sensors

Full Paper

Calibration of a Sensor Array (an Electronic Tongue) for Identification and Quantification of Odorants from Livestock Buildings

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Abstract: This contribution serves a dual purpose. The first purpose was to investigate the possibility of using a sensor array (an electronic tongue) for on-line identification and quantification of key odorants representing a variety of chemical groups at two different acidities, pH 6 and 8. The second purpose was to simplify the electronic tongue by decreasing the number of electrodes from 14, which was the number of electrodes in the prototype. Different electrodes were used for identification and quantification of different key odorants. A total of eight electrodes were sufficient for identification and quantification in micromolar concentrations of the key odorants n-butyrate, ammonium and phenolate in test mixtures also containing iso-valerate, skatole and p-cresolate. The limited number of electrodes decreased the standard deviation and the relative standard deviation of triplicate measurements in comparison with the array comprising 14 electrodes. The electronic tongue was calibrated using 4 different test mixtures, each comprising 50 different combinations of key odorants in triplicates, a total of 600 measurements. Back propagation artificial neural network, partial least square and principal component analysis were used in the data analysis. The results indicate that the electronic tongue has a promising potential as an on-line sensor for odorants absorbed in the bioscrubber used in livestock buildings.

Keywords: electronic tongue, odorants, principal component analysis (PCA), partial least squares (PLS), back propagation artificial neural network (BPNN)

1. Introduction

An odour is defined as a sensation resulting from reception of a stimulus by the olfactory sensory system [1], and it is an important environmental pollution issue [2]. Odorants are the compounds responsible for imparting an odour, and their molecular mass is between 30 to 300 Daltons [3,4]. These odours lead to environmental and health problems. It was found that neighbours of livestock buildings suffer from depression, negative emotions, greater mood disturbance, more tension, an overall feeling of less vigour, anger, fatigue and confusion compared with people living far away from livestock buildings [5].

There are many methods to reduce odours emission from livestock building, *i.e.* physical, chemical and biological. Biological methods are considered environmentally friendly [6]. One of the biological methods is the bioscrubber which is used for air treatment in different industrial and agricultural applications [7,8]. The bioscrubber comprises two main parts: an absorption column and a bioreactor. The absorption column is placed inside the ventilation channel in the livestock building, where odour substances (odorants), ammonia and dust particles are absorbed by water droplets. Water droplets are introduced to the absorption column through nozzles, which receive water recycled from the bioreactor. The bioreactor can be placed at floor level and can supply water for several absorption columns [6].

Odours are measured sensorally or analytically. Sensory methods measure odours, while analytical methods measure odorants. Examples of analytical methods include purge and trap (P&T), solid phase micro extraction (SPME), direct aqueous injection–gas chromatography (DAI-GC) and solvent extraction [9-12]. Analytical methods have the advantages of objectivity, repeatability and accuracy [13].

Characterization of a mixture of odorants, in absorption column or in bioreactor, gives information about the absorbed odorants and the efficiency of the bioreactor. An electronic tongue (ET) has a high potential for this application. A calibrated ET inserted before or/and after the bioreactor characterizes the odorants on-line. ET is an analytical instrument containing an array of electrodes, with partial specificity for different components in liquids and an appropriate pattern recognition or multivariate calibration tool for identification and quantification of even complex liquid mixtures. It measures the compounds in a liquid with high sensitivity [14,15]. It was already used in many applications including characterization of different types of mineral water and wine [16], monitoring of fermentation process [17,18] and food quality [19,20].

There is a need to test ETs in different applications, *e.g.* health services, environmental technology and quality control. Therefore, research should become less focused on the relation between the ET signals and human sensory panels [14,21,22]. However, ET is a recently developed method, and it has not yet reached its full potential for application outside the laboratory [14,23]. Nevertheless, ET's ability should neither be underestimated [14] nor overestimated [22], and more research should address new applications.

There are many advantages in using ET compared to other methods, such as GC, high performance liquid chromatography (HPLC) or mass spectrometry. The key advantages are: rapidity, simplicity, low cost and simultaneous on-line determination of several components of very different chemical

properties in the liquid. Furthermore, ET provides information about ions and compounds that are found in aqueous phase only (*e.g.* compounds having a low vapour pressure) [18,22,23].

There are huge numbers of odorants in the livestock building. Approximately 300 different odorants have been identified [1], many of them have a very low detection threshold of 0.001 mg/m^3 or less [24]. A representative selection of these odorants was used in this study.

The pH plays an important factor in the bioscrubber application. The transfer of odorants from the gas (*i.e.* in the air) to the liquids phase in the absorption column and the microbial activity in the bioreactor are strongly dependent on pH. The optimum pH in the bioreactor is in the interval of 4 to 8 [25]. However, most microbial growth occurs near neutral pH [26]. In this study, an ET based on potentiometric cross-sensitive electrodes was used to study the characterization of four test mixtures of selected odorants, *i.e.* two different mixtures of key odorants, at two different acidities (pH 6 and pH 8).

This study serves a dual purpose. The first purpose is to investigate the possibility of using ET to identify and/or quantify key odorants, and the second purpose is to simplify the ET by decreasing the number of electrodes from 14, which was the number in the prototype to a lower but sufficient number. The present study is an example of application of the ET for monitoring environmental and industrial processes.

2. Experimental

2.1. Sensor array, i.e. the electronic tongue (ET)

A custom made prototype ET was purchased from Analytical Systems, Ltd., St. Petersburg – Russia. It was designed to have a cross-sensitivity for the selected key odorants tested in this study. It consists of 14 potentiometric electrodes. Eleven polymer (PVC) plasticized membrane electrodes containing different active substances (no. 1-11), two chalcogenide glass electrodes (no. 12-13) and one wire electrode (no. 14). The electrodes were numbered in order to identify the individual electrodes that were sufficient for identification and quantification of different key odorants.

A pH glass electrode was also included in the sensor array in addition to a conventional Ag/AgCl reference electrode. Potentiometric measurements were performed using a high-input impedance multichannel voltmeter connected to a PC for data acquisition. The electrode response comprises ionic, redox or molecular interaction at the membrane/liquid interface. Pattern recognition and multivariate calibration methods were used to deconvolute these complex signals, producing quantitative and qualitative information about multicomponent liquids [16,27].

