	– –	20×3 mm I.D., same polymer as in separation column, 8 μm Particle size

n.d.: no data available.

[a] Polystyrene–divinylbenzene polymer.

column outflow was mixed with *o*-phthaldialdehyde (OPA) reagent (prepared according to the recommendations of Pickering) in a T-piece and conducted through to a reaction coil. A constant reagent flow-rate was achieved applying a Dionex RP-1 low pulse mono piston pump.

2.3. Methods

In order to achieve improved determination of aliphatic carboxylic acids, two separation conditions were defined for both analytical systems. Thus it was possible to use the different responses (shift of retention times) of mono-, di- and hydroxycarboxylic acids to changes of column temperature and proton (eluent) concentration for their separation and identification. The utilized ground-laying retention principles as well as practical applications are described and discussed in earlier works [18,19].

Specific calibration standards with different substance combinations were prepared for each separation condition. Calibrations were performed by analysis of five concentration levels of standard mixtures, each injected twice. Calibration functions were calculated using curve fitting by means of linear regression analysis. Table 3 contains the retention times, k' values (void time measured with totally excluded compounds) and calibrated concentration ranges of the reference compounds, determined with both analytical systems.

Amino acids were analyzed according to a conventional method. Details including the gradient

Table 3
Retention times (t_R) and capacity factors (k') of organic acids under various chromatographic conditions

Acid	System Dionex				System Gynkotek			
	0.4 m*M* PFBA 60°C		1.6 m*M* PFBA 10°C		5 m*M* H_2SO_4 45°C		50 m*M* H_2SO_4 10°C	
	t_R (min)	k'	t_R (min)	k'	t_R (min)	k'	t_R (min)	k'
Acetic	13.88[d]	1.89	16.85[d]	2.44	13.94[d]	1.74	13.75[d]	1.81
Adipic	32.12[d]	5.39	n.m.		15.66[d]	2.08	19.65[d]	3.01
n-Butyric	39.80[d]	7.29	77.88	14.89	20.30[d]	3.00	20.73[d]	3.23
Citric	6.70	0.40	10.26	1.09	7.63	0.50	7.70	0.57
Formic	10.25[d]	1.14	12.54[d]	1.56	12.81[d]	1.52	12.72[d]	1.60
Glutaric	20.18[d]	2.98	44.40[d]	8.06	13.22[d]	1.60	15.47[d]	2.16
Glyceric	8.22[d]	0.71	9.73[d]	0.99	10.19[d]	1.01	9.98[d]	1.04
Glycolic	9.37[d]	0.95	11.08[d]	1.26	11.95[d]	1.35	11.36[d]	1.32
Glyoxylic	6.88	0.43	7.91	0.61	8.97[d]	0.77	8.66	0.77
2-Hydroxybutyric	14.87[d]	2.10	20.70	3.22	13.53	1.66	13.02	1.66
3-Hydroxybutyric	14.95	2.11	19.14	2.91	12.92	1.54	12.28	1.51
2-Hydroxyisobutyric	13.25	1.76	16.11[d]	2.29	12.23	1.41	11.26	1.30
2-Hydroxyisovaleric	22.68	3.73	38.36	6.83	15.38	2.03	14.92	2.04
2-Hydroxy-*n*-valeric	25.60[d]	4.33	47.71	8.74	17.02	2.35	17.32[d]	2.53
Isobutyric	35.32[d]	6.36	62.57	11.77	18.74[d]	2.69	18.61[d]	2.80
Isocitric	6.88	0.43	10.13	1.07	7.73	0.52	7.91[c]	0.61
Isovaleric	70.83	13.22	n.m.		23.54[d]	3.63	24.31	3.96
Lactic	10.85[d]	1.26	13.12[d]	1.68	11.49[d]	1.26	11.49	1.34
Malic	7.82	0.63	10.79	1.20	9.12	0.80	9.32	0.90
Malonic	6.77	0.41	9.94	1.03	9.36	0.84	10.19	1.08
Oxalic	4.98	0.04	5.20	0.06	5.73[a]	0.13	10.43	1.13
Propionic	21.62[d]	3.50	32.06[d]	5.54	16.55[d]	2.26	16.43[d]	2.35
Pyruvic	5.75[d]	0.20	7.63[d]	0.56	8.59[b]	0.69	9.06[c]	0.85
Succinic	12.80[d]	1.67	21.35[d]	3.36	11.27[d]	1.22	11.96[d]	1.44
Tartaric	7.29[c]	0.48	8.27	0.69	8.06	0.59	8.36	0.71
Tartronic	5.42	0.13	6.15[c]	0.26	7.22[c]	0.42	8.12	0.66
n-Valeric	n.m.		n.m.		28.22[d]	4.56	n.m.	

