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On-line monitoring of continuous beer fermentation process using automatic membrane inlet mass spectrometric system

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Abstract

A fully automatic membrane inlet mass spectrometric (MIMS) on-line instrumentation for the analysis of aroma compounds in continuous beer fermentation processes was constructed and tested. The instrumentation includes automatic filtration of the sample stream, flushing of all tubing between samples and pH control. The calibration standards can be measured periodically. The instrumentation has also an extra sample line that can be used for off-line sample collection or it can be connected to another on-line method. Detection limits for ethanol, acetic acid and eight organic beer aroma compounds were from $\mu g\,l^{-1}$ to low $mg\,l^{-1}$ levels and the standard deviations were less than 3.4%. The method has a good repeatability and linearity in the measurement range. Response times are shorter than or equal to 3 min for all compounds except for ethyl caproate, which has a response time of 8 min. In beer aroma compound analysis a good agreement between MIMS and static headspace gas chromatographic (HSGC) measurements was found. The effects of different matrix compounds commonly present in the fermentation media on the MIMS response to acetaldehyde, ethyl acetate and ethanol were studied. Addition of yeast did not have any effect on the MIMS response of ethanol or ethyl acetate. Sugars, glucose and xylose, increased the MIMS response of all studied analytes only slightly, whereas salts, ammonium chloride, ammonium nitrate and sodium chloride, increased the MIMS response of all three studied compounds prominently. The system was used for on-line monitoring of continuous beer fermentation with immobilised yeast. The results show that with MIMS it is possible to monitor the changes in the continuous process as well as delays in the two-phase process.

Keywords: On-line measurement; On-line analysis; Membrane inlet mass spectrometry; Continuous beer fermentation; Matrix effects

1. Introduction

Controlling of industrial scale fermentation processes is based mainly on chemical and physical analysis of process control samples. In order to control a process effectively, one needs to know exactly when and where changes in the process come up. This creates a need for fast, accurate and high throughput analytical methods. Off-line methods do not usually meet these demands; they are time consuming and prone to errors. Therefore, various on-line analytical methods, such as flow injection analysis (FIA), liquid chromatography (HPLC), infrared spectroscopy (IR), gas chromatography (GC) and mass spectrometry (MS), have become more and more popular in fermentation monitoring during the past decades [1].

Membrane inlet mass spectrometry (MIMS) is a specific and sensitive method of analysis of volatile organic compounds (VOCs) in aqueous as well as in gaseous samples. It is a technique based on the separation of volatile organic analytes from a water or gas sample by a thin membrane, which is installed between the sample and the ion source of a mass

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spectrometer [2]. Organic compounds dissolve in the membrane, permeate through it and finally evaporate into the mass spectrometer. Silicone membrane, which is the type of membrane most commonly used, is highly selective for organic compounds relative to water or the major constituents of air and 10- to 100-fold of enrichment of the organic compounds compared to water or air can be obtained [3].

Being a relatively simple and rugged technique with high chemical specificity and high sensitivity for VOCs, MIMS can readily be applied for the on-line monitoring of fermentation processes. It does not need any sample pre-concentration and can be easily adapted to various sample introduction systems and to different mass analysers. A great advantage of MIMS is that different fermentation products can be monitored simultaneously on-line [4,5].

Reuss et al. were the first to report an application of MIMS for fermentation monitoring in 1975 [6]. Since then the use of MIMS in the area of fermentation and bioprocess monitoring has been increasing steadily, as evidenced by recent reviews [7–9]. For example, MIMS has been used to monitor ethanol [10] and acetone–butanol [11] fermentations, brewing [12], production of cider [13] and cheese [14] as well as processes of respiration [15], methanogenesis [16] and denitrification [17,18].

In favourable cases MIMS can be used for the on-line monitoring of biological reactions at the extremely low ng l^{-1} level [19]. MIMS instrumentation can quite easily be adapted for operation in a harsh industrial product environment [20]. All operations of MIMS on-line monitoring systems can be automated and it is also possible to connect automated feedback control to the monitoring instrumentation. The feedback control system can be employed e.g. to automate substrate addition [5,21].

MIMS is very comparable to the purge-and-trap gas chromatography mass spectrometry (P&T) [22] and static headspace gas chromatography (HSGC) in the determination of VOCs in aqueous samples [22–24]. The main advantages of MIMS method compared to the more conventional P&T and HSGC methods are low detection limits, short analysis times and applicability for continuous on-line monitoring [4,5,19]. The drawback of the MIMS method is the lack of chromatographic resolution of components and a multicomponent spectrum of all analytes that pass through the membrane is obtained. This can cause difficulties when biological samples containing a large amount of different compounds are analysed. This problem can be resolved in most cases with Solver deconvolution program [25].

When MIMS is utilised for precise quantitative analysis, the knowledge of sample matrix is essential. Different matrix compounds present in the sample can change permeation rates and ionisation efficiencies of analytes, cause long-term memory effects and change membrane properties [26–29]. Although it is obvious that matrix compounds present in the sample play an important role in the MIMS analysis, rather few studies on the effect of matrix compounds has been done.