2.2. Preparation of test mixtures of key odorants

It is an impossible task to include all odorants from livestock buildings in the calibration. Therefore six key odorants were selected in this study as representative odorants. They represented a variety of chemical groups, *i.e.* volatile fatty acids (VFAs), indoles, phenols and ammonia. The selected key odorants were: n-butyric acid, iso-valeric acid, 3-methyl indole (skatole), phenol, 4-methly phenol (p-cresol) and ammonia. The chemical and physical properties of the selected key odorants are shown in Table 1. The *pKa* for neutral skatole is unavailable in literature. The *pKa* for indole, *pKa* = 16.7, was

used to give a rough estimate of the dissociation of skatole [28]. Despite the very low dissociation of skatole in water, skatole was added to the test mixtures of key odorants. This was done to mimic mixtures of odorants in livestock buildings, where skatole is one of the most important components of odour nuisance problems [29].

Many researchers investigated the concentrations of these key odorants in air samples from livestock buildings. O'Neil and Philips [24] and Schiffman *et al.* [1] reviewed concentration intervals which are used as the main reference for the minimum and maximum concentrations of these key odorants (Table 2). The lowest minimum and the highest maximum concentrations reported in these two reviews were used in the test mixtures of key odorants in this work.

In the bioscrubber, odorants are present in the liquid phase. Henry's constant *(H)* is the ratio of the partial pressure of the analyte in the gas phase to the equilibrium concentration in the water (expressed in: atmosphere × liter / mol) and is used for calculating the concentrations of odorants in the liquid phase. The dimensionless air-water partition coefficient *(K_{AW})* is equal to *H/RT,* and it is the air to water concentration ratio at equilibrium [30]. The value of the dimensionless air-water partition coefficient expresses the volatility of the odorant. An odorant with K_{AW} of 0.05 or larger is volatile, whereas those with a lower K_{AW} occur predominantly in the water phase [31]. All of the key odorants have K_{AW} lower than 0.05 and they will occur predominantly in the liquid phase. The concentrations of the key odorants in air, the equivalent equilibrium concentrations in the liquid phase and the interval of concentrations used in our experiments are shown in Table 2. The interval of concentrations of each key odorant was subdivided into seven intervals (Table 3), to get as many combinations as possible in the test mixtures of key odorants used in calibration experiments.

Stock solutions with different concentrations were prepared separately for each key odorant. Phenol and skatole were obtained as solids, with purities of 99.5% and 98%, respectively. The purity of n-butyric acid, iso-valeric acid and p-cresol was 99%. These key odorants were purchased from Sigma-Aldrich (Schnelldorf, Germany). Ammonium hydroxide (25%, v/v) was purchased from J. T. Baker (Deventer, Holland). All key odorants were diluted in deionised water, except skatole which was dissolved in hot deionised water [32]. All key odorants were used without any further purification.

Table 1. Chemical and physical properties of key odorants.

No.	Odorant	Chemical abstract service (CAS #)	Molecular formula	Molecular mass (g mol^{-1})	Solubility in H$_2$O at 25°C (g l^{-1})	pKa	Henry's constant (H) atm. l. mol^{-1}	Vapour pressure at 25°C (mm Hg)	Octanol-water partition coefficient (log p)	Melting point ^{o}C	Boiling point ^{o}C
1.	n-butyric acid	107-92-6	$C_4H_8O_2$	88.11	60	4.82	5.35×10^{-4}	1.65	0.79	-5.7	163.7
2.	iso-valeric acid	503-74-2	$C_5H_{10}O_2$	102.13	40.7	4.77	8.33×10^{-4}	0.44	1.16	-29.3	176.5
3.	phenol	108-95-2	C_6H_6O	94.11	82.8	9.99	3.33×10^{-4}	0.35	1.46	40.9	181.8
4.	4-methyl phenol (p-cresol)	106-44-5	C_7H_8O	108.14	21.5	10.3	1×10^{-3}	0.11	1.94	35.5	201.9
5.	3-methyl indole (skatole)	83-34-1	C_9H_9N	131.18	0.498	≈ 16.7 [a]	2.13×10^{-3}	0.00555	2.60	97.5	266
6.	ammonia	7664-41-7	NH_3	17.03	482	9.25	1.61×10^{-2}	7510	0.23	-77.7	-33.4

Reference of properties: Syracuse Research Corporation [33]

[a] pKa for indole [28]

Table 2: Concentration of key odorants in air and water.

Odorant	Dimensionless air-water partition coefficient (K_{AW})[b]	Minimum key odorant concentration in air [c] mg/m^3	Maximum key odorant concentration in air [c] mg/m^3	Minimum equivalent equilibrium key odorant concentration in water [d, e] mg/m^3		Maximum equilibrium key odorant concentration in water [d, e] mg/m^3		Interval of concentrations used in experiments Minimum (M)	Maximum (M)
				mg/m^3	M	mg/m^3	M	Minimum (M)	Maximum (M)
n-butyric acid	2.19×10^{-5}	0.001	0.7	46	5.2×10^{-7}	32×10^3	3.6×10^{-4}	10^{-7}	10^{-3}
iso-valeric acid	3.40×10^{-5}	0.002	0.21	59	5.8×10^{-7}	62×10^2	6.0×10^{-5}	10^{-7}	10^{-4}
phenol	1.36×10^{-5}	0.001	0.0078	73	7.8×10^{-7}	57×10^1	6.1×10^{-6}	10^{-7}	10^{-5}
p-cresol	4.09×10^{-5}	0.002	0.041	49	4.5×10^{-7}	10×10^2	9.3×10^{-6}	10^{-7}	10^{-5}
skatole	8.70×10^{-5}	0.00049	0.003	5.6	4.3×10^{-8}	34	2.6×10^{-7}	10^{-8}	10^{-6}
ammonia	6.54×10^{-4}	0.01	18	15	8.9×10^{-7}	27×10^3	1.6×10^{-3}	10^{-7}	10^{-3}

[b] $K_{AW} = H / RT$, where: R: gas constant = 0.0821 atm. l. / (mol. K), T: degree Kelvin
   $K_{AW} = H$ (atm. l. / mol) / 24.47

[c] According to O'Neil and Philip [24] and Schiffman *et al.* [1]

[d] Equivalent equilibrium concentrations in water calculated using K_{AW} [30]:
   K_{AW} = Concentration in air (C_a) / Concentration in water (C_w) $\Rightarrow C_w = (24.47 \times C_a) / H$ (atm. l. /mol)

[e] M (mole/l.) = $10^{-6} \times$ concentration (mg/m^3) / molecular mass (g/mole)

Table 3. Mixture of odorants and concentration intervals of key odorants.