Calibrated concentration ranges (μmol l^{-1}), [a] 10–500, [b] 30–1500, [c] 50–2500, [d] 100–5000, [e] 250–5000.
n.m.: not measured capacity factors calculated with void volume of totally excluded compounds.

program and statistical evaluation of the repeated analysis of the Pierce hydrolysate standard are given in [20].

2.4. Sampling and sample pretreatment

Leachates of a hazardous waste disposal site (S1) and of a sewage sludge disposal site (S2) were directly taken from a seepage drainage pipe. Samples attributed to the composting plant (S3) were taken from a seepage collection tank. In the case of the fermentation plant, which is a multi-stage plant including a hydrolysis reactor and a methanization reactor, operated on a pilot scale, process waters (S4) were sampled from the hydrolysis reactor. The samples were filled in PE screw top tubes and frozen at −18°C. Immediately before analysis, the thawed samples were centrifuged for 30 min at about 9500 rpm. in a Biofuge 22R (Heraeus-Sepatech, Osterode, Germany) and filtered through 0.1-μm cellulose nitrate membrane filters. Dionex OnGuard-P polyvinylpyrrolidone (PVP) filter cartridges were used for the removal of strongly adsorbing, humin-like substances before injection into the LC eluent stream.

3. Results

3.1. Basic properties of leachates

Some basic properties of the leachates including heavy metal concentrations are listed in Table 4. Due to the different formation conditions, the pH value varies between 5.78 and 10.60. The high pH value of the sewage sludge disposal site leachate is caused by the addition of lime for sludge stabilization and sterilisation. All leachates contain high amounts of organic carbon. The maximum DOC was determined in the composting plant seepage water. With exception of nickel in leachates of the both disposal sites, the concentration of potentially environmental harmful metal ions has not reached critical levels.

3.2. Carboxylic acid content of sewage sludge dump leachate

The obtainable separation performance of the various chromatographic conditions and instrumentations for the analysis of aliphatic carboxylic acids is exemplarily demonstrated with the sewage sludge dump leachate. The chromatograms (Figs. 1–4) exhibit significant differences in resolution, peak

Table 4
Basic properties of leachates and seepage waters

	Sample code			
	S1	S2	S3	S4
Origin	Hazardous waste disposal site	Sewage sludge disposal site	Composting plant	Fermentation plant
pH	8.15	10.60	7.57	5.78
DOC ($mg\ l^{-1}$)	1038	5908	6734	1569
Conductivity ($mS\ cm^{-1}$)	41.4	10.4	17.9	12.3
Metal ions ($\mu g\ l^{-1}$)				
Cu	<80[a]	132	97	167
Zn	1026	6262	1592	5850
Cd	<99[a]	<99[a]	<99[a]	<99[a]
Pb	<200[a]	<200[a]	<200[a]	<200[a]
Ni	2975	4039	719	382
Cr	221	<73[a]	204	88
Fe	1109	1654	3080	6010
Mn	2155	<76[a]	7916	16 100

[a] Limit of determination.

shape and elution sequences. Generally a higher yield of chromatographic information is gained operating with the Gynkotek/Shimadzu system. More importantly, a combined evaluation of all chromatograms is necessary to get the full chromatographic information.