In the MIMS analysis of aqueous solutions of ethanol–acetoin (3-hydroxybutan-2-one) high concentrations of ethanol enhanced the permeation of acetoin while the permeation of ethanol was unaffected by the presence of acetoin. When measurements were done in chemical ionisation mode at high concentrations the signals of both compounds in the ternary solutions were lower than those for binary solutions [26]. Polyethylene glycol (PEG) present in the solution of 1-octanol was found to decrease the MIMS response of 1-octanol in two different ways. Firstly, PEG adsorbed on the surface of silicone membrane and secondly, PEG formed non-volatile PEG–octanol complexes with 1-octanol [27]. It has also been observed that addition of sugars, sugar alcohols or yeast cells to the ethanol solution dramatically reduces the pervaporation flux of ethanol [28]. On the other hand according to Kasthurikrishnan and Cooks, [29] seawater, which is a very complex mixture containing e.g. salts, dissolved gases and organic matter, had no significant effect on the signal response, response times and detection limits of several VOCs compared to pure water.

Although MIMS has been utilised in the on-line monitoring of different types of fermentations, it has seldom been applied for on-line monitoring of beer fermentations [12]. In our present study MIMS instrumentation and the methodology for on-line analysis of continuous beer fermentation processes were developed. The design of the on-line monitoring system with the analytical performance data is presented in this paper. Beer fermentation media are complex mixtures containing e.g. sugars, yeast cells, dissolved gases and minor amounts of different salts that may affect the MIMS analysis. In our study the matrix effects of sugars, salts, carbon dioxide and yeast on the MIMS response of selected organic compounds were also studied. A preliminary account of some of this work has been published by Mattila et al. 2003 [30].

2. Experimental

2.1. Chemicals and reagents

The stock solutions of beer aroma compounds used in the testing of the on-line instrumentation were made by weighing 1 g of compound into 100 ml of methanol (Riedel-de Haën, Seelze, Germany, 99.8%). All further dilutions were made in deionised water. Aqueous standard solutions used in matrix effect studies were prepared by directly dissolving pure standard compounds into deionised water.

The standard compounds used were acetic acid [64-19-7] 99.8%, hydrochloric acid [7647-01-0] 37% and sodium chloride [7647-14-5] from Riedel-de Haën (Seelze, Germany); ethyl acetate [141-78-6] 99.9%, 1-propanol [71-23-8] 99.8%, 2-methylpropanol [78-83-1] 99%, ammonium chloride [12125-02-9] and ammonium nitrate [6484-52-2] from Merck (Darmstadt, Germany); ethanol [64-17-5] 99.5% from Primalco Oy (Rajamäki, Finland); 3-methylbutyl acetate 97.0% [123-92-2], ethyl caproate 99% [123-66-0],

2-methylbutanol 99.5% [1565-80-6], acetaldehyde 99.5% [75-07-0], 3-methylbutanol 98.5% [123-51-3], glucose [50-99-7] and D-xylose [58-86-6] from Fluka Chemie AG (Buchs, Switzerland); carbon dioxide [124-38-9] from Oy AGA Ab (Riihimäki, Finland); yeast from Suomen Hiiva Oy (Lahti, Finland).

2.2. On-line instrumentation

A schematic picture of the experimental set-up for the on-line MIMS-measurement of fermentation medium is presented in Fig. 1.

The sampling system and mass spectrometer are controlled manually or automatically by a portable computer. A DT9802 data acquisition module, DO-board, (Data Translation Inc., Marlboro, MA, USA) handles the data transport between the computer and the rest of the analytical system. Measurement routines are automated with a visual-programming environment HP VEE (Hewlett Packard, Palo Alto, CA, USA) with DT VPI (Data Translation Inc., Marlboro, MA, USA).

The fermentation medium is pumped continuously by a peristaltic pump P1 (Cole-Parmer 7554-85; Cole-Parmer, Vernon Hills, IL, USA) at 50 ml min^{-1} to the filter unit. To achieve a sterile and cell free sample for the analysis the sample taken from the fermentor is filtered through a 0.22 μm autoclavable hydrophilic filter unit (A-SEP, Applikon, Applicon Dependable Instruments, Schiedam, The Netherlands). With this sampling system it is also possible to take samples alternately from four different fermenters and then also four filters are needed. A peristaltic pump P4 (Ismatec Reglo MS-2/8-160; Ismatec, Ismatec SA, Switzerland) sucks culture medium through the filter and to the membrane inlet at a flow rate of 0.3 ml min^{-1}. The two valves used in the instrumentation are a six-position valve V1 (Valco C25Z-3186E, Valco, Schenkon, Switzerland) and a four port two-position valve V2 (Valco E4C6WE, Valco, Schenkon, Switzerland). The six-position valve V1 has four ports for sample streams, one port for flushing liquid and one port for periodical measurement of standard solution in between the fermentation samples for calibration purposes (usually once a day). The four port valve V2 directs the sample stream either to MIMS or to off-line sample collection. A peristaltic pump P2 (Cole-Parmer 7544-06; Cole-Parmer, Vernon Hills, IL, USA) is needed for the sample collection for off-line analysis. It is also possible to connect more than one on-line method to this sample line. The continuously working, programmable syringe pump P3 (Harvard Apparatus PHD2000; Harvard Apparatus Inc., Holliston, MA, USA) is used to pump 0.1 M HCl solution to the sample stream at a flow rate 0.3 ml min^{-1}. pH control is needed when analytes are in dissociated form and do not pervaporate through the membrane. This is the case when acids, such as acetic acid or lactic acid, are measured from a neutral culture medium. Before MIMS analysis the sample and HCl-solution are mixed together in a mixing coil (marked with ^^^^ in Fig. 1) that is made of Teflon (length 1.5 m, 1.6 mm (1/16″) o.d., 1 mm i.d., Teflontalo Irpola, Turku, Finland). After every sample the tubing and the membrane inlet are flushed with water (and HCl).