Odorant	Mixture containing ammonium	Mixture containing p-cresolate	Minimum	Concentration numbers [g]				Maximum	
			1	2	3	4	5	6	7
			M	M	M	M	M	M	M
n-butyrate	X [f]	X	10^{-7}	10^{-6}	10^{-5}	5×10^{-5}	10^{-4}	5×10^{-4}	10^{-3}
iso-valerate	X	X	10^{-7}	5×10^{-7}	10^{-6}	5×10^{-6}	10^{-5}	5×10^{-5}	10^{-4}
phenolate	X	X	10^{-7}	3×10^{-7}	5×10^{-7}	10^{-6}	3×10^{-6}	5×10^{-6}	10^{-5}
p-cresolate		X	10^{-7}	3×10^{-7}	5×10^{-7}	10^{-6}	3×10^{-6}	5×10^{-6}	10^{-5}
skatole	X	X	10^{-8}	3×10^{-8}	5×10^{-8}	10^{-7}	3×10^{-7}	5×10^{-7}	10^{-6}
ammonium	X		10^{-7}	$5 \times 10^{-6\,h} / 10^{-6}$	10^{-5}	5×10^{-5}	10^{-4}	5×10^{-4}	10^{-3}

[f] X: presence of key odorant in mixture

[g] Concentration numbers were used to randomize concentration intervals of key odorants. Method was explained in experimental design section

[h] Concentration of 5×10^{-6} M was included in concentration interval of ammonium in test mixtures of key odorants in deionised water, *i.e.* pH 6

2.3. Experimental design

Four groups of experiments were carried out separately: two mixtures of key odorants at two different acidities. In the first group of experiments, the mixture of key odorants contained: n-butyrate (n-butanoate), iso-valerate, phenolate, skatole and ammonium. In the second group of experiments, ammonium was replaced with p-cresolate. Deionised water was solvent at pH 6. The pH of the mixtures was adjusted by addition of sodium hydroxide or hydrochloric acid. At pH 8, a buffer of KH_2PO_4 (3.7×10^{-3} M) and Na_2HPO_4 (78×10^{-3} M) was used. After pH adjustment, the acidity remained constant throughout the experiment. Each group of experiments comprised 50 measurements in triplicates, totally 150 measurements. This number of measurements was chosen according to preliminary experiments, which showed that 50 measurements constitute a sufficient number of combinations of mixtures of key odorants. In each group of experiments, the mixtures of key odorants were measured in random order. Microsoft office Excel 2000 (Microsoft Corporation, USA) software was used to randomize the intervals of concentrations in the test mixtures in each group of experiments, using a randomization and uniform distribution function. Williams [34] suggested that samples for calibration should be collected with uniform distribution of composition within the anticipated interval. In uniform distribution, each treatment has an equal probability of being observed. The method for randomization of 50 measurements using Excel program was: use the tools option, data analysis, random number generation, distribution: uniform, number of variables was 5 (since we had five key odorants in each mixture), parameter was between 1-7 (since we had seven intervals of concentrations) and number of measurements was 50 (since we had 50 experiments). The randomized group of experiments comprised 50 rows (experiments), with 5 columns (five key odorants) and in each row there were five numbers between 1 to 7, which is related to the concentration of each key odorant. For example, if the digits for one row (experiment) were: 1, 7, 5, 3, 4 and if we follow the order in Table 3 for the test mixture containing ammonium, we will mix concentration no. 1 of n-butyrate (10^{-7} M), concentration no. 7 of iso-valerate (10^{-4} M), concentration no. 5 of phenolate (3×10^{-6} M), concentration no. 3 of skatole (5×10^{-8} M), and concentration no. 4 of ammonium (5×10^{-5} M).

The ET was submerged in the test mixture of key odorants in a 100 ml Teflon container with a magnetic stirrer. Five minutes were sufficient for electrodes to reach stable potential in all cases. Electrodes were washed several times with deionised water between measurements to reach initial potential readings.

2.4. Multivariate data analysis

Multivariate data analysis, including pattern recognition and calibration methods, is reviewed in many papers [27,35-39].

Pattern recognition includes a variety of methods, *e.g.* principal components analysis (PCA), linear discrimination analysis (LDA) and self organizing map (SOM). Calibration methods include partial least squares (PLS), principal component regression (PCR), multiple linear regression (MLR) and back propagation artificial neural network (BPNN) [27,36]. In this study, we mainly used PCA, PLS and BPNN.

PCA is a well known method for processing of multidimensional data. It is an unsupervised data reduction method and it describes variations of multivariate data in terms of a set of uncorrelated variables. The original data matrix is projected from a high dimensional space into a less dimensional space, with as little loss of information as possible. The matrix is decomposed into scores (which describes the relationship among samples) and loadings (which describes the relationship among variables). The principal components (PCs) are determined on basis of the maximum variance criterion, and they are orthogonal. The first PC contains most of the variance of the data. In addition, PCA results are comparatively easy to comprehend and interpret [16,27].

PLS projects the original data to latent structures. It correlates two matrices, *e.g.* X (the response of the electrodes) and Y (the concentration of the key odorant), by a linear multivariate model. It has the ability to analyse noisy, collinear and incomplete variables in both matrices [27,40]. There are two types of PLS regression. PLS-1, where only one Y-variable is used, and PLS-2 where more than one Y-variable is used. It was suggested that PLS-1 gave better results than PLS-2 [41].

The root mean square error of prediction (RMSEP) is an estimate of the prediction error, which should be as small as possible. Also, the correlation, the lowest numbers of PCs and the lowest difference between the RMSEP and the root mean square error of calibration (RMSEC) were considered in modelling [42]. Outliers were identified and handled.

The Unscrambler (v. 9.2, Camo, Oslo, Norway) software was used for PCA and PLS analysis. Full cross validation was used for the averaged triplicates of each sample.

2.5. Artificial neural networks (ANNs)

Artificial neural networks (ANNs) are networks of simple processing elements, *i.e.* neurons, operating within their local data range and communicating with other elements. The architectures of ANN are inspired by the structure of the brain, but have developed away from their biological inspiration [37,39]. ANNs have many applications, for instance in spectroscopy, process control, protein folding, analytical chemistry and electrochemical systems [38,39].

The BPNN (also called feed forward network), which is one type of ANNs, is the most widely used network and was used in this study as well. It comprises many processing elements that are arranged in layers: an input layer, an output layer, and one or more layers in between, called hidden layers. In BPNN, the inputs are introduced and weighted, then received by each node in the next layer. The weighted inputs are summed and passed through a non-linear transfer function to produce the node output, which is also weighted and passed to the processing elements in the next layer. The output from the network is compared with the actual value and the error between the two values is calculated. This error is then used to adjust the weights until the network finds a set of weights that will produce the input-output mapping with the smallest possible error [35,37]. Principal components are used as inputs for the neural network model in order to reduce the risk of overfitting [41].

