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On-line monitoring of biotechnological processes by gas chromatographic–mass spectrometric analysis of fermentation suspensions

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Abstract

An on-line monitoring system has been developed for the control of a bioreactor for the anaerobic pretreatment of an industrial waste water. The monitoring system is based on a process mass spectrometer with a temperature controlled membrane inlet. The membrane introduction mass spectrometer (MIMS) is coupled with a resistively heated metal gas chromatography capillary column, which serves as a transfer line between the bioreactor and the MIMS. Sampling and injection is performed by means of a pneumatically driven membrane probe, which enables monitoring of soluted and gaseous substances in the fermentation broth. The system can also be coupled to other processes.

Keywords: Fermentation; Membranes; Process monitoring; Gas chromatography–mass spectrometry

1. Introduction:

Membrane introduction mass spectrometry (MIMS) is extensively used for environmental analysis as well as for fermentation monitoring [1–4]. In the field of fermentation control, a flow-by and a flow-through configuration have been used so far [4]. For the flow-by configuration, the inlet membrane is mounted inside the fermenter broth, evacuated and directly coupled to the vacuum chamber of the mass spectrometry (MS) system. For the flow-through configuration, a liquid sample is continuously withdrawn from the fermenter and transported to the inlet membrane mounted inside or near the ion source. Hail and Yost [5] and Jain and Phillips [6] describe the separation of semi-volatiles and volatiles on resistively heated gas chromatography (GC) capillary columns. The GC runs described by the authors take about 2 min for the semi-volatiles and 2 s for the volatiles, the columns are up to 5 m long. Thermal programming is performed by a computer-controlled d.c. power supply.

Matz and Kesners [7] developed a new technique for the membrane extraction of organic carbons from aqueous matrices: the thermal membrane desorption application (TMDA).

Like the solid-phase microextraction (SPME) technique [8] the TMDA allows enriching organic carbons in a polymer and setting the trapped compounds free by a heat pulse. In contrast to SPME, TMDA enables the measurement of gases simultaneous with semi-volatile and volatile hydrocarbons and separates the liquid phase from the carrier gas stream.

The aim of this work has been to set up a reliable on-line monitoring system for gases and semi-volatile compounds in an industrial process.

The presented membrane probe adapts the TMDA

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technique to a biotechnological process and performs automatic sampling procedures in a fermenter. The two sampling membranes are installed in a membrane module, which is easily exchanged. A 25 m long metal GC capillary column has been mounted into a wire reinforced PTFE tube. The capillary column is resistively heated and combines sample transfer to the MS and chromatographic separation of the sample mixture. The MS used is equipped with a membrane inlet. The inlet membrane is thermostated to an adjustable temperature to maintain constant permeation conditions at the inlet.

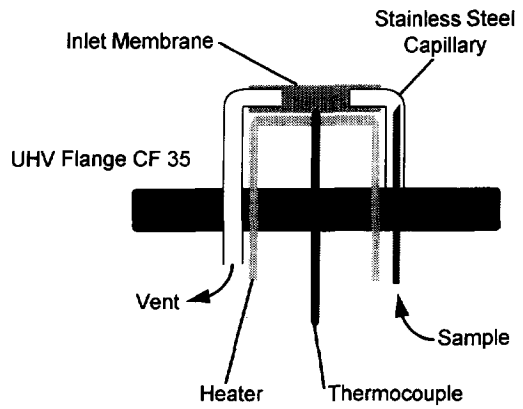

Fig. 1. Temperature controlled membrane inlet.

2. Experimental

2.1. Detector

An electron ionization (70 eV) quadrupole mass spectrometer (Leda Mass, Stoke-on-Trent, UK) with range 1–200 u was used for this work. The secondary electron multiplier was set at 1026 V. The MS system was equipped with a laboratory made membrane inlet.

2.2. Construction of the membrane inlet

The membrane inlet was assembled on a CF-35 ultra high vacuum (UHV) flange. The inlet membrane was a 2 mm long tubular polydimethylsiloxane (PDMS) membrane of 200 μm wall thickness and has an I.D. of 1 mm (Reichelt Chemietechnik, Heidelberg, Germany). It was stretched on stainless steel capillaries with an O.D. of 1.59 mm. The membrane was heated conductively by a coaxial heater (Philips, Hamburg, Germany). A Philips thermocouple measured the temperature at the membrane's surface. The membrane temperature was adjustable between 50–175°C, in the present work 110°C was been chosen. The passages of capillaries, heater and thermocouple into the vacuum chamber were tightened with silver solder. Fig. 1 shows a sketch of the membrane inlet.