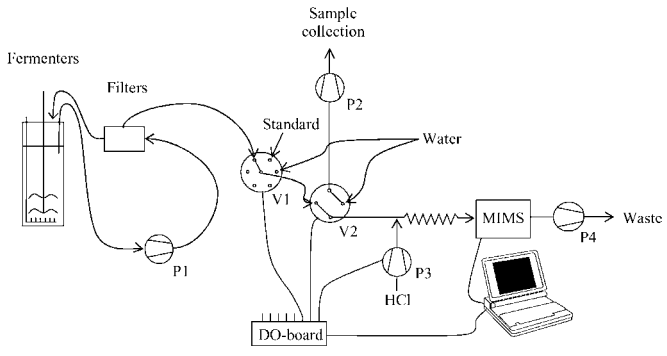

Fig. 1. The experimental set-up for on-line measurement of fermentation medium by MIMS.

The mass spectrometer used was a Balzers QMG 421 quadrupole mass spectrometer equipped with an open cross-beam electron impact (70 eV) ion source and a secondary electron multiplier detector (Balzers Aktiengesellschaft, Balzers, Liechtenstein). A custom-made sheet membrane inlet was used in the experiments [31]. In order to decrease response times and to achieve comparable results with different samples, the inlet temperature was set to 80 °C. The membrane material was polydimethylsiloxane (SSP-M100C, Specialty Silicone Products Inc., Ballston Spa, NY, USA), with thickness of 70 μm and contact area of 8 mm^2.

Matrix effects were studied using a Faraday cup detector and polydimethylsiloxane membrane (SSP-M100, Specialty Silicone Products Inc., Ballston Spa, NY, USA) of thickness 125 μm. Each matrix compound was added to the vessel containing 300 ml of the aqueous standard solution of organic analyte so that the concentration of glucose, xylose, sodium chloride, ammonium chloride or ammonium nitrate in the solution was 0, 0.1, 0.5, 1.0, 2.5, 5.0, 7.0 and 9.0% (w/w). When glucose, sodium chloride and ammonium nitrate were simultaneously added in equal amounts to the aqueous standard solution containing ethanol and ethyl acetate the combined concentrations of the matrix compounds were 0, 0.3, 1.5, 3.0, 7.2, 13.2, 18.6 and 23.4% (w/w). With yeast as a matrix compound the concentrations of fresh yeast were 0, 0.05, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% (w/w). Test solution was stirred with a magnetic stirrer and during the operation of the system a stream of aqueous test solution was continuously supplied to the membrane inlet via a peristaltic pump (Ismatec, Ismatec SA, Switzerland) at a flow rate of 1.5 ml min^{-1}. Analysed test solution was recycled back to the vessel. Data were collected continuously by using the selected ion monitoring (SIM) method. Characteristic ions of each studied compound were chosen for the MS monitoring; *m*/*z* 43 for acetaldehyde, *m*/*z* 45 for ethanol and *m*/*z* 43 or *m*/*z* 61 for ethyl acetate (*m*/*z* 61 used when measured together with ethanol). For testing the effect of carbon dioxide development in the fermentation medium on the MIMS response of organic analytes, carbon

dioxide was bubbled directly into the aqueous standard solutions of studied compounds and the SIM-ion chromatograms were recorded simultaneously.

Detection limits, linearities, repeatabilities and response times were measured by selected ion monitoring mode (SIM). Data were acquired at 0.5 s amu^{-1}. Beer samples were analysed by full scan mode, *m*/*z* 46–150, data were acquired at 0.5 s amu^{-1}. Ethanol concentration was so high that it was impossible to measure it with the same detector amplification setting as the rest of the spectrum and ethanol was measured separately using SIM mode with ion *m*/*z* 46. There was no pre-treatment of the samples prior to MIMS analysis. The beer bottles had been stored in room temperature for the last 24 h before the analysis.

2.3. Practical testing of the sampling system

After the construction of the instrumentation the operation of the sampling system was tested. After tests a continuously working glass syringe pump was chosen for addition of acid (pump P3 in Fig. 1). The peristaltic pump tested was too unstable and the HPLC pump tested did not work properly in the conditions of our sampling system. The sample and acidic solutions were mixed well in a mixing coil (^^^^ in Fig. 1) before entering the membrane inlet. A mixing coil was chosen over a simple T-piece to ensure thorough mixing of sample and acidic solutions.

All tubing used in the sampling system had i.d. of 1 mm. Tubing with smaller inner diameter were unsuitable because they easily got blocked and had higher counterpressure that worsened the flowing conditions. The tubing and the membrane inlet were flushed with water (and 0.1 M HCl) after every sample in order to avoid accumulation of contaminants and to provide a constant background for the mass spectrometer. If only ambient temperature water was used for flushing, some deposition collected in the tubing and the tubing got easily blocked. Therefore, the flushing started with 80 °C water ensuring effective cleaning and minimising the formation of deposition, after which it continued with cold water. The flushing time required depends on the type of medium used in the fermentation. Ten-minute flushing has been found to be adequate for most cases. For small laboratory scale fermentations longer periods between measurements were practical and spared the consumption of culture medium.