We used a neural network software 'Predict' (v. 3.13, NeuralWare, Pittsburgh, USA) employing BPNN for modelling in the framework of Microsoft Excel. The 'Predict' program is powerful and easy to use [43]. The models in the program contain one hidden layer with different numbers of nodes. Despange and Massart [35] concluded that models with one hidden node are stable. The models employ hyperbolic tangent and sigmoid transfer functions in the hidden and output layers, respectively. These functions are commonly used, differentiable, fit a large number of non-linearities and have the appropriate slope behaviour for data extremes [35,43]. Direct connections between input and output nodes were also allowed, which enables the models to evaluate the need for a hidden layer. The model employs an adaptive gradient learning rule. Also, it reduces overfitting by including a weight decay method. The default parameters suggested by the program were used. Maier and Dandy [44] suggested that inclusion of default parameters is acceptable. The default parameters and mathematical explanation of the functions are beyond the scope of this communication but they are described elsewhere [45].

In all BPNN models, the rule of thumb that the number of samples in the training set is at least twice the total number of weights in the BPNN topography [35] was followed in our analysis. Each measurement in triplicates was treated as one sample. This triplicate was used either in train, in test or in validation set.

Data were centred and scaled (*i.e.* by multiplying each element in the matrix by the term 1/standard deviation) before modelling in both PLS and BPNN, so each variable will have the same importance in the analysis [40]. Calibration models were carried out separately for each key odorant.

During data analysis, different electrodes were examined for their contribution in identification and quantification of key odorants. The aim was to achieve the best recognition and calibration results, taking into consideration the rules of thumbs in multivariate data analysis. The total number of electrodes in the electronic tongue was reduced without any loss of analytical information. This was done before by others in many applications of ET, *e.g.* Legin et al. [16] and Auger *et al.* [20]. Moreover, a dimensionless parameter called: ratio of standard error of performance to standard deviation (RPD) can be used to assess the calibration model in both PLS and BPNN. RPD is the standard deviation of the validation set of the dependent variable divided by RMSEP. As a rule of thumb, an acceptable model has RPD larger than 2.5, and an excellent model has 10 or larger [34,46].

3. Results and discussion

In this study, we calibrated an ET using four test mixtures of selected key odorants in concentrations within the interval of minimum and maximum concentrations of key odorants given in two reviews [1,24]. However, it is emphasized that there is a huge variation in the concentrations of odorants in livestock buildings caused by environmental factors, composition of feed, construction of livestock building including ventilation, sources of sample and measuring methods [29].

The four test mixtures comprised two mixtures of key odorants at two different acidities (pH 6 and pH 8). One mixture of key odorants contained: n-butyrate (n-butanoate), iso-valerate, phenolate, skatole and ammonium. In the other mixture of key odorants ammonium was replaced with p-cresolate (Table 3). The choice of ammonium and p-cresolate was due to their importance in the odour problems in livestock buildings [29,47]. The four groups of experiments (two mixtures of key odorants at two acidities) were carried out separately, and they can be considered independently with regard to experimental design and number of samples in each interval. The possibility to identity and/or quantify key odorants in different mixtures is discussed below.

3.1. Test mixtures of key odorants containing ammonium at pH 6

Standard deviation of triplicate measurements was between 0 - 11 mV and 0 - 5.6 mV when electrodes no. 1-14 and no. 2, 5, 6, 7, 8, 9 were used, respectively. The relative standard deviation (RSD = (standard deviation / mean) $\times$ 100), was between 0 - 4.8% and 0 - 3.4% when electrodes no. 1-14 and no. 2, 5, 6, 7, 8, 9 were used, respectively. It was noticed that the most interfering ions were ammonium and n-butyrate.

PCA score plot of all samples (Fig. 1) indicates that it is possible to monitor ammonium in the mixture of key odorants. The two PCs accounted for 96% of the variation. Six electrodes were sufficient (no. 2, 5, 6, 7, 8, 9). Samples containing high ammonium concentrations, *i.e.* 10^{-4} - 10^{-3} M, are surrounded by the dashed line.

Due to the complexity of the test mixture, it was difficult to model any key odorant reasonably in their entire interval of concentrations. Data were sorted in ascending and descending orders, according to concentrations of key odorants, in an attempt to find a trend in the data. We could identify ammonium, when the concentration of n-butyrate was below 10^{-4} M. The number of samples which has concentration of n-butyrate below 10^{-4} M was 34 samples. The PCA score plot of these samples is shown in Fig. 2. Two PCs accounted for 97% of the variation. Six electrodes were sufficient (no. 2, 5, 6, 7, 8, 9). The figure shows that the concentration of ammonium decreases diagonally, which indicates that ET is able to monitor ammonium in the mixture of key odorants.
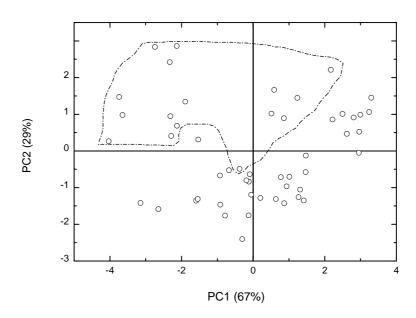
Figure 1. PCA score plot of all samples in test mixtures of key odorants containing ammonium at pH 6. Samples surrounded by dashed line (16 samples) contain high ammonium concentration (10^{-4} to 10^{-3} M). Full cross validation was used and six electrodes were sufficient.

Samples having ammonium concentrations equal to and higher than 5×10^{-6} M (23 samples including one outlier) could be modelled reasonably. PLS-1, full cross validation and two principal components were used and six electrodes (no. 2, 5, 6, 7, 8, 9) were sufficient. The principal components accounted for 92% and 93% of total validated variance of X and Y, respectively. Slope, correlation (r), RMSEP and RPD of the calibration curve were 0.93, 0.95, 0.26 and 3.35, respectively (Fig. 3). The model is an acceptable model, since the RPD is greater than 2.5, and it has a good slope and correlation. For modelling ammonium using BPNN, 23 samples in triplicates (69 samples) were split into train, test and validation sets, *i.e.* 33, 18 and 18, respectively. The BPNN used 6, 3, 1 nodes. Six electrodes were sufficient (no. 2, 5, 6, 7, 8, 9). Slope, correlation, RMSEP and RPD of the calibration curve were 0.92, 0.98, 0.18 and 4.40, respectively (Fig. 4). It is noticed that slope, correlation, RMSEP and RPD showed an improvement in the BPNN model compared to the PLS-1 model.
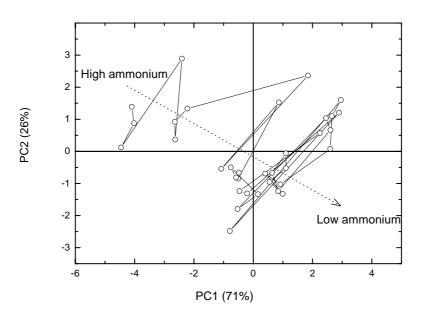
Figure 2. PCA score plot of 34 samples in test mixtures of key odorants containing ammonium at pH 6. Concentration of n-butyrate was below 10^{-4} M. Full cross validation was used and six electrodes were sufficient.
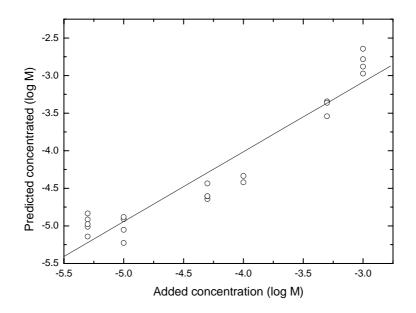