2.3. Construction of GC transfer capillary

A metal GC capillary column (Restek MXT-5) with a 3 μm DB-5 stationary phase and an I.D. of 530 μm was used as transfer capillary. For electric insulation, the capillary column was covered with an PTFE shrinking tube AWG 24 (Detacta, Hamburg, Germany). Four PTFE spacing discs per meter were placed on the 25 m long column. This set up was passed through a wire reinforced PTFE tube (TUBOFLEX, Hamburg, Germany). Fig. 2 outlines the set up. The capillary column was heated by a d.c. voltage from a computer programmable XKW 300-3.5 d.c. power supply (Xantrex Technology, Burnaby, Canada).

2.4. Construction of membrane probe

The membrane probe was developed at the institute and manufactured by the university's mechanic workshop. The 45 cm long probe was equipped with two tubular PDMS sampling membranes. The membranes (Reichelt Chemietechnik) were 10 cm long with an inner diameter of 1 mm and a wall thickness

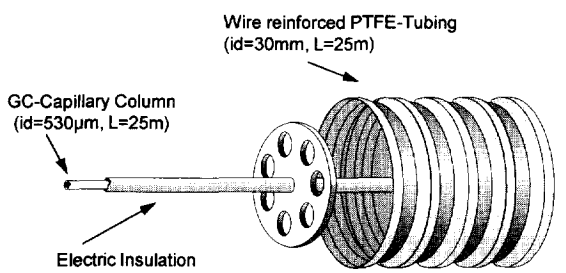

Fig. 2. GC transfer capillary column for sample transfer and chromatographic separation.

of 200 μm. They were mounted in an exchangeable membrane module, 20 mm in diameter. The module exchange was possible without interrupting the fermentation process. The module carried two membranes in order to combine different membrane materials and to vary membrane area. A schematic sketch of the membrane probe is shown in Fig. 3. Components like carrier gas supply valves and charcoal filters were placed inside the pneumatic piston. Pneumatic valves for probe operation were fixed under the pneumatic cylinder.

2.5. Chromatographic conditions

The carrier gas was nitrogen of 99.999% purity with a flow-rate of 6 ml/min.

The injection was performed by thermal desorption of the sampling membranes at 180°C for 60 s while the GC column was at ambient temperature. After the injection, a simple two stage temperature program is carried out: after 90 s, the column temperature reached 120°C; after another 60 s, it reached 200°C where it was held for 300 s.

2.6. Solubility in PDMS

Solvent solubility in PDMS was studied by static sorption experiments as published by Blume et al. [9]. Polymer samples with 33% (w/w) of silica filler were desorbed in a heating cupboard and weighed. Afterwards, the samples were submerged for one week in bottles containing liquid solvent. Mass uptake was determined by weighing a polymer sample after drying by paper blotting.

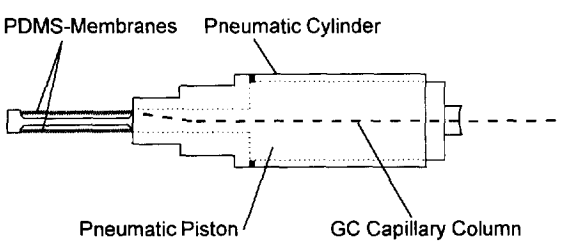

Fig. 3. Membrane probe: total length 450 mm; diameter at top 20 mm.

3. System operation

A complete analysis cycle comprised sampling followed by injection and chromatographic separation.

For sampling, the membrane was immersed into the fermentation broth. Gases and volatile organic compounds (VOCs) permeate the membrane, whereas semi-volatile organic compounds (SOCs) dissolve in the membrane material and are enriched. Other substances hardly interact with the membrane, especially particles and ions are excluded. During the sample phase, carrier gas flowed with a rate of 6 ml/min at the membrane interior and carried gases to the MS, VOCs are trapped on the capillary column. As a consequence gases are detected on-line (Fig. 4a).