Highest chromatographic efficiency was reached working with the Gynkotek/Shimadzu system under condition I (high separation temperature, low eluent concentration, Fig. 1). Eleven analytes could be identified by retention times and ten were quantified. Due to the coelution of the substance pairs glycolic/lactic acid and formic/glutaric acid, two peaks could not be clearly assigned.

Separation condition II (Fig. 2) is characterized by a lower deprotonation of the acids, stronger hydrophobic and van der Waals interactions between the analytes and the stationary phase, and slower kinetics of processes regulating the distribution of analytes between the mobile and the stationary phase. Therefore peak broadening and tailing increases and the retention times are generally higher. In contrast to condition I, succinic acid elutes after glycolic/lactic acid and formic acid does not coelute with glutaric acid.

Compared with the Polyspher OA-HY column, the IonPac HPICE AS6 offers enhanced retention of hydroxy- and dicarboxylic acids. Under separation condition I (high temperature, low eluent concentration, Fig. 3) glycolic acid elutes before lactic acid and glutaric acid elutes baseline separated after formic and acetic acid. The beginning separation of lactic from formic acid is indicated. The quantification of both compounds is possible at equal concentrations.

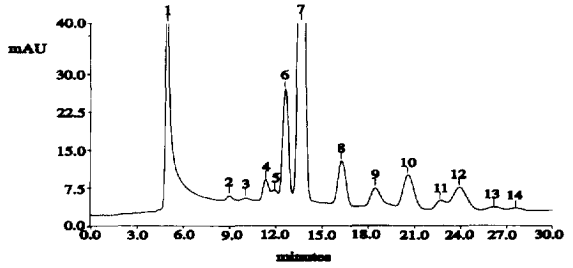

Fig. 2. Chromatogram of sewage sludge dump leachate. Conditions: Merck Polyspher OA-HY column and pre-column; temperature 10°C; flow-rate 0.5 ml min^{-1}; eluent 50 m*M* H_2SO_4; UV-detection (210 nm). Compounds (acids): 1=void volume; 2=pyruvic; 3=glyceric; 4=glycolic/lactic; 5=succinic; 6=formic; 7=acetic; 8=propionic; 9=isobutyric; 10=butyric; 11=unknown; 12=isovaleric; 13/14=unknown.

Analogously to the relation between chromatographic condition I and II, realized with the Gynkothek/Shimadzu system, the decrease of separation temperature and the increase of the proton concentration leads to an increase of the retention times and to a partial change of the elution sequence applying the HPICE-AS6 column (Fig. 4). Whereas

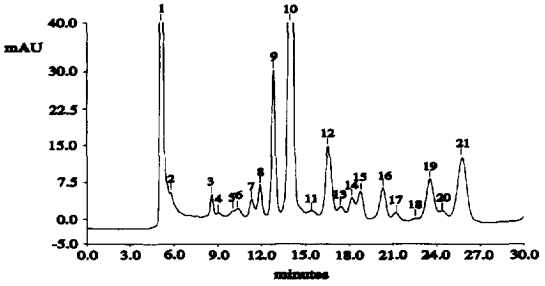

Fig. 1. Chromatogram of sewage sludge dump leachate. Conditions: Merck Polyspher OA-HY column and pre-column, temperature 45°C; flow-rate 0.5 ml min^{-1}; eluent 5 m*M* H_2SO_4; UV detection (210 nm). Compounds (acids): 1=void volume; 2=oxalic, 3=pyruvic; 4=glyoxylic; 5=unknown; 6=glyceric; 7=succinic; 8=glycolic/lactic; 9=formic/glutaric; 10=acetic; 11=adipic; 12=propionic; 13/14=unknown; 15=isobutyric; 16=butyric; 17/18=unkown, 19=isovaleric; 20/21=unknown.