Figure 3. Calibration curve of ammonium from 5×10^{-6} to 10^{-3} M at pH 6. PLS-1, full cross validation for 22 samples and two PCs were used and six electrodes were sufficient. Concentration of n-butyrate was below 10^{-4} M.
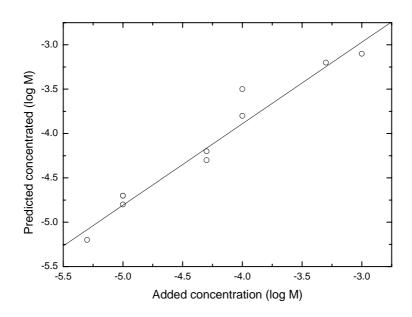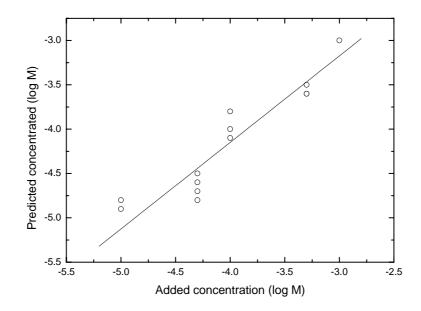
Figure 4. Calibration curve (18 samples) of ammonium from 5×10^{-6} to 10^{-3} M at pH 6. BPNN used 6, 3, 1 nodes. Concentration of n-butyrate was below 10^{-4} M.

It was possible to model n-butyrate, if the concentration of ammonium was below 5×10^{-4} M, and the concentration of n-butyrate was equal to or higher than 10^{-5} M. The 29 samples in triplicates (87 samples) were split into train, test and validation sets, *e.g.* 48, 21 and 18, respectively. The BPNN used 6, 8, 1. Six electrodes were sufficient (no. 2, 5, 6, 7, 8, 9). Slope, correlation, RMSEP and RPD of the calibration curve were 0.97, 0.94, 0.28 and 2.56, respectively (Fig. 5).

Figure 5. Calibration curve (18 samples) of n-butyrate at pH 6. BPNN used 6, 8, 1 nodes. Concentration of ammonium was below 5×10^{-4} M.

In quantification of both ammonium and n-butyrate, we found modelling limitations. It was noticed that ammonium could be modelled if the concentration of n-butyrate was below 10^{-4} M (between 10^{-7} to 5×10^{-5} M). Also n-butyrate could be modelled if the concentration of ammonium was below 5×10^{-4} M (between 10^{-7} to 10^{-4} M). Considering these limitations, the sample number was decreased from 50 to 27. When modelling ammonium from 5×10^{-6} to 10^{-4} M, the number of samples decreased to 16. PLS-1, full cross validation and two principal components were used and six electrodes (no. 2, 5, 6, 7, 8, 9) were sufficient. The two PCs accounted for 94% and 89% of the total calibrated variance of X and Y, respectively. The PLS-1 score plot (Fig. 6 a) shows that ET can monitor ammonium. Samples with high ammonium are located to the right side of the figure. Slope, correlation, RMSEP and RPD of the calibration curve were 0.86, 0.92, 0.20 and 2.5, respectively (Fig. 6 b). These results indicate that the ET can monitor ammonium in the presence of the other key odorants, if the concentration of n-butyrate is below 10^{-4} M.

3.2. Test mixtures of key odorants containing p-cresolate at pH 6

Standard deviation of triplicate measurements was between 0 - 17.3 mV and 0 - 6.8 mV when electrodes no. 1-14 and no. 1, 2, 4, 5, 8 were used, respectively. The RSD was between 0 - 15.5% and 0 - 3.5% when electrodes no. 1-14 and no. 1, 2, 4, 5, 8 were used, respectively.

In this test mixture, all samples of key odorants containing high concentrations of n-butyrate (5×10^{-4} - 10^{-3} M) were identified. PLS-1 and full cross validation were used and five electrodes (no. 1, 2, 4, 5, 8) were sufficient. The PLS-1 scores plot (Fig. 7) identifies these samples (10 samples) at the upper right side of the figure. This indicates that the ET can monitor high n-butyrate concentrations (5×10^{-4} - 10^{-3} M) in the test mixture.
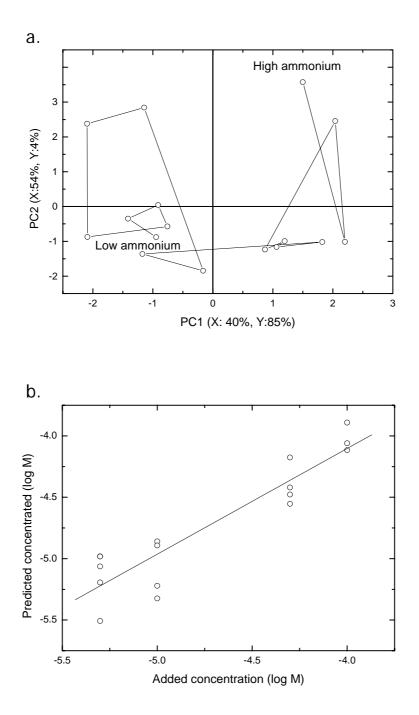
a.

b.

Figure 6. a: PLS-1 score plot for 16 samples of ammonium, considering limits of modelling in test mixtures of key odorants containing ammonium at pH 6. b: Calibration curve of identical ammonium samples. PLS-1, full cross validation and two PCs were used and six electrodes were sufficient.
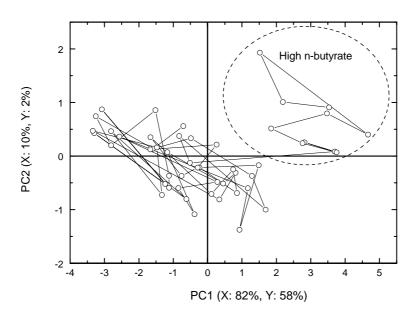
Figure 7. PLS-1 score plot of all samples in test mixtures of key odorants containing p-cresolate at pH 6. Samples (10 samples) with high concentrations (5×10^{-4} - 10^{-3} M) of n-butyrate are surrounded by dashed line. Full cross validation was used and five electrodes were sufficient.