After finishing the sample phase, the membranes were pneumatically driven out of the fermenter and the fermentation suspension was removed. The membrane was heated to a temperature of 180°C by a titanium heating wire mounted inside the membrane. As a consequence, the sampled organics were set free, flushed onto the GC transfer column by the carrier gas and trapped at the column head (Fig. 4b). The injection split ratio was adjusted by throttle valves. Injections in the present work were performed in the splitless mode.

Simultaneous to the subsequent GC-run, a backflush stream flowed through the heated membrane and cleaned it of residual organics. This significantly reduced memory effects of the membrane. Fig. 5 shows the different operating modes. After completion of the GC run, the next analysis cycle was started.

4. Characterisation of GC transfer column

No insulating material was used for the set up of the transfer capillary column. The first reason was, that as long as no convection occurs, the air between column and outer tube is a good insulator in comparison to, for instance, asbestos ($\lambda_{AIR}(200°C)=0.02$ W/mK, $\lambda_{ASB}(200°C)=0.2$ W/mK [10]). Additionally, air has a low heat capacity and enables, in combination with the capillary column's low heat capacity, fast temperature programs. Finally, it was

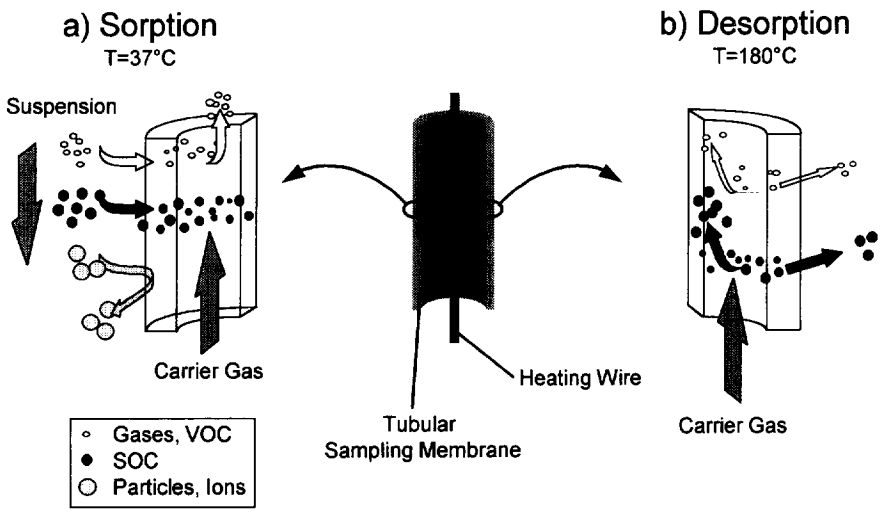

Fig. 4. Sampling with TMDA.

shown in [11], that an insulation with an outer diameter less than a critical diameter (d_{crit}) does not reduce but increases the heat flow by increasing the heat exchange area. The critical diameter is defined as follows:

$$d_{crit} = 2 \cdot \frac{\lambda}{\alpha} \quad (1)$$

with λ the heat conductivity of the insulation and α the heat transfer coefficient at the insulation surface. The critical diameter for the presented application is approximately 5 cm.

The linear temperature dependence of the column resistance was evaluated and was used for temperature control of the capillary column.

The way the column temperature follows a set temperature has been investigated with time response experiments. The d.c. current was varied stepwise

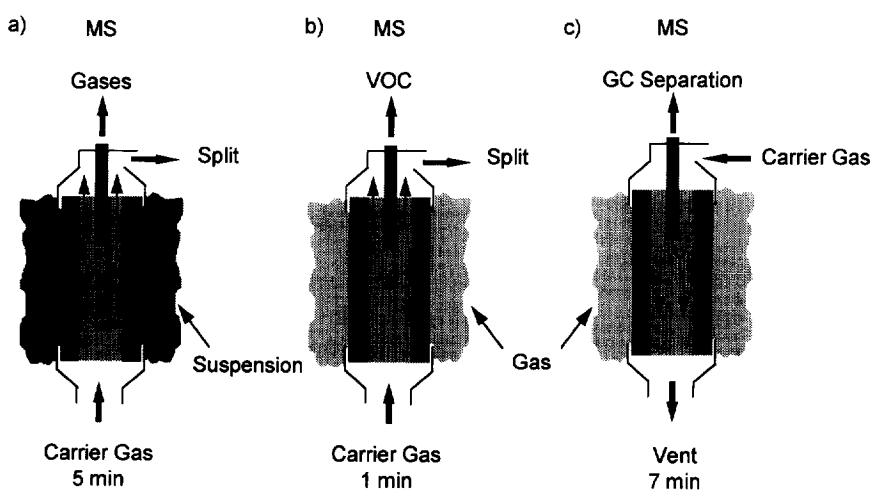

Fig. 5. Membrane probe operating modes: (a) migration into and through the membrane; (b) thermal desorption for injection; (c) thermal desorption for cleaning.