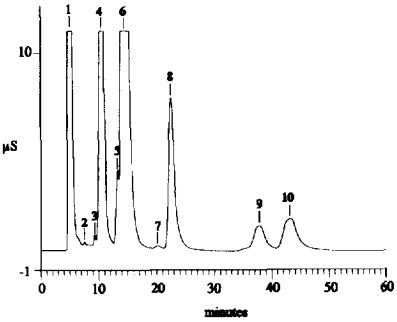

Fig. 3. Chromatogram of sewage sludge dump leachate. Conditions: Dionex IonPac HPICE-AS6 column; temperature 60°C; flow-rate 1 ml min^{-1}; eluent, 0.4 m*M* perfluorobutyric acid; conductivity detection. Compounds (acids): 1=void volume; 2=unknown; 3=glycolic; 4=formic; 5=succinic; 6=acetic; 7=glutaric; 8=propionic; 9=isobutyric; 10=butyric.

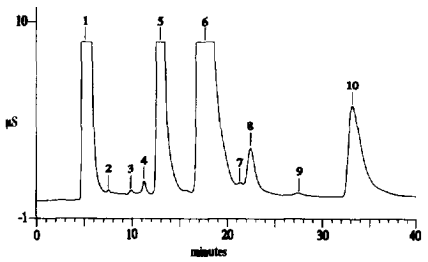

Fig. 4. Chromatogram of sewage sludge dump leachate. Conditions: Dionex IonPac HPICE-AS6 column; temperature 10°C; flow-rate 1 ml min^{-1}; eluent, 1.6 m*M* perfluorobutyric acid; conductivity detection. Compounds (acids): 1=void volume; 2=pyruvic; 3=glyceric; 4=glycolic; 5=formic/lactic; 6=acetic; 7=succinic; 8=unknown; 9=unknown; 10=propionic.

peak broadening and tailing are unfavourable, the condition II offers a broader analytical window for keto- and hydroxy acids with small k' values. Therefore pyruvic acid, glyceric acid and glycolic acid are well separated. The retention of succinic acid is strongly enhanced. It elutes now after acetic acid.

Interferences with bicarbonate did not arise operating the Gynkotek system and were proved to be minimal applying the Dionex system. The analysis of pure bicarbonate solutions by the latter chromatographic unit resulted in very small detection signals, correlated with retention times of 16.9 min (condition I) and 17.5 min (condition II). However these interferences can be eliminated by acidifying the samples followed by ultrasonic treatment.

The combined quantitative results of the leachate analysis are listed in Table 5. In total 15 compounds could be detected and 13 compounds quantified. Main components are the homologous straight-chain and branched-chain fatty acids. Additionally five di- and trifunctional acids such as glyceric and succinic acid are present in concentrations greater than 100 µmol l^{-1}.

Five acids were quantitated with both analytical

Table 5
Concentrations (µmol l^{-1}) of carboxylic acids in a sewage sludge landfill leachate

Acid	Separation unit					
	Dionex		Gynkotek/Shimadzu		SV/MV (µmol l^{-1})	R.S.D. (%)
	0.4 m*M* PFBA, 60°C	1.6 m*M* PFBA, 10°C	5 m*M* H_2SO_4, 45°C	50 m*M* H_2SO_4, 10°C		
Oxalic	[c]	[c]	[b]	[c]	–	–
Pyruvic	[b]	[b]	64	57	60	–
Glyoxylic	[d]	[d]	351	[d]	351	–
Glyceric	[b]	160	[b]	159	159	–
Glycolic	321[a]	378	[c]	[c]	350	–
Formic	9900[a]	[c]	[c]	9278	9589	–
Lactic	[b]	[c]	[c]	[c]	–	–
Acetic	53 100[a]	[b]	54 090	48 564	51 917	5.7
Succinic	543[a]	[b]	542[a]	526[a]	537	1.8
Propionic	5735	6589	6520	6273	6279	6.1
Glutaric	53	56	[c]	[d]	54	–
Adipic	127	[e]	136	[d]	132	–
Isobutyric	2145	[e]	2161[a]	1961	2089	5.3
Butyric	2713	[e]	2999	2726	2813	5.7
Isovaleric	[e]	[e]	4068	4001[a]	4035	–

[a] Not baseline separated.
[b] Detectable, because of a strong peak overlapping not quantifiable.
[c] Complete coelution.
[d] No chromatographic signal recorded.
[e] Not detectable (retention time>run time).
SV/MV: single method value/mean value.
R.S.D.=Relative standard deviation.

systems and three chromatographic conditions. The variation coefficients of these data sets vary between 1.8 and 6.1 and express a good correspondence between the single results.