BPNN was used for modelling n-butyrate from 10^{-5} to 10^{-3} M. Thirty-nine samples in triplicates (117 samples) were split into train, test and validation sets, *i.e.* 60, 30 and 27, respectively. The BPNN used 5, 2, 1. Five electrodes were sufficient (no. 1, 2, 4, 5, 8). Slope, correlation, RMSEP and RPD of the calibration curve were 1.02, 0.93, 0.28 and 2.61, respectively (Fig. 8).
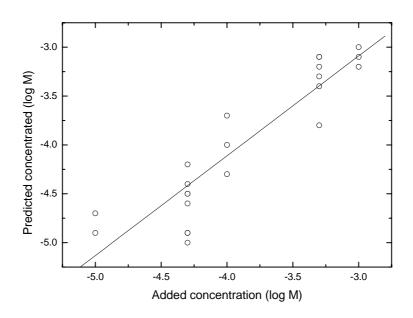
Figure 8. Calibration curve (27 samples) of n-butyrate in test mixtures of key odorants containing p-cresolate at pH 6. BPNN used 5, 2, 1 nodes.

3.3. Test mixtures of key odorants containing ammonium at pH 8

Standard deviation of triplicate measurements was between 0 - 2.6 mV and 0 - 1.6 mV when electrodes no. 1-14 and no. 1, 2, 4, 5, 7, 8 were used, respectively. The RSD was between 0 - 8.4% and 0 - 0.7% when electrodes no. 1-14 and no. 1, 2, 4, 5, 7, 8 were used, respectively. The standard deviation of triplicate measurements and RSD were between 0 - 1.6 mV and 0 - 0.7% when electrodes no. 1, 5, 7, 8 were used.

The PLS-1 score plot of n-butyrate (Fig. 9), shows that ET can monitor all samples (15 samples) containing a high n-butyrate concentration (5×10^{-4} - 10^{-3} M) in the mixture. PLS-1 and full cross validation were used and six electrodes (no. 1, 2, 4, 5, 7, 8) were sufficient. It was noticed that the number of samples with these concentrations, *i.e.* 15 samples, was different from the number of samples in the same mixture in deionised water, *i.e.* 10 samples (Fig. 7). This is because the design of each experiment was carried out independently. However, in both experiments we used uniform distribution.

BPNN was used for modelling n-butyrate. Thirty-six samples were prepared with n-butyrate concentrations from 10^{-5} to 10^{-3} M. These triplicate samples (108 samples) were split into 60, 27 and 21 as train, test and validation sets, respectively. The BPNN used 6, 0, 1. Six electrodes were sufficient (no. 1, 2, 4, 5, 7, 8). Slope, correlation, RMSEP and RPD of the calibration curve were 0.88, 0.94, 0.22 and 2.67, respectively (Fig. 10). This indicates that ET can monitor and model n-butyrate at pH 8, in the presence of the other key odorants in the test mixture of odorants.

It was impossible to model the ammonium concentration. This is most likely explained by the decrease of the ammonium-ammonia ratio in combination with the increased ionisation of the other added key odorants in the test mixture when pH was increased from 6 to 8.
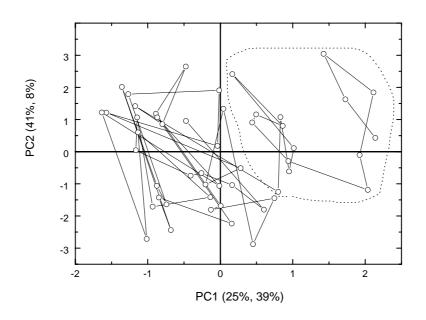
Figure 9. PLS-1 score plot of all samples in test mixtures of key odorants containing ammonium at pH 8. Samples (15 samples) with high concentrations (5×10^{-4} - 10^{-3} M) of n-butyrate are surrounded by dashed line. Full cross validation was used and six electrodes were sufficient.
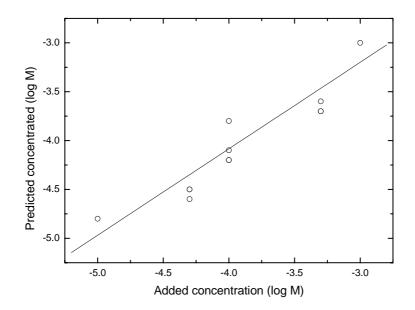

Figure 10. Calibration curve (21 samples) of n-butyrate in test mixtures of key odorants containing ammonium at pH 8. BPNN used 6, 0, 1 nodes.

Phenolate was modelled from 10^{-6} to 10^{-5} M, when the concentration of both n-butyrate and ammonium was below 5×10^{-4} M. Seventeen samples in triplicates (51samples) were split into 24, 15 and 12 for train, test and validation sets, respectively. The BPNN used 4, 4, 1. Four electrodes were

sufficient (no. 1, 5, 7, 8). Slope, correlation, RMSEP and RPD of the calibration curve were 0.89, 0.91, 0.15 and 2.62, respectively (Fig. 11). This indicates that ET has a potential for prediction of phenolate concentration, when the concentrations of ammonium and n-butyrate are low. This result needs further investigations, since the calibration curve covers a rather a small interval of concentrations compared to other key odorants. Nevertheless, it indicates that ET has a potential as a sensor for phenolate as well.
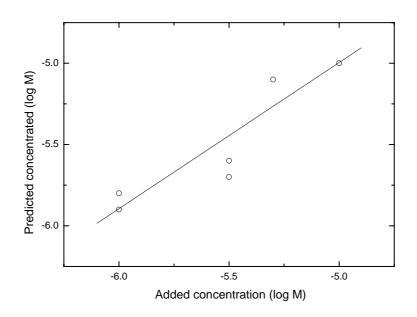

Figure 11. Calibration curve (12 samples) of phenolate in test mixtures of key odorants containing ammonium at pH 8. BPNN used 4, 4, 1 nodes.