2.4. Gas chromatography

Gas chromatographic measurements were done with a Perkin-Elmer Autosystem XL gas chromatograph that was equipped with a FID detector and a headspace sampler Perkin-Elmer HS-40 (Perkin-Elmer Corporation, Norwalk, CT, USA). The column used was PE-5, 50 m, i.d. 0.32 mm, film thickness 1.0 μm (Perkin-Elmer Corporation, Norwalk, CT, USA). Carrier gas, as well as make-up gas was helium with a flow rate of 0.9 ml min^{-1}. Injector temperature was 225 °C and detector temperature was 250 °C. The temperature program was 40 °C $\overset{12\,^{\circ}\mathrm{C\,min^{-1}}}{\longrightarrow}$ 100 °C, 3.5 min $\overset{12\,^{\circ}\mathrm{C\,min^{-1}}}{\longrightarrow}$ 150 °C, 5 min. Out salting with NaCl was used to improve the sensitivity.

2.5. Liquid chromatography

The high-performance liquid chromatographic apparatus from Waters (Milford, MA, USA) consists of a system controller Alliance 2690 and a Waters 410 differential refractometer. Measurements of ethanol were performed on a BIO-RAD Cation-H precolumn (Hercules, CA, USA) followed by a BIO-RAD Aminex HPX-87H ion exclusion column (300 × 7.8 mm i.d.) at an eluent flow rate of 0.6 ml min^{-1}. The mobile phase used for the separation was 5 mM sulphuric acid. Peak areas were quantified by Waters Millennium software using standard solutions of known concentrations and an internal standard (sorbitol 250 mg l^{-1}).

2.6. Fermentation

The MIMS instrumentation was tested by monitoring the primary fermentation of brewer's wort using immobilised yeast. The fermentation process has been described in detail earlier [32]. An industrial yeast strain VTT A-63015 and a commercial wort from a Finnish brewery were used. The wort was autoclaved before use. The yeast in this experiment was immobilised on beech chips. This setup enables continuous long term operation without a need to replace the yeast after each fermentation batch.

3. Results and discussion

3.1. Matrix effects

Beer fermentation medium is a complex mixture consisting of water, yeast, sugars, higher oligosaccharides, protein, some lipids, ethanol and several aroma compounds. Also various salts are present at concentrations up to about 1% (w/w) [33]. Higher concentrations of salts can be found in other fermentations (e.g. up to 20% (w/v) in traditional Japanese soy sauce fermentation) [34] and that is why the matrix effects of different salts on the MIMS response of organic compounds are of special interest.

When salt is dissolved in a solution consisting of two or more volatile, compatible components, the formation of complexes or other structural changes in the liquid phase caused by the added salt affects the activities of the volatile components [35,36]. As the concentration of the salt increases, also the activity coefficients of the organic compounds in the solution increase causing a decrement in the solubility of organic analytes in the solution, and consequently the analytes are salted out. Decreased solubility stimulates the pervaporation of organic compounds through the silicone membrane and the increment of permeation flux is directly

seen as an increasing MIMS response of studied organic analytes.

It has been shown that different matrix compounds affect the MIMS responses of organic compounds [26–28]. To get accurate results with MIMS one has to know which compounds are present in the fermentation medium and how these compounds affect on the MIMS response of compounds of interest. The effect of addition of matrix compounds on the MIMS response of organic analytes was studied using the three different analytes and seven matrix compounds presented together with the description of the observed effect in Table 1.

Addition of yeast as a matrix compound did not affect the MIMS response of ethanol and ethyl acetate, whereas the addition of glucose or xylose increased the MIMS response of acetaldehyde, ethanol and ethyl acetate only slightly and the addition of salts increased their responses prominently. The effect of additions of sodium chloride on the MIMS response of ethanol can be seen from SIM-ion chromatogram in Fig. 2. When sodium chloride was added as a matrix compound, the intensity of the MS-signal rose with every addition of salt. Average standard deviation of the signal of SIM-ion chromatogram in Fig. 2 was 1.5% and average standard deviation of duplicate measurements was 1.1%.

Effects of additions of glucose and different salts on the MIMS response of studied organic compounds are summarised in Table 2. As can be seen from Table 2, the effect of glucose addition was very minor. The effect of xylose addition was almost identical to that of glucose and was therefore excluded. Also the addition of ammonium nitrate caused only slight increase in the responses of acetaldehyde, ethanol and ethyl acetate, but the addition of sodium chloride or ammonium chloride significantly increased the MIMS responses of all studied organic analytes. This can also be seen in Fig. 3, in which the effects of additions of glucose, sodium chloride, ammonium chloride and ammonium nitrate on the MIMS response of ethyl acetate are presented in detail.

Table 1
Measurements made for studying the matrix effects together with the observed effect of each matrix compound

Analyte	Matrix compound	Observed effect[a]
Ethanol	Glucose	+
	Sodium chloride	+
	Ammonium nitrate	+
	Ammonium chloride	+
	Carbon dioxide	−
	Yeast	None
	Glucose, sodium chloride and ammonium nitrate	+
Ethanol (solution saturated with CO_2)	Glucose	+
	Sodium chloride	+
Ethyl acetate	Glucose	+
	Xylose	+
	Sodium chloride	+
	Ammonium nitrate	+
	Ammonium chloride	+
	Carbon dioxide	+
	Yeast	None
	Glucose, sodium chloride and ammonium nitrate	+
Acetaldehyde	Glucose	+
	Xylose	+
	Sodium chloride	+
	Ammonium nitrate	+
	Ammonium chloride	+
	Carbon dioxide	+

[a] Effect of the matrix compound marked as follows: none, no effect on MIMS response; +, increases the MIMS response; −, decreases the MIMS response.