3.3. Carboxylic acid content of further leachates and process waters

The chromatogram of the fermentation plant process water, recorded with the Gynkotek/Shimadzu system, condition I (Fig. 5), gives an impression of the different composition of the various samples. In contrast to the chromatogram shown in Fig. 1, the main detection signal in Fig. 5 is that of lactic acid. The homologous fatty acids are present in lesser concentrations. Dicarboxylic acids, except oxalic acid, are missing. A relatively intense signal at 18.1 min (peak No. 12), which occurs also with lesser peak area in chromatogram 1 (peak No. 14) could not be identified.

A general survey of the carboxylic acid contents of leachates and process waters is provided in Table 6. All samples were analyzed in the same manner as the sewage sludge landfill leachate. In total 16 organic acids could be detected including 9 compounds possessing more than one functional group (di-, hydroxy- and keto-acids). Both the sewage sludge landfill leachate and the composting plant seepage water are characterized by a high substance diversity, whereas only 8 compounds were identified in the hazardous waste landfill leachate. Three (acetic, formic and iso-valeric acid) from seven VFAs were found in all samples. Except the fermentation plant process water, the VFAs account for more than 90% of the mole sum of the dissolved organic acids. The concentrations of acetic, propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acid are highest in the composting plant seepage water. Even the mole percentages of propionic, *n*-butyric and *n*-valeric acid, related to the amount of organic acids, reached maximum values in this seepage water. The highest formic acid concentration was determined in the sewage sludge landfill leachate. Despite the differences in the absolute amounts of the components in the latter leachate and in the fermentation plant process water, the proportion of the individual acids on the mole sum of the VFAs increases in the same way following the sequence: *n*-butyric < isovaleric < propionic < formic < acetic acid.

The highest mole percents of acetic and isovaleric acid were found in the hazardous waste landfill leachate.

Lactic acid is the most important compound within the group of multifunctional acids. Because of signal interferences with formic acid, an exact quantification was not always possible. Similar problems hindered the quantification of oxalic acid.

The following multifunctional acids were present in concentrations of 100 $\mu mol\,l^{-1}$ or higher in at least three of the four seepage waters: glyoxylic, glyceric, glycolic and adipic acids.

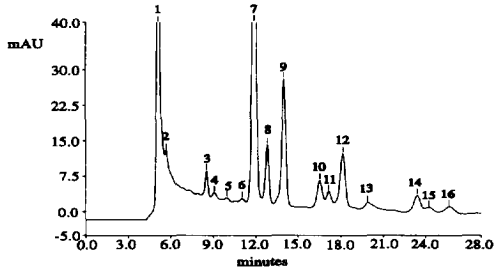

Fig. 5. Chromatogram of fermentation plant process water. Conditions: Merck Polyspher OA-HY column and pre-column; temperature 45°C; flow-rate 0.5 ml min^{-1}; eluent 5 m*M* H_2SO_4; UV detection (210 nm). Compounds (acids): 1=void volume; 2=oxalic, 3=pyruvic; 4=glyoxylic; 5=glyceric; 6=unknown; 7=lactic; 8=formic; 9=acetic; 10=propionic; 11/12=unknown; 13=butyric; 14=isovaleric; 15/16=unknown.

3.4. Amino acid contents

A typical chromatogram of an amino acid-rich leachate, 250-fold diluted prior to injection, is depicted in Fig. 6. Most of the components are well separated allowing quantification without compensation of matrix interferences.