3.4. Test mixtures of key odorants containing p-cresolate at pH 8

Standard deviation of triplicate measurements was between 0 - 2.1 mV and 0 - 1.6 mV when electrodes no. 1-14 and no. 2, 5, 6, 7, 8, 9 were used, respectively. The RSD of glass electrodes was high when electrodes no. 1-14 were used, since the potential readings and standard deviations of triplicate measurements were very small, *e.g.* 0, -0.2 mV. When omitting the glass electrodes from the array, the RSD was between 0 - 0.9%. The RSD was between 0 - 0.4% when electrodes no. 2, 5, 6, 7, 8, 9 were used. It is noticed that the standard deviation of triplicate measurements in the mixture of key odorants in phosphate buffer at pH 8 was lower than the standard deviation of triplicate measurements in the same mixture of key odorants in the deionised water with pH 6. This is because the buffered mixture contains higher and stabilized concentrations of ions.

Figure 12 shows that samples with high n-butyrate concentration (5×10^{-4} - 10^{-3} M) in the mixture, can be monitored using PLS-1 score plot. PLS-1 and full cross validation were used and six electrodes (no. 2, 5, 6, 7, 8, 9) were sufficient. It was possible to model n-butyrate from 5×10^{-5} to 10^{-3} M. Twenty-nine samples in triplicates (87samples) were split into 48, 21 and 18 as train, test and validation sets, respectively. The BPNN used 6, 9, 1 nodes. Six electrodes were sufficient (no. 2, 5, 6,

7, 8, 9). Slope, correlation, RMSEP and RPD of the calibration curve were 0.83, 0.97, 0.14 and 3.22, respectively (Fig. 13).
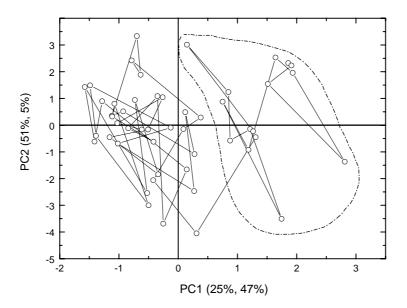

Figure 12. PLS-1 score plot of all samples in test mixtures of key odorants containing p-cresolate at pH 8. Samples (15 samples) with high concentrations (5×10^{-4} - 10^{-3} M) of n-butyrate are surrounded by dashed line. Full cross validation was used and six electrodes were sufficient.
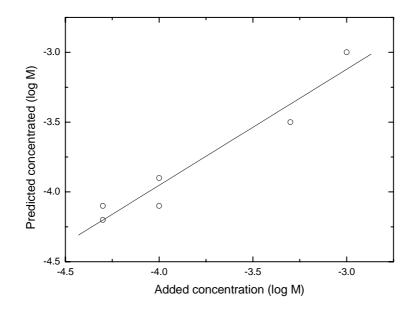

Figure 13. Calibration curve (18 samples) of n-butyrate in test mixtures of key odorants containing p-cresolate at pH 8. BPNN used 6, 9, 1 nodes.

3.5. Potential of ET for on-line measurement of odorants

A summary of results of all experiments is shown in Table 4. The modelling using BPNN was preferred in most of the analytical procedures. This was due to the non-linear relation between the response of electrodes (independent variables, or predictors) and the concentration of the key odorants (dependent variables) [48]. The non-linear response of the electrodes results from interferences between ions in the test mixtures [18]. PCA and PLS can explain linear models only. They are able to show the linear projection of samples, and to model the concentration of key odorants in a linear way. If PLS is used for modelling of non-linear relations a high number of principal components is required, which may lead to overfitting. Therefore, the BPNNs were preferred for modelling, and they could model concentrations in the range below what was possible using PCA or PLS score plots. PCA and PLS score plots show the linear relation between samples in two dimensions. BPNN used all dimensions of the inputs, *i.e.* electrode signals, for non-linear modelling of concentration.

In all modelling, it was noticed that inclusion of measurements of the wire and the two glass electrodes decreased the quality of models. Therefore we excluded these electrodes from all our models *i.e.* PCA, PLS and BPNN.

The ET used in this study was a custom made prototype, which was used for the first time. This study served a dual purpose. The first goal was to test the ET in identification and quantification of key odorants, and the second goal was to simplify the array by decreasing the number of electrodes. Both goals have been achieved. It was possible to reduce the number of electrodes sufficient for modelling without any loss of analytical information (Table 4), since the calibration curves of different key odorants had a high correlation coefficient, reasonable slope, small RMSEP and an acceptable RPD. The reduction of number of electrodes was achieved using multivariate data analysis. For example, six electrodes only were sufficient for all identification and quantification models in the test mixtures of key odorants containing ammonium at pH 6. Six and four electrodes were sufficient to model n-butyrate and phenolate, respectively in the test mixture of key odorants containing ammonium at pH 8. By inclusion of individual electrodes sufficient for analysis of all four test mixtures of key odorants, it is seen that eight electrodes (no. 1, 2, 4, 5, 6, 7, 8, 9) were sufficient for identification and quantification of n-butyrate, ammonium and phenolate. The decreased, but sufficient number of electrodes improved the repeatability, since the standard deviation and the RSD decreased, as shown in Table 5.

ET measured mainly ions in the mixtures [49]. The percentage of ionised n-butyric acid, iso-valeric acid, phenol, p-cresol, skatole and ammonium at pH 6 is: 94%, 94%, 0.01%, 0.005%, 0 % and 100%, respectively. For comparison, the percentage of ionised n-butyric acid, iso-valeric acid, phenol, p-cresol, skatole and ammonium at pH 8 is: 100%, 100%, 1%, 0.5%, 0% and 95%, respectively.

The ET could identify and quantify different key odorants, *i.e.* ammonium, n-butyrate and phenolate, in different mixtures at different acidities. These results are promising for the application in bioscrubbers, since most existing bioscrubber designs focus on the removal of one single type of compound only [50], or removal of one single compound only [51].

Table 4. Summary of results for different test mixtures of key odorants at pH 6 and pH 8.