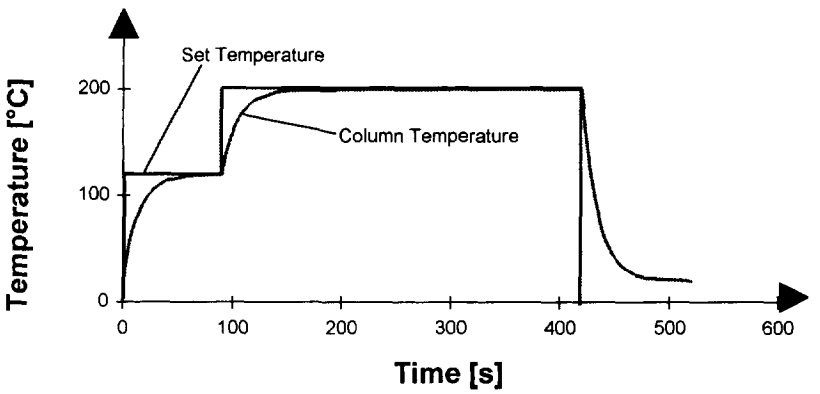

Fig. 6. Temperature program applied to transfer capillary column.

and the lapse of the d.c. voltage over the column was monitored. It showed that the capillary column can be described as a first-order lag element with a time constant t_c of 14.4 s. This means that a set temperature value is approximated to 63% after 14.4 s and after 43.2 s to 95%.The temperature response is expressed as:

$$T(t) = T_{SET} \cdot \left[1 - \exp\left(-\frac{t}{t_c} \right) \right] \quad (2)$$

In this work, a simple two step temperature program was applied and is depicted in Fig. 6. The reproducibility of temperature can be expressed in terms of reproducibility of compound retention times. Average retention times and their standard deviation are given in Table 1 for several compounds.

The poor reproducibility of compound retention times is tolerable in our case, because the composition in compounds of the fermentation suspension does not change suddenly and compounds are identified by their mass spectra.

Table 1
Reproducibility of compound retention times

Compound	Retention time (s)	Standard deviation (s)
Dimethylsulfide	139	7 ($n=10$)
Butanethiol	195	10 ($n=10$)
Phenol	304	16 ($n=10$)
Cresol	369	20 ($n=10$)
Indole	427	23 ($n=10$)

5. Characteristics of membrane sampling

The TMDA sampling process has been earlier described as membrane permeation process [7]. Permeation can be split into two processes with different characteristics: sorption, which is an equilibrium driven process and diffusion, which is a rate driven process. The method detection limit depends on both processes. Whereas diffusion determines the rate of reaching a steady state or equilibrium, sorption determines the maximum amount of substance available for detection in equilibrium or at steady state. The sorption of an analyte in a polymer depends on analyte–polymer interaction or solubility. Solubility in polymers can be described with dependence on a multitude of dimensions. Usually the critical temperature of a substance is related to its solubility in a polymer [12]. Another important dimension for sorption in PDMS is the analyte polarity as displayed in Fig. 7.

The mass transfer process into and through the sampling membrane is similar to pervaporation. Pervaporation at steady state is described with a three resistances model [13]. The three resistances are:

1. liquid to membrane transfer resistance
2. membrane transfer resistance
3. membrane to gas phase transfer resistance.