Due to the high dilution ratio, only major components were detected. Fourteen of nineteen detection signals could be identified. Only one of the unidentified compounds (peak No. 14) showed a higher signal intensity. A closer view on the baseline elucidates that some trace components are present additionally.

The concentrations of the determined amino acid are listed in Table 7. The sample specific total amino

Table 6
General survey: aliphatic carboxylic acid content of seepage waters

Acid	Origin											
	Sewage sludge disposal site			Composting plant			Fermentation plant			Hazardous waste disposal site		
	SV/(MV) (μmol l^{-1})	S.D.	mol^{-1}%	SV/(MV) (μmol l^{-1})	S.D.	mol%	SV/(MV) (μmol l^{-1})	S.D.	mol%	SV/(MV) (μmol l^{-1})	S.D.	mol%
Oxalic	n.q.	–	–	n.q.	–	–	n.q.	–	–	n.q.	–	–
Pyruvic	(60)	5	0.1	n.q.	–	–	79	–	0.2	n.d.	–	–
Glyoxylic	351	–	0.4	490	–	0.3	269	–	0.7	269	–	1.2
Glyceric	(159)	1	0.2	594	–	0.4	222	–	0.5	n.d.	–	–
Glycolic	350	–	0.4	3145	–	1.9	n.d.	–	–	224	–	1.0
Formic	(9589)	482	12.2	1151	–	0.7	(3533)	158	8.7	102	–	0.5
Lactic	n.q.	–	–	603	–	0.4	(20 585)	483	50.6	n.d.	–	–
Acetic	(51 918)	2947	66.3	(76 386)	5181	45.9	(11 650)	404	28.6	(14 909)	231	67.2
Succinic	(537)	10	0.7	<25[a]	–	–	(119)	1	0.3	n.d.	–	–
Propionic	(6279)	380	8.0	(20 950)	902	12.6	(2020)	99	5.0	n.d.	–	–
Glutaric	(54)	3	0.1	119	–	0.1	n.d.	–	–	629	–	2.8
Adipic	(132)		0.2	<25[a]	–	–	n.d.	–	–	302	–	1.4
Isobutyric	(2089)	111	2.7	(2318)	132	1.4	n.d.	–	–	n.d.	–	–
Butyric	(2813)	162	3.6	(30 575)	2092	18.4	(903)	58	2.2	n.d.	–	–
Isovaleric	(4035)	47	5.1	7575	–	4.6	1331	–	3.3	3548	–	16.0
Valeric	n.d.	–	–	22 420	–	13.5	n.d.	–	–	2208	–	9.9
MolΣ	78 366			166 326			40 711			22 191		

n.d.: not detectable: n.q.: detected, not quantified.
[a] Detectable, concentration below determination limit.
SV/(MV): single method value/(mean value); S.D.: standard deviation.

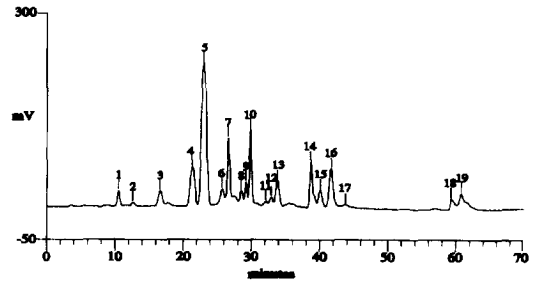

Fig. 6. Amino acid contents of a sewage sludge landfill leachate. Conditions: cation exchange column (Pickering); temperature 50°C; flow-rate 0.4 ml min^{-1}; gradient of Pickering buffer solutions (pH 3.15–7.4) and 0.2 *M* NaOH; fluorescence detection. Peaks: 1=Asp; 2=Ser; 3=Glu; 4=Gly; 5=Ala; 6=unknown; 7=Val; 8=Met; 9=Ile; 10=Leu; 11=unknown; 12=Tyr; 13=Phe; 14=unknown; 15=Lys; 16=NH_3; 17=His; 18/19=flushed impurities.