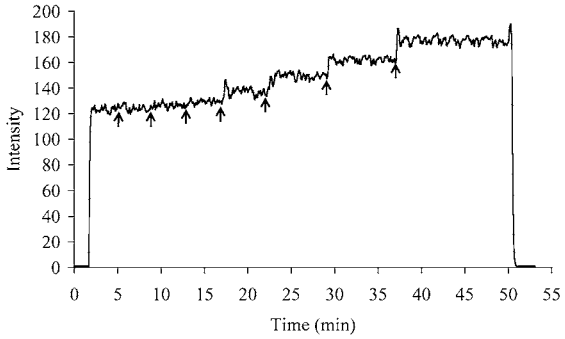

Fig. 2. SIM ion chromatogram showing the effects of additions of sodium chloride on the MIMS response of ethanol. Additions of sodium chloride are marked with arrows. Concentrations of sodium chloride were 0, 0.1, 0.5, 1.0, 2.5, 5.0, 7.0 and 9.0% (w/w).

The observed effects of sodium chloride and ammonium chloride additions on the MIMS response of studied organics were almost equal. This agrees well with the results of Banat and his group [37], who reported that sodium chloride and ammonium chloride have almost identical salting out effects on the vapour–liquid equilibria (VLE) of the propionic acid–water mixture. The stronger effect of sodium chloride and ammonium chloride on the MIMS response of studied analytes compared to that of ammonium nitrate can be explained by the different radii of the ions. According to electrostatic theories, the electrostatic forces are stronger when the ion radius is smaller and more intense electrostatic forces lead

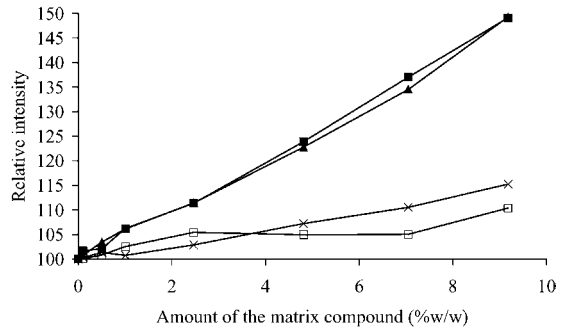

Fig. 3. The effects of additions of glucose, sodium chloride, ammonium chloride and ammonium nitrate on the MIMS response of ethyl acetate. Different compounds are marked with the following symbols ((□) glucose, (■) sodium chloride, (▲) ammonium chloride and (×) ammonium nitrate). Relative intensity is compared to the intensity before matrix additions.

Table 2
Effects of glucose and salt additions on the MIMS response of ethanol, ethyl acetate and acetaldehyde

Analyte	Relative intensity[a] as percent after addition of glucose or specific salt				
	Glucose[b]	NaCl[b]	NH_4NO_3[b]	NH_4Cl[b]	NaCl, NH_4NO_3, Glucose[c]
Acetaldehyde	114 ± 3	199 ± 27	124 ± 7	185 ± 3	nm[d]
Ethyl acetate	110 ± 2	149 ± 2	115 ± 2	149 ± 7	212 ± 16
Ethanol	112 ± 5	155 ± 5	118 ± 2	132 ± 2	207 ± 18

[a] Compared to the intensity before glucose or salt additions.
[b] Concentration of glucose or salt 9% (w/w).
[c] Concentration of NaCl 8% (w/w), concentration of NH_4NO_3 8% (w/w), concentration of glucose 8% (w/w), together 24% (w/w).
[d] Not measured.

to stronger salting out effects [38]. The size of the cation is commonly used to explain the strength of salting out effects, because cations are smaller in size than anions. The radius of ammonium ion is 1.43 Å and the radius of sodium ion 0.97 Å [39] and therefore sodium chloride has stronger effect than ammonium nitrate on the MIMS responses of studied compounds. The difference between the effects of ammonium chloride and ammonium nitrate additions can not be explained by the size of the cation, because both salts have the same positive ion. In this case the radii of the anions are crucial. The radius of chloride ion is 1.81 Å [39]. The exact measure for the radius of nitrate ion can not be found in the literature. However, based on the shape of the nitrate ion, which is a planar triangle (nitrogen in the middle, oxygens in the three corners), can be estimated that the nitrate ion is larger in size than the chloride ion and so the salting out effect caused by ammonium chloride is stronger.

Simultaneous addition of glucose, sodium chloride and ammonium nitrate matrices to the ethanol or ethyl acetate standard solution increases the MIMS response even more than the addition of either salt alone as can be seen from Table 2. This observation agrees well with the studies of Balaban et al. [35], who reported that in the quaternary system containing 2-propanol, water and two salts, the salting out effect was higher than that estimated in approximation of additive contributions of two salts.