pH	Test mixture of key odorants	Sufficient electrodes out of 14	Key odorant [i]	Identified (I) and quantified (Q) key odorant
6	Containing ammonium	2, 5, 6, 7, 8, 9	ammonium	I. between 10^{-4} - 10^{-3} M (Fig. 1)
		2, 5, 6, 7, 8, 9	ammonium	I. between 10^{-7} - 10^{-3} M, when concentration of n-butyrate was $< 10^{-4}$ M (Fig. 2)
		2, 5, 6, 7, 8, 9	ammonium	Q. between 5×10^{-6} - 10^{-3} M, when concentration of n-butyrate was $< 10^{-4}$ M (Fig. 3 and Fig. 4)
		2, 5, 6, 7, 8, 9	n-butyrate	Q. between 10^{-5} - 10^{-3} M, when concentration of ammonium was $< 5 \times 10^{-4}$ M (Fig. 5)
		2, 5, 6, 7, 8, 9	ammonium	I. between 5×10^{-6} - 10^{-4} M, when concentration of n-butyrate was $< 10^{-4}$ M, and concentration of ammonium was $< 5 \times 10^{-4}$ M (Fig. 6 a)
		2, 5, 6, 7, 8, 9	ammonium	Q. between 5×10^{-6} - 10^{-4} M, when concentration of n-butyrate was $< 10^{-4}$ M, and concentration of ammonium was $< 5 \times 10^{-4}$ M (Fig. 6 b)
6	Containing p-cresolate	1, 2, 4, 5, 8	n-butyrate	I. between 5×10^{-4} - 10^{-3} M (Fig. 7)
		1, 2, 4, 5, 8	n-butyrate	Q. between 10^{-5} - 10^{-3} M (Fig. 8)
8	Containing ammonium	1, 2, 4, 5, 7, 8	n-butyrate	I. between 5×10^{-4} - 10^{-3} M (Fig. 9)
		1, 2, 4, 5, 7, 8	n-butyrate	Q. between 10^{-5} - 10^{-3} M (Fig. 10)
		1, 5, 7, 8	phenolate	Q. between 10^{-6} - 10^{-5} M, when concentration of n-butyrate and ammonium were $< 5 \times 10^{-4}$ M (Fig. 11)
8	Containing p-cresolate	2, 5, 6, 7, 8, 9	n-butyrate	I. between 5×10^{-4} - 10^{-3} M (Fig. 12)
		2, 5, 6, 7, 8, 9	n-butyrate	Q. between 5×10^{-5} - 10^{-3} M (Fig. 13)

[i] Key odorant identified (I) and/or quantified (Q)

Table 5. Standard deviation (StDev) and relative standard deviation (RSD) of triplicate measurements with total and sufficient numbers of electrodes.

pH	Test mixture of key odorants	Electrode no.	StDev [j] (mV)	RSD [j] (%)
6	Containing ammonium	1-14	0 - 11	0 - 4.8
		2, 5, 6, 7, 8, 9	0 - 5.6	0 - 3.4
6	Containing p-cresolate	1-14	0 - 17.3	0 - 15.5
		1, 2, 4, 5, 8	0 - 6.8	0 - 3.5
8	Containing ammonium	1-14	0 - 2.6	0 - 8.4
		1, 2, 4, 5, 7, 8	0 - 1.6	0 - 0.7
		1, 5, 7, 8	0 - 1.6	0 - 0.7
8	Containing p-cresolate	1-14	0 - 2.1	high [l]
		1-11, 14	0 - 2.1	0 - 0.9
		2, 5, 6, 7, 8, 9	0 - 1.6	0 - 0.4

[j] StDev: Standard deviation of triplicate measurements

[k] RSD: Relative standard deviation of triplicate measurements

[l] high: potential readings and standard deviation were very small, which results in high value of RSD

The ability to monitor ammonium indicates that ET has a potential as an alarm system for ammonium in livestock buildings, for which there is a demand [47,52].

ET could reasonably identify and quantify n-butyrate, in most cases. This indicates that n-butyrate can be used as a representative odorant of the mixture of odorants. Moreover, n-butyric acid is considered as an important odorant [29].

By comparing the results of quantification using ET and gas chromatography (GC) [9], it is seen that ET could model phenolate from 10^{-6} to 10^{-5} M. The GC method showed a rectilinear correlation with concentration of phenol from 1.6×10^{-6} to 8.0×10^{-5} M. ET could model n-butyrate from 10^{-5} to 10^{-3} M. Also, the GC had a rectilinear correlation with concentration of n-butyric acid from 1.1×10^{-6} to 5.7×10^{-5} M. These results indicate that ET is comparable to GC in terms of sensitivity. However, ET is the method of preference since it has the potential as an on-line sensor.

Above it is reported that model limitations for both ammonium and n-butyrate were observed. However, it is unlikely that these limitations will have any significance, when the ET is used as an on-line sensor in the bioscrubber. In the case of ammonium, the model limitation occurred in quantification of ammonium when the concentration of n-butyrate was above 5×10^{-4} M. Literature values for minimum and maximum equivalent equilibrium concentrations in water for n-butyrate are 5.2×10^{-7} M and 3.6×10^{-4} M, respectively, so the model limitation concentration range for quantification is marginal compared to the total concentration range.

As far as model limitations for n-butyrate at high ammonium concentration are concerned, the same considerations are valid.

Even in extreme cases the model still identifies both ammonium and n-butyrate with an accuracy sufficient for application of ET in an alarm function. Due to the all limitations, n-butyrate and ammonium can be simultaneously quantified in narrow range: $5 \times 10^{-6} – 5 \times 10^{-4}$ and $10^{-5} – 10^{-4}$ M, respectively.

The simultaneous on-line measurement of ammonium, n-butyrate and phenolate makes ET an obvious candidate for objective characterization of odour emission from livestock buildings. Of equal importance is the application of ET in control of the bioscrubber. By measurement of key odorants in

the liquid after the bioreactor it is possible to optimize the function of the bioscrubber, *i.e.* keeping dissolved odorants below suitable threshold values. By doing this, a sufficient driving force for transport of odorants from the gas to the liquid is maintained. This control is a prerequisite for optimization of the water flow through the nozzles in the absorption column, which is the most energy consuming part of the bioscrubber.

4. Conclusion

This study served a dual purpose. The first purpose was to identify and/or quantify key odorants occurring in livestock buildings using ET. The second purpose was to simplify the construction of the ET and the data analysis by decreasing number of electrodes in ET as much as possible. The ET was calibrated using 4 different test mixtures, each comprising 50 different combinations of key odorants in triplicates, a total of 600 measurements. The ET was able to quantify ammonium and n-butyrate using six electrodes only in the test mixtures of key odorants at pH 6. In the test mixtures containing ammonium at pH 8, n-butyrate and phenolate were quantified using six and four electrodes, respectively. Initially 14 electrodes were investigated in different PCA, PLS and BPNN models, which showed that eight electrodes were sufficient for all identifications and quantifications of n-butyrate, ammonium and phenolate. The decreased, but sufficient number of electrodes improved the performance of the ET because the standard deviation and relative standard deviation of measurements in triplicates decreased in comparison with the array comprising 14 electrodes. Limitations were taken into consideration during identification and quantification of key odorants. These limitations are related to the interference of different ions at different conditions, *i.e.* odorants present in mixtures at different acidities. Further research with more cross-sensitive electrodes is needed. However, the results indicate that ET has a promising potential as an on-line sensor for measurement of odorants in livestock buildings as a prerequisite for control of odorant emission from livestock buildings.

Acknowledgements

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