acid concentrations decrease as follows (total concentrations in mmol l^{-1}): sewage sludge landfill leachate (15.2)>composting plant seepage water (3.75)>fermentation plant process water (0.64)> hazardous waste landfill leachate (0.16). The latter one has an extremely poor amino acid content. Only histidine could be identified, but its concentration was higher than in the other samples. Except the hazardous waste landfill leachate, the samples are characterized by high concentrations of aliphatic monoaminomonocarboxylic acids without further functional groups such as alanine, glycine, valine and leucine.

3.5. DOC balance

The proportions of the various groups of organic acids on the DOC of the leachates and process waters are summarized in Table 8.

More than three quarters of the total DOC content were identified in the case of hazardous waste leachate, fermentation plant process water and composting plant seepage water. In the case of the seepage sludge leachate about a half of the DOC remained unidentified. Except the fermentation plant process water, the main fraction of the identified DOC is apportioned to the group of volatile fatty acids. Nearly half of the DOC of the fermentation plant water belongs to the fraction of multifunctional aliphatic acids with lactic acid as the main component. Multifunctional acids contribute also to a

Table 7
Amino acid concentration in leachates and seepage waters

Amino acid	Origin							
	Sewage sludge disposal site		Composting plant		Fermentation plant		Hazardous waste disposal site	
	(μmol l^{-1})	mol%	(μmol l^{-1})	mol%	(μmol l^{-1})	mol%	μmol l^{-1}	mol%
Aspartic acid	366.1	2.4	296.5	7.9	48.2	7.5	n.d.	–
Threonine	n.d.	–	167.5	4.5	24.5	3.8	n.d.	–
Serine	95	0.6	198.2	5.3	27.2	4.2	<5[a]	–
Glutamic acid	515.8	3.4	442.4	11.8	58.5	9.1	n.d.	–
Glycine	1983.6	13.0	270.2	7.2	55	8.6	n.d.	–
Alanine	7060.9	46.4	991.5	26.4	255.1	39.8	n.d.	–
Valine	1272.6	8.4	330.3	8.8	53.7	8.4	<5[a]	–
Methionine	269.8	1.8	82.2	2.2	6.2	1.0	n.d.	–
Isoleucine	398	2.6	199.8	5.3	24.5	3.8	n.d.	–
Leucine	1585.5	10.4	295.3	7.9	38.7	6.0	n.d.	–
Tyrosine	314	2.1	97.2	2.6	9.5	1.5	n.d.	–
Phenylalanine	801.5	5.3	106.3	2.8	13.4	2.1	n.d.	–
Lysine	499	3.3	198.7	5.3	26.3	4.1	n.d.	–
Histidine	61.8	0.4	43.7	1.2	n.d.	–	160.3	100.0
Arginine	n.d.	–	37.7	1.0	n.d.	–	n.d.	–
MolΣ	15 224		3757.5		640.8		160.3	

n.d.: not detectable.

[a] Detectable, concentration below determination limit.

Table 8
DOC balance

	Sewage sludge disposal site	Composting plant	Fermentation plant	Hazardous waste disposal site
DOC (mg l^{-1})	5908	6734	1569	1038
DOC fraction	(%) of DOC			
Volatile fatty acids (up to 5 C atoms)	34.98	88.70	33.13	67.86
Multifunctional aliphatic carboxylic acids	1.07	2.06	48.70	6.87
Amino acids	12.69	2.98	1.97	0.94
Total calculated DOC (%)	48.74	93.74	83.80	75.67
Not identified DOC (%)	51.26	6.26	16.20	24.33

certain portion of the DOC of the hazardous waste leachate.

Generally amino acids are of lesser importance in relation to its DOC proportions. A maximum amino acid DOC portion of 12.7% was determined in the sewage sludge leachate.