The addition of carbon dioxide into test solutions of the studied organic compounds decreased the MS-signal of ethanol by 10%, whereas the MS-signals of ethyl acetate and acetaldehyde increased by 100 and 300%, respectively. The permeation fluxes of compounds in the water phase through silicone membrane are slower than the permeation fluxes of compounds in a gas phase [40]. Numerous different factors have an effect on the evaporation rates of volatile compounds from the liquid phase into the carbon dioxide gas phase. Among other things the molecular characteristics and the concentrations of the volatile compounds [41] as well as the air-water partition coefficients and the mass transport of compounds in both phases may have an effect [42]. Also the concentration- and species-dependent interactions between the silicone membrane and the studied compounds may partly create the observed effects of carbon dioxide addition on the MS-signals [43]. One possible explanation is that acetaldehyde, ethyl acetate and ethanol evaporate from the aqueous standard solutions into carbon dioxide bubbles at different rates depending on the volatilities of the individual compounds. The volatility of a certain compound can be described with its Henry's law constant. The larger the Henry's law constant is, the more volatile the compound is. The Henry's law constant of acetaldehyde at 25 °C is 6.67×10^{-5} atm-m^3 mole^{-1}, that of ethyl acetate is 1.34×10^{-4} atm-m^3 mole^{-1} and that of ethanol is 5×10^{-6} atm-m^3 mole^{-1} [44]. As can be seen from Henry's law constants acetaldehyde and ethyl acetate are more volatile than ethanol and therefore evaporate more readily to the carbon dioxide gas phase than ethanol. However, the real reasons for this behavior remain unclear and will be studied further in the future.

3.2. Detection limits, linearities, repeatabilities and response times

Detection limits, linearities, repeatabilities and response times were measured for ethanol, acetic acid and eight typical beer aroma compounds in deionised water. The results are presented in Table 3. Detection limits were from μg l^{-1} to low mg l^{-1} levels and they all are well below the normal concentrations of these compounds found in beer fermentations. Linearities were tested with six samples for every aroma com-

Table 3
Detection limits (LODs), relative standard deviations (R.S.Ds.) of six consecutive measurements and response times of ethanol, acetic acid and eight typical beer aroma compounds

Compound	LOD (mg l^{-1})[a]	R.S.D. (%)	Response time (min)[b]
Acetaldehyde	0.5	nm[c]	1.0
Acetic acid[d]	30	0.9	3.0
Ethanol	5	0.9	1.0
1-Propanol	0.5	1.7	1.5
2-Methylpropanol	1	1.7	1.5
2-Methylbutanol	0.5	2.2	1.5
3-Methylbutanol	0.2	2.2	1.5
Ethyl acetate	0.05	3.4	0.5
3-Methylbutyl acetate	0.03	2.2	2.5
Ethyl caproate	0.05	2.1	8

[a] $S/N = 3$.
[b] Response times are defined as signal rise times from 10 to 90%.
[c] Not measured.
[d] pH has been adjusted to 2 with 0.1 M HCl.

pound studied. The correlation coefficients were better than 0.9921 for each compound indicating acceptable linearity in the measurement range. The repeatability of the method was tested with six consecutive measurements of the same sample. Relative standard deviations better than 3.4% show that the method has a good repeatability. Response times are defined as the time that it takes for the signal to rise from 10 to 90% of the final response. The times are shorter than or equal to 3.0 min for all compounds except for ethyl caproate (8 min). Based on the response time measurements, an analysis time of 10 min was selected for all further measurements.

3.3. Off-line analysis of beer samples

The calibration of the MIMS-system was tested off-line by measuring eight commercial beer samples. Two calibration methods were used for the quantification. First, calibration standards were made by dissolving pure compounds in deionised water, but as expected from the matrix effect studies, this calibration method gave unsatisfactory results compared to headspace gas chromatography (HSGC). The reason for the difference in MIMS and HSGC results is believed to be the high amount of carbon dioxide in beer samples. Carbon dioxide interferes the MIMS analysis, but it is impossible to remove it from beer samples without loosing some of the VOCs at the same time. HSGC is a reliable standard method for determination of VOCs and for this reason, one sample and its HSGC results were chosen for the calibration.

Mass spectrometric results of aroma compounds have been calculated from a single mass spectrum using the Solver deconvolution program [25]. Solver identifies and quantifies compounds from a multicomponent mass spectrum by a nonlinear asymmetric error function-based least mean square method (NALMS).

After calibration, eight commercial beer samples, with one triplicate sample (beer 1), were analysed by MIMS and HSGC. Ethanol concentrations were analysed by MIMS and HPLC. The results are presented in Table 4 together with ethanol concentrations given on the beer bottle labels. As can be seen from Table 4, the results obtained by MIMS are mostly in good agreement with HSGC results. For 3-methylbutyl acetate, ethyl acetate, 3-methylbutanol and 2-methylbutanol the average error of all beer samples analysed was between 12 and 17% and for 1-propanol, ethyl caproate and 2-methylpropanol the average error was between 29 and 36%. The largest errors in the results are from the measurements in which the measured concentrations are close to the detection limits. Standard deviations of the concentrations of all measured compounds from triplicate beer sample (beer 1) were between 1.8 and 9.8%. The results in Table 4 show that MIMS method is suitable for the monitoring of continuous beer fermentation, because if the fermentation is somehow disturbed the concentrations of the monitored compounds will change prominently.