Applying the above model to TMDA one has to consider that sampling and desorption are transient

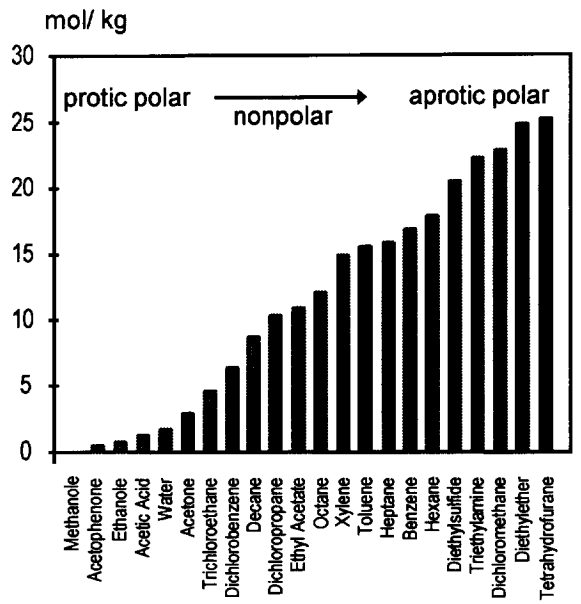

Fig. 7. Solubility in PDMS. Own data obtained according to Ref. [9].

processes. Transient diffusion is described by Fick's second law. The complete set of model equations for sampling with a planar membrane are as follows:

$$\frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial x^2} \tag{3}$$

$$-D \cdot \frac{\partial C}{\partial x} = k_l \cdot \left(C_{\text{Feed}} - \frac{C}{K^{\text{L}}_{\text{Mem}}} \right) \tag{4}$$

$$-D \cdot \frac{\partial C}{\partial x} = k_g \cdot \left(\frac{C}{K^{\text{G}}_{\text{Mem}}} - C_{\text{Gas}} \right) \tag{5}$$

Eq. 3 describes the diffusional mass transport within the membrane material. Eq. 4 is the border condition at the outer membrane surface and describes the convective mass transport from the bulk solution to the membrane surface. The convective mass transfer coefficient k_l is obtained from [14]. The membrane surface is assumed to be in equilibrium with the solution next to the surface. The equilibrium is described similar to Henry's law with the distribution coefficient $K^{\text{L}}_{\text{Mem}}$, which is obtained from [15]. Similar to Eq. 4, Eq. 5 is the border condition at the inner membrane surface, which is in contact with the

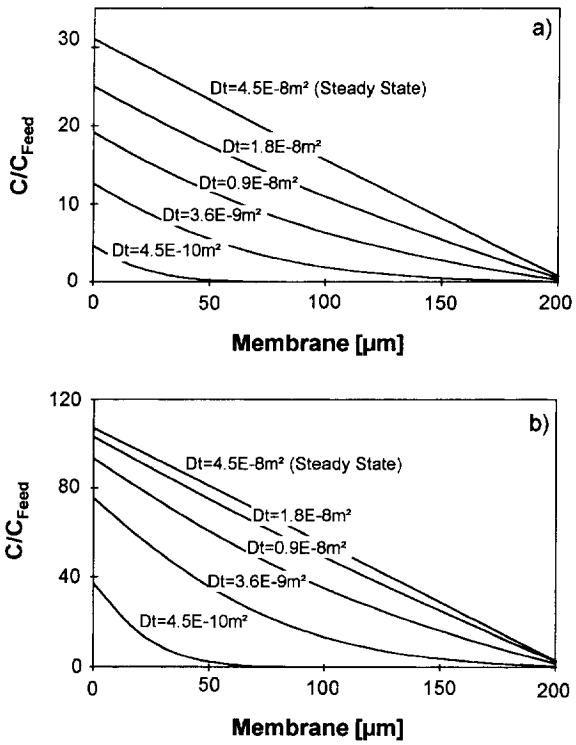

Fig. 8. Solution of Eqs. 3–5. Equilibrium sorption at $C/C_{\text{Feed}} = 145$.

carrier gas. The coefficients k_g [16] and $K^{\text{G}}_{\text{Mem}}$ [15] are applied analogically.

The above set of equations has been solved to determine an optimum sampling time and to estimate the possible extent of sorption. Fig. 8a and b shows the result for a 200 μm thickness membrane with two different values of k_l, all other coefficients are unchanged. Values for all coefficients are given in Table 2.

Three points are worth mentioning in respect to Fig. 8a and b.