4. Discussion

The parallel operation of two ion-exclusion chromatography analytical systems, different in chromatographic separation properties and in the detection mode, provided complementary chromatographic information, resulting in an enhanced certainty of substance identification and in a higher flexibility for peak separation and quantification. With both systems wide linear calibration ranges, high analytical reproducibilities and minimized matrix interferences were achieved. The columns exhibit high separation specificities for aliphatic carboxylic acids, no cross-sensitivities for aromatic acids occurred.

A direct juxtaposition of the analytical properties of the applied separation systems for carboxylic acids reveals the following peculiarities:

(a) Gynkotek/Shimadzu system (including Merck Polyspher OA-HY column): (1) smaller peak widths, (2) lower capacity factors and therefore shorter retention times of volatile fatty acids (k' of isovaleric acid on Merck Polyspher OA-HY column, condition I: 3.63, ditto on Dionex HPICE-AS6, condition I: 13.22) and (3) lower eluent pH value reduces the dissociation of stronger acids such as oxalic or pyruvic acid and enables a higher retention of these compounds.

(b) Dionex DX-500 (including HPICE-AS6): (1) the detection mode (conductivity detection) is more specific for ionizable compounds eg. organic acids, (2) lower matrix influences on detection sensitivity and component separation and (3) higher flexibility to alter the retention of most compounds by changes of column temperature and eluent concentration.

Further improvements of the chromatographic methods to increase the resolution between lactic and formic acid on HPICE-AS6 and to select an appropriate analytical window for separation of oxalic acid on Polyspher OA-HY column seem to be necessary. The chromatography of acids with longer chain lengths (eg. caproic acid) within an acceptable time span should be possible after addition of an organic modifier (e.g. methanol) to the eluent.

As follows from the comparison of our findings with those of other authors, almost exclusively conducting GC or GC–MS analysis, the main advantage of the leachate analysis by IEC techniques is the capability to determine and quantify simultaneously volatile fatty acids (including formic acid!) and multifunctional aliphatic acids such as keto-, hydroxy-, di- and tricarboxylic acids. Possibly caused by the preference of the GC methods, no report has been published dealing in detail with the presence of multifunctional acids in landfill leachates up to date.

One of the limitations of some GC methods was mentioned by Beihoffer and Ferguson [12]. They stated that simple dicarboxylic acids (oxalic, malonic, succinic) did not chromatograph on a DB-FFAP column—a column which is widely used for the direct determination of carboxylic acids in water samples without derivatization [8].

Several papers give quantitative data about the VFA composition of sanitary landfill leachates

[2,3,15]. Due to remarkable differences in the formation conditions of the leachates, the following details should provide a rough orientation only. The DOC of the leachates spanned from 800 mg l^{-1} to 20 000 mg l^{-1}. The proportion of VFAs on the DOC content ranged from 49–87%. Always the predominant acids were butyric and acetic acid, followed by caproic or propionic acid. A certain correspondence between these data and our findings is only ascertainable in the case of the composting plant seepage water. The hazardous waste leachates differs in the absence of butyric and propionic acid, whereas the sewage sludge leachate contains higher amounts of formic acid.

The determination of polyfunctional and amino acids enabled us to identify higher DOC proportions even of such waters, whose organic fractions do not mainly consist in volatile fatty acids. Nevertheless, remarkable DOC proportions of the sewage sludge leachate and the hazardous waste leachate remained unidentified. The above cited investigations indicate, that aliphatic acids with longer carbon chains (hexanoic, heptanoic acids), amines, alcohols (ethanol), carbohydrates, unhydrolyzed proteins and humin-like substances may contribute together with various xenobiotics to the leachate DOC.

The main implications of our findings are under environmental analytical aspects:

1. To date, the content of polyfunctional acids and amino acids in landfill leachates and similar waters is underestimated or remains unconsidered.
2. Polyfunctional acids and amino acids may contribute to the metal complexation and mobilization capacity of leachates to a high degree. Therefore more attention should be paid to its presence in seepage waters of unsealed landfills and abandoned waste sites.

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