3.4. On-line analysis of continuous beer fermentation

The MIMS instrumentation was tested by measuring for 15 days primary fermentation of brewer's wort by immobilised yeast. Samples from prereactor and main reactor were taken once in every 75 min. The fermentation system consisted of two packed bed reactors and a buffer tank between the reactors (Fig. 4). The sampling points for MIMS were on the top of the prereactor and the main reactor and off-line sam-

Table 4
MIMS, HSGC and HPLC analysis of eight commercial beer samples

Compound	Beer 1	Beer 2	Beer 3	Beer 4	Beer 5	Beer 6	Beer 7	Beer 8
MIMS-analysis (mg l^{-1})[a]								
3-Methylbutyl acetate		1.2	0.4	2.3	0.7	1.1	0.1	1.2
1-Propanol		11.0	8.5	10.3	15.5	13.7	7.5	9.9
Ethyl caproate		0.1	0.0	0.1	0.1	0.1	0.0	0.1
Ethyl acetate		13.3	4.5	15.7	10.8	9.2	5.1	7.5
3-Methylbutanol		33.2	34.3	33.7	36.5	60.3	18.9	58.7
2-Methylpropanol		9.7	8.0	16.6	4.6	14.4	4.2	19.2
2-Methylbutanol		16.0	9.6	16.9	11.8	14.2	3.7	14.0
GC-analysis (mg l^{-1})								
3-Methylbutyl acetate	1.0	1.1	0.4	2.3	0.6	1.0	0.2	1.2
1-Propanol	9.1	7.7	6.9	8.5	10.8	18.4	7.0	16.9
Ethyl caproate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ethyl acetate	13.3	15.1	5.8	16.1	11.1	10.5	8.4	10.2
3-Methylbutanol	36.7	35.9	30.8	41.4	29.5	49.7	19.5	54.0
2-Methylpropanol	11.4	10.9	13.3	11.6	6.6	19.6	9.9	29.1
2-Methylbutanol	11.6	14.7	12.9	14.4	10.1	13.1	5.3	15.6
Ethanol measurements (vol-%)[b]								
MIMS measurement		3.9	2.6	4.3	4.2	3.7	3.8	3.4
Ethanol from labels	4.5	4.1	2.5	4.1	4.3	3.8	4.1	4.0
HPLC measurement	4.3	4.0	2.7	4.2	4.1	3.7	3.8	3.7

[a] MIMS results have been calculated by Solver program using beer 1 GC results for calibration.
[b] MIMS results have been calculated by using beer 1 HPLC results for calibration.

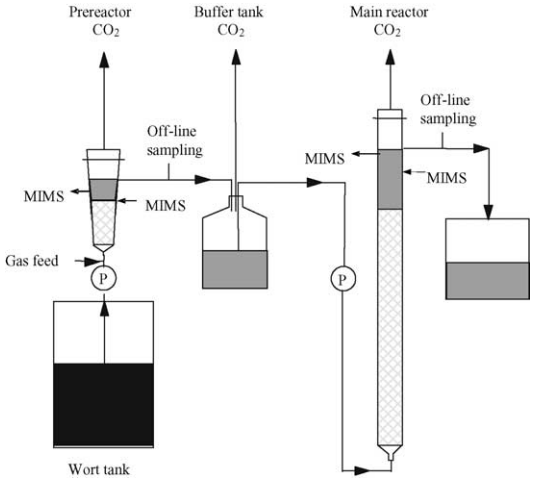

Fig. 4. Immobilised yeast system [32] used in primary fermentation of brewer's wort. Sampling points for on-line MIMS were on the top of the prereactor and the main reactor. Off-line samples were taken from the outlets of the reactors.

ples were taken periodically from the outlets of the reactors. MIMS results were calculated with Solver deconvolution program and are presented in Figs. 5 and 6. 3-Methylbutyl acetate and ethyl caproate were also measured, but their concentrations were below 1 mg l^{-1} level. The results show that MIMS is capable of following the changes in the process conditions. Minor changes in the continuous process and also the delays in two-phase process can be clearly seen in Figs. 5 and 6. For example, the concentrations of ethanol and 3-methylbutanol decrease in both reactors after the flow speed of the process was increased from 500 to 750 ml h^{-1} on the 27th November. In prereactor the changes of the monitored concentrations are faster and more distinct than in the main reactor due to the different conditions in those reactors, e.g. in the prereactor oxygen is present and the growth of the yeast is oxygen limited. The delay between the prereactor and the main reactor is 7 h. Four samples were taken for HSGC analysis. The results are presented in Tables 5 and 6 together with MIMS results from the same time points. The first HSGC result was used for calibrating MIMS and the calibration was checked daily

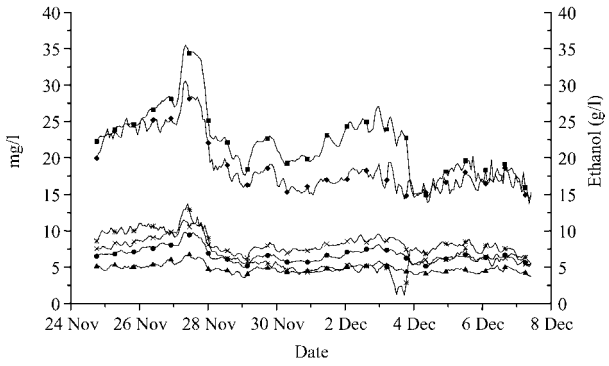

Fig. 5. On-line measurement results of the prereactor with an automatic MIMS-system. Different compounds are marked with the following symbols ((×) propanol, (*) ethyl acetate, (♦) 3-methylbutanol, (▲) 2-methylpropanol, (●) 2-methylbutanol and (■) ethanol). 3-Methylbutyl acetate and ethyl caproate were also measured and their concentrations were below 1 mg l^{-1} level.