1. As the membrane to gas phase mass transfer has a

Table 2
Coefficients of numerical solution for Fig. 8a and b

	k_L (m/s)	k_G (m/s)	$K^{\text{L}}_{\text{Mem}}$ (-)	$K^{\text{G}}_{\text{Mem}}$ (-)
Fig. 8a	$1 \cdot 10^{-5}$	0.2	213 000	145
Fig. 8b	$1 \cdot 10^{-4}$	0.2	213 000	145

much higher rate than the liquid to membrane mass transfer the concentration at the inner membrane surface remains near zero.
2. The ratio of the diffusion coefficient D and the mass transfer coefficient k_l have a strong impact on the extent of sorption at steady state and the steepness of the concentration profile across the membrane.
3. The diffusion coefficient D mainly determines the time to reach steady state.

By dividing the expression D_t in Fig. 8a and b by a diffusion coefficient, the concentration profile at time t is obtained. The time to reach steady state for n-butanol ($D = 6.5 \cdot 10^{-11}$ m^2/s) and chloroform ($D = 6.5 \cdot 10^{-10}$ m^2/s) is derived from $D_t = 4.5 \cdot 10^{-8}$ m^2 to be 11.5 min and 70 s, respectively.

Equilibrium will be attained if, for instance, no mass transfer at the membrane interior is admitted.

The sampling time often is laid down by application requirements. In connection with the control of a bioreactor analyses have to be performed every quarter of an hour. This constrains the sampling time to 5 min.

6. Measurements

The GC–MS system has been developed for the Biogas-Tower reactor described in Ref. [17]. Waste water from a yeast production line is anaerobically pretreated and biogas is produced. Measurements have been performed in the fermentation suspension of this bioreactor. Fig. 9 shows a total ion chromatogram of a suspension analysis. Substances are identified by their mass spectra. Dimethylsulfide and butanethiol are typical metabolites in the presence of sulfur reducing micro organisms. Phenol and cresol are metabolites and toxic at concentrations higher than 500 mg/l [18]. Ethylphenol has been detected in the feed as well, no degradation has been observed. Indole is a degradation product of tryptophan.

The time constants of the reactor processes are about 30 min. This affords analysis times under 15 min. Fig. 10 shows two subsequent analysis cycles in aqueous solution and demonstrates the ability to

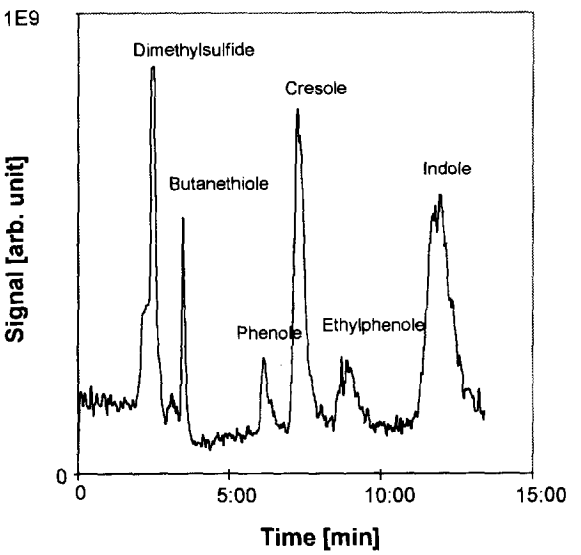

Fig. 9. Total ion chromatogram of fermentation suspension. Analysis performed with the presented set-up.

meet this demand. The measurements are divided into sampling phase and GC run. Gases are detected on-line in selected ion monitoring (SIM) mode during the first and SOCs are detected in scan mode during the second phase. For process control, it is important to detect quantitatively compounds relevant to the process. Table 3 gives data characterising the linear response of the analysis system for several compounds. The detection limit for phenol and cresol is 10 mg/l. TMDA detection limits for typical environmental pollutants are given in Ref. [19]. The relative standard deviation of signal intensities at constant conditions in aqueous solution has been investigated to be less than 20% (Table 4).

7. Conclusion

The TMDA sampling technique combined with MS was applied to a biotechnological process. Analysis cycles were performed within 15 min. Gases and semi-volatile organic carbons were detected quantitatively within one analysis cycle. The method detection limit is in the range of some ppm and depends on sampling time and solubility of the respective substance in the applied sampling membrane.

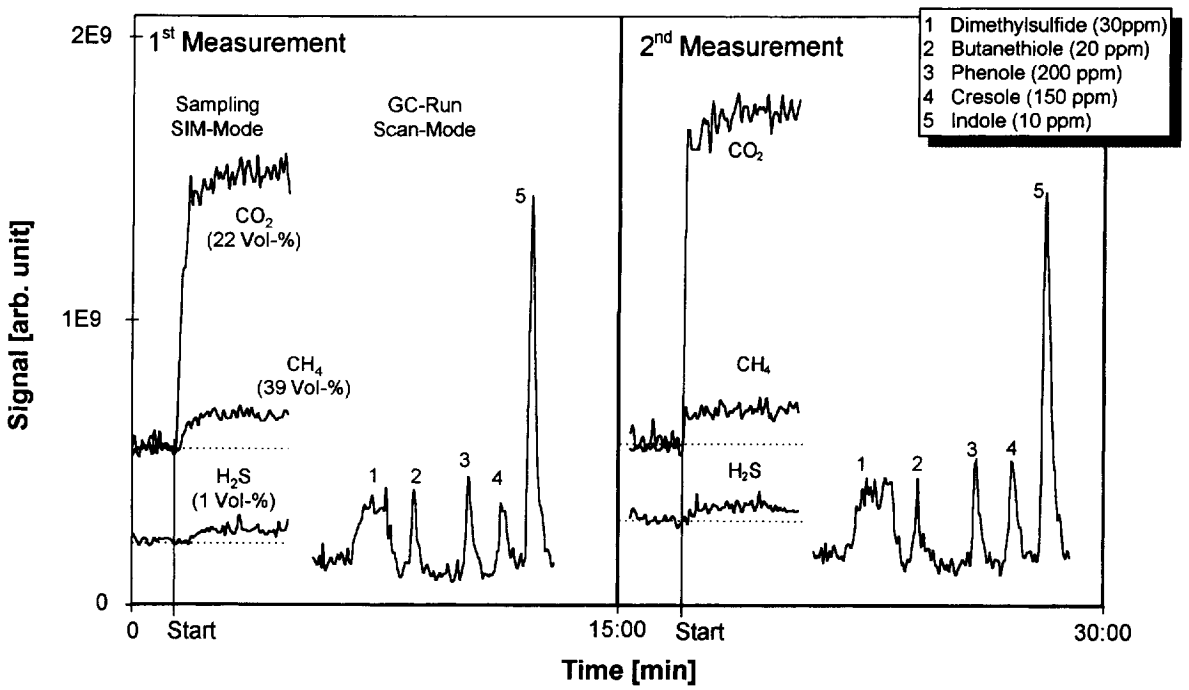

Fig. 10. Two subsequent analysis cycles performed with the presented system in aqueous solution. SIM: selected ion monitoring.

Table 3
Linearity of response for several compounds

Compound	Range of concentration (mg/l)	Response [counts/(mg/l]	Correlation coefficient	Data points
Dimethylsulfide	10–100	$2.06 \cdot 10^7$	0.93	14
Butanethiol	10–100	$4.18 \cdot 10^7$	0.92	13
Phenol	10–600	$0.45 \cdot 10^7$	0.95	13
Cresol	10–450	$0.57 \cdot 10^7$	0.94	14
Indole	10–100	$7.01 \cdot 10^7$	0.92	13

Table 4
Reproducibility of signals for several compounds at constant conditions

Compound	Concentration (mg/l)	R.S.D. at pH = 7.7 (%)	R.S.D. at pH = 3.4 (%)
Dimethylsulfide	65	14.2	5.4
Butanethiol	42	15.8	12.5
Phenol	307	5.2	5.0
Cresol	302	12.0	7.6
Indole	23	7.4	7.3

8. List of symbols

C	Concentration in the membrane
C_{Feed}	Concentration in the feed suspension
C_{Gas}	Concentration in the carrier gas
d_{crit}	Critical diameter
df	Thickness of stationary phase
D	Diffusion coefficient in the membrane
I.D.	Inner diameter
O.D.	Outer diameter
K^{G}_{Mem}	Distribution coefficient membrane–carrier gas
K^{L}_{Mem}	Distribution coefficient liquid phase–membrane
k_g	Mass transfer coefficient membrane–carrier gas
k_l	Mass transfer coefficient liquid phase–membrane
T	temperature
T_{SET}	Set temperature
t	Time
t_c	Time constant
α	Heat transfer coefficient
λ	Thermal conductivity
λ_{AIR}	Thermal conductivity of air
λ_{ASB}	Thermal conductivity of asbestos

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