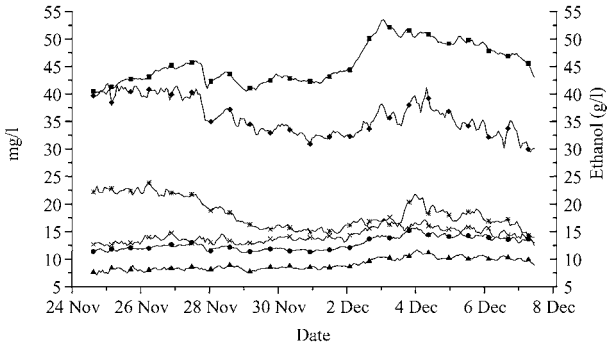

Fig. 6. On-line measurement results of the main reactor with an automatic MIMS-system. Different compounds are marked with the following symbols ((×) propanol, (*) ethyl acetate, (♦) 3-methylbutanol, (▲) 2-methylpropanol, (●) 2-methylbutanol and (■) ethanol). 3-Methylbutyl acetate and ethyl caproate were also measured and their concentrations were below 1 mg l^{-1} level.

by measuring a standard solution. It should be noted, that different sampling points for HSGC and MIMS may cause differences in results. The experiment demonstrated that MIMS instrumentation can be operated reliably for long periods of

Table 5
HSGC and on-line MIMS results for prereactor (mg l^{-1})

Compound	26.11 (Calibration)[a]		30.11		3.12		7.12	
	HSGC	MIMS	HSGC	MIMS	HSGC	MIMS	HSGC	MIMS
3-Methylbutyl acetate	0.5	0.5	0.4	0.4	0.4	0.4	0.4	0.4
1-Propanol	8.9	8.9	7.6	7.5	7.1	9.0	7.7	6.1
Ethyl caproate	<0.1	0.1	<0.1	0.1	<0.1	0.1	<0.1	0.1
Ethyl acetate	10.4	10.4	7.5	5.0	8.0	5.0	8.8	7.0
3-Methylbutanol	25.2	25.2	19.7	16.0	19.5	19.4	20.8	16.8
2-Methylpropanol	5.3	5.3	4.3	4.4	4.1	5.4	4.6	4.6
2-Methylbutanol	7.8	7.8	6.4	5.7	6.3	7.3	6.8	6.3
Ethanol (g l^{-1})	nm[b]	26.9	19.6[c]	19.6	18.3	25.2	21.0	17.7

[a] MIMS results have been calculated by Solver program using 26.11. HSGC results for calibration.
[b] Not measured.
[c] Used for ethanol calibration.

Table 6
HSGC and on-line MIMS results for main reactor (mg l^{-1})

Compound	26.11 (Calibration)[a]		30.11		3.12		7.12	
	HSGC	MIMS	HSGC	MIMS	HSGC	MIMS	HSGC	MIMS
3-Methylbutyl acetate	0.9	0.9	0.8	0.8	0.8	0.9	0.8	0.8
1-Propanol	14.0	14.0	13.7	13.4	13.7	17.3	13.5	14.8
Ethyl caproate	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Ethyl acetate	22.2	22.2	19.1	15.0	22.2	16.8	22.0	14.2
3-Methylbutanol	39.9	39.9	35.0	32.8	35.4	36.4	34.1	31.1
2-Methylpropanol	8.1	8.1	6.8	8.3	7.3	9.8	7.2	9.6
2-Methylbutanol	12.5	12.5	11.3	11.6	11.9	13.8	11.6	13.5
Ethanol (g l^{-1})	nm[b]	44.2	42.8[c]	42.8	46.8	52.1	43.7	46.0

[a] MIMS results have been calculated by Solver program using 26.11. HSGC results for calibration.
[b] Not measured.
[c] Used for ethanol calibration.

time even in high humidity at 10 °C for monitoring of a stable process such as a continuous beer fermentation.

4. Conclusions

A new automatic on-line membrane mass spectrometric instrumentation for the monitoring of continuous beer fermentations was designed, constructed and thoroughly tested. Both off-line and on-line testing of the instrumentation have shown that the method is very suitable for the on-line determination of volatile organic aroma compounds in continuous beer fermentation processes. Detection limits for aroma compounds that are produced by the yeast at very low, yet physiologically significant levels range from μg l^{-1} to low mg l^{-1}. Good linearities in the measurement range and repeatabilities better than 3.4% show that the method is accurate and reliable. Short response times for changes in beer fermentation process enable fast and accurate controlling of the process. It has been shown that different salts and carbon dioxide present in the analysis solution in varying concentrations affect significantly the MIMS response of organic compounds, whereas glucose and xylose have only minor effect on the MIMS response of studied compounds and yeast has no effect on the MIMS response of ethanol and ethyl acetate. The results show that MIMS with Solver is adequate for a qualitative analysis of continuous beer fermentation media and for a precise quantitative analysis the calibration should be made in the same matrix as the sample. For that reason the precision of the developed method can be improved in the further studies by using a standard-addition-method for the calibration. It is also possible to automate the calibration procedure